Is Neonatale Screening op Mucoviscidose aangewezen in België?

KCE reports 132A

Federaal Kenniscentrum voor de Gezondheidszorg
Centre fédéral d'expertise des soins de santé
2010

Het Federaal Kenniscentrum voor de Gezondheidszorg

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Acknowledgements:	Wij willen volgende personen danken voor hun waardevolle hulp bij het verzamelen en bespreken van gegevens uit de Belgische en Franse Mucoviscidose Registers: Muriel Thomas, Simeon Wanyama en Herwig Jansen van het Wetenschappelijk Instiuut Volksgezondheid dat momenteel belast zijn is het Belgisch Mucoviscidose Register, Sophie Ravilly ("Vaincre la Mucoviscidose"), Lydie Lemonnier ("Vaincre la Mucoviscidose") en Gill Bellis (Institut National d'Etudes Démographiques, Paris).
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Conflict of interest:	Geen gemeld.
Disclaimer:	De externe experten werden geraadpleegd over een (preliminaire) versie van het wetenschappelijke rapport. Nadien werd een (finale) versie aan de validatoren voorgelegd. De validatie van het rapport volgt uit een consensus of een meerderheidsstem tussen de validatoren. Dit rapport werd unaniem goedgekeurd door de Raad van Bestuur. Alleen het KCE is verantwoordelijk voor de eventuele resterende vergissingen of onvolledigheden alsook voor de aanbevelingen aan de overheid.
Lay-out:	Verhulst Ine

Brussel, 15 juli 2010

Studie nr: 2009-13

Domein: Health Technology Assessment (HTA)

MeSH: Cystic Fibrosis ; Newborn Screening

NLM Classificatie: WI 820

Taal: Nederlands, Engels

Formaat: Adobe® PDF™ (A4)

Wettelijk depot: D/2010/10.273/41

Dit document is beschikbaar van op de website van het Federaal Kenniscentrum voor de gezondheidszorg.

Hoe refereren naar dit document?

Proesmans M, Cuppens H, Vincent MF, Palem A, De Boeck K, Dierickx K, Nys H, Neyt M, Vinck I, Lebecque P. Is Neonatale Screening op Mucoviscidose in aangewezen België? Health Technologie Assessment (HTA). Brussel: Federaal Kenniscentrum voor de Gezondheidszorg (KCE). 2010. KCE reports 132A. D/2010/10.273/41

VOORWOORD

Mucoviscidose of Cystic Fibrosis (CF) is een relatief zeldzame erfelijk overdraagbare aandoening waarbij het kind van beide ouders een gemuteerd CF gen heeft geërfd. Vooral de werking van de pancreas en de longen is aangetast door ophopingen van taai slijm, wat aanleiding geeft tot een onvoldoende voedselopname en chronische longinfecties. Een halve eeuw geleden stierven de meeste van deze kindjes in de kinderjaren. Door een betere en intensieve behandeling is de levensverwachting intussen aanzienlijk toegenomen. Wie vandaag met deze ziekte geboren wordt, mag hopen de leeftijd van vijftig jaar te bereiken. Hoe vroeger de diagnose wordt gesteld, hoe vroeger ook een aangepaste behandeling kan worden gestart. Zo vermijd je een lange diagnostische zoektocht voor ouders en kind, en ook – zo hoopt men – voorkomt men dat reeds vroegtijdig longschade optreedt, of groeiachterstand.

Kan een systematische screening van alle pasgeborenen deze belofte waarmaken, en heeft ze een gunstige invloed op de levensverwachting? Maar ook de vraag wat een dergelijk programma als risico's inhoudt, en wat het zou kosten. En is het dus zinvol om 120 000 pasgeborenen te screenen om de diagnose met enkele maanden of een jaar te vervroegen voor een 20-tal kinderen? En zo ja, hoe doe je dit best? Dit zijn de vragen waarover dit rapport zich buigt.

We wensen hierbij de multidisciplinaire equipe te danken die dit rapport grotendeels geschreven heeft, alsook de externe experts en validatoren die een kritische en waardevolle bijdrage leverden.

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Samenvatting

INTRODUCTIE

MUCOVISCIDOSE

Mucoviscidose (Cystic Fibrosis, CF) is een aangeboren ziekte die autosomaal recessief wordt overgeërfd. CF komt meer voor bij kaukasische bevolkingsgroepen. In België worden elk jaar ongeveer 35 kinderen gediagnosticeerd met CF. Om aangetast te zijn moeten de kinderen een ziekte veroorzakende mutatie dragen in beide genen die coderen voor het 'Cystic Fibrosis Transmembrane Conductance Regulator (CFTR)' gen. Dit resulteert in een tekort aan functioneel CFTR eiwit. Dit eiwit komt vooral voor in de celmembraan van de luchtwegen, de gastro-intestinale tractus, de zweetklieren en de reproductieve tractus. De afwezigheid van functioneel CFTR eiwit verhoogt de viscositeit van de exocriene secreties. De meest ernstige gevallen vertonen een meconioum ileus de eerste dagen van het leven. De hoofdkenmerken van de ziekte zijn insufficiëntie van de exocriene pancreas en chronische infecties van de luchtwegen. Colonizatie met *Pseudomonas aeruginosa* komt frequent voor en gaat gepaard met een achteruitgang van de longfunctie.

BEHANDELING EN OVERLEVING

De mediane leeftijd bij overlijden van CF patiënten is sterk gestegen gedurende de voorbije decennia, van minder dan 5 jaar in 1950 tot 25 jaar vandaag. Voor CF kinderen vandaag geboren wordt een mediane levensverwachting van 50 jaar voorspeld (dit moet echter nog worden aangetoond). Dit komt vooral door een verbeterde "symptomatische" behandeling zoals die wordt opgestart en regelmatig wordt opgevolgd in gespecialiseerde centra bij CF kinderen vanaf een zeer vroege leeftijd. De behandeling omvat voedingssupplementen, het vrijmaken van de luchtwegen (oa via fysiotherapie van de longen), het bestrijden van luchtwegen infecties (inclusief intraveneuse antibiotica) en het onderdrukken van de ontsteking. Het nadeel blijft de omvangrijke tijdsinvestering voor deze behandelingen (ongeveer 2 uur per dag gemiddeld). Indien een longtransplantatie kan plaatsvinden op het juiste moment kan dit ook de overleving verhogen. In België zijn er zeven CF referentie centra. Ze bevinden zich allemaal in een universitair ziekenhuis en ontvangen sinds 1999 financiële steun van het RIZIV via een conventie. Deze centra behandelen de meeste CF patiënten in het land en registreren een aantal klinische gegevens in het Belgische CF register.

DIAGNOSE EN SCREENING

Patiënten met CF hebben verhoogde concentraties van natrium en chloor in hun zweet en een 'zweettest' wordt gebruikt voor de diagnose van CF bij kinderen met tekens en symptomen van de ziekte. In contrast met deze klassieke diagnostische benadering, worden bij CF NBS vooral asymptomatische pasgeborenen opgespoord. In dit geval zal een CF DNA test die een groot aantal mutaties kan detecteren vaak als tweedelijnstest worden uitgevoerd. Gezien het klinische fenotype voor een groot aantal minder frequente mutaties niet goed gekend is kan de diagnose van CF dus best moeilijk zijn bij tot 15% van de gevallen die via screening gedetecteerd worden.

NEONATALE SCREENING PROGRAMMA'S

Neonatale screening programma's voor CF zijn bestudeerd sinds 1985 en werden daarna geïntroduceerd in veel westerse landen of regio's, inclusief een groot aantal staten in de US. In België werd tot nu geen systematisch CF NBS programma opgestart. Deze programma's zijn momenteel gebaseerd op de detectie in het bloed van 'immunoreactief trypsinogeen' (IRT), een merker van pancreas schade. Het nut van het toevoegen van een tweede merker voor pancreas schade, 'pancreatic associated protein' (PAP), is onder studie. Er is geen extra bloedafname nodig voor deze eerste screening test gezien gedroogde bloedspots gebruikt kunnen worden. Deze worden in de routine gecollecteerd bij pasgeborenen voor de detectie van aangeboren metabole ziekten. Zelfs gebruik makend van een zeer hoge IRT cut-off waarde, zullen de meeste pasgeborenen die positief testen echter geen CF hebben. Een tweedelijnstest is daarom nodig. In de meeste algoritmen volgt dan een DNA test op de originele bloed spot. In sommige programma's wordt een tweede bloedstaal afgenomen en wordt de IRT test herhaald, dit om DNA testen op grote schaal te vermijden. Nochtans kan de effectiviteit van het programma hieronder lijden gezien het verkrijgen van een tweede staal niet mogelijk blijkt bij een klein deel van de doelgroep.

ONDERZOEKSVRAGEN EN METHODES

De volgende onderzoeksvragen werden bestudeerd:

- Welke gegevens zijn gekend ivm de klinische voor- en nadelen van neonatale screening voor CF?
- Is CF NBS kosteneffectief?
- Welke organisatorische, ethische, legale, en budgetaire aspecten dienen te worden meegenomen bij de introductie van CF NBS in België.

Het project werd grotendeels uitgevoerd door een multidisciplinair team van pediaters met ervaring in CF zorg, wetenschappers en experts in neonatale screening, in ethiek en in medisch recht. De voortgang van het project werd regelmatig geëvalueerd door bijkomende externe experts in CF NBS en vertegenwoordigers van de gemeenschappen die voor preventieve zorg verantwoordelijk zijn.

Een systematisch literatuuronderzoek werd uitgevoerd naar de effectiviteit en de kosteneffectiviteit van CF NBS. De organisatorische, ethische, legale en budgetaire aspecten zijn gebaseerd op bijkomende verzamelde gegevens, gepubliceerde data en analyses van het Belgische CF register van het Wetenschappelijk Instituut voor Volksgezondheid.

VOORDELEN EN NADELEN

Het doel van CF NBS is de grote meerderheid van de CF patiënten te diagnosticeren voor de leeftijd van 2 maand. Door een vroegtijdige diagnose en het instellen van een optimale behandeling kan de longfunctie bij CF patiënten beter bewaard worden tot het moment dat ze hopelijk voordeel kunnen halen uit toekomstige behandelingen die het basisdefect corrigeren. Een vroege diagnose zal ook een potentieel lange en kostelijke diagnostische lijdensweg helpen vermijden.

DE GERAPPORTEERDE VOORDELEN

De op studies gebaseerde ondersteuning van CF NBS is niet zo sterk als men zou verwachten, wetende dat er toch twee grote gerandomiseerde studies (RCTs) werden opgezet om CF NBS te evalueren. Het ontwerp van de UK RCT (1985-1989) was ondermaats en deze studie werd daarom niet weerhouden in een recente Cochrane review. De Wisconsin RCT (1985-1995) had wel een correcte design en toonde een significant voordeel van CF NBS op het gebied van voeding en groei (gewicht en lengte). Op het gebied van longfunctie kon echter geen voordeel van CF NBS aangetoond worden op de lange termijn gezien de longen van gescreende CF babies vroeger gekoloniseerd werden door *Pseudomonas aeruginosa* in vergelijking met klinische gediagnosticeerde kinderen. Dit werd verklaard door een gebrek aan hygiëne-maatregelen zoals het gebrek aan een strikte scheiding van gekoloniseerde en niet gekoloniseerde patiënten in een van de twee deelnemende centra.

De conclusies die men kan trekken op basis van klinische gegevensbanken en enkele cohortestudies zijn beperkt door twee redenen. Ten eerste, de "symptomatische" behandeling is duidelijk verbeterd over de tijd en dit had een dramatische impact op de overleving. Ten tweede, bij screening hebben ongeveer 15% van de gedetecteerde kinderen een zeer mild fenotype. Zonder screening zouden ze nooit meegeteld zouden worden in de CF patiëntengroep. Beide redenen maken dat de groep van gescreende patiënten over het algemeen een betere gezondheidstoestand hebben in vergelijking met historische controles die klinisch gediagnosticeerd werden. De echte bijdrage van CF NBS blijft daarom moeilijk om in te schatten. Op basis van de observationele data wordt er gesuggereerd dat CF NBS aanleiding geeft tot een verbeterde longfunctie en dat het de therapielast vermindert.

Het is vermeldenswaardig dat de hoogste mediane overleving bij CF gezien wordt in Denemarken en Zweden, zonder screening, maar gebruik makend van dure en intensieve zorgprogramma's.

DE GERAPPORTEERDE NADELEN

De detectie door screening van kinderen met twijfelachtige ('equivocal') test resultaten die een "variante" vorm van CF suggereren kan voor de familie onzekerheid en angst veroorzaken en resulteren in een potentieel onnodige behandeling zonder duidelijk voordeel. Dit probleem is echter nog niet afdoende onderzocht in de literatuur.

Afhankelijk van het gekozen algoritme in het screeningsprogramma maar ook van de beslissing om al of niet asymptomatische pasgeborenen met een intermediaire zweettest verder te onderzoeken, zullen deze zogenaamde 'equivocal' gevallen tot 15% van de "CF" kinderen uitmaken die gedetecteerd worden via screening. Dit is nu het geval in Frankrijk. Deze gevallen geven aanleiding tot meerdere niet beantwoorde vragen. Bovendien zijn de voordelen van CF NBS voor kinderen met een milde vorm van de ziekte nooit aangetoond. Ongeveer de helft van deze gevallen zijn gerelateerd aan de detectie van de R117H mutatie waarvoor recent een zeer lage graad van penetratie werd aangetoond. Het lijkt aangewezen om het aantal van zulke 'equivocal' gevallen te beperken.

Een ander potentieel risico verbonden aan het invoeren van CF NBS is dat artsen de diagnose van CF niet meer zouden overwegen bij patiënten die zich presenteren met suggestieve symptomen.

CF ZORG IN BELGIË

De gegevens voor België zijn gebaseerd op publicaties door de CF centra en analyses van het Belgische CF register. Volgens internationale richtlijnen dienen kinderen bij wie CF vermoed wordt of die via CF NBS gedetecteerd zijn, onmiddellijk doorverwezen worden naar een gespecialiseerd CF centrum voor diagnose, opstarten van de behandeling en regelmatige opvolging. De studie van de plaats van de CF centra en de andere pediaters en huisartsen in de opvolging van de CF patiënten was niet opgenomen in de scope van deze studie. Toch moet vermeld worden dat in 2004 nog 30% van de CF kinderen pas meer dan 2 jaar na diagnose voor het eerst doorverwezen werden naar een CF centrum.

In België worden 16% van de CF kinderen reeds gedetecteerd via niet gestandaardiseerde lokale CF NBS programma's. Voor sommige van deze lokale programma's werd klinische follow-up na screening als een probleem gerapporteerd en een aparte analyse van CF kinderen gediagnosticeerd via screening bleek niet mogelijk. Bij de andere CF patiënten zou screening de mediane diagnoseleeftijd kunnen doen dalen van 10 maand naar minder dan 2 maand.

In België bestaan er nog steeds variaties per centrum in de kwaliteit van de zorg voor CF. Niettegenstaande deze variatie en de afwezigheid van een georganiseerde neonatale screening hebben CF patiënten in België een mediane overleving die goed overeenkomt met die in andere westerse landen.

Ongeveer de helft van de CF patiënten in België zijn homozygoot voor de frequente F508del mutatie en 70% van de patiënten draagt deze mutatie op minstens een van de allelen.

HET ALGORITME EN DE GEVOLGEN

In lijn met de meeste CF NBS algoritmen die vandaag in gebruik zijn start het voorgestelde algoritme met de IRT test voor de initiële screening, gevolgd door een CF DNA test in IRT positieve gevallen. Indien zou beslist worden CF NBS te implementeren, dient rekening te worden gehouden met de bestaande infrastructuur voor neonatale screening die zowel voordelen alsook een aantal nadelen kan hebben bij de invoering van CF NBS.

De informatiebrochures voor de ouders kunnen makkelijk aangepast worden om hen te informeren over het uitgebreid screening programma. Alvorens bloed afgenomen wordt van de pasgeborene dienen (een van) de ouders expliciete mondelinge toestemming te geven na het ontvangen van de nodige informatie over het uitgebreid neonataal screening programma, inclusief CF, en het risico op vals positieve en vals negatieve resultaten. Geschreven geïnformeerde toestemming is nodig indien men overweegt het bloedstaal ook voor onderzoeksdoeleinden te gebruiken. Het routinematig afgenomen bloedspot staal kan gebruikt worden voor de initiële testen (IRT en CF DNA) en volgens het voorgestelde algoritme zal het slechts uitzonderlijk nodig zijn een tweede staal af te nemen.

Dit screening algoritme vereist echter meer standaardisatie van de methodes over de deelnemende laboratoria dan wat vandaag het geval is. Tevens introduceert het algoritme formeel een DNA test als tweedelijnstest.

De uitdaging van het gebruik van de 99,5 percentiel voor IRT.

De initiële screening is gebaseerd op het gebruik van de percentiel 99,5 als cut-off waarde voor IRT. IRT is een eerder goedkope test om uit te voeren maar voor de neonatale screening laboratoria die deze test wensen uit te voeren houdt dit een volledige standaardisatie in van de analytische methode tussen de laboratoria (protocol, reagentia merk en batch, en afleesinstrument). De aankoop van een gemeenschappelijk systeem kan best gebeuren via publieke aanbestedingsprocedure. Het voordeel van het integreren van de PAP test in het algoritme is nog onder studie. Deze test zou potentieel het aantal DNA analyses nog verder kunnen doen dalen, en dus ook het aantal gedetecteerde gezonde dragers en 'equivocal' mutaties. Resultaten van een pilootstudie hebben recent geleid tot een aanbeveling door de Gezondheidsraad in Nederland om te starten met CF NBS op basis van IRT en PAP.

DNA testen voor CF en gerelateerde problemen

Volgens het voorgestelde screening algoritme, zal de tweede screening stap, een CF DNA test, uitgevoerd worden op ongeveer 600 bloedspot stalen (0,5% van de ongveer120 000 neonaten elk jaar). De mutaties die veel voorkomen in de Belgische bevolking zijn opgenomen in de mutatie panels van commercieel beschikbare test kits. Voor immigranten (en als de kost significant daalt misschien voor alle stalen in de toekomst) is sequencing van de CFTR allelen de meest gepast methode. Het laboratorium zou enkel dienen te screenen voor mutaties die geassocieerd zijn met een significant risico op ernstige ziekte. De voordelen van het screenen op 'equivocal' mutaties zijn niet duidelijk en de communicatie van positieve resultaten zonder klinisch belang zou psychologische schade kunnen veroorzaken. De periode tussen het informeren van de familie van een positief screening resultaat en het onderzoek nodig voor een diagnose dient zo kort mogelijk te zijn.

Het voorstellen van CF DNA analyse aan de ouders dient met de nodige voorzichtigheid te gebeuren, gezien uit de test soms het kan blijken dat de vader niet de natuurlijke vader is. Hierbij dient opgemerkt dat een ongekende fractie van de 15000 CF DNA testen die elk jaar terugbetaald worden in België gebruikt worden voor opportunistische carrier screening bij toekomstige ouders. In tegenstelling tot CF NBS, zijn de voor- en nadelen, de kosten en de ethische aspecten van deze opportunistische vorm van screening van toekomstige ouders niet goed gedocumenteerd. Het is duidelijk dat het nut van CF NBS grotendeels wegvalt indien beide ouders reeds negatief testten op CF DNA.

De Zweettesten

Wegens hun belang bij de diagnose en hun technische moeilijkheidsgraad zouden zweettesten enkel mogen uitgevoerd worden in laboratoria die strikt de internationale richtlijnen volgen en ISO 15189 geaccrediteerd zijn voor deze test.

GEZONDHEIDSECONOMISCHE OVERWEGINGEN

Er zijn te weinig betrouwbare gegevens die als input kunnen dienen van economische modellen van CF NBS. Vooral het effect van screening los van de verbeterde behandeling over de tijd is onduidelijk. Na een systematisch literatuuronderzoek werden geen kosteneffectiviteitanalyses van CF NBS weerhouden die en robuust en ook nog actueel waren. Sommige analyses veronderstellen een verbetering in kwaliteit van leven bij kinderen gedetecteerd via screening, zonder dat daar echter betrouwbare metingen rond bestaan. Een recente kosteneffectiviteitanalyse voor Nederland was gebaseerd op de veronderstelling dat na CF NBS de 6% mortaliteit bij CF kinderen onder de 10 jaar zou dalen met 1.5%. Deze aanname is gebaseerd op mortaliteitsdata uit het verleden en is niet langer actueel. Momenteel is de mortaliteit bij CF kinderen onder de leeftijd van 10 jaar 2% in België. Veronderstellen dat na CF NBS er nog een daling komt met 1,5% in mortaliteit lijkt weinig realistisch.

De budget impact van NBS CF in België werd ingeschat op €340 000 – €635 000, enkel gebaseerd op de kosten voor het uitvoeren van IRT, DNA, een mogelijke tweede IRT en de zweettesten. Een groot aantal andere items (bvb kosten voor het behalen van ISO 15189 accreditatie, kosten voor coördinatie en supervisie, kosten voor genetische counseling, prijsonderhandelingen, enz.) kunnen deze budget impact schatting verhogen of verlagen. Gebaseerd op expert opinie kan het totale budget wellicht onder de €1 miljoen blijven.

Een belangrijke vaststelling is dat de reële kosten en de waarde van de RIZIV terugbetaling voor die activiteit sterk kunnen verschillen, zoals voor CF DNA testen (€150 kost t.o.v. €320 terugbetaling) en zweettesten (€126 kost t.o.v. €72 terugbetaling voor test in duplo). Het aantal testen in de context van CF NBS vertegenwoordigen voor CF DNA (n=600, plus mogelijks detective van dragers van het gen) en zweettesten (n = 75 pasgeborenen x 2 testen per kind) slechts een kleine fractie van de in totaal 15000 CF DNA testen en 6400 aparte zweettesten die elk jaar terugbetaald worden, zelfs rekening houdend met een klein aantal klinische CF diagnoses die gemist werden door de screening en het testen van broers en zussen van geïdentificeerde CF kinderen.

De budget impact voor het implementeren van CF NBS is klein in vergelijking met het budget dat elk jaar gespendeerd wordt voor het terugbetalen van de CF DNA testen, grotendeels voor het detecteren van dragers van het gen op ingestuurde stalen. Alleen al de terugbetaling van CF DNA testen aan de reële kost zou een jaarlijkse besparing opleveren van meer dan €2 miljoen, of meer dan het dubbel van het budget dat nodig is om CF NBS te financieren.

CONCLUSIE

De invoering van een programma voor CF neonatale screening programma in België zal een soms langdurige en dure diagnostische lijdensweg helpen vermijden en bij de CF kinderen die zo vroeger gediagnosticeerd worden de groei in lengte en gewicht bevorderen. Mogelijks is er ook een voordeel van CF NBS op longfunctie en wordt de therapie last minder zwaar, maar dit is niet aangetoond in RCTs. De voordelen naar overleving toe zijn waarschijnlijk niet langer aantoonbaar gezien de verbeterde zorg de vroegtijdige sterfte al sterk verminderd heeft. De voordelen van CF NBS zijn enkel groter dan de nadelige effecten, bvb door ‘equivocal’ CF diagnoses, indien een aantal kwaliteitscriteria voor screening strikt gevolgd worden.

AANBEVELINGEN DOOR HET KCE[a]

- **Alle kinderen gediagnosticeerd met CF zouden onmiddellijk na diagnose (gebaseerd op screening of klinisch) moeten kunnen genieten van de hoogst kwalitatieve zorg (die nog betaalbaar is), gezien zulke verbeterde zorg een dramatische impact heeft op de overleving.**
- **Teneinde de kwaliteit van de zorg in de CF centra naar een uniform hoog niveau te brengen, dienen kwaliteitsindicatoren regelmatig te worden gemeten en dient de deelname aan initiatieven tot kwaliteitsverbetering te worden verplicht voor CF centra met een conventie.**
- **Het opstarten van lokale CF NBS programma's zonder evaluatie van patiëntenuitkomsten is een gemiste kans en is te vermijden.**
- **De publieke financiering van twee grotendeels overlappende screening initiatieven dient in vraag gesteld (CF NBS programma en het lopende grootschalige opportunistische screenen op CF dragerschap van toekomstige ouders).**
- **De beslissing om CF NBS te in te voeren hangt voornamelijk af van de balans van de voor- en nadelen. De voordelen van CF NBS zijn groter dan de nadelen enkel indien volgende voorwaarden strikt vervuld zijn:**
  - **Alvorens bloed afgenomen wordt van de pasgeborene dienen (een van) de ouders expliciete mondelinge toestemming te geven na het ontvangen van de nodige informatie over het uitgebreid neonataal screening programma, inclusief CF, en het risico op vals positieve en vals negatieve resultaten.**
  - **De nodige aandacht dient besteed te worden aan een optimale doorstroming van de informatie wanneer een zweettest aangewezen is bij een pasgeborene met een positief screeningsresultaat. De periode tussen het informeren van de familie van een positief screening resultaat en de diagnostische bevestiging dient zo kort mogelijk te zijn.**
  - **De nadelen van screening kunnen tot een minimum beperkt worden indien enkel gescreend wordt voor mutaties die geassocieerd zijn met een significant risico op ernstige ziekte.**
  - **Voor de via screening gedetecteerde kinderen dient de huidige zorgstandaard van strikte scheiding van patiënten op basis van hun microbiologische status te worden gegarandeerd om vroegtijdige kolonisatie met *Pseudomonas aeruginosa* te vermijden.**
  - **Het voorgestelde screening algoritme (gebruik van de 99.5 percentiel als cut-off) vereist dat de IRT testen uitgevoerd worden via eenzelfde test systeem en een enkele batch van reagentia voor gans België. De neonatale screening laboratoria dienen te beschikken over een ISO 15189 accreditatie voor deze tests. Een gezamelijke aankoopprocedure dient te worden overwogen voor de aanschaf van een gemeenschappelijk diagnostisch systeem.**

[a] Alleen het KCE is verantwoordelijk voor de aanbevelingen aan de overheid.

- **De CF DNA testen in de context van screening dienen te worden gestandaardiseerd en enkel te worden uitgevoerd in laboratoria die over een ISO 15189 accreditatie beschikken voor deze test. Bovendien moet de 'test turnaround time' het screening proces respecteren en mag niet meer dan 1 week bedragen. Een verlaging van het terugbetaalde bedrag in lijn met de reële kosten dient te worden overwogen.**
- **Zweettesten mogen enkel uitgevoerd worden in laboratoria die over een ISO 15189 accreditatie beschikken voor deze test en die de internationale richtlijnen voor deze test respecteren. Een verhoging van het terugbetaalde bedrag in lijn met de reële kosten dient te worden overwogen.**
- **Artsen moeten geïnformeerd worden van het belang van een vroege diagnose en doorverwijzing van CF patiënten en ervan bewust zijn dat zelfs na implementatie van CF NBS een aantal gevallen van CF nog steeds gediagnosticeerd moeten worden op basis van ziektetekens en symptomen.**
- **Gezien de complexiteit van CF NBS, de nood voor standaardisatie tussen de centra, en de nood om de outcome van de patiënten op te volgen wordt de oprichting van een permanente multidisciplinaire stuurgroep aanbevolen.**

- **Onderzoeksagenda**
  - **Het evalueren van de integratie van de PAP test in het screening algoritme gebaseerd op de resultaten van de lopende studies en de recente aanbeveling te starten met zulk een screening algoritme in Nederland.**
  - **Het evalueren van de beste praktijk voor de opvolging van de CF patiënten, met inbegrip van de rol van het CF centrum, de pediater en de huisarts.**

Scientific summary

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ABBREVIATIONS

ABLM	Association Belge de Lutte contre la Mucoviscidose
AFDHPE	Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant
BAL	bronchoalveolar lavage
BCXR	Brasfield chest X-ray
bIRT	IRT at birth
BMI	body mass index
BMR	Belgisch Mucoviscidose Register
BVSM	Belgische Vereniging voor de Strijd tegen Mucoviscidose
CBAVD	congenital bilateral absence of the vas deferens
CDC	Centers for Disease Control
CEA	Cost effectiveness analysis
CF	cystic fibrosis
CFF	Cystic Fibrosis Foundation
CFGAC	Cystic Fibrosis Genetic Analysis Consortium
CF NBS	cystic fibrosis newborn screening
CFTR	cystic fibrosis transmembrane conductance regulator
CI	confidence interval
CG	clinical group
CSI	cognitive skills index
CT	computed tomography
CV	coefficient of variation
ECFS	European Cystic Fibrosis Society
ESCF	Epidemiologic Study of Cystic Fibrosis
EQA	external quality assurance
FEV1	forced expiratory volume in 1 second
FVC	forced vital capacity
GP	general practitioner
HAS	Haute Autorité de Santé
HTA	health technology assessment
ICER	Incremental cost-effectiveness ratio
IRT	immunoreactive trypsinogen
LF	lung function
MI	meconium ileus
MRSA	methicillin resistant *Staphylococcus aureus*
NS	neonatal screening
OR	odds ratio
P	percentile

PA	*Pseudomonas aeruginosa*
PAP	pancreatitis associated protein
PERT	pancreatic enzyme replacement therapy
PI	pancreatic insufficiency
PTC	premature termination codon
QNS	quantity not sufficient
RBM	Registre Belge de la Mucoviscidose
RCT	randomized controlled trial
rIRT	resampling IRT
RPN	risk priority number
SG	screened group
SK score	Shwachman-Kulczycki score
WCXR	Wisconsin chest X-ray

1 GENERAL INTRODUCTION

1.1 CYSTIC FIBROSIS

Cystic Fibrosis (CF) is the most common lethal genetic disease in white populations with an incidence throughout the world of 0.25- 5 per 10 000 live births.[1] In other races CF is also found but with a lesser frequency. The disease was first recognized as a separate disease entity by a pathologist in 1938.[2]

CF is a complex multi-organ disease with a variable clinical spectrum. The most typical CF patient has pancreatic insufficiency and recurrent pulmonary symptoms together with an abnormal sweat test. A five-fold excess of chloride in the sweat of CF patients was demonstrated in 1953[3] and permitted the development of the sweat test[4] which remains the gold standard diagnostic tool in CF. Although the vast majority of CF patients are diagnosed through the combination of classic signs and symptoms of the disease and corroborative laboratory results, the diagnosis is not as clear-cut in approximately 5% of individuals with CF. A recent Cystic Foundation Consensus Report summarizes current recommendations and includes detailed guidance for the diagnosis of both infants with positive newborn screening and older patients with less typical clinical picture.[5]

CF is caused by a mutation in a gene that encodes the 'Cystic Fibrosis Transmembrane Conductance Regulator' (CFTR) protein, which is expressed in many epithelial cells. The CFTR gene[6] is located on the long arm of chromosome 7. The corresponding protein is a chloride channel regulating ion and water balance across epithelia.[7, 8]

Absence of functional CFTR results in increased viscosity of exocrine secretions. The hallmarks of the disease are exocrine pancreatic insufficiency and chronic lung infection.

More than 1600 mutations have been identified (see the CFTR mutation database: http://www.genet.sickkids.on.ca/cftr/app). One common mutation (F508del) accounts for about 70% of all mutant CFTR alleles while 10-20 less common mutations represent a further 10-15% of all mutant alleles.

According to their impact on the CFTR protein, mutations have been grouped into 5 classes:[9]

- *Class I* includes mutations which result in disruption of the CFTR protein synthesis and typically lead to the creation of premature termination codons (G542X, W1282X …).
- *Class II* mutations are associated with defective protein processing (F508del, N1303K …). F508del is by far the most common mutation and its prevalence in Belgian CF patients is 67%. These mutations result in the synthesis of a misfolded CFTR protein that will be almost totally degraded in the endoplasmic reticulum (>99% vs 75% in normal protein).
- *Class III* mutations lead to the production of proteins which reach the plasma membrane. However their regulation is defective: they cannot be activated by ATP or cyclic AMP (G551D …).
- Mutations in the two remaining classes, *class IV* (defective conductance: D1152H …) and *class V* (reduced function/synthesis - mainly including splicing mutations: 3849+10kbC→T …) produce CFTR variants with residual expression and/or function.

The prognosis of a child born with cystic fibrosis in the fifties was very grim with most children dying before their 5th birthday from pancreatic insufficiency (PI) and malnutrition. In the United States, the median survival of an individual with CF is 36.5 years, data published in 2006,[10] with more than 90% of patients dying from lung-related disease. A recent United Kingdom report studied CF mortality in consecutive three year birth cohorts.[11] Comparison of the 1968-1970 cohorts with recent birth cohorts shows that survival has increased dramatically. The median life expectancy for the CF population born in 2000-2003 is estimated at 50 years.

For babies with CF born nowadays, the disease is - although still life shortening - primarily a chronic disease with a high burden of therapy and complex care. This should not mask the impressive list of complications of this disease, several of which are life-threatening. In Belgian as in several western countries close to 50% of current CF patients are 18 years or older. In the next decade the majority of people with CF will be adults. Here too, however, it is clear that this progress comes with prices:

- a number of "new" complications are now emerging in adults, including high prevalence of CF related diabetes of > 30% above 30 years[12]
- a high prevalence of early osteoporosis[13]
- an increased incidence of digestive tract cancers[14] and iatrogenic diseases.

1.2 CF TODAY: AN EXTREMELY DEMANDING SYMPTOMATIC TREATMENT

As a result of a more comprehensive approach of symptomatic treatment, the survival of CF patients improved markedly over the past decades. The drawback is the tremendous burden of therapy: a recent US study in CF adults reported that the daily mean time spent on treatment activities was 108 minutes (± 58).[15] A large fraction of these activities consists of nebulation of mucolytics and inhaled antibiotics together with chest physiotherapy.

Such complex care is best implemented in specialized centres (see chapter 5), which has been recognized more than 40 years ago in North-America.

Four pillars of the symptomatic treatment have long been established. They include nutritional repletion, relief of airway obstruction, treatment of airway infection and suppression of the inflammation.[16]

Yet, CF is a progressive disease of which the natural course that we are just trying to slow still leads to the destruction of the lungs. In end-stage lung disease, life can be extended by lung transplantation. Recent follow-up data on transplanted CF patients show survival rates between 60% and 70% five to seven years after transplantation.[17, 18] It should be kept in mind that the intensity of follow-up and burden of therapy remains high after transplant. CF lung disease is exchanged for post transplant disease with risk of bronchiolitis obliterans, opportunistic infections and post-transplant malignancy.

The most important issue concerning transplantation is timing. Being transplanted too early may shorten survival, while postponing too long means that they may die while on the waiting list. The relative contribution of lung transplant to overall CF survival statistics is currently under debate.[19-21]

1.3 THE FUTURE: TOWARDS A CURATIVE TREATMENT

Advances in symptomatic treatment will continue but new approaches to therapy attacking the basic defect are now required to both further improve the prognosis and reduce the burden of the treatment. The major aim of CF search is now to find a cure.

Since the discovery of the CFTR gene in 1989, CF has been considered a prime candidate for gene therapy by inhalation. However, the search for an efficient vector and the problem of thick mucus that forms a barrier to reach the epithelia have proved to be major obstacles. Additionally, host-specific immune responses generated against a virus-derived vector may pose a problem.[22, 23] While initially promising, research has now been limited to few centres because of disappointing results so far.[24, 25]

Two pharmacological alternative strategies are now explored,[26] some examples of which are detailed below:

- Circumventing the CF-related ion transport defect, by increasing chloride secretion and/or inhibiting excess sodium reabsorption. Several medications aim to restore airway surface liquid without targeting CFTR. Phase 2 trials of MOLI1901 and SPI-8811 are ongoing while Phase 3 trials of hypertonic saline, denufosol, bronchitol have begun.

- Developing allele-specific therapies to correct the specific defects in mutant forms of CFTR.

Class I mutations are characterized by the presence of a premature termination codon (PTC) resulting in a truncated and often non-functional protein. Agents that suppress the normal proof reading function of the ribosome can overcome diseases caused by PTCs. In preclinical settings high dose aminoglycosides induce such a translational 'read-through' and restore protein function,[27] however the potential renal and ototoxicity limit their clinical use. ATALUREN (formerly known as PTC124) has been studied in healthy volunteers and in CF.[28, 29] Results of phase 2 studies have recently been published[30] and a phase 3 study is now ongoing.

Similarly, encouraging data have been reported using VX-770 in adults with CF and at least one copy of the mutation G551D. This "potentiator" is believed to help to open the chloride channel in CF cells. In a randomised, double-blind, placebo-controlled study, this agent appeared to be safe and was associated with significant improvements in terms of the forced expiratory volume in 1 second (FEV1), nasal potential difference measurements and sweat electrolytes. Two Phase 3 studies began in the summer of 2009.

VX-809 is a "corrector" that is expected to improve CFTR cell trafficking and its function as a chloride channel. A large Phase 2a trial of this compound is ongoing in patients homozygous for the F508 del mutation.

1.4 CF NEWBORN SCREENING (CF NBS) IN THIS CONTEXT

Perspectives of curative treatment have modified the objectives of CF dedicated physicians. An important part of the challenges they are now facing is to preserve lung function of many children (and a much smaller proportion of adults) by maximising current treatment regimens, so that they can benefit fully from future therapies that could correct the basic defect.[31]

A number of studies using bronchoalveolar lavage (BAL) markers of infection and inflammation but also lung function tests and more recently computed tomography of the chest have documented that significant lung damage occurs very early in many, even asymptomatic CF infants.[32-35]

Universally coupled with immediate referral to CF centres, CF NBS has expanded rapidly to most western countries including all US states except one within a few years. By permitting earlier diagnosis and optimal treatment, it has the potential to be one of the factors helping many patients to reach the key moment of "the cure discovery" with clear enough lungs. Centre-based[36-38] and/or wider scale quality improvement initiatives[39, 40] are obviously necessary too.

1.5 PURPOSE OF THIS REPORT

This work aimed to address the following issues around CF NBS:

- Clinical effectiveness: summary of current evidence
- Possible harms and how to minimize them
- Current guidelines for implementation and care after screening
- Belgian data: current outcome and room for improvement
- Choosing a screening algorithm
- Ethical and legal aspects
- Economical aspects

The issue of community-wide screening for cystic fibrosis carriers is beyond the scope of this report.

1.6 SEARCH STRATEGY

The search strategy is described in detail below (Appendix 1). The electronic databases Medline and Embase were searched for NBS and CF, using 17 keywords. The priority of this step was to encompass the issues addressed in chapters 2, 3 and 4. Some specific aspects of other chapters necessitated the addition of articles derived from the list of references in relevant publications and the authors' collection of references. Selection of articles and the quality appraisal was performed by two authors of this report (M. Proesmans and P. Lebecque).

- For the review the effectiveness of CF NBS 55 abstracts were selected for which the full texts were read. In the references of these articles 19 additional references were found as possibly relevant. For these 55+19 references (read as full text) the following selection criteria were used: studies comparing outcome parameters of a screened versus a non screened group, RCT (1), non-RCT (2), and reports from CF registries (3). Additionally, systematic reviews were retrieved together with articles on guidelines after screening. Were not selected: non-systematic reviews, letters and comments, studies not specific on screening, studies not comparing a screened versus an non screened group.
- Using these selection criteria 32 articles were selected for chapter 2 (clinical effectiveness). Quality appraisal was performed for the non-RCT studies. For the RCTs the quality appraisal as performed by the authors of the Cohrane review was used. After a quality appraisal (see text and tables in appendix 4, four articles were rejected (detailed in section 2.2.1.4), leaving 28 articles.

1.7 ABOUT A FEW KEY REVIEWS

A few references identified by the search strategy provide such an exhaustive and comprehensive background on the topic of CF NBS that they unavoidably serve as a basis for a large part of the current report and will often be cited. The reader's attention is especially drawn to 5 papers,[41-45] briefly commented in Appendix 2.

2 CLINICAL OUTCOME AFTER NEONATAL SCREENING

2.1 INTRODUCTION

Neonatal screening for CF (CF-NBS) has been in use in some areas as early as the seventies. Initially test quality was insufficient (measuring meconium protein levels). Later, 'immunoreactive trypsinogen' (IRT) in blood, a marker of pancreatic injury was introduced. Infants with CF have a raised IRT level at birth that remains elevated in the first weeks of life. The drawback of the IRT test is the high number of false positives. Combining the IRT with DNA analysis or another biochemical marker (for example 'PAP' pancreatic associated protein) increases test performance. For systematic review, technical aspects and references on neonatal screening algorithms we refer to chapter 6.

Several studies have shown that starting therapy very early (also in asymptomatic children at time of diagnosis) improves clinical outcome and prognosis, 'early' being specified as before 2 months of age.[46, 47] The median age at diagnosis for children with CF diagnosed on clinical ground in Belgium however is 6 months (Belgian CF registry data; see chapter 5).

The rationale of CF-NBS is thus to lower the age at diagnosis and institute adequate treatment and follow-up also in pre-symptomatic infants. The ultimate goal is to improve long term prognosis.

Knowing that CF patients are generally well nourished at birth but develop PI in 90% (around 60% within the first weeks of life[48]), delayed diagnosis will lead to severe malnutrition. The CF lung is normal at birth but lung disease usually develops as early as 2 months. *Pseudomonas aeruginosa* (PA) infection and colonization occur in one out of 3 undiagnosed patients.

The aims of CF-NBS are :

- Early treatment with pancreatic enzyme replacement therapy (PERT), diet and fat soluble vitamins to improve long term nutritional status
- Early therapy to prevent and treat lung infection by close monitoring of sputum cultures in order to slow down progression of lung disease and to diminish treatment burden [49-52]
- Avoiding a diagnostic odyssey with resulting parental stress
- Allowing genetic counselling for future pregnancies
- Reducing burden and thus cost of treatment by preventing complications.

NBS may however carry risks:

- Parental stress around false positive diagnosis and dubious diagnosis
- In order to set-up the necessary early follow-up and treatment, infants even when presymptomatic, may be exposed to nosocomial infections, and
- Late diagnosis in initial false negative tested children (see also chapter 3.)

In the following part we summarize existing literature on clinical outcome. Possible harms are reviewed in Chapter 3.

2.2 SUMMARY OF RELEVANT LITERATURE

Randomized controlled trial (RCTs) are in theory the most reliable source of evidence for evaluating effectiveness of newborn screening. However, these trials are difficult to set up and large numbers of newborns need to be screened in order to have sufficient diagnoses to compare. Additionally, depending on the unblinding and the randomization procedure, the screened group (SG) may not be comparable to the clinical group (CG) because of missed cases in the latter: this may lead to overrepresentation of more severely diseased patients in the CG. Also the close monitoring of patients in RCTs can lessen the validity of the results. Therefore data of observational studies are valuable as well. Interpretation of this type of studies needs to be done with care. Even more than with RCT, CG and SG may not be comparable, mild cases being missed in the CG. For studies reporting comparison with historical controls, it is important to distinguish between the effect of screening *versus* the effect of increased awareness and improved diagnostic and therapeutic options over time. Finally, analysis of large patient registries gives information over long term evolution of SG and CG.

There are only 2 RCTs found on CF-NBS. Each trial resulted in several reports with varying length of follow-up and reporting on different aspects of outcome (UK trial and USA Wisconsin trial). Observational data are discussed for Australia (New South Wales), north of France, USA (Connecticut) and Italy (Piemonte). Registry data for UK and US have been published.

Results are summarized per outcome parameter. The tables in appendix 3 summarize the publications separately. The quality appraisal of the non-RCT trials discussed here is detailed in appendix 4. The Cochrane review rejected one of the two RCTs (the UK trial) based because of ascertainment bias and the absence of intention to treat analyses. Because it concerns a very large trial, we have included the UK RCT in this document. We have mentioned its shortcomings and did not consider it to for the final conclusions on effectiveness.

2.2.1 General overview

2.2.1.1 Wisconsin trial

CF- NBS was performed from 1985-1994 (initially IRT, after 1991 IRT/F508del testing). Only half of the positive screened children (those with odd digit number on the Guthrie card) were recalled for sweat testing. Data were un-blinded when the children reached the age of 4. CF presenting with meconium ileus (MI) at birth was excluded from the analysis. Follow-up data are available for 15 years. Mean age at diagnosis was 12 weeks (SD 37) versus 72 (SD 106) weeks. Median age at diagnosis was 7 weeks (range 4-281) in the screened group (SG) (n=56) versus 23 weeks (3-372) in the control group (CG) (n=40).[53] By chance the SG differed at baseline from the CG with more PI patients (79% vs. 58% p=0.012) and more F508del mutations (53% homozygotes *vs.* 43% p=0.012) (*i.e.* more 'severe' mutations).[54]

2.2.1.2 UK RCT trial

From 1985-1989 infants born in Wales and West Midlands were screened with IRT on an alternate week basis, thus identifying 2 groups in the same area. For both groups babies with MI or a sibling with CF were excluded. Screened (n=58) and clinically diagnosed CF children (n=44) were followed annually for 4 years. Nine children diagnosed clinically after false-negative screen were analysed in the clinical group (CG 44= 35+9). MI was excluded from the analysis. Mean age at diagnosis was 9.1 (SD 3.1) weeks for the SG versus 50.7 (SD 60.5) weeks for the CG. Median age at diagnosis was not reported. From a figure in the publication it can be deduced that the range was 4-22 weeks and 5-230 weeks respectively. Follow-up was not necessarily in an accredited CF centre and there was no uniform treatment protocol.[55]

2.2.1.3 Methodological concerns about the 2 RCT studies

Cochrane reviewers identified six trials potentially fulfilling search criteria of RCT or nearly-controlled randomized study about NBS outcomes but only two were retained for further analyses of data based on strict eligibility and quality criteria of Cochrane's method selection.[42] The first RCT is the Wisconsin CF Neonatal Screening project which randomly assigned 650 341 neonates born in Wisconsin during 1985-1994 to either a screened (n = 325 171) or control group (n = 325 170).[53] The second RCT is the UK trial where screening took place on alternate weeks from 1985 to 1989 (screened group: n = 230 076, control group: n = 243 510).[55, 56]

From the latest published reports of these trials, Cochrane reviewers identified 108 CF patients (57 in the screened group, 51 in the control group) and 176 CF patients (86 in the screened group and 90 in the control group) respectively.[42] Risks of bias of these studies were carefully analysed in this updated Cochrane review[42] and were most critical for the UK trial where they included among others, a lack of consistency for an intention-to-screen analysis and ascertainment bias (including a too heterogeneous management in SG). The UK study finally was removed from the Cochrane analysis, which left the Wisconsin study as unique RCT validated.

Some methodological remarks have to be made about the Wisconsin study too. First, to avoid a risk of lead time bias in this RCT, only data after the unblinding of the control group at four years of age were analysed and reported.[42] Thus, some important comparative data before the age of 4 were considered not comparable and were thus not evaluated by Cochrane reviewer as for example, the demographic data in both groups at the time of diagnosis.[53]

Another methodological issue- revealed by the unexpected high survival rate even in the CG (no death prior to 10 years) - needs to be considered. The Wisconsin study was not powered to evaluate mortality as an endpoint.[57, 58] However, the absence of death before age 10 - unlike in the general paediatric population with CF in the respective time period- is most probably secondary to the close follow-up provided in this RCT which did not take place in the UK randomized trial.[58] Thus, although the results of that RCT have internal validity, they may lacking external validity, which is not an uncommon problem with RCTs.[58] The usual close monitoring of patients in RCT can lessen the external validity or 'generalizability' of results to individuals receiving care in the community.[57] In the Wisconsin study, several aspects of this unusually close monitoring of infants and young children involved in this trial can be outlined. First, parents from both groups were well informed about CF and NBS[54] and they knew that they could get premature access to the assignment of their child if needed. It is remarkable that (before unblinding) the median age at diagnosis in the CG was reduced by 13-14 weeks compared to 'usual' time-to-clinical-based diagnosis in daily practice before start of the trial (22-23 weeks instead of 36 weeks).[53] Moreover, the median age of diagnosis continuously decreased along the randomization period.[50, 59, 60] This does not mean that mothers brought numerous milder CF patients to clinical practice centre, but they brought clinically suspected CF patients earlier than they would have done, if uninformed about the disease.

Another ascertainment bias was generated by the interventions of the coordinators of the study towards general practitioners (GP). GPs were regularly called and motivated to detect as soon as possible any false-negative of NBS or any new CF cases from the control group.[54]

The Wisconsin Trial is however a landmark study and it should be realized that the above described bias concordantly could have partially masked the benefits of CF NBS. The same holds also true for the trend to higher proportion of pancreatic insufficient patients and F508del homozygote in the screened group despite the randomization process.[54] Even more importantly, the lack of consistent patient segregation in one of the 2 led to a higher rate of *Pseudomonas* acquisition in the SG, again possibly masking other possible screening advantages. The latter point has such prognostic implications that it is further discussed elsewhere (2.3.2.6.1).

2.2.1.4 Other (non-RCT) studies

Several retrospective observational studies compare the outcome of CF patients diagnosed with NBS with either recent historical controls or contemporary CF patients, clinically diagnosed (Netherlands, Italy, France, New South Wales Australia, Connecticut USA).

The oldest observational data on nutritional and respiratory parameters come from the Netherlands.[61-63] These data concern patients born from 1973 to 1979 and diagnosed based on measurement of meconium protein levels, a test with even less specificity and sensitivity compared to IRT. Historical controls were used as comparison. Based on these arguments the authors decided that the relevance of these data in the context of current CF care is low. Results of these three reports are therefore not further discussed.

Nutritional outcome has been reported for CF patients diagnosed after screening from 1983 to 1992 in Veneto, Italy compared to CF children with a clinical diagnosis born in the same time period in Sicily.[64] Although according to the report they were treated following the same protocol, the 2 regions are geographically far apart and differ significantly in socio-economic status, a factor known to influence clinical outcome in CF. For these reasons, this report is not further discussed. For the other retrospective observational studies included in this report a quality appraisal was performed (see Appendix 4). Data from these studies are discussed further in this document.

Finally, CF patient registries provide data comparing results of screened versus unscreened patients (UK and US – Cystic Fibrosis Foundation (CFF) registry) (see also quality appraisal).

2.2.2 Summary of effect of NBS on clinical outcome

2.2.2.1 Growth: weight /height

In the UK trial, no differences were found in height- or weight z-score at any age up to age 3.[55]

In the Wisconsin trial however a clear nutritional benefit for the screened group was documented. In the report of 1997,[53] data on 56 screened group (SG) and 48 clinical group (CG) children are reported. Height and weight z-scores were significantly better at diagnosis for SG and this advantage persisted during the 10 year follow-up (repeated measurements analysis) with the largest differences for weight during the first 5 years. Number of patients evaluated at age 10 were however only 4 (SG) and 9 (CG). A follow-up report[54] evaluating patients until age 13, confirmed a significantly better height but marginally significant weight advantage in the screened group (numbers evaluated at age 10 being only 17 and 13). Expressing height and weight as proportion of patients with values below the 10th percentile (P10), showed better values for the SG. The odds ratio (OR) for having a weight below P10 compared to the SG was 4.12 (95% CI 1.64-10.38) for CG patients while for height OR was 4.62 (95%CI 1.70-12.61). No differences in dietary intake were demonstrated. Additionally smaller head circumference at diagnosis was documented in the non-screened group.[54]

A Cochrane review analysing the Wisconsin RCT[42] concludes that severe malnutrition is less common among screened patients.

IRT screen was initiated in New South Wales, Australia from 1981. CF children diagnosed with CF and born in the 3 years before 1981 (n=57) were compared to CF diagnosed after newborn screening (NS) between 1981-1984 (n=60).[65, 66] Since historical controls are used, data have to be interpreted with care (see also quality appraisal). At 1 year screened patients were significantly heavier but not taller, while at 5 and 10 years they are taller but not heavier.[65] At age 15 years (n = 48 non screen and n= 52 screen) both groups had comparable height and weight z-scores.[66]

In a French cohort study, screened and non-screened patients born from 1989 to 1998 from adjacent regions with comparable living standards were followed for a 10 year period.[67] Initially NBS testing consisted of IRT, from 1995 combined IRT/DNA testing was used. Seventy seven CF patients diagnosed after NBS in Bretagne (Brittany) were compared with 36 patients clinically diagnosed in Loire-Atlantique. Patients were followed in a CF centre and treated according to the same protocols. Screened patients were significantly heavier at 1 year and 8 years and significantly taller at age 1, 3 and 5 years. At 10 years of age, these nutritional advantages were no longer maintained. However, the data in the latter age group were based on very low numbers: at 10 years 9 and 7 patients in each group respectively.

A retrospective observational study from Connecticut, US compared a SG (n=48) and a CG (n=50) born between 1983 -1997 and followed in the same CF centre until 2005. From the 48 SG and 50 CG, 34 and 21 were evaluable. Weight and height (expressed as % for age) were significantly better in the SG at diagnosis and body mass index (BMI) was better at 10 and 15 years of age.[68]

The US CF registry reported growth data for CF children. Cross sectional data analysis of children born between 2000 throughout 2002 and diagnosed before 12 months of age showed that only 9% of the SG (n=250) had a height below P3 compared to 26% of the CG (n=1023). Similar differences were found for weight < P3. For all CF patients up to age 20 years in the 2002 database (n=14647), the percentage of patients with a weight below P3 remained significantly lower for the SG compared to the CG in all age categories.[69]

UK registry data were analysed for patients 1-9 year old in 2002. Data of 184 SG patients were compared to 950 CG patients. Lower height *z*-scores were found for SG compared to the CG but weight *z*- scores were comparable.[70]

2.2.2.2 *Vitamin status*

In the context of CF-PI, low levels of the fat soluble vitamins A, E, D are often found at diagnosis. Institution of PERT and vitamin substitution can correct these deficiencies. Long term deficiencies of vitamin A and E can lead to neurological and haematological abnormalities. Osteoporosis in CF patients may be related to early vitamin D deficiency.[71]

In the Wisconsin trial α-tocopherol levels below 300 mcg/dl were present in 73% of CG *vs.* 49% of the SG (p=0.045) and this deficiency often persisted for more than 6 months.[60] This analysis only concerned PI, non-MI patients.

An important question is the relevance of these deficiencies. The combination of lower head circumference with vitamin E deficiency led the Wisconsin group to investigate the possible effect of low α-tocopherol levels at diagnosis on later cognitive outcome. At age 7-16 years the 'Cognitive Skills Index' (CSI) measured in the CG (n= 47) and SG (n=42) were significantly correlated with indicators of malnutrition.[72] As a whole CSI was not different between the CG and SG (and comparable to the normal population). However, after adjusting for co-variables, there was a significantly lower CSI score in the CG (n= 37) compared to the SG (n= 34) in the subgroup with vitamin E deficiency at diagnosis.[73]

Apart from the older data of the Netherlands,[61] a study we did not retain for this report, there are no data on vitamin status from other observational studies nor from registry data.

2.2.2.3 *Pulmonary outcome*

Several studies have shown a relation between nutritional status and pulmonary outcome in CF,[74, 75] suggesting that nutritional intervention may slow the decline in pulmonary function. CF lung disease may be present from the first weeks of life documented by abnormal lung function,[51] presence of pulmonary inflammation and infection[52] and early computed tomography (CT) changes.[76] A study comparing patient data between different US CF centres report the forced expiratory volume in 1 second (FEV1) of children followed in 2003 and aged 6-12 years. Centres with a mean FEV1 in the upper quartile range had more patients diagnosed as infants based on NBS or family history, again suggesting that early diagnosis favourably influences pulmonary outcome.[77]

In the Wisconsin trial, pulmonary function data have been documented from age 7 until the age of 16.[78] Overall, there were no differences in lung function (LF) parameters between the 2 groups with most patients having a lung function within the normal range. The absence of a pulmonary benefit for the screened group may be explained by 2 factors. Firstly, the screened group (SG) had more PI patients than the CG (see above). Secondly, the SG acquired *Pseudomonas aeruginosa* (PA) infection earlier and more frequently. Chronic PA infection is known to adversely influence outcome.[79] This has been attributed to lack of segregation in one of the 2 centres were the children were followed. Even more worrying is the finding that the X-ray score (Chrispin-Norman) although better in the SG the first 5 years, was worse compared to the CG after the age of 10- 12 years.[80] A report on a subgroup of the Wisconsin study, only reporting on PI (MI excluded) (49 screened vs. 31 non screened) confirmed these worse X-ray scores after 10 years in the SG together with a significantly lower lung function. Growth advantages on the contrary persisted until age 16 years.[60]

In the UK trial, X-ray scores (Chrispin-Norman) were comparable in the 2 groups at 1 and 4 years follow-up.[55].

A Cochrane review analysing reports of the Wisconsin RCT[42] concludes that at the age of 7 years, 88% of SG and 75% of CG had lung function parameters of at least 89% predicted. Over time chest radiograph scores were worse in the screened group (WCXR p=0.017 and BCXR p=0.041). In screened patients PA colonization occurred at a younger age.

This finding stresses the importance of strict hygienic measures to prevent asymptomatic children to become infected by nosocomial bacteria. Another important comment is that in the light of current CF treatment, spirometry is most likely not sensitive enough to measure differences between SG and CG especially in the first decade. More sensitive methods like 'Lung Clearance Index' may make it possible in the future to monitor this more closely.[81]

The Australian observational data report significantly higher lung function parameters (forced vital capacity, FVC and FEV1) in the SG at age 5 and 10 years[65] and this advantage was maintained at age 15 years (FEV1 12.3% higher (95%CI 2.9-21.7%; p< 0.001) in the screened group).[66] At 10 years the mean difference in FEV1 was 9.4% (95%CI 0.8-17.9). While in this study X-ray scores were not different at age 5 and 10 years, Shwachman-Kulczycki score (SK score, a composite scoring system for overall clinical well being including the following: general activity, clinical examination, nutritional status, X-ray score) was 5.2 points higher (95%CI 1.2 – 9.4) in the SG.[66]

Observational data from France show an FEV1 decline that was more pronounced in the CG although differences were not significant.[67] This may be due to small patient numbers in the analysis, especially after the age of 5 years. Brasfield X-ray score (higher score means less lung disease) was found to be significantly higher in the SG at all ages up to age 10, except at age 1 ($p<0.05$). Also the Shwachman score was better in the screened group at all ages ($p<0.05$).

In the observational study from Connecticut,[68] FEV1 was higher at all ages in the SG (p=0.03) but only when controlling for age, gender, inhaled tobramycin and Pa colonization. In a univariate analysis, FEV1 was significantly better in SG *versus* CG at age 15 (90% vs. 74% p=0.15). FEV1 decline was less in the SG between age 6-15 years (p=0.01). Brasfield X-rays scores and age at acquisition of PA infections were not different.

For patient data in the USA CF registry, an FEV1 below 70% was 1.32 times more likely in CG (0.77-1.71; p=0.11) but this difference compared to the SG did not reach statistical significance.[82] Recent cross sectional data (2002 registry data on 14 647 patients) show an advantage for FEV_1 in the SG in age category 6-10 (97% vs. 89% , p< 0.05, CI not stated) and 11-20 years (87% vs. 82% p< 0.05, CI not stated).[69]

The UK registry data (cross sectional data analysis in 2002; n=183 screen and n=950 CG) documented no LF differences between SC and CG. X-ray scores (Northern score, lower is less disease) were significantly lower in the SG aged 1-3 (p=0.05) and 7-9 years (P=0.05). Shwachman scores were higher for NS in the age range of 1-3 (p=0.05) and 7-9 (p=0.05) years.

2.2.2.4 Complications

In the US CF patient registry the proportion of patients with more than 1 hospitalisation for complications was 70% in CG versus 29% in SG (p< 0.0001). Patients studied were diagnosed under age 1 and born in the period 2000-2002.[69]

2.2.2.5 Burden of therapy

Burden of therapy is related to severity of CF lung disease especially when looking at the need for intravenous (IV) antibiotics and hospital admission.[83]

In the Wisconsin trial, there was no difference in the use of intravenous or inhaled antibiotics, nor for the days spent in hospital per year between the groups.[84] This may be due to the earlier acquisition of PA in a subset of the screened group.

Most observational studies report lower treatment burden in CF patients after screening.

In the UK trial, patients in the SG were on average 1.3 (sd 1.9) times admitted in hospital in the first year while this was 3.2 (SD 2.7) times for the CG (CI not stated; p< 0.01).[55]

In the French study, the proportion of children requiring at least one hospital admission before age 10 years was 86% (CG) versus 49% (P< 0.001) (SG).[67] Despite this difference, use of IV and inhaled antibiotics was not different.

In the UK registry data treatment burden was much higher in the CG, despite comparable LF. This may suggest that LF is maintained in the CG at the cost of more treatment burden.[70, 85]

Again in the UK registry, cross sectional data on treatment intensity have been reported for CF patients born after 1994 and younger than 10 years (NS n=184; CG n=950).[85] Long term therapies (prescribed for longer than 3 months) were fewer in SG *vs.* CG (2 versus 3; p=0.005). In the first 3 years of life, 42% of the CG needed IV antibiotics versus 20% of the SG (p=0.05). At age 7-9 the figures were 38% versus 15 % respectively (p= 0.05).

In the year of diagnosis 22% (CI 18-28) of SG were hospitalized versus 64% (CI 60-67) of the CG according to the data of the US CF registry (OR 3.7 (95%CI 2.3-5.9) and p< 0.001).[69]

2.2.2.6 *Survival and mortality*

With improving CF care, life expectancy is progressively improving and therefore CF related deaths are rare before age 10 years. It is thus not easy to document favourable effects of NBS on mortality.

A systematic review and analysis of all studies published between 1997 and 2003 addressing the impact of NBS on survival in CF has been performed.[86] The conclusion of this systematic review is that the absolute difference in mortality risk is modest in size (difference in mortality of 1.5 - 2 per 100 children at 10 years of age, MI excluded) and comparable to NBS for certain other genetic disorders.

In the Wisconsin RCT there was no mortality in either group before age 10. In the UK trial there were 4 CF deaths (5% in the CG), while no deaths in the SG group before age 5 (MI excluded from the analysis) ($p< 0.05$).[56] Two of the CG deaths occurred however before age 3 months, thus even despite relatively early diagnosis.

In the Australian data, mortality was 9.1% and 0%, up to age 10 in the CG *vs.* the SG ($p< 0.05$) [66]. Mortality was 12% and 7% at age 15, but this was no longer statistically significant.

Survival advantage after NS is also suggested by CFF registry data.[87] On cross sectional data from 13687 registered patients born after 1986 the risk for shorter survival was 1.76 fold higher (CI 1.24-2.48; $p<0.001$) for the CG vs. the SG. [82]

2.3 CONCLUSION

Only 2 RCTs have been published (spread over several reports) comparing the outcome after NBS versus clinical diagnosis. NBS lowers the age at diagnosis and has beneficial effect on growth parameters throughout childhood. It prevents long term vitamin E deficiency thus possibly avoiding effects on cognition.

A beneficial effect on pulmonary outcome has not been documented in these RCTs. This is in part due to the uneven distribution of PI and severe mutations with disadvantage for the SG. Additionally, the NBS grouped has been exposed to risk for earlier PA infection with resulting worse pulmonary course.

With modern CF care, following guidelines in CF centred care, this harm can most likely be prevented.

In several observational studies and registry analysis, a pulmonary benefit has indeed been documented for the SG and this was associated with lower treatment burden and less complications.

Although these non RCT studies carry an inherent risk for bias, the large number of independent reports provides an additional argument in favour of screening, also concerning pulmonary outcome.

Several sources document a modest effect on mortality.

A very important message for a screening programme is that benefits of NBS can only be assured if excellent clinical follow-up is provided in accredited CF centres and according to international standards. Consensus guidelines of CF-NBS screening and follow-up after screening have been published.[5, 43, 44] A consensus report specifically on early treatment after NBS is in preparation.

Key points:

- **CF NBS should be followed by immediate follow-up and appropriate management.**
- **CF-NBS decreases the median and mean age at diagnosis. This finding has been confirmed by all studies on this subject, RCT as well as non-RCT.**
- **Patients diagnosed by CF-NBS followed by immediate treatment and follow-up have a long term nutritional advantage over clinically diagnosed patients. This has been confirmed by RCT and non-RCT. Data on the advantage for fat soluble vitamin status and cognitive function are based on low number of patients.**
- **RCTs have not shown an advantage for respiratory disease after early diagnosis in the context of screening but this may be linked to non-segregation of patients after screening in this trial leading to more Pseudomonas infections. Non-RCT and register data do however suggest that with current standards of care, NBS will improve pulmonary status and decrease the burden of therapy.**
- **NBS possibly decreases CF mortality based on RCT and non-RCT data. Since mortality below the age of 10 years is low, these data are only based on low numbers of deceased CF patients.**

3 CF NBS – POSSIBLE HARMS

3.1 RISK FOR PSEUDOMONAS ACQUISITION

CF NBS is universally coupled with immediate referral to a specialized CF centre. It is long known that without currently widely accepted standards of care, centralized CF-care might be associated with an increased isolation rate of PA in respiratory secretions[88, 89] as well as with outbreaks of infection with multiresistant strains of this pathogen.[90-92] This is especially worrying as chronic PA colonization has been associated with the loss of nearly a third of life expectancy,[93] a less favourable FEV1 and an accelerated decline of this parameter,[94, 95] an increased treatment load affecting quality of life and much higher treatment costs.[96]

The earlier acquisition of PA in the SG compared to the CG in one of the 2 centres involved in the Wisconsin RCT raised concern but was shown to be primarily due to absence of cohort segregation for PA positive and negative CF patients.[78] Cross sectional data after 9 years follow-up showed a PA prevalence of 56.7 % in the SG versus 46.3 % in the CG, this difference not being significant. When looking at the 2 centres separately the median PA free survival time was significantly shorter only in 1 centre for the SG compared to the CG (52 weeks versus 234 weeks; p = 0,0003). In the other centre the median time to Pa acquisition was 130 weeks in the CG versus 289 weeks in the SG.[84]

This finding of earlier acquisition of PA in CF NB screened patients was not confirmed in subsequent studies.[67, 97] In fact, several studies including long term data from the US CF registry suggests that the risk for acquiring PA is lower in children detected by CF NBS.[70, 82, 98]

This is not surprising. It is actually now realized that a link between attendance at a centre and earlier acquisition of PA is not a fatality, neither during regular specialist CF care nor in the context of neonatal screening.[68, 97] In reality it is largely manageable with a policy of patient segregation based on bacteriology (applied to ambulatory as well as hospitalised care), regular systematic bacteriological surveillance and early intervention once this organism is detected.[99-103]

Such policies have been put into practice in the 6 Belgian centres studied (the 7th centre was not included in this study), for more than 17 years in one centre, for more than 7 years in 4 others, and only recently in the 6th centre, contributing to a low number of clusters[104] and a rather low (though variable in different centres) prevalence of PA in our country.[105] Moreover, another Belgian study showed that "early" referral of CF patient to an accredited CF centre (defined as a delay less than 2 years) was associated with a significantly better FEV1 (86.7% pred. ± 19.4 vs 77.2% pred. ± 22.4, p<0.01) as well a with a lower prevalence of PA (17.5 % vs 36.6%, p< 0.005).[106]

3.2 PSYCHOLOGICAL ISSUES

Three detailed reviews of the psychosocial issues related to CF NBS are available,[42, 107, 108] including a very recent Cochrane review that only considered the Wisconsin trial. There is little data about long-term consequences. Overall, data suggest that CF NBS - although creating short term anxiety among parents awaiting definitive diagnostic tests - has the potential of reducing long-term adverse psychosocial effects resulting from delayed diagnosis. Persistent false beliefs (ie, vulnerable child syndrome) by parents about healthy children who had false-positive results are a recurring finding in NBS programs. Identification of children without pancreatic insufficiency or with ambiguous DNA and sweat test results that suggest a "variant" form of CF may subject the family to uncertainty, anxiety, and potentially unnecessary treatment without a clear compensating benefit. Yet this issue has not been addressed in the literature.[109] The most commonly measured variables are knowledge, anxiety and depression.[108, 110]

3.2.1 Parental affects and NBS

Both cohorts (with or without NBS) found the diagnosis emotionally traumatic but all considered NBS as an advantage.[107] There has been concern about the evolution of the mother-baby relationship in the case of parents confronted to a pre symptomatic disease and/or the carrier status of their other children. NBS could mediate a rupture of this relationship or could induce excessive protective attitudes, designating the child as suffering from disease when it is not yet the case ('Stigmatisation'). However, no such excessive maternal reaction has been described in CF NBS literature up to now.

3.2.2 Parental knowledge about NBS

One year after the communication process, 8% of family with false-positive result still did not fully understand that their child definitely did not have CF compared to 5% at the time of result. On the contrary, families with true positive result improve their understanding of the issue from 90% at three months to 97% at one year. These results are considered unsatisfactory by some[108] and remarkable by others.[111] In RCT, families with disclosed result delayed at four years (cohort group) could not manage the information as well as families from the screening group. Most expressed anger regarding hidden NBS results and worried for potential deleterious effect of the late diagnosis for their child's health. The majority of families knew what a newborn screening program was, but there were still 22% of parents with no idea. Finally, in all cases, telephone call failed to correctly transmit the information compare to face-to-face communication. Table 1 in Appendix 5 summarizes the findings of the main studies.[108]

3.2.3 Feelings of families with a CF child : comparison between NBS and clinical CF diagnosis

Diagnostic delays are experienced as extremely stressful for all parents after true positive CF diagnosis but particularly, for parents in screening group[108] (Table 4 in Appendix 4). Grob et al.[112] collected interviews to further investigate this issue. The 'disturbing' feeling generated by NBS is well expressed by the author as "Is my sick child healthy? Is my healthy child sick?". Shock and strong emotional responses are described in all NBS programs.[108] "Reactions from the family are never the same, as a positive result is almost always unexpected".[113] Tables 2 and 3 in Appendix 5 summarize data about feeling of families at the time of diagnosis. Ways to reduce the psychological stress at several steps of the process are described in Figure 1 of Appendix 5. The period between informing the family of a positive screening test result and the definitive diagnostic assessment should be as short as possible. According to most algorithms, the crucial next step will be the sweat test. Sensitive approach around it is described in chapter 4 but is worth mentioning here too: it includes rapid appointment after the initial contact, information about the possibility of cystic fibrosis the day before the sweat test, contacting the families in the first three days of the week in order to avoid leaving them unsupported over the week-end preceding the test, provision of the results of the sweat test by the lead CF physician within one hour or two.[114]

3.2.4 Psychological impact of false positive screening test

Depression and potentially strong emotional shock have been reported at the announcement of an increased IRT level. However, this feeling quickly resolved in false-positive results and parents did not experience stress anymore three months later.[108] 43% of parents expressed depressive feelings while waiting for sweat test results and > 70% suffered from anxiety.[111] Such feelings almost always fully resolve after announcement of results of the normal sweat test. There is no publication of mother-baby relationship disruption due to NBS. Anxiety is the predominant psychological side effect. Parents of babies with a low Apgar score at birth are at risk to keep on worry despite normal sweat test (they have concerns about their child's health because of the 'bad' entry in the life). The potential psychological harm effects of 'unexplained' false-positive NBS results directly impact on parents' acceptance of being screened or not as carrier.[111]

3.2.5 Feelings of families with a child having an inconclusive diagnosis

No study specifically assessed this issue. Tluczek describes feelings of these parents as a combination of feelings similar to the true-positive group and to the false-positive results when diagnosis is finally ruled out.[115] Anxiety is not milder but decreases along the time, while they observe their child is doing well despite inconclusive results. Emphasis is made to definitely avoid pronouncing the name "cystic fibrosis" in front of the parents until the diagnosis is clearly established or excluded. "Suspected CF" seems more appropriate than "inconclusive case" for reducing the feeling of 'uncertainty' which always increases parental anxiety. These parents are not numerous and the feeling of being misunderstood and isolated is high since they even cannot share their experience with parents of definitive CF diagnosis.

3.2.6 Psychological impact of disclosing carrier status

According to the literature, three considerations may justify a CF NBS process with DNA analysis resulting in the identification of healthy carriers:[107]

- A protocol without DNA analysis would identify much more false positive results than number of carrier status.
- Family can get the information through appropriate genetic counselling and use it in reproductive planning.
- The generation of false-positive cases is the inevitable price some families will have to pay to enable screening programmes to operate.

In his review, Hayeems compared the effect of disclosing carrier results induced by NBS for sickle cell anaemia and cystic fibrosis.[111] A 'CF carrier' is first 'flagged' as positive in the screening protocol unlike other screened genetic diseases. Doing so, we are the first responsible of child 'stigmatisation'. As stated by the author, "it is not surprising that parents are stressed while awaiting sweat test results". The result of the screen should be given in a thoughtful and empathetic manner in order to reduce anxiety which is a common side effect generated by CF NBS in parents of a child being revealed as a carrier (Table 5). One year after the announcement of CF carrier status, stress score and parent's worrying about their child are still significantly different from other parents with 10% of parents thinking about this result once a week or more and nearly 30% of parents worrying about the health of their child. The first community-wide newborn screening programs for CF started in 1981, in New Zealand and New South Wales. Yet, studies assessing the impact at various ages (childhood, adolescence, adulthood) of being detected as a carrier are still lacking. From the child's perspective, there is a consensus that the knowledge of being a carrier is not of direct and immediate benefit, and as the child could not decide whether he/she wished to be tested, this can be considered as a violation of the "right-not-to know". Finally, parental CF DNA analysis can be offered to identify the carrier parent or to distinguish the 1-carrier couple from the 2-carrier couple. This issue should be addressed sensitively, because parental testing can result in detection of misattributed paternity.

3.3 DELAYED DIAGNOSIS IN CASE OF FALSE NEGATIVE NBS

The true incidence of false negative NBS is very difficult to assess. The quality of the associated surveillance program and issues related to difficult cases are critical. Increasing the number of mutations looked for when IRT/DNA algorithms are used improve the sensitivity of the program. In the Wisconsin program (IRT/DNA protocol with a panel of 23 CFTR mutations)[116] and without taking into account false-negative inherent to technical issues or administrative reasons, at least 3.8% of CF patients will be missed. Even after excluding patients with meconial ileus, a rare patient homozygous for the F508del mutation may present with a neonatal IRT level below the 95th percentile.

So the message to be delivered to primary care physicians is clear: "Not all cases of CF will be detected through CF NBS and any patient with suggestive symptoms should be investigated accordingly".[117]

Two papers[116, 117] highlight possible troublesome situations, including:

- meconial ileus: true-false negative IRT levels in some CF newborns with meconial ileus and a CF diagnosis should be excluded in these cases, regardless of IRT values.
- blood transfusions: their effect on IRT are unclear. A repeat sample should therefore, be taken after a minimum of 72 hours has elapsed. As white cells are removed prior to transfusion, misleading results with mutation analysis are unlikely.
- viral infection leading to fever and acute gastroenteritis or respiratory illness has sometimes been associated with a falsely negative screening result.
- falsely negative results have also been reported in some premature or small for dates babies.
- some seasonal variation of IRT dosages which might also be due in part to reagent lot.
- black newborns may have higher IRT levels

Trivial risk factors for "non-screening" include baby changes from maternity to intensive care soon after birth or transfers to another hospital and modifications of parent's address or no phone number to reach parents for giving results. Such babies should be clearly identified as "non-screened for CF".

3.4 INCONCLUSIVE NBS RESULTS

3.4.1 Diagnosis of CF: the grey zone is increasing

CF NBS is a screen, not a diagnostic test, and thus only identifies newborns at risk for CF.

At the present time, false positive and false negative are unavoidable, inasmuch as it is increasingly realized that the diagnosis of CF itself might be difficult.

In 1998, a CFF consensus about the diagnosis of CF stated that although the vast majority of persons with CF are diagnosed through classic signs and symptoms of the disease and corroborative laboratory results, the diagnosis was not as clear-cut in approximately 2%.[118] Ten years later, an update of this consensus extended this grey zone to 5% to 10% of individuals with CF.[5] It is in fact expected to be even larger in infants identified through CF NBS as many of them (± 50%) are asymptomatic. This makes reliable assessment of specificity and sensitivity of CF NBS programs difficult.

Debate is still ongoing around terms such as "atypical CF", "non-classic CF", "CFTR dysfunction", "CFTR related disorder" and most appropriate algorithms. In practice, the wide range of CF phenotypic variability is increasingly recognized. Of note, the last CCF consensus report[5] recommends a diagnosis of CF to be made in any patient carrying 2 CF-causing mutations in trans, regardless the results of the sweat tests. Using these criteria, 47% (28/59) of a group of patients classified under the terms "CFTR dysfunction" according to a diagnostic algorithm formulated and then validated by an international group of experts after a European consensus conference[119, 120] should be considered as CF.

All current CF NBS protocols rely on IRT as the primary test and on sweat test for confirming or excluding the diagnosis of CF. Determining the cut-off neonatal IRT is thus the first crucial issue. As seasonal and reagent lot variations of this dosage have been documented,[116] it seems more meaningful to use a floating IRT cut-off adjusted daily to an IRT percentile rather than a fixed cut-off (such as 65 µg/L in France).

After an abnormal IRT value is identified, most NBS programs perform DNA testing to identify known CFTR gene mutations (IRT/DNA strategy), while other programs repeat the IRT measurement.[43] Various combinations of these two are also possible.

Increasing the number of mutations in the initial panel aims to improve the sensitivity of the program (ie lower rate of false negative). Drawbacks include increased costs and identification of a larger number of healthy carriers.

Doing so while including some mutations of uncertain clinical significance can further increase the sensitivity of the program but will be associated with lower specificity and detection of an increased number of equivocal cases (implying additional costs for assessment and at times for follow-up, possible psychological harms, difficult genetic counselling and unwarranted treatment). On the other side, it has the potential to improve our knowledge of the severity of a number of mutations.

3.4.2 Mutations of uncertain significance

The pathogenic potential of the vast majority of CFTR mutations is unclear or unknown. This is most often related to their rareness. According to the CFF, only 23 mutations have been demonstrated by direct or empirical evidence to cause sufficient loss of CFTR function to confer CF disease and thus can be recommended as conclusive genetic evidence for diagnostic purposes.[5] This list includes several class IV or V mutations that can be associated with rather mild (though often very variable) phenotype and at times intermediate (or more rarely even normal) sweat chloride values. Most patients who are compound heterozygotes for a Class I, II or III / Class IV or V mutation are pancreatic sufficient. A recent European consensus suggested a list of 34 CF-causing mutations but acknowledged that 6 of them (among which R117H-5T and R117H 7T) could at times be associated just with a CFTR-related mono-symptomatic disorder (congenital bilateral absence of the vas deferens (CBAVD), idiopathic pancreatitis …).[121] Seven of the added mutations were class I, II or III. Table 1 provides a list of these mutations.

Table 1. CF-causing mutations according to the CFF consensus[5] and a European consensus[121]

Class I,II or III		Class IV or V
1078delT	F508del	2789+5G→A *
1717-1G→A	G542X	3849+10kbC→T
1677delTA	G551D	A455E
1898+1G→A	G85E	*D1152H*
2184delA	I507del	*L206W*
2184insA	N1303K	*R117H*
3120+1G→A	**R1158X**	R334W
3659delC	R1162X	R347P
621+1G→T	R553X	*TG13-T5*
711+1G→T	R560T	
711+3A→G	**S549N**	
E822X	W1282X	

*usually PI; Underlined: mutations common to both lists; Bold: mutations mentioned only in the European list; Italic: mutations mentioned in the European list as at times causing CF disease, at times resulting in a CFTR-related disorder.

There are several ways to try to list the most common problematic mutations. Yet, it should also be emphasized that a substantial phenotypic variability exists and constitutes a well acknowledged limitation of this approach. Modifiers genes, environmental factors, quality of care, medical non compliance can all contribute to this variability.

3.4.2.1 Mutations that can be associated with milder forms of the disease

Several studies have looked at the effects of genotype on phenotype. The largest study on the impact of class IV or V mutations investigated in 15 651 patients enrolled in the US CFF Registry.[122] Median age at death for patients carrying at least 1 class IV or V mutation was 37.6 years while it was 24.2 years for the high-risk CFTR genotype group ($p<0.001$). Given the considerable variation in the CF phenotype that occurs between patients with the same CFTR genotype, the authors stressed that this finding is of little value at the individual level.

A few studies focusing on specific mutations support the conclusion that they are "globally" associated with a milder (though variable) form of the disease. Such mutations include A455E,[123] 3849+10kbC->T,[124, 125] 2789+5G->A,[125] D1152H,[126, 127] R334W,[128] 3272-26A->G,[129] as well as R117H and 5T that will be discussed later.

3.4.2.2 Mutations that can be associated with suggestive symptoms of CF and borderline (30-60 mmol/L) or normal (<30 mmol/L) sweat chloride values

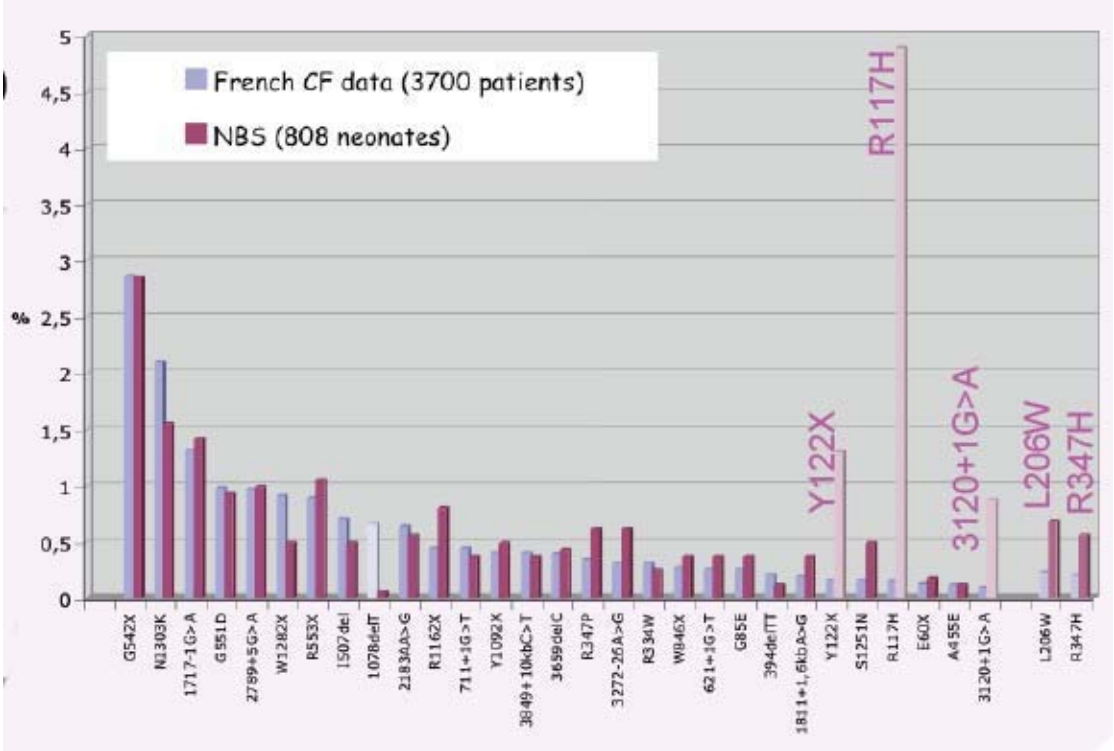
According to the literature, the Class V mutation 3849+10kbC→T and the Class IV mutation D1152H seem to be the most common of these mutations.[120, 123-126, 130-133] Interestingly, compound heterozygotes for a severe CFTR mutation and R117H were reported to have abnormal sweat chloride values in most cases (78%) when a 5T allele was present, and in a few cases only when the background was 7T.[134] In addition, sweat tests in 56 CF NBS infants carrying the F508del/R117H genotype (almost always on the 7T background), yielded normal (<30 mmol/L) sweat chloride values in almost half of the cases and intermediate (30-59 mmol/L) results in 46%.[135]

Table 2. Mutations that can be associated with suggestive symptoms of CF and normal or intermediate sweat chloride values beyond neonatal period

Sweat chloride values (mmol/L)	
30-59 mmol/L	**< 30 mmol/L**
3849+10kbC→T D1152H, L206W, R117H-7T R117H-5T, 5T, 3272-26A→G, S1235R, D945L, G149R, R1070W, R117C-7T, G91R, S977F, S945L, L165S, L159S,	3849+10kbC→T D1152H R117H-7T R117H-5T

3.4.2.3 *Mutations that are clearly overrepresented in CF screened infants as compared with those identified in CF patients diagnosed on the basis of clinical symptoms*

Figure 1. Mutations that are clearly overrepresented in CF screened infants as compared with those identified in CF patients diagnosed on the basis of clinical symptoms



French authors compared the frequency of mutations identified in CF patients on the basis of clinical symptoms (2000) and in the first 808 CF newborns identified through the national CF NBS program implemented in 2002-2003 (A. Munck et al. Personal communication).

The 2 main findings are that:

- 2 mutations with specific ethnic distribution were identified more often (Y1222X: Reunions Island; 3120+1G→A: Black Africa). Diagnosis of CF in these patients was probably often overlooked before CF NBS.
- R117H was identified 33 times more often through CB NBS as this mutation was included in the initial panel of mutations.

3.4.2.4 *Mutations that are clearly overrepresented in patients with monosymptomatic CFTR related disease*

Among these disorders, CBAVD is by far the best studied. A review of data concerning 449 men with CBAVD concluded that about 16% of them were carriers of two CFTR changes and roughly half were heterozygotes for one CFTR change many of whom (67%) have the 5T variant on the chromosome not carrying the CFTR mutation.[136] While the frequency of the 5T allele is estimated at 5-10% in the general population, roughly one quarter of CBAVD patients carry this variant. When looking at the genotypes of CBAVD patients carrying two CFTR mutations, it was evident that the R117H mutation which was found in almost half of the cases was grossly overrepresented. Desideri-Vaillant et al. [137] investigated 291 CBAVD patients and showed that 25% of them carried 2 mutations, 22% carried one CFTR mutation and the 5T allele in trans, 19% carried only a CFTR mutation or only the 5T allele. Seventy different mutations were identified, most differing from those seen in the CF population. R117H was found in 7% of the cases.[137] Another study of 182 CBAVD patients showed that 83% of them carried 2 mutations and 8.8% carried only one.[138]

Here, the 5T variant was considered as a mutation and accounted for 17% of the detected CFTR changes. The frequency of R117H in this study was much lower (3.02%), similar to that of D1152H (3.3%) and L997F (3.02%). L206W and R347H were found in 1.92% and 1.37% of the cases, respectively.

The reasons for the discrepancies between these studies are unclear but it can be concluded that R117H is overrepresented in patients with CBAVD.

3.4.2.5 Mutations that are overrepresented when a diagnosis of CF is made in adulthood (> 18 y)

A recent cross-sectional study of 9 766 new diagnoses of CF reported to the US CFF Registry between 1995 and 2005 compared features of patients diagnosed before (91.7%) or after (8.3%) 18 years of age.[139] It showed that adults present with commonly described CF respiratory disease (PA infection and reduced lung function) but have lower sweat chloride values and lower frequency of F508del mutation. Median age of adult diagnoses was 33 years with a first FEV1 around 69% predicted. 54.8% of them were pancreatic sufficient. When available, sweat chloride values were lower than 60 mmol%L in 24 % (!) of patients and lower than 40 mmol/L in 11 %.

By contrast, in the 2005 Cystic Fibrosis Foundation Patient Registry, only 3.5% of patients with a diagnosis of CF had a sweat chloride value <60 mmol/L, and only 1.2% had a value <40 mmol/L.[140] Table 2 is adapted from this paper and lists the 10 most commonly reported non-F508 del mutations, as the prevalence of the remaining were extremely low. All except two (W1282X: class I, L206W: class II) are class IV or V mutations.

Table 3. CFTR Mutation frequency (%) in patients diagnosed ≥ 18 years of age [137]

	Class	Diagnosis ≥ 18years % (n=655)	Relative frequency in patients diagnosed in adulthood *
F508de	II	74.1 (485)	X 0.84
R117H	IV	15.6 (102)	X 6
3849+10kbC->T	V	7.8 (51)	X 4.8
D1152H	IV	6 (39)	X 30
W1282X	I	4.6 (30)	X 2.1
2789+5G->A	V	3.5 (23)	X 3.9
R334W	IV	3.2 (21)	X 8
A455E	IV (V?)	2.4 (16)	X 4.8
L206W	II	1.1 (7)	X 5.5
I148T	IV	0.9 (6)	X 0.4
R347H	IV	0.9 (6)	X 4.5

*for each of these mutations, the frequency was significantly different according to the age at diagnosis (< or ≥ 18y).

3.4.2.6 Mutations for which a low penetrance has clearly been demonstrated

When found in trans with a severe CFTR mutation, the 5T allele usually results in normal phenotype. Yet, it may be associated with CBAVD or recurrent pancreatitis. Very few cases have been reported of adults who were either compound heterozygotes for the F508del mutation and 5T[141, 142] allele or homozygous for the 5T allele on a TG11 or TG12 and M470V background[143] and presented with late respiratory symptoms suggestive of CF, abnormal or intermediate sweat chloride values and abnormal nasal potential. Given its frequency in normal subjects (5-10%), it is clear that the penetrance of this variant is very low. The molecular basis for this has been described.[143, 144]

Recent work from French authors convincingly demonstrated that the penetrance of the R117H mutation was very low too.[135] It is known that its severity is modulated by the polypirimidine variant in the intron 8 acceptor splice site (T7 or T5) in cis with R117H. However, only 4% of 173 individuals carried the F508del/R117H mutation combined with the 5T allele (96% carried 7T). In this series, 37% of the subjects were identified through CF NBS. The median age at diagnosis of the 121 remaining patients was high (30 years, IQR 26-34 years). Most of them (89.3%) were males as CBAVD led to the identification of this genotype in 74.4% of this group. The penetrance of classical CF for the F508del/R117H-7T genotype was estimated at 0.03% and that of severe CF in adulthood at 0.06%.

3.4.3 How important is the problem?

In every CF NBS program based on the IRT/DNA strategy, the importance of equivocal cases is strongly related to the choice of the mutations looked for. According to the 5-year French experience, as much as 15% of CF NB screened infants belong to this category, with R117H accounting for more than half of the cases.[145] In the Massachussets program, 11% of detected infants fall in this diagnostic dilemma category[146] and R117H is often involved too.[44]

Such inconclusive cases raise unanswered questions. Definitely informing parents whether or not their infant will develop signs and symptoms of CF is currently impossible. This ambiguity is likely to generate psychological harmful effects but no study aimed to assess them. Genetic counselling is very difficult too. Panels of experts have provided recommendations for the clinical follow-up of these infants[5, 147] but these have still to be validated.

In Australia where all but one state only include class I, II or III in the initial panel of mutations, only 1-2% of infants with a positive NBS result (defined as neonatal IRT >99th percentile and at least one CFTR mutation) are reported to have a borderline sweat test (Cl: 30-59 mmol/L).[148] According to the Australasian consensus, repeat clinical assessment and sweat testing at 3-6 months of age is recommended but it is considered that parents need closure of the issue and should not be left with the lingering doubts about a diagnosis of CF. "Missing" some infants who may not have clinical evidence of CF for many years may be the price to pay but is deemed an acceptable limitation of a program that is by nature a screening program.

3.4.4 The case of R117H

There is no doubt that compound heterozygotes for a severe CF mutation and R117H may present symptoms of CF (usually with pancreatic sufficiency). This risk is higher when a 5-thymidine sequence in the intron 8 is found in cis with R117H. Though very rarely, symptoms of CF have also been reported on a 7T background[149], even at times in young children.[150, 151] In such cases, the sweat test may yield normal, intermediate or abnormal results though the latter is rare with the 7T background. Association of R117H and 5T seems very unusual in France (but could be more frequent in UK and Australia).[135] The French experience also shows that

- R117H accounts for the majority of equivocal cases following CF NBS implementation in this country
- the penetrance of classical CF or severe CF in adulthood for the genotype F508del/R117H -7T is very low (less than 1%).

3.4.5 Trying to limit the number of equivocal cases?

Population-based newborn screening for CF is not a comprehensive diagnostic program. By definition, any newborn screening programme will miss some infants with the disease in question. Which children it is permissible to miss is open for debate. Most current CF NBS programs are based on an IRT/DNA strategy. Whether to include so-called mild CF mutations (mainly classes IV and V) in the panel of screened mutations remains controversial for several reasons that include the implications of making a genetic diagnosis of CF in newborns who might not become symptomatic for years, if at all. Most of the controversy is around the R117H mutation. While current US consensus is that it is worth gaining knowledge by regular follow-up of compound heterozygotes carrying this mutation,[5] French authors convincingly plead for its withdrawal from the initial panel.[135, 152] The Australian approach is even more pragmatic[148] and suggests that inconclusive diagnoses are not acceptable and that CF NBS should only focus on class I, II or III mutations.

3.5 KEY POINTS

- **Applying standards of good care for CF infants detected by CF NBS is crucial to avoid early *Pseudomonas* acquisition. If well applied this should result in less *Pseudomonas* acquisition in CF NBS patients as compared to patients detected on the basis of clinical symptoms.**
- **Psychological side effects of CF NBS seem mild and acceptable but sensitive communication is essential. There is little data about long-term consequences. The impact of equivocal case has not been studied.**
- **It should be made clear for the physicians that not all cases of CF will be detected through CF NBS so that any patient with suggestive symptoms will still need appropriate investigations.**
- **Depending on the choice of the algorithm including the decision to investigate whether or not asymptomatic infants with intermediate sweat chloride values, equivocal cases may represent – as in France - up to 15 % of "CF" infants detected by a NBS program. Such cases raise several unanswered questions. In addition, the benefits of CF NBS for mild forms of the disease have not been established. Roughly half of these cases are related to the R117H mutation for which a very low degree of penetrance has recently been demonstrated. It seems wise to try to limit the number of such equivocal cases.**

4 CONSIDERATIONS FOR IMPLEMENTATION.

4.1 GUIDELINES FOR IMPLEMENTATION OF CF NEWBORN SCREENING PROGRAMS – "NO SYSTEM IS STRONGER THAN ITS WEAKEST LINK".

Several guidelines for implementation of CF NBS have been published[43, 44, 148] as well as an updated CFF consensus about the diagnosis of CF taking into account specific aspects related to NBS.[5] In addition, an in-depth critical assessment of the wide-scaled French experience is now available.[45] Appendix 6 Tables 1 and 2 summarize important information derived from the Australasian[148] and French[45] experiences. Tables 3-5 are tables of contents of the US and European guideline papers,[5, 43, 44] allowing to easily locate valuable information. These documents resulted from workshops organized by the Cystic Fibrosis Foundation and the European Cystic Fibrosis Society respectively. No data are available concerning the methodology used by the panel of experts.

In addition, a flowchart and 5 tables of particular interest derived from these articles are presented in Appendix 6. They detail :

- The diagnostic process for screened newborns[5] – Flowchart 1.
- Components of successful CF NBS programs[5] – Table 6.
- Workgroups topics for successful CF NBS programs[5] – Table 7.
- Items to be recorded for quality control[5] – Table 8.
- Recommendations for counselling about positive NBS results[43]– Table 9.
- Recommendations for counselling parents when NBS identifies carrier infants[43] – Table 10.

The goal of NBS has been defined to harvest over 90% of **truly** afflicted cystic fibrosis cases permitting their entry into a treatment programme in the first 2-month window but without alarming the families of healthy babies.[44, 153]

At the present time, such an ideal CF test does not exist. Numerous false positives will be identified before sweat testing and it remains critical to minimize the risk of either a wrong diagnosis or an ambiguous result. In addition, false-negative tests are unavoidable too, which stresses the need for primary care physicians to remain vigilant. Regardless of CF NBS results, a diagnosis of CF should still be considered and excluded by appropriate evaluation including sweat testing whenever a patient presents with a positive family history or suggestive clinical symptoms.[5] Among these, meconial ileus (which can be associated with low neonatal IRT levels) or a history of prenatal echogenic bowel, pancreatic insufficiency, chronic cough and bronchiectasis are especially worth mentioning.

4.1.1 Determining the spectrum of CF disease to be identified by the CF NBS program

Carefully determining the spectrum of CF disease that a NBS program will aim to identify is one of the first essential tasks. It has major implications regarding the choice of any IRT-DNA algorithm, its sensitivity and specificity, the number of equivocal cases to be managed (see chapter 3) and costs. Early detection of mild forms of the disease may raise unresolved questions in terms of genetic counselling for example. Moreover, its benefit is not established. Accordingly, there is an emerging consensus that programs using an IRT-DNA algorithm should focus on mutations commonly associated with severe lung disease. Australian authors even suggested that only class I, II or III mutations should be looked for.[148]

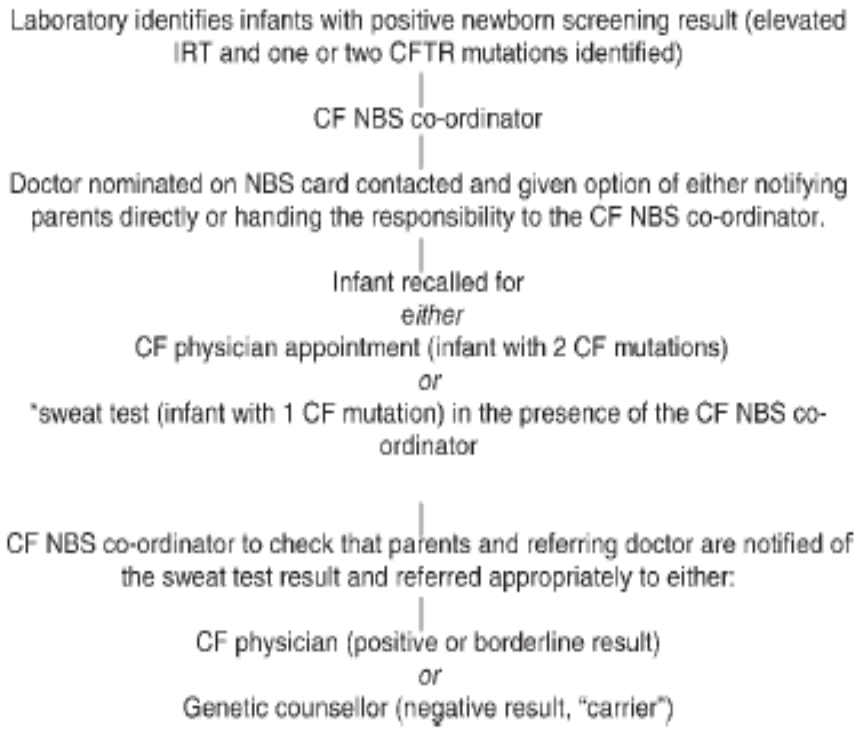
In practice however, this complex issue remains controversial. It is usually accepted that IRT-DNA algorithms should include a panel of mutations detecting at least 80% of mutations in a selected population. Data from the Belgian registry show that it would be difficult to reach this level without including some Class IV or V mutations. This seems acceptable in some cases. It is now recognized that a few well-studied CF mutations which globally result in a rather mild phenotype such as D1152H,[126] A455E,[123] 2789+ 5 G→ A and 3849 +10kb C→T[125] are in fact associated with a broad clinical spectrum quite commonly including early lung disease. Moreover, though these patients are often diagnosed later, severe lung disease with chronic colonization by PA at the time of diagnosis is not uncommon.[126, 139, 154] The case of the R117H (class IV) mutation has been discussed in detail in chapter 3. It is suggested that this mutation should not be included in the initial panel of mutations. Though currently recommended,[5, 147] extensive investigation of asymptomatic infants with elevated IRT (D3), 1 CFTR mutation and repeatedly intermediate sweat chloride values is also debatable as even if they are found to carry 2 CFTR gene changes, most of them are unlikely to develop significant respiratory symptoms in early childhood.

4.1.2 Defining an operational network

Algorithms will be discussed in chapter 6. Whatever the choice, guidelines recommend to clearly identify and assemble those who will have operational responsibility, typically including all of the CF centre directors and a CF newborn screening coordinator for each screening centre. The role of the primary care physician and the regional paediatrician, if any, should also be defined.

Pathway for result notification should be well defined as illustrated in Figure 2 which summarize the current Australian approach.[148]

Figure 2. Pathway of notification and follow-up of positive results from NBS for CF (Australia).



* Approach in New Zealand is to recall all infants with one or two mutations for sweat test before referral to CF physician.

Belgium is a very small country with a limited number of well-established accredited CF centres (n=7, academic hospitals) and neonatal screening programs (n=6: St-Jan Bruges, UCL St-Luc Brussels, CHU Liège, PCMA Antwerp, ULB Erasme Brussels, UZ Ghent). This should facilitate greatly CF NBS implementation. The diagnostic pathway for CF could be similar to the existing pathways for metabolic disorders detected using neonatal screening.

Flowcharts 2-5 in Appendix 6 suggest a model of operational network, based on the experience abroad, which could be considered in our country and specify the respective interactions between neonatal screening labs, CF Centres and a supervising structure that should be implemented. Note that in contrast to France where the nearby CF centre is alerted if case of a screen positive result, in Belgium according to the proposed protocol the pediatrician of the maternity will be alerted, who will inform the parents.

4.1.3 Sweat tests.

The sweat test is a key component of any NBS protocol for CF. False-negative results and anxieties created by false-positive results are known to be common in hospitals without large experience and engagement in quality-assurance programs. The test might be especially challenging in this age group and has to be performed and interpreted according to specific guidelines.[5, 148, 155-157] Around 4 weeks of age, sufficient sweat can generally be collected. Because of the anxiety experienced by families when they learn of a positive NBS result, bilateral sweat sampling is recommended if the baby is at least 2 kg and more than 36 weeks gestation at birth.[5] Tests resulting in insufficient sweat samples should be repeated. In Belgium, it is already a mission of the 7 accredited centres to provide accurate diagnosis for CF and it seems wise to perform all these diagnostics tests in such centres, inasmuch as it will be their tasks to communicate adequately about the results and to initiate a treatment without delay if a CF diagnosis is confirmed. If exceptions are to be considered then care should be taken to ensure that adherence to specific requirements for sweat testing required for accreditation is actually met and that referral to a CF centre is not delayed.

As for all technical aspects of CF NBS programs (IRT, DNA analysis), quality control is necessary. In the US, it is recommended that NBS positive newborns undergo sweat testing only at a Cystic fibrosis Foundation-certified laboratory. Specific accreditation is mandatory.[5, 156] Of note, it is not considered appropriate to perform the sweat test using conductivity.[156] Specific requirements such as a very low incidence of insufficient (QNS) samples (below 5% for patients older than 3 months), positive and negative controls with every sweat analysis run and documenting sweat testing proficiency (three specimens mailed twice a year to laboratories and feed-back provided concerning their performance) are especially worth mentioning. Such external quality assessment structure for sweat tests is currently lacking in Belgium. Finally, current reimbursement of sweat test in our country is notoriously insufficient.

4.1.4 Communication

Optimized communication is also considered essential at all steps of the process,[43, 44, 148, 158, 159] which should be fluent. For instance, the UK Newborn screening program centre states that the newborn screening laboratory must initiate clinical referral within 24 hours of the mutational analysis results being received and that a baby must be seen within five working days of the specialist centre being informed of positive screening results.[160] Sensitive approach around the sweat test includes rapid appointment after the initial contact, information about the possibility of cystic fibrosis the day before the sweat test, contacting the families in the three first days of the week in order to avoid leaving them unsupported over the week-end preceding the test, provision of the results of the sweat test by the lead CF physician within one hour or two.[114] The appropriateness of genetic counselling is also of great importance, not only as a vital part of follow-up care but also for parents of infants identified as healthy carriers. A recent French study emphasized that a sweat test should be arranged for all first-degree siblings of screened babies diagnosed with CF (even if older children are symptom-free) with gene analysis prescribed only in case of a positive sweat test result.[161]

The latter recommendation (the law in France does not allow to perform genetic testing in a child, ie sibling, less than 18 years of age unless it provides personal benefit) aims to avoid early identification of children with genotypes of uncertain significance but should presumably be restricted to asymptomatic children. The US guidelines also recommend a sweat test for any half-siblings who have signs or symptoms of CF or who have 2 parents known to be carriers.[44]

4.1.5 Quality assurance

In this context of rapidly expanding CF NBS programs, quality assurance is now a major issue. Integrated screening programs are recommended, which include centralization of outcome data from all CF centres and allow a monitoring structure to evaluate and modify screening algorithms if needed.[45, 117, 156] In France, this surveillance committee is a small, well-funded structure derived from the Association Française pour le Dépistage et la Prévention des Handicaps de l'Enfant (AFDHPE, French Association for the Screening and Prevention of Infants Handicaps) which is mandated by the Ministry of Health to organize CF NBS within the same framework as other screening tests. It includes a senior CF physician working part time (one day/week) and centralizing data from the 22 regional associations. They provide the following data on a quarterly basis to the national structure: 1. Data on each "positive IRT test" (ie IRT value, date of sampling, genetic finding, sweat test result, conclusion of the centre) and 2. Summary items form completed by the CF centre physician (date of initial consultation at CF centre, CF status of the child, and in case of confirmed CF: family history for CF, personal symptoms). Reimbursement of IRT laboratories (n=23) and CFTR laboratories (n=9) is performed quarterly after the national structure has checked the the adequacy of the IRT results/sampling. In addition, the monitoring structure is also collecting through a yearly questionnaire to the accredited CF centres data on false-negative cases diagnosed on the basis of clinical symptoms. A questionnaire is also sent to the regional associations in charge of collecting IRT values. The national structure has a technical committee with quarterly meeting. One person is responsible for IRT evaluation and one for molecular testing. It also plays an important role in adjusting IRT cutoff values to maintain the percentage of positive screens at around the 0.5% target and continuously trying to improve the algorithm. For example, from February 2010 a prospective study has started to assess in 500 000 newborns within one year the possible role of PAP measurements – in parallel to the current algorithm – with the main objective to reduce the rate of carrier detection.

A further step towards quality assurance has recently been reported by North-American authors who comprehensively tracked and rated 112 potential errors in the whole CF NBS programs. It appeared that 60 % of the most serious potential errors are related to communication challenges.[162] Table 4 summarizes the very concrete critical points identified in this study. The authors generated a list of 120 of potential errors throughout the whole CF NBS program, each of which was then rated on a 1 to 10 scale for severity, occurrence probability, and detectability. The product of these is the Risk Priority Number or RPN *. Of note, two of the 7 most severe identified problems are related to the role of primary care physicians.

Table 4. Most severe potential problems of CF NBS programs identified by a comprehensive approach

		RPN *
1	parents misunderstand genetic counselling information	203
2	missed babies due to neonatal intensive care unit transfer	180
3	IRT cutoff errors leading to false negative outcome	165
4	babies with confusing or similar names	160
5	primary care physicians not understanding NBS results and being unable to accurately explain them to parents	159
6	birth hospitals entering the wrong data, i.e., clerical errors	158
7	primary care physicians understanding the NBS results but not how to communicate properly without a scripted message	157
8	babies never screened	153
9	nurses or lab technicians obtaining and processing the wrong NBS card	153
10	false negative NBS test results	148

4.1.6 The issue of false-negative screens

Even in well-implemented CF NBS programs, this issue remains difficult. This is in part related to the grey-zone of diagnosis such as discussed in chapter 3 (3.4.1). It is also very dependent on the choice of the algorithm. Anyway it is important to try to limit the delay for clinical diagnosis in case of a false negative screen. The medical community should be made clear that

- screening is not a diagnosis
- even if an algorithm is chosen aiming to mainly identify severe forms of the disease, a few of them will still be missed (see Chapter 3.3) in addition to usually milder phenotypes.

This implies that any patient with suggestive symptoms should still be investigated appropriately regardless of NBS results. An even more specific information could be delivered to primary care physicians and adult pulmonologists about milder, yet at times very progressive, forms of the disease that are more likely to present in adulthood and will probably have be missed by such a CF NBS program. Educational tools are to be developed for this. The Belgian CF Association (ABLM, BVSM) can play a role in this context, by contributing to share relevant information.

In addition, tracking these false-negative patients is an important component of quality assessment of the program. The small number of accredited CF centres in Belgium (n=7) and the high degree of CF care centralization already achieved in our country should facilitate their identification and notification to a monitoring structure.

4.2 GUIDELINES ON CARE AFTER SCREENING

CF centre care and the availability of necessary medications are essential prerequisites before the introduction of NBS programmes. Both are met in Belgium. Clearly defining practical modalities ensuring rapid referral of CF screened infants to specialist CF services is a crucial part of any CF NBS program. All newborns identified by NBS should be managed according to internationally accepted guidelines.[102, 163, 164]

The implementation of a dedicated CF NBS programme shifts the focus of CF health care from control of disease manifestations in symptomatic children to health keeping in a pretty large proportion of presymptomatic infants. In the future, this is likely to involve some specific recommendations. Expert opinions on the clinical management of infants identified following CF NBS are in the process of being collected, through a modified Delphi methodology.[165] A general agreement on aspects of standard care is likely to be reached but the lack of an evidence-base approach will make it difficult to produce definitive statements. Unresolved issues need to be tackled through the implementation of specifically designed longitudinal studies and randomized clinical trials.[43, 164]

Key Points

- **Current guidelines for implementation of CF newborn screening programs invariably stress the importance of carefully considering each step of the process, keeping in mind that "No system is stronger than its weakest link". It is also necessary to identify and assemble those who will have operational responsibility.**
- **Carefully determining the spectrum of CF disease that a NBS program will aim to identify is one of the first essential tasks.**
- **Physicians should understand the limits of the screening and remain vigilant to identify false-negative cases: suggestive symptoms should still lead them to suspect a diagnosis of cystic fibrosis.**
- **Quality assurance programs for the sweat test remain a key component of any CF NBS program.**
- **Optimized communication at all steps of the process should be fluent.**
- **Centralization of outcome data from all CF centres should allow a monitoring structure to evaluate and modify screening algorithms if needed**

5 BENEFITS FOR THE BELGIAN CF PATIENTS

Benefits of CF NBS are supported by a number of mostly observational studies and have been reviewed in chapter 2. There is no reason to suspect that these findings would not apply to the Belgian CF population. Based on 'real life data' using the Belgian CF registry, we further discuss here how the project of NBS could improve outcome for Belgian CF patients (ie excluding benefits related to genetic counselling) and how to optimize this ultimate goal.

The Belgian CF Registry started in 1998 and was based on the US model. It is now for years a well-established tool,[166-170] estimated to cover more than 90% of Belgian patients with CF. From 2006, the Registry is sponsored by the National Institute for Health and Disability Insurance (INAMI-RIZIV) and has been transferred to the Operational Direction of Public Health and Surveillance, the scientific serve of Health Services Research of the Institute of Public Health (IPH). An annual report is provided to the accredited centres. The supply of data by centres is obligatory and directly impacts on their funding. It is supervised by a board consisting of physicians from all CF centres and scientific collaborators of the IPH. For the year 2007, a first public version of the annual report was available on request (http://www.iph.fgov.be/epidemio/epien/index20.htm).

Selected data from this report are listed in Table 5.

Table 5. Annual Belgian CF Registry report (2007, public version) - Selected data.

Demographics	n	1057
	Median age (years)	17.4
	M (Males) (%)	51.4
	Adults (≥ 18 years) (%)	49.3
	Patients ≥ 40 years (%)	7.3
	Deaths reported	8
Diagnosis	New diagnoses	32
	Median age at diagnosis	7 months
	Diagnosis < 1year of age (%)	66
	Diagnosis > 18 years of age (%)	7
Anthropometry	Height <P3 – children – M (%)	12.6
	Height <P3 – children – F (%)	9.2
	Weight <P3 - children – M (%)	12.5
	Weight <P3 – children – F (%)	14.3
	BMI < 18.5 – adults – M (%)	21
	BMI < 18.5 – adults – F (%)	18.9
Spirometry	FEV1 < 70% pred. : moderate to severe lung disease (%) *	
	6-11 years (n:192)	7.9
	12-17 years (n:161)	19
	18-29 years (n:272)	54.4
	≥ 30 years (n:140)	65
Treatment	Pancreatic enzymes (%)	87
	Insulin therapy (%)	9.7
	≥ 1 treatment with IV antibiotics in 2007 – children (%)	27.9
	≥ 1 treatment with IV antibiotics in 2007 – adults (%)	48.5
	Number of lung transplants performed in 2007	17
Genetics	Patients homozygous for the F508del mutation (%)	47.9

* excluding lung transplanted patients

Some additional data are available from a published Belgian multicenter study.[106]

The following items will be considered: 1) earlier diagnosis 2) earlier referral to a CF centre 3) optimizing quality of CF care in Belgium.

5.1 EARLIER DIAGNOSIS

5.1.1 How important is it ?

Early diagnosis is associated with improved outcome and a recent study is especially worth mentioning.[46] Using the United Kingdom Cystic Fibrosis Database, this retrospective cross-sectional analysis looked at the outcome of 990 patients homozygous for the F508 del mutation, attending specialist CF centres, and 1 to 10 years of age between 2000 and 2010. The study compared subjects diagnosed via NBS with subjects diagnosed via clinical symptoms at an early age (before 2 months) or at a later age (after the age of 2 months). Two meaningful analyses were performed. When compared to late clinical diagnosis via symptoms (median age at diagnosis 9 months), CF NBS led to improved growth, reduced morbidity and lower treatment burden for at least the first ten years. Early clinical diagnosis via symptoms (before the age of 2 months) resulted in a nutritional status that was as good as the one in patients diagnosed via NBS but was still associated with an increased treatment burden. As up to and including 2002, a reactive rather than proactive approach was used in the treatment of CF in the United Kingdom, treatment burden was a reasonable and justifiable surrogate marker for increasing disease severity (ie, lower SK score) rather than poor pulmonary function, which may manifest later. Of interest, the benefits of early diagnosis could even have been underestimated in this study as the percentage of patients with at least 3 positive PA cultures was similar in the three groups while more recent studies suggest that optimal centralized care should nowadays result in a lower rate of chronic colonization by PA (see Chapter 3.1).

An important conclusion of this landmark article is that making the diagnosis before the age of 2 months - which is achievable by CF NBS - can be considered as a critical objective.

5.1.2 International data about age at diagnosis

Specific information about age at diagnosis is available from several large national registries.

In 2000, median age at diagnosis in Australia (where 70% of cases were already diagnosed through the NBS) was 1.8 months. Corresponding values in UK (20% of infants screened), France and US (smaller proportions of screened infants) were 4, 4 and 6 months respectively.[171]

A more refined analysis was available in the USA for the years 2000-2002.[69] The median age at diagnosis was 2 weeks for screened babies and 14 ½ months for those clinically diagnosed (excluding meconium ileus). Corresponding values in the UK were 1 and 6 months respectively.[85]

In France, systematic newborn screening was introduced in 2002 and implicated in all centres by the end of 2003. Very recent results of this nationwide CF NBS program show that the median age at effective referral in CF centres for 987 screened CF infants was as low as 35 days. Interestingly enough, 54 % of them were already symptomatic (digestive complaints: 69%, growth failure: 44%, respiratory symptoms: 24%) but without CF NBS many of them would have gone through the well described long and sometimes traumatic journey to a diagnosis.[172] These data prove that NBS can reduce the median age at diagnosis to below 2 months of age.

5.1.3 Current age at diagnosis in Belgium

The Belgian CF registry data report 2007 (N=1057) shows that the median age at diagnosis is 7.03 months overall.[170] These data include 16.2 % of the subjects in whom the diagnosis was made via newborn screening because several Belgian hospitals implement systematic neonatal screening using IRT on a local basis. The data also include 13.8% of subjects in whom the diagnosis is made at birth because of meconium ileus. Omitting these subjects from the calculations would thus increase the median age at diagnosis. If one excludes all subjects in whom the diagnosis is made after the age of 10 years (thereby excluding subjects with milder disease forms, but also some late diagnoses of patients with classical severe forms of disease hence 'missed diagnoses') the median age at diagnosis is 4.4 months and thus still double the 'necessary age for benefit' of 2 months. If, from these subjects with diagnosis before age 10 years one excludes 16 % of the cohort with diagnosis made via NBS, the median age at diagnosis rises to 6.9 months and if one further excludes 14% presenting with meconium ileus the median age at diagnosis rises to 9.5 months. The analysis was then further refined to include only subjects F508del homozygous diagnosed via symptoms before the age of 10 years and excluding subjects presenting with meconium ileus (n=92). Their median age at diagnosis was 7.7 months.

When limiting the analysis to subjects with a new clinical (i.e. through symptoms) diagnosis of CF up to the age of 10 years in the last 5 years of data collection (n=135) the median age at diagnosis was 10.9 months and when restricting this analysis further to subjects homozygous for F508 del (n=35) the median age at diagnosis was 7.3 months. In the latter analysis subjects presenting with meconium ileus are also excluded.

A very strict way of assessing subjects with potential benefit by NBS in the Belgian population is by evaluating the age at diagnosis in all subjects homozygous for F508del in the registry (N=492) diagnosed through symptoms, and excluding those presenting with meconium ileus at birth (n=93): the median age at diagnosis is then 8.5 months. In 256 subjects the diagnosis was later than age 2 months and taking into account their specific ages at diagnosis a total of 8086 patient months of benefit can be gained in the Belgian population. If looking only at the 82 subjects diagnosed in the last 10 years the median age was 5.8 months, amongst them 66 diagnosed after 2 months with 1950 patient months in excess of the benefit.

The above-mentioned 16.2% proportion of Belgian CF patients diagnosed by CF NBS could suggest that some useful information can be derived from a comparison of this group and unscreened patients. For several reasons however, this is not the case. Little is known about these local programs: different algorithms have been used, these local initiatives were not structurally funded and some have evolved over time. Most importantly, no data about quality control is available.

5.2 EARLIER REFERRAL TO A CF CENTRE.

5.2.1 How important is it ?

CF NBS is universally coupled with immediate referral to a specialist centre. Guidelines and international consensus all emphasize that this is a key point for a program to be efficient.[43, 44] The French experience is of particular interest in this context. Ten years ago, it was apparent that the outcome of CF patients in France was less favourable than in the US and other European countries with similar economic status but with an organized CF care systems. For instance, the median life expectancy, median age at death and number of pregnancies in CF women all were lower in France.[173] French experts in the field then pleaded for CF NBS implementation but this was subordinated to the creation of a network of CF Resource and Skill Centers, allowing the multidisciplinary approach to this complex disease with standardized follow-up protocols.[173, 174]

Even outside the context of CF NBS, early referral to a specialist healthcare centre is considered as an important prognostic factor as highlighted in consensus reports on optimal management of CF.[102, 175, 176] Though this is long supported by a number of common sense reasons and has been mentioned as a model for the management of complex diseases,[177] most published studies concerning the impact of this centralization on lung disease are either biased due to comparison with historical controls, and/or probably underpowered.[63, 178-182] Two studies avoid these pitfalls.

Mahadeva et al.[89] compared two groups of adults who had either received continuous care from paediatric and adult CF centres (n=50) or had received neither paediatric nor adult centre care for their CF (n=36). Excluding body mass index as a covariate, FEV1 was significantly better in the first group.

More recently, a Belgian retrospective multicenter study clearly showed that earlier referral of children suffering from CF to specialist care is associated with a significant pulmonary benefit.[106] This work should have been conducted by the Belgian CF Registry team but this proved impossible at the time (2003) so the directors of 6 out 7 accredited centres accepted to supply their data. This carefully designed study demonstrated that children referred early (defined as patients referred less than 2 years after the diagnosis) had a better FEV1 (86.7% pred.±19.4 *vs* 77.2% ± 22.4, p=0.01) and a lower prevalence of PA (17.5% *vs* 38.6 %, p<0.05) than patients referred later. Assuming a mean annual decline in FEV1 of 1–3%, a 10% difference in this major outcome variable between "early" and late referred children at a mean age of 13 years is impressive and is likely to have survival implications.

5.2.2 Room for improvement in Belgium

In Belgium, several centres have for quite some time now been functioning according to the North American model organization of care. A further crucial step in this regard occurred in 1999 when seven CF centres were officially accredited and financially supported by the national health system. All are based in academic hospitals. Centres had to fulfil a number of qualitative criteria (facilities and resources) closely derived from the Cystic Fibrosis Trust's 1996 guidelines.[183] A minimum of 50 patients on full care was required. Shared care is not considered as an option in this small country. The last annual report of the Belgian CF Registry included data of about 1057 patients at the end of the year 2007[170] and all these patients were at least known in an accredited CF Centre (which provided the data). An independent listing of CF patients known by the Belgian CF Association was similar, confirming the high coverage of the Belgian Registry. This is an encouraging point but cannot mask the fact that at the end of 2003, the delay between the age at diagnosis and the age at referral to a CF Centre still exceeded 2 years in 31% of patients.[106] Obviously, implementing CF NBS coupled with a sine qua non immediate referral to a specialized centre will result in substantial reduction of this delay.

5.3 OPTIMIZING QUALITY OF CF CARE IN BELGIUM

5.3.1 Caveat : comparing data from different countries is difficult.

Numerous methodological issues make it difficult to compare results of national registries and actually will seriously dent many of such comparisons.

These include (but are not limited to):

- The coverage or exhaustivity of a registry, an item which might be difficult to assess and is not always available
- The choice of reference equations used to normalize anthropometric and spirometry data (several sets can be used and they are not even always specified)
- The type of subjects included in the registry: an increasing number of patients with milder forms of the disease are now being identified in some countries (children as well as adults),[184] through wide access to CFTR gene sequencing and nasal potential difference measurements, or via CF NBS. An example of the latter is the increasing percentage of genotypes including the R117H mutation in the French CF Registry.[185] This allowed French authors to study a large group of such patients, demonstrating a very low classical-CF penetrance of this mutation[186] and supporting the suggestion that this mutation should be withdrawn from the panel of CFTR mutations used for screening purpose in this country.[152]
- A lack of uniform definitions of many clinical conditions (ie hepatopathy, CF related diabetes, pulmonary exacerbation …)
- Differences in set-up (longitudinal versus cross-sectional)
- Differences in age stratification (in UK, adults are defined as patients ≥ 16 years …)
- Issues related to quality control and missing data
- In or exclusion of lung transplanted patients ("another disease")
- Bacteriology: crucial differences in frequency of sampling and laboratory practice in isolating and reporting pathogenic organisms
- Spirometry: FEV1 has been recorded as the last value of the year, the best value of the year, the mean value of the year, the average of the best value for each quarter of the year … Normal FEV1 has been defined as ≥ 80 or 90% of the predicted value in different registries
- Wide heterogeneity of CFTR mutations throughout the world

There is an obvious need for standardization in data collection if we are to compare different registries meaningfully. It has recently been suggested that evaluating cohorts on the basis of the presence or absence of pancreatic insufficiency could help to overcome some of the current limitations.[166] Limiting analysis to patients homozygous for the F508 del mutation could be even "cleaner".

5.3.2 Selected data

Overall, Belgian data compare very well with those from other western countries.

Reliable, well-established national CF registries with a high coverage are scarce. Such registries, all originate from countries with a high level quality of care in this field. This is confirmed as preliminary data continues to appear in congresses from other European or even developing countries: their outcomes (variables) are clearly less favorable. On the other hand, it is to be mentioned that detailed yearly data from Canadian and Scandinavian national registries are lacking, even though the latter are known to have the best results in the world.

In order to circumvent some of the above mentioned drawbacks of registry data, a few indicators have been chosen. It should be realized that improvement in the median age and proportion of adults over time can is indeed correlated both to increased survival and to new diagnoses in adults. However, continuous improvement is clearly documented on a very wide scale in the US Registry over the past 4 decades using the same items, median life expectancy and anthropometric as well as spirometric data, and this has been true before the recent development of new tools for diagnosing milder forms of the disease and CF NBS. In addition Kulich et al.[187] specifically addressed this issue, taking advantage of the huge amount of data from this registry which is estimated to contain information about at least 85% of patients diagnosed with CF in the United States. In-depth statistical analysis of the whole cohort and specific subgroups provided evidence that most of the observed improvement in survival among CF children was related to progress in care.

Comparisons with data from the US Registry are especially meaningful as the Belgian Registry was initially based on its model.

5.3.2.1 Median age of patients (Belgium vs other countries)

Table 6 summarizes published data from 6 well established national registries.

Table 6. Median age of CF patients in 6 industrialized countries.

Country	Year	Estimated population (millions)	Number of patients	Median age (yrs)	Ref
UK	2004	60	7046	16.4	[188]
France	2006	63	4994	15	[185]
Australia	2005	20	2472	15.3	[189]
Belgium	2003	10.5	860	16	[167]
Belgium	2005	10.5	950	17	[168]
Belgium	2006	10.5	1036	17	[169]
Belgium	2007	10.5	1057	17.4	[170]
Ireland	2007	4.3	1170	17	[190]
Germany	2007	82	4926	17.2	[39]

5.3.2.2 Proportion of adults (Belgium vs other countries)

Another index of survival is the proportion of patients now reaching adulthood. This percentage is steadily increasing in western countries where it is expected that within the next few years most patients will actually be adults. This is already the case in Scandinavia (unpublished data).

Table 7. Proportion (percentage) of adult patients (≥ 18 y) in selected western countries.

	Belgium	USA	France	Germany	Sweden	Australia	Canada	Ireland
Year								
1999	36.4				45[191]			
2000	38.4	38.7[192]						
2001	35.3	39.5[193]						
2002	39.6	40.2[194]					47.6[195]	
2003	40.5	40[196]						
2004	42.7	41.8[197]						
2005	44.0	43[198]	40.7[199]	43.4[40]		41.1[189]		
2006	48.4[169]	44.6[200]	41.3[186]					
2007	49.3[170]	44.4[201]		47.6[191]				49[190]

These data are in keeping with those derived from a recent report of the European Cystic Fibrosis Society (ECFS) concerning a few national registries with high rates of exhaustivity (Table 8).[202].

Table 8. Proportion of adult patients (≥ 18 y) in selected western countries at the end of 2006.[202]

	Denmark	Belgium	Israel	Germany	Ireland	Czech Republic
N	438	1.036	447	4.894	830	470
≥ 18 yrs (%)	52.3%	48%	46.8%	46.6%	45.3%	38.3%

5.3.2.3 Proportion of children (6-<18y) with a normal FEV1 (≥ 90% predicted) (Belgium vs US).

As the respiratory course has a major impact on the prognosis of CF and FEV1 is as yet the best marker, the proportion of CF children with a "normal" FEV1 is an index of great interest. In 2003, this percentage was 45.2 in US[196] and 51.7 in Belgium,[167] using the same reference values by Knudson.[203] The difference between the 2 countries is in fact somewhat underestimated as the mean of best FEV1 per quarter was retained in US while the last value of the year (fall or winter) is used in Belgium. Unfortunately this proportion is no longer detailed in the more recent annual reports of US Registry.

5.3.2.4 Median FEV1 in selected age groups (Belgium vs Denmark and Germany).

Detailed FEV1 data of non transplanted patients by the end of 2006 (according to Wang[204] & Hankinson[205]) were available for 3 European countries in the report of the ECFS [202] and are summarized in Table 9.

Table 9. Median FEV1 % predicted values for selected age groups in Denmark, Belgium and Germany (at the end of 2006).

Age group	Median FEV_1 (% pred.)		
	Denmark	Belgium	Germany
10-14 yrs	102	93	88
15-19 yrs	98	78	74
20-24 yrs	84	72	63
25-29 yrs	73	59	56

5.3.2.5 Comparison of recent anthropometric data of children 2-3 years old in French and Belgian CF Registries.

In France, CF NBS is fully implemented from 2004. While writing this chapter, we wondered whether there could be a difference in anthropometric data of young children (2-3 years since Centers for Disease Control (CDC) reference values for BMI below 2 years of age are not available) in the French and Belgian registries. The CF Registry coordinators from these 2 countries kindly accepted to provide the requested mean data within a few days. Pancreatic sufficient patients were excluded from analysis as well as patients diagnosed after meconium ileus. Belgian infants diagnosed by CF NBS were excluded too. 120 French children 2-3 y old by the end of 2006 were compared to 53 Belgian children 2-3 y old by the end of 2007, 2006 and 2005. These data demonstrate a nutritional advantage in the French (screened) patients (Table 10).

Table 10. Recent anthropometric data of pancreatic insufficient 2-3 years-old children without meconium ileus (after exclusion of the NBS Belgian patients): France vs Belgium.

	FRANCE	BELGIUM	P
N	120	53	
Mean age (years)	2.42	2.43	
Mean age at diagnosis (months)	<2	7.6	
Height Z score	0.02 (± 0.92)	-0.57 (± 0.87)	<.0011
Weight Z score	-0.36 (± 0.86)	-0.93 (± 1.26)	<0.001
BMI Z score	-0.46 (± 0.88)	-0.74 (± 1.43)	NS

Data are presented as mean (± SD).

5.3.3 Scandinavia : the best outcomes without NBS programs.

Even though published data are limited, Scandinavian countries are currently considered as the reference in terms of outcomes for CF patients.[202, 206] It should be emphasized that good outcome in Denmark and Sweden is achieved without CF NBS. This cannot be attributed to less severe genetic background since Denmark is reporting the highest percentage of patients homozygous for the F508 del mutation. Data are lacking to explore in-depth the factors involved and such analysis is well beyond the scope of this work. Yet, it demonstrates that improving the quality of care is very important. Possibly, key features of these 'success' countries include very high economic status, a long tradition of centres of reference, more frequent visits to the centres (monthly), a very aggressive and costly approach for patients chronically colonized by PA (2 weeks hospitalization quarterly for intravenous antibiotic treatment) in Denmark, cultural importance of physical exercise, attention to nutritional status (Sweden). …

5.3.4 Quality of CF care in industrialized countries is heterogeneous

5.3.4.1 A long known fact & Selected examples

Large differences between outcomes variables obtained from the different centres have been recognized for a long time.[207]

A few striking illustrations of this heterogeneity are provided by recent data from the 2007 US Registry,[201] allowing for comparisons between centres:

1. mean FEV1 (% predicted) for CF children 6-17 years - : national average: 92.6%, range: 75-103 % (reference values : Wang[204] & Hankinson[205]
2. percent with BMI < 5th Percentile for CF patients 2-20 years - national average: 52.7, range: 32-83
3. MRSA infection rate – national average: 21.2%, range: 6 -42%

Other recent similar features can be derived from the CF German Registry where a quality management program with an overall coverage of 82% for the year of 2005 confirmed considerable difference between centres in terms of key parameters[39]. For instance, the percentage of children (6 - <18 years) with an FEV1 above 80% of the predicted value (according to Zapletal[208] ranged from 20 to 100% in centres treating less than 50 patients and from 35% to 100% in larger centres. Globally, the mean FEV1 in this age group was 88% of the predicted value.

5.3.4.2 A reality in Belgium too

A recent Belgian multicenter study confirms wide differences in outcome between the reference centres in our small country.[106] Neither the climate, nor the distance to the centre, nor the genetic heterogeneity could be relevant in Belgium. The role of socioeconomic factors cannot be excluded, yet the quality of the social security system of this country is expected to limit their impact. A comprehensive attempt to identify specific care patterns associated with better outcome was beyond the scope of this study.

5.3.4.3 An opportunity for quality improvement initiatives

Differences between outcomes variables obtained from different centres are now drawing considerable attention as they may provide an opportunity to develop quality improvement initiatives.[39]

The Epidemiologic Study of Cystic Fibrosis (ESCF) was an observational database collecting prospective information from a large number of CF patients. North American data from 1995 through 1996 were analyzed in order to identify care patterns associated with better respiratory outcomes.[209] The 194 participating sites all cared for at least 50 patients. They were ranked on the basis of median values for FEV1 within each of three age groups (6 to 12 years, 13 to 17 years, and > 18 years). The study confirmed substantial differences in lung health across different CF care sites. For 6-12 yrs children, mean values of FEV1 (% predicted according to Knudson[203] were 93% and 74% in upper and lower quartiles respectively. Corresponding values for children 13-17 yrs were 85 and 62%. Patients who were treated at higher ranking sites had more frequent monitoring of their clinical status, measurements of lung function, and cultures for respiratory pathogens. These patients also received more interventions, particularly IV antibiotics for pulmonary exacerbations.

A more recent ESCF study led to the identification of a link between better lung function in 6 to 12 year-old children and clinical care patterns in infants in previous years.[77]

Patient registries are essential to identify significant differences in clinical state and outcomes. A recent comprehensive editorial emphasized the importance of collecting very reliable data for quality management.[210]. This necessitates complete datasets probably through obligatory (as in Belgium) rather than voluntary supply of data, standardisation of sampling moments and procedures but also for regular audits of the centres by the registration committee. The latter has still to be implemented in Belgium. The author also underlines the importance of structure indicators evaluating the care-delivery system ("Is the dedicated team appropriate?" – the answer to this question has funding implications) and process indicators (including a number of important items such as the quality of microbiological evaluations, local policies for segregation of patients on a bacteriological basis …).

This should lead to sharing expertises and eventually facilitate a more standardized and efficient approach[177, 211-213] with the ultimate goal being the improvement of the services delivered to patients. This fits in the scope of the cystic fibrosis quality assurance project in Germany.[39, 40] A few key interrelated outcome parameters such as BMI, FEV1 and PA infection rate can be used to qualitatively describe the results achieved by different centres. Benchmarking diagrams have been derived. Auditing and quality groups have then been created to enable CF centres "to learn from the best", an option currently endorsed by the new European CF Registry organised by the European CF Society (ECFS) and Euro Care CF. It has been suggested that such data could also serve as a basis for political action in the health care system.

Centre-based quality improvement using a clinical microsystems approach might also be helpful.[36-38] Involving a number of critical components (ie assessment of care processes, standardization of care, patient involvement, communication, developing a culture of continuous improvement …) it has been shown effective in improving pulmonary function of pediatric patients at the US CF centre of the Akron Children's Hospital.[36]

Key-points

- **Implementing a systematic CF NBS program in Belgium has the potential to lower the median age at diagnosis in patients with typical CF but without meconium ileus from about 10 months to less than 2 months.**
- **Immediate referral to a specialized CF centre after CF NBS is required to have early access to optimal care. Both CF NBS and immediate referral have been shown to improve the outcome of CF patients.**
- **A number of methodological issues flaw comparisons between different national registries. Nevertheless, it might be derived from current data that the quality of care in CF patients in Belgium is already high on average.**
- **Considerable variability of outcomes between centres has long been documented and is also observed in Belgium. It offers an opportunity for promoting quality improvement initiatives which would help to maximize the benefits from CF NBS. The Scientific Institute of Public Health could play pivotal role in this context.**

6 SCREENING STRATEGIES

6.1 LITERATURE

We identified numerous recent reviews and guidelines dealing with CF NBS strategy. Three health technology assessment studies were published.[110, 214, 215] There is no universally agreed approach to screening for CF. The primary studies are heterogeneous (strategy, IRT cut off values, number and type of mutations analysed, CF prevalence) and results in term of sensitivity, specificity and predictive values are difficult to compare. Several studies have been conducted to compare strategies. They were reviewed in 2007.[216]

A document that describes European best practice guidelines for CF NBS was recently published.[43] This working document is a true state-of-the-art democratic consensus among experts with respect to guidelines for CF NBS.

Indeed, these guidelines were obtained as follows. Altogether, 37 experts in the field of CF NBS from different countries of Europe and the United States were invited to participate. They were divided in working groups. Each of these working groups had pre-conference consultations and drafted a preliminary document. A meeting was then organized in Garda, Italy, on March 28–29, 2008, in which 31 of the 37 experts participated. The individual working groups had then formal discussions within their working group and concluded a working document. In a next phase, all participants took part in a general discussion of all working documents and reached a group consensus. Post-conference consultations were then performed in subsequent months by all participants to generate the final consensus guidelines for CF NBS.[43] This document, containing a chapter devoted to strategies, is thus an excellent basis for evaluating, and possibly setting up, a CF NBS program in Belgium.

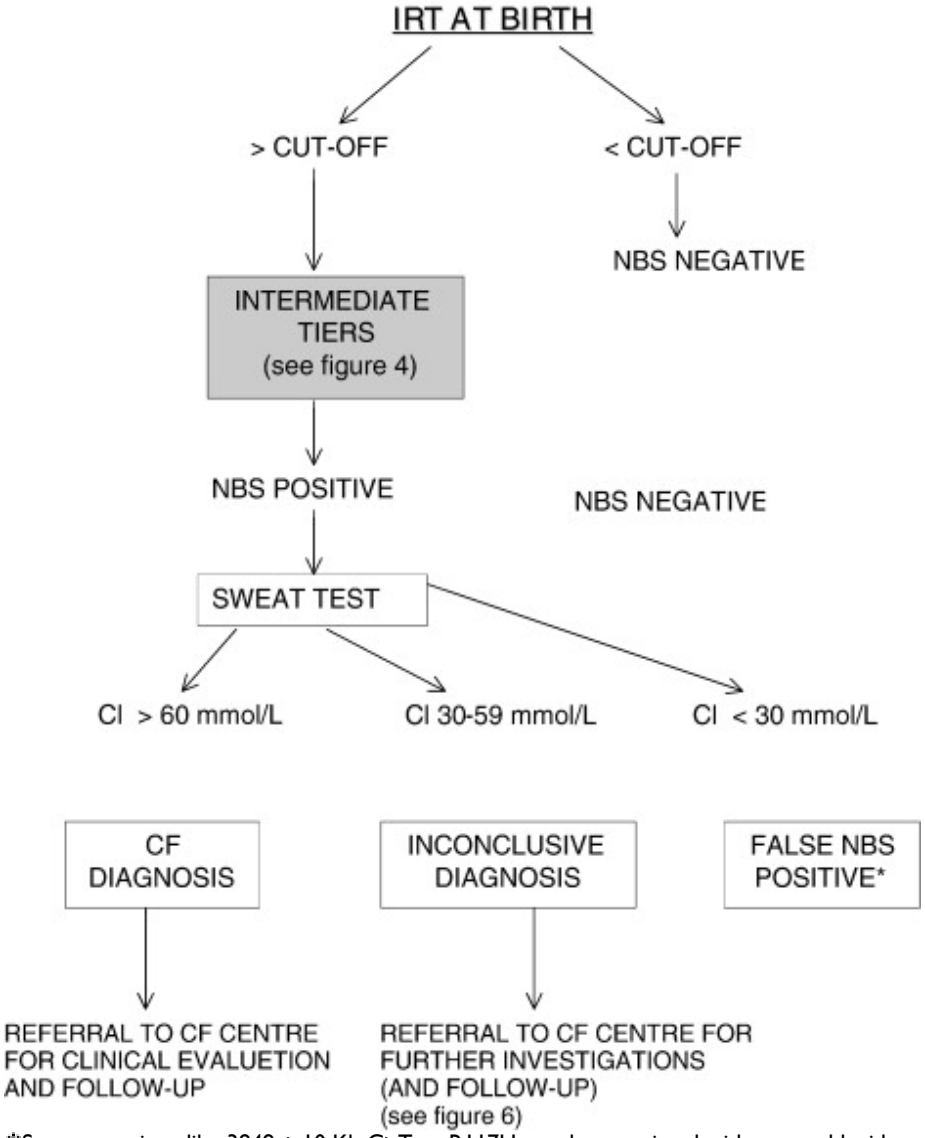
6.2 DESCRIPTION OF THE VARIOUS PROTOCOLS

Screening allows the detection of children with an elevated risk for CF. Screening is thus not a diagnostic test.

A screening test is valid if it detects most people with the target disorder (high sensitivity) and excludes most people without the disorder (high specificity), and if a positive test usually indicates that the disorder is present (high positive predictive value).

Multiple protocols and algorithms are used to screen newborns for CF. In Europe, more than 25 screening programs have been developed, with quite marked variation in protocol design.[43] An algorithm for a standard CF NBS procedure is shown in Figure 3.

Figure 3. Standard CF NBS procedure



*Some mutations like 3849 + 10 Kb C>T or R117H may be associated with sweat chloride values < 30 mmol/L. Reproduced from [43].

A referral is given to Figure 4 (taken from [43]), which is included as Figure 4 in this KCE report. Figure 6 referred to in the above flowchart is included as Figure 8 in this KCE report.

CF NBS protocols currently used in Europe are shown in Table 10. This reflects (1) the ethnic mix of populations, (2) the structure of current NBS protocols (for instance the ability to organize a second heel prick test), and (3) variations in healthcare provision and resources. While harmonization of protocols is desirable, it is not realistic to hope for a single European scheme, and probably this is not desirable. However, it is important to compare rigorously the performance of different available protocols, in order to find the protocol best suited for a given region or country in Europe.

Table 11. CF NBS protocols used in Europe

Areas	Average screened population per year	2nd tier	3rd tier	4th tier	Specifics
Two-tier protocols					
Liguria (I)	12,000	ST	–	–	
Three-tier protocols					
Czech Rep	76,000	MUT	ST	–	PS; Feb 2005–Nov 2006
Emilia Romagna (I)	40,000	rIRT	ST	–	
Calabria (I)	18,000	rIRT	ST MUT	–	
Lombardy (I)	98,000	MUT rIRT	ST	–	rIRT if bIRT > 97.5° centile MUT if bIRT > 99° centile
Marche (I)	14,000	MUT rIRT	ST	–	PS; rIRT if bIRT > 97.5° centile MUT if bIRT > 99.8° centile
Tuscany (I)	33,000	MP rIRT	ST	–	
Piedmont (I)	39,000	MUT rIRT	ST	–	PS; rIRT if bIRT > 98.6° centile MUT if bIRT > 99.6° centile
Lazio 1 (I)	28,000	rIRT	ST MUT	–	
Lazio 2 (I)	37,000	MUT rIRT	ST	–	
Sicily (I)	54,000	MUT rIRT	ST	–	
Austria	77,000	rIRT	ST	–	
Catalunya (SP)	83,000	rIRT	MUT ST	–	
Castilla-Leon (SP)	18,000	rIRT MUT	ST		
Galice (SP)	21,000	rIRT	MUT ST	–	PS
Wales (UK)	34,000	MUT	ST		
Dresden (GER)	15,000	MUT			Since January 2008 IRT PAP
Heidelberg (GER)	40,000	MUT PAP	ST		PS (started April 2008)
Russia	1,300,000	rIRT	ST		
The Netherlands	180,000	PAP	MUT		PS (started 1-1-2008)

Areas	Average screened population per year	2nd tier	3rd tier	4th tier	Specifics
Four-tier protocols					
France	809,000	MUT	rIRT	ST	rIRT if MUT-tive and bIRT > 100 µg/l
Poland	210,000	MUT SEQ	ST		
England (UK)	655,000	MUT	rIRT MUT	ST	rIRT if 1 mutation at MUT or bIRT > 99.9th centile Expanded MUT if one mutation at first panel
Northern Ireland (UK)	24,000	MUT	rIRT MUT	ST	rIRT if 1 mutation at MUT or bIRT > 99.9th centile Expanded MUT if one mutation at first panel
Scotland (UK)	58,000	MUT	rIRT MUT	ST	rIRT if 1 mutation at MUT or bIRT > 99.9th centile Expanded MUT if one mutation at first panel
Veneto	56,000	MUT	rIRT	ST	rIRT if MUT and MP-tive and bIRT twice the cutoff
Trentino Alto-Adige (I)		MP			

Notes:
- 1st tier is always IRT (bIRT).
- abbreviations: rIRT = IRT resampling; MUT = genetic analysis by mutation-specific tests; MUT SEQ = genetic analysis by sequencing of exons (6 regions of the CFTR gene, recently upscaled to .. regions of the CFTR gene, Norek A. personal communication); MP = meconium proteins dosage; ST = sweat test; NA = not available; PS = pilot study; PAP = pancreatitis associated protein.
- more than one test per tier is considered if tests are performed at the same time.
Modified and updated from [43, 217, 218].

All current protocols are multi-stage testing and rely on IRT as the primary test and on a sweat test for confirming or excluding the diagnosis of CF. Intermediate tiers are required to achieve an acceptable combination of sensitivity and specificity and are nearly always used. These tiers may either consist of a further analysis on the first blood spot (CFTR mutation or Pancreatitis-associated protein), or a second IRT test on another blood spot collected later on, or various combinations of these three. Each of these protocols have their own advantages and drawbacks.

6.2.1 IRT issues

Serum immunoreactive trypsine (IRT) levels are raised in neonates with CF compared with unaffected neonates due to back leakage from blocked pancreatic exocrine duct. A single IRT protocol involves measurement of IRT on a heel prick blood sample during the first week of life, followed by a DNA test or sweat chloride test in infants with elevated IRT levels to confirm the diagnosis. It has been used in the early years of newborn screening for CF. Since elevated IRT levels during the first week of life are not only found in infants with CF but also in healthy infants (ethnicity, CF carrier status and perinatal health), the major problem associated with this protocol is its low positive predictive value due to a high number of false positives.[110] It is now generally accepted that a single IRT stage is not an adequate screening strategy.

6.2.1.1 IRT cut offs

The initial IRT centile cut-off has the greatest effect on NBS performance, and therefore optimization of cut-off levels depends on the relative importance attached to sensitivity and, particularly, specificity. Early experience with the two-stage IRT–IRT protocol[219, 220] showed that a good sensitivity can be achieved with a 99.5th centile cut-off, although the use of a lower cut-off (e.g. 99th centile) is also common.[221] A retrospective study of false-negative cases suggests that when the sample is taken relatively late (day 5), lowering the cut-off would have little effect on sensitivity (Heeley, unpublished data taken from Castellani[43]). Furthermore, the use of low IRT cut-offs around the 95th centile, prior to mutation analysis, reduces the positive predictive value of a single CFTR allele with regard to making a subsequent diagnosis of CF.

The range of cutoff values (min-max) reported to the Newborn Screening Quality Assurance Program is very large for IRT (32/170 ng/mL whole blood).[222] This is a reflection both of differences between IRT tests being marketed and the between-lot variations. Unfortunately, no standardized reference material is available for this important test.

In general, a policy of replication in duplicate should be adopted for all samples with IRT values above a preliminary threshold, usually set 10 ng/ml below the final cut-off.[43] This is to minimize effects of volumetric variability of the punched discs, day-to-day variation in IRT assay calibration, and to detect contamination of the sample with faeces or possibly sample misidentification.

6.2.1.2 Quality control

There are significant differences among commercially available IRT assay kits in terms of the value assigned to the initial cut-off and possibly also in the rate of decline of measured IRT in positive cases during the first few weeks of life.[223, 224] This could result in a different IRT cut-off for different kits. Despite such variability, all the current commercial IRT assays seem to produce satisfactory results. However, this should not pose any problem since in a given lab cut-off centiles are used. The performance of the various commercially available IRT assays is shown in Table 12.[222]

The total coefficient of variation (CV = SD / mean) reaches more than 50% for some brands of IRT around cut-off. However the averages within lab CV remains at acceptable levels.

Since commercially available trypsin preparations differ between companies and batches in immunoreactivity (see 6.2.1.7 IRT1/IRT2 isoforms), protein concentration, or tryptic activity, the production of quality control material for IRT assays is extremely difficult.[225] Traditional external quality assurance (EQA) schemes are of limited use for calibrating IRT assays used in screening since there is too much volumetric variability in the control spots themselves and in the calibrant spots used to construct the standard curve. A large number of spots assayed in several different daily runs would be needed in order to produce accurate results.

An alternative approach is to use the population distribution of IRT values to monitor assay performance. Centile plots of IRT results from routine screening are useful as a retrospective check on assay calibration, and will detect quite small differences between individual batches or between different laboratories.[226] This overcomes difficulties associated with prepared blood spots and, provided that a sufficient number of results is available (at least over 2000), has greater statistical validity than EQA based on relatively few blood spots.[226]

Table 12. IRT : 2008 Quality Control Data Summaries of Statistical Analyses (ng IRT/mL whole blood) (adapted from [222])

METHOD	N	Mean	Average Within Lab SD	Average within lab CV (%)	Tot SD	Tot CV (%)	Y-Intercept*	Slope
Lot 791 – Assayed 16.1 ng/mL blood								
MP Biomedicals ELISA	20	29.4	3.1	11	10.6	36	1.7	1.4
Delfia	419	16.1	2.4	15	2.9	18	-0.3	1.1
AutoDelfia	1043	16.9	1.7	10	2.0	12	-1.7	1.2
Bio-Rad Quantase	90	13.2	2.7	20	4.5	34	-2.4	0.9
Bioclone ELISA	29	13.1	1.6	12	1.9	15	-7.1	0.9
Other	29	16.5	2.0	12	2.1	13	1.6	0.9
Lot792 Assayed 38.8 ng/mL blood								
MP Biomedicals ELISA	20	55.5	9.5	17	18.5	33	1.7	1.4
Delfia	409	40.3	4.6	11	5.8	14	-0.3	1.1
AutoDelfia	1043	43.4	4.2	10	5.0	12	-1.7	1.2
Bio-Rad Quantase	90	31.2	4.4	14	16.4	53	-2.4	0.9
Bioclone ELISA	30	28.1	2.5	9	6.3	22	-7.1	0.9
Other	30	38.6	5.6	15	9.0	23	1.6	0.9
Lot793 Assayed 69.2 ng/mL blood								
MP Biomedicals ELISA	20	86.5	10.6	12	11.7	14	1.7	1.4
Delfia	410	74.8	7.9	11	10.6	14	-0.3	1.1
AutoDelfia	1058	81.7	8.4	10	10.3	13	-1.7	1.2
Bio-Rad Quantase	88	55.0	7.4	13	26.6	48	-2.4	0.9
Bioclone ELISA	30	52.2	12.3	24	16.7	32	-7.1	0.9
Other	30	67.5	6.5	10	8.9	13	1.6	0.9
Lot 794 – Assayed 133.7 ng/mL blood								
MP Biomedicals ELISA	20	190.7	17.6	9	41.7	22	1.7	1.4
Delfia	408	140.3	13.4	10	17.3	12	-0.3	1.1
AutoDelfia	1068	155.3	14.1	9	18.2	12	-1.7	1.2
Bio-Rad Quantase	86	115.4	21.3	18	51.7	45	-2.4	0.9
Bioclone ELISA	29	122.8	27.2	22	48.3	39	-7.1	0.9
Other	30	128.1	12.3	10	24.7	19	1.6	0.9

*Estimated by performing a weighted linear regression analysis of mean reported concentrations versus enriched concentrations and extrapolating the regression to the Y-axis.

6.2.1.3 Age at sampling

The age at which the blood samples are taken seem to have little direct effect on the effectiveness of screening. Some US programs sample on day one of life and report reasonable results, as does the relatively late day 5 sampling.[54, 224] The introduction of CF screening does not require a change in current blood sampling practice except for additional emphasis on avoiding fecal contamination.

6.2.1.4 Stability of IRT in blood spots

IRT in dried blood spots is stable over the short term, but it is not advisable to rely on a screening result from a sample that has been significantly delayed in transit. When stored for 10 weeks in dark at room temperature and in a dry location inside a cardboard box, 'Guthrie' blood samples from normal babies lose approximately two-thirds of their IRT as measured by Sorin® reagents, which measure mainly trypsinogen and give a screening cut-off ~ 80 ng/ml.[227] When samples are stored at + 4 °C for 4-8 months, using the Boehring RIA-gnost® neonatal trypsin kit (which detects both trypsin, trypsinogen and inhibited forms of the enzyme), with a screening cut-off of approximately 900 ng/ml,[228] samples from babies with non-CF hypertrypsinaemia lost approximately 25% of their activity after 4 months, and almost 45% after 8 months. Unlike samples from normal neonates, samples from CF cases showed a bimodal decay curve suggesting a different mix of IRT species.

6.2.1.5 Non-CF causes of increased IRT

A wide variety of physiological or medical conditions have been associated with hypertrypsinaemia in the neonatal period. Increased IRT has been noted in trisomies 13.[82, 229] Perinatal stress has also been reported to be a significant factor in hypertrypsinaemia,[230] and a cohort of 372 sick infants on the neonatal intensive care ward had significantly increased blood-spot IRT compared to normal infants irrespective of the diagnostic category.[231] However, neonatal infection as such was reported not to have any effect.[232] Elevated IRT levels have been found in association with congenital infections, renal failure and bowel atresia[223] and in a case of nephrogenic diabetes insipidus.[233]

In the absence of any of the common CF-causing mutations, and particularly if the mutation panel has a sufficiently high population specific detection rate in the newborn ethnic group, the likelihood of CF in such cases is low.

The population distribution of blood IRT concentrations in the newborn period is slightly higher in babies of North African parentage[234] and in African-Americans[235] than in babies of North European origin, which should be taken into consideration if newborns have an ethnic origin from these regions.

6.2.1.6 IRT in special situations

A tendency for CF neonates with meconium ileus to have IRT values within the normal range was noted early in the history of newborn screening, though its basis is obscure.[145, 224, 236]

Hyperechogenic bowel on ultrasound imaging in utero in the second trimester of pregnancy may indicate CF, with risk of the disease in the fetus ranging from 1.5% to 25% depending on the grade of echogenicity observed.[237, 238]

Neonates presenting with meconium ileus, who have had hyperechogenic bowel in utero, should be regarded as high risk and should be investigated independently, in parallel with the normal NBS process.

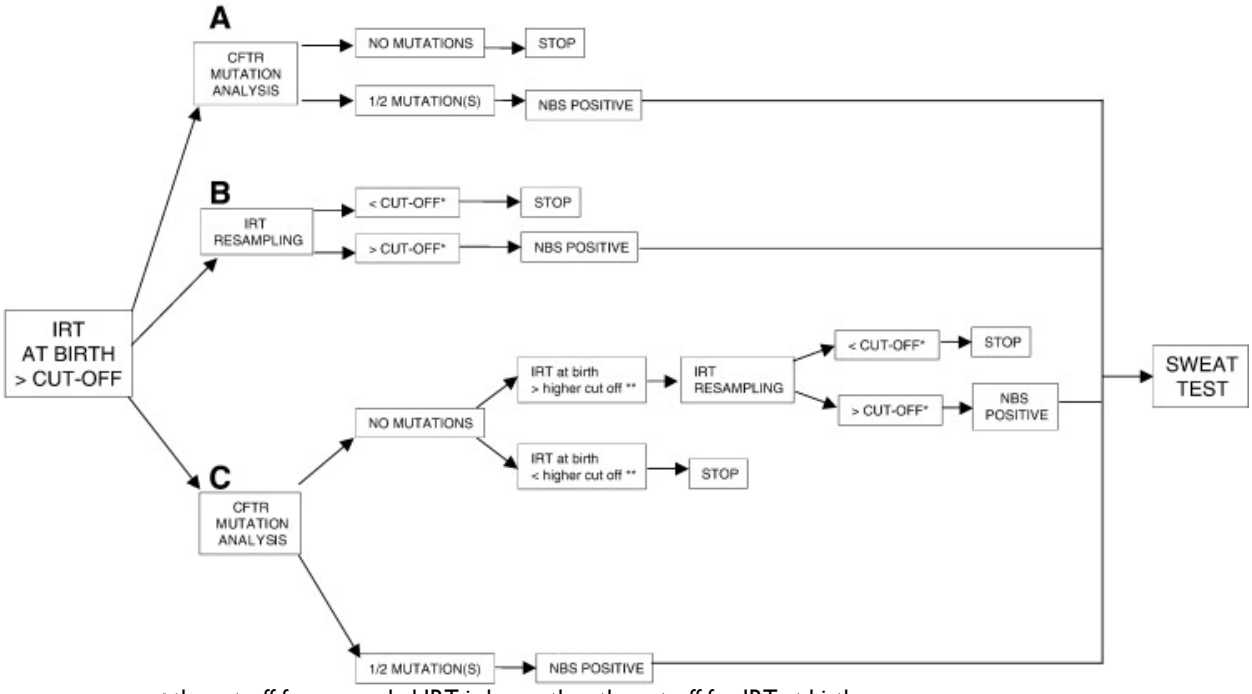
6.2.1.7 IRT1/IRT2 isoforms

There are several molecular forms of IRT; the 2 major forms secreted by exocrine cells of the pancreas are trypsinogen 1 (cationic trypsinogen or IRT1) and trypsinogen 2 (anionic trypsinogen or IRT2).[239, 240] A multiplex method with specificity for 2 isoforms of IRT has been described and has performance comparable to that of a standard IRT method and the advantage of improved standardization by detection of the 2 isoforms.[241]

6.2.2 Screening algorithms

Some examples of intermediate tiers strategies are shown in Figure 4. A comparison of the main used protocols is presented in Table 13.

Figure 4. Three examples of intermediate tier strategies.



*the cut-off for resampled IRT is lower than the cut-off for IRT at birth.
**if IRT at birth exceeds a cut-off higher than the one used to start the procedure, the protocol proceeds to resampling even though no mutations were detected. Reproduced from [43].

Table 13. Comparison of different CF screening strategies (adapted from Wilcken[216])

Strategy	Countries/ references	Likely benefits	Likely drawbacks
IRT/IRT	Austria, part of Italy, part of USA	Good specificity and sensitivity after second test No genetics testing - No carriers detected	Need for repeat blood sample Poor specificity first test: thus, more families with anxiety Need for second IRT cut off
IRT/DNA	Merelle 2006	Single sample Good sensitivity	Lower specificity Unwanted carrier identification Unwanted detection of mild CF Increased cost
IRT/DNA/failsafe step	Comeau, 2004	Single sample Increased sensitivity compared with IRT/DNA or IRT/DNA/IRT strategies	Increased number of sweat tests
IRT/DNA/IRT	UK, Irland, Scotland, France	Increased specificity compared with IRT/DNA Reduced number of sweat tests	Repeat blood sample for a small number of babies False-negative result possible if no mutations detected, or if second IRT is normal (2 severe mutations, one undetected) Some carriers are identified, and offered genetic counselling. Some of these could have another mutation
IRT/PAP	Sarles 2005 Pilot studies Holland, Germany	Single sample Same or equal sensitivity than IRT/DNA	Cheap Easy to implant No sample splitting

6.2.2.1 IRT/IRT

The concentration of blood/serum IRT declines with age much faster in false positive cases than in infants with CF,[242] and therefore raised IRT at about one month of age has a high positive predictive value. Most programs select a slightly lower cut-off for this second sample.

The rate of decline of IRT in babies with CF is variable. In the CF group as a whole, IRT is still increased at one year of age,[243, 244] but such patients typically have non-detectable levels of IRT by the age of five years.[243-245] However, a significant proportion of CF patients show a more rapid decline in IRT: Rock[246] found that of 24 neonates who were positive on the initial screen, 10 had IRT values below the cut-off in samples taken between 41 and 62 days of age. Retrospective genotyping of babies with a raised IRT value in the initial sample, but an IRT value below the cut-off in a second sample taken at 27 days of age, showed that approximately 1% of these were compound heterozygotes for the F508del mutation and another CFTR mutation, the majority being R117H.[247]

This was the initial protocol adopted by most screening programs in Australia, New Zealand, Europe, and the US.[248]

A major disadvantage of this protocol is that a second blood sample will be required from 1 in 200 to 250 of all babies screened. A considerable number of unaffected babies will be subjected to a sweat test, generating a great deal of parental anxiety.[248, 249]

Other disadvantages of CF screening protocols with an algorithm requiring a second sample are possible delays to obtain this additional sample or cases where no sample is obtained.[250]

6.2.2.2 IRT/DNA

Cystic fibrosis is caused by mutations in the CFTR (ABCC7) gene.[6, 251] The most common mutation is F508del, previously termed ΔF508, which accounts for approximately two thirds of all CFTR alleles in patients with CF, with a decreasing prevalence from Northwest to Southeast Europe.[252, 253] The remaining third of alleles are substantially heterogeneous, with fewer than 20 mutations occurring at a worldwide frequency of more than 0.1% (http://www3.genet.sickkids.on.ca/cftr/app and www.who.int/genomics/publications/en/). Some mutations can reach a higher frequency in certain populations, due to a founder effect in religious, ethnic or geographical isolates.[254, 255] The combinations of these mutations mostly explain at least 90% of all mutant CFTR genes in Northern- and Western European countries.

Although the majority of CFTR mutations have been associated with European-derived populations,[252] there are also CFTR mutations in non-European populations, such as African and East Asian populations,[256] but no alleles have reached the high frequency of F508del.

At this moment, more than 1600 mutations have been identified in the CFTR gene and additional mutations are continuously found and updated in the Cystic Fibrosis Genetic Analysis Consortium (CFGAC) database (http://www3.genet.sickkids.on.ca/cftr/app). Missense mutations account for 42%, frameshift mutations for 15%, splicing mutations for 12%, nonsense mutations for about 10%, inframe insertions/deletions for 2%, large insertions/deletions for 3%, promoter mutations for 0.5%, and sequence variations which are not predicted to be disease-causing for 15% of all alleles. De novo mutations and uniparental disomy of chromosome 7 bearing a mutated CFTR gene are exceptional events.

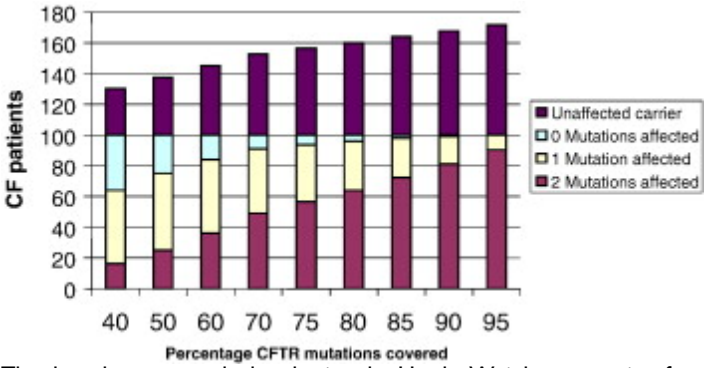
It is generally advised in Europe to screen for all mutations that are found at an incidence of at least 0.5% in the mutant CFTR genes from which the tested individual originates.[43, 121, 257]

Most NBS protocols use a panel of the most common CF-causing mutations only on samples with a raised IRT.[257] Homozygotes and compound heterozygotes can be assessed promptly. Management can then often be initiated in the first three weeks of life. It is mandatory to confirm the findings of the DNA test with a confirmatory positive sweat test.

Infants carrying only one identified mutation proceed in the protocol, usually through a sweat test, in order to distinguish affected individuals from carriers. DNA-testing should always be linked to genetic counselling in order to provide sufficient advice to the family and eventually offer testing of the index case's relatives if they are within the reproductive age.

A second tier NBS panel should be designed in order to include well known CF-causing mutations represented in the local CF population, including those alleles frequently occurring in ethnic minorities. However, population genetic calculations based on the Hardy–Weinberg law provide evidence that increasing mutation coverage much above 80% has little effect on the proportion of CF patients in whom no CF-causing mutation is found (false negative rates). One should also keep in mind that the number of unaffected carriers detected keeps increasing in proportion to the mutation detection rate (Figure 5). In areas with ethnic diversity and a tendency to marry within ethnic groups, the Hardy–Weinberg equation does however not apply. Here, increasing the number of mutations, i.e. including the ethnic specific mutations, has a clear benefit on screening performance. Additionally, in such areas there is also an ethical and political dimension to ensure that minority groups are not disadvantaged.

Figure 5. Effect of CFTR mutation coverage on yields of CF patients and CF carriers.



The data shown are calculated using the Hardy–Weinberg equation for a population where 0.5% of the initial screened samples are sent for a single-stage mutation analysis. It is assumed that carriers for all the mutations examined are over-represented to the same extent as carriers of F508del. Reproduced from [43].

It is still a matter of discussion whether the R117H mutation should be included in a second tier NBS panel or not. R117H can be in cis, i.e. on the same parental allele, with a stretch of either 5 or 7 thymidines located in intron 8, conventionally named T5 or T7.[258] These polymorphic tracts affect the extent of correct splicing of CFTR exon 9: T5 stretches give rise to less efficient splicing than T7.[259-261] Thus, R117H-T5 will result in less functional CFTR than R117H-T7.[260] In clinically diagnosed CF patients populations, when found in compound heterozygosity with a CF-causing mutation R117H-T5 generally results in pancreatic sufficient CF, whilst R117H-T7 may result in a mild form of CF, obstructive azoospermia, or no disease at all.[121] In the French NBS program,[145, 262, 263] up to 7% of the newborns who had an elevated IRT test and two CF alleles were compound heterozygous for R117H-T7 and a CF-causing CFTR mutation, a much higher proportion than in the CF population diagnosed clinically.[152] These children have shown no major signs of CF in their pediatric lifespan, although the longterm outlook is difficult to predict and there are reports of significant chest involvement with this genotype and manifestations of CF disease in adulthood.[149]

But all by all, when a baby is found to be compound heterozygous for R117H-T7 and a CF-causing CFTR mutation, without any signs of clinical CF symptoms, it is much more likely that the child does not develop pediatric CF disease. In a follow-up study of 184 F508del/R117H individuals it was concluded that the R117H mutation should be withdrawn from mutation panels in CF screening programs.[135]

There are indications that in a small fraction of patients, CF or CF-like disease may be caused by mutations in (a)nother gene(s) than CFTR.[264, 265] There is evidence that mutations in the subunit genes coding for the amiloride sensitive epithelial sodium channel may explain disease in a minority of these patients by a monogenetic mechanism, an oligogenetic system with CFTR (CF carriers), or even a polygenetic system.[266-268] Additional studies are needed, and currently it is not appropriate to screen for mutations in other genes than CFTR.

Functional studies are needed to determine if a CFTR mutation is a CF-causing mutation rather than a variant. For more than 80% of the more than 1600 different CFTR mutations, functional studies have not been performed. Although the pathological consequences of nonsense mutations and frameshift mutations can be accurately predicted (representing about 25% of all CFTR mutations identified), the functional consequences of the remainder cannot be accurately predicted. Such mutations might be found when the complete CFTR gene is analyzed for the presence of mutations, such as by sequencing. Up till now, many clinicians therefore advise to perform mutation-specific testing only of mutations of which the CF-causing status has been clearly established.

To overcome this problem, the CFTR project (Garry Cutting, personal communication, http://www.genet.sickkids.on.ca/cftr2ComingSoon.html)[269] has been set up. The goal of the CFTR2 project is to assess the disease liability of CFTR mutations that have been reported in CF patients. The CFTR2 database currently has clinical data on 1099 mutations that occur in about 40 000 CF patients. A committee of CF clinical experts approved the clinical data set obtained for every patient using uniform criteria. Sweat chloride level was selected as the primary metric. The first application of this project has been to study mutations that occur in 9 or more patients worldwide. These 160 mutations account for 96% of the CF alleles in CFTR2. To complement clinical evidence of CF pathogenicity, the CFTR2 team is evaluating the functional consequences of the 160 mutations using 4 steps: 1) the nature of the mutation 2) studies of mutation effect on RNA splicing 3) studies of mutation effect upon CFTR processing and function and 4) absence in "non-CF" CFTR genes (CFTR genes in normal subjects). The results of the aforementioned methods are also being used to construct an algorithm that predicts the disease liability of the 160 mutations. The development of a predictive algorithm will provide an objective assessment of disease liability that will be used to 1) corroborate clinical and functional data, 2) evaluate the disease potential of the remaining rare mutations and 3) enable prediction of disease causing potential of mutations discovered after the CFTR2 project has been completed.

In a second phase, the number of mutations will be extended to 260 mutations, i.e. mutations that occur in 5 or more patients worldwide and that account for 98% of the CF alleles in CFTR2.

This CFTR2 database will thus be of utmost importance in future genetic counselling.

Sequencing technologies are indeed rapidly evolving and it is predicted that by 2015 a complete human genome can be sequenced for less than 1000 €.[270] It is therefore very likely that complete sequencing of a gene may become the standard genetic test format in the near future. Moreover, such sequencing tests might be offered by private companies (outside Belgium) in which genetic tests simply can ordered through the internet, e.g. by families in which the newborn had a positive IRT test, but in whom only 1, or even no, CF-causing mutation was found. In the end, professionals of a Belgian CF NBS screening program then still have to deal with this potential problem of problematic counselling.

Given the decrease in sequencing cost, sequencing of the CFTR gene in IRT-positive newborns in Belgium from other ethnic minorities, may become a reasonable option in the future. Indeed, screening for ethnic mutations of other countries than the country in which the individual currently lives and in which the screening is performed is practically not feasible. Sequencing may be a valuable practical and economical option in these individuals. An alternative would be to use another biological marker like PAP (see below).

It should be noted that there are currently already CF NBS screening programs in place in which sequencing of the majority of the CFTR gene, or even the complete CFTR gene, is performed. In these populations, the spectrum of CF-causing CFTR mutations is very heterogeneous so that mutation-specific tests do not reach a sufficient sensitivity, such as in Poland[218] and in the state of California in the US because of the many Latin-American immigrants.[271]

Requirements regarding parents' consent to potential genetic analysis at the moment of the blood collection vary from country to country but may be very onerous.[272] Usually an "opt out" scheme is utilized at maternity wards that explains the entire CF NBS scheme, including the potential of a secondary "CFTR-gene specific" genetic test. We refer to chapter 8 for a detailed discussion.

Compared to an IRT/IRT algorithm, an IRT/DNA protocol is associated with increased sensitivity, specificity, and positive predictive value and has the advantages of an earlier diagnosis and of eliminating the need for a second blood sample. A potential disadvantage of DNA analysis is unwanted carrier identification which results in the increase in the number of unaffected infants referred for a sweat test.[249]

6.2.2.3 IRT/DNA/IRT

To address the drawbacks of the IRT/DNA protocol, the IRT/DNA/IRT protocol which includes a second IRT measurement after DNA testing, significantly reduced the number of second IRT tests compared to the IRT/IRT protocol and also the number of (negative) sweat tests.[248] This protocol is used in France and UK (England) with some differences as shown respectively in Figure 6 and Figure 7.

Figure 6. French protocol (from[45])

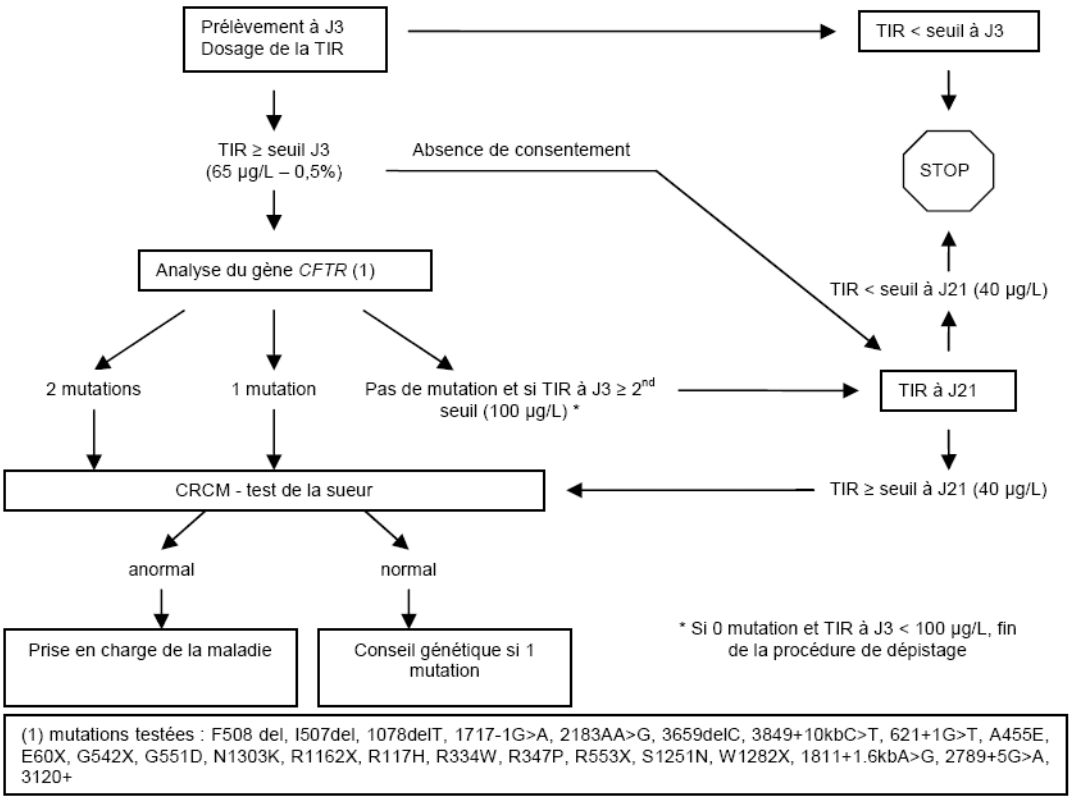
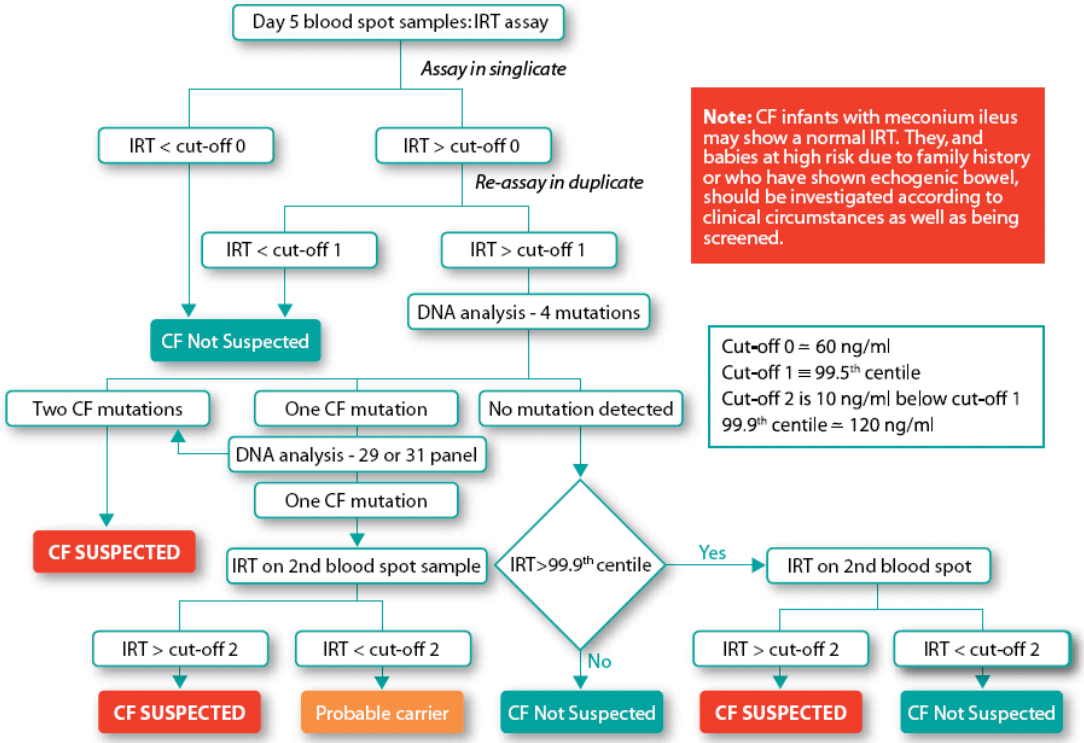


Figure 7. UK protocol (from [117])



Cut-off 0 : approximately 10 ng/ml less than cut-off 1 (typically a value of 60 ng/ml) ; Cut-off 1: 99.5th centile (70 ng/ml) ; Cut-off 0 : approximates to 0.1 % (120 ng/ml)

- In both programmes the cut-off for the sweattest is > 60 mmol/l for CF diagnosis, < 30 mmol/l for normal and from 30 to 60 mmol/l for inconclusive result.
- In both programmes, replications in duplicate for all samples with an IRT above a preliminary threshold, set 10 ng/ml below the final cut-off.

The main differences between these two protocols are:

- Number of mutations screened: 30 in France and 4, extended to 29 or 31 if one mutation found initially, in the UK. However, it should be noted that these 4 mutations have a much higher sensitivity in the UK than in other European populations, which makes a two-phase mutation screening protocol less feasable in most European populations.
- Different attitudes if one mutation is found: sweat test in France, and second IRT in the UK.

6.2.2.4 *IRT/PAP or IRT/PAP/DNA*

The use of the pancreatitis-associated protein (PAP) as a second tier, or even combined with IRT in the first tier is being explored. This approach would avoid the issues raised by CFTR mutation analysis or the need for a second blood sample. PAP measurement is technically feasible on the same sample as other neonatal screenings tests (phenylketonuria, hypothyroidism). CF neonatal screening with PAP alone performs similarly to screening with IRT alone, with less carrier detection. Combining PAP with IRT for CF neonatal screening may be as efficient as an IRT/DNA strategy, but would be cheaper and would result in a lower number of CF carriers. If obtaining informed consent for DNA testing poses ethical or practical problems, the PAP/IRT strategy would be an alternative to the IRT/DNA strategy.

It should be noted that the use of the IRT/PAP approach will allow one to detect patients with ethnic-specific mutations in ethnic minorities. This is an issue for countries and/or large cities with multiple ethnicities.

A combined IRT+PAP assay kit is being developed and pilot studies are in progress in the Netherlands[273], Germany[274, 275], France, Australia,[276] and in Belgium.[277] In the Netherlands, the results of a pilot trial recently resulted in a recommendation of the Health Council of the Netherlands to start CF NBS using IRT+PAP (http://www.gezondheidsraad.nl/en/publications/neonatal-screening-cystic-fibrosis), thus avoiding many of the 'equivocal' CF cases seen when CF DNA tests are used as second line screening test.

6.3 DIAGNOSIS THROUGH CF NEONATAL SCREENING

Screening tests identify apparently healthy individuals who have a high probability of having a specific disorder, which justifies a subsequent diagnostic procedure.[278] Following a positive NBS for CF, a major component of the subsequent assessment is a sweat test, which can usually distinguish between true and false positives. Despite recognition of the *CFTR* gene defect, the sweat test is still considered by consensus groups as the 'gold standard' for a diagnosis of CF. Infants with a positive NBS test and a raised sweat chloride (above 60 mmol/L) are considered to have a diagnosis of CF, even in the absence of any clinical features.[5, 119]

After a positive screening test result is reported, the time to diagnostic assessment should be as short as possible. This requires efficient cooperation between screening laboratories, maternity units, community health care providers and CF centers. It is essential that these pathways are established before implementation of a CF NBS program. In particular, attention should be given to ensure that the assessment and sweat testing are carried out promptly to minimize parental stress, which is known to be extreme in the period following an abnormal screening test result.[279]

6.3.1 Sweat test

The sweat test is a key component of any NBS protocol for CF, providing a physiological confirmation of the screening result, or reassurance that a carrier is not a compound heterozygote with an as yet unidentified *CFTR* mutation. However, sweat testing in this age group is challenging and has to be performed according to specific guidelines.[5, 280]

The following must be taken into account when undertaking a sweat test in infants following a positive screening result:

- Sweat chloride concentration is the "gold standard" analytical measure to confirm a diagnosis of CF in NBS positive infants. With rare exceptions, a diagnosis of CF can be made when chloride levels exceed 60 mmol/L, and excluded when they are below 30 mmol/L.[5, 119] Results which are not physiologically compatible should be questioned (i.e. chloride or sodium > 150 mmol/L).
- Sweat collection and analysis should be performed in a laboratory with adequate experience. International standards suggest that a laboratory should be undertaking at least 50 tests per year.[156, 280]
- When using a 2 × 2-inch gauze or filter paper, the minimum sweat weight should be 75 mg. When using the coiled capillary system (Macroduct™), the electrodes and stimulation area are smaller and the minimum acceptable sample is 15 µL (calculated to ensure a rate of sweat secretion greater than 1 g m^{-2} min^{-1}). A macoduct system is the preferred method for a NBS programme: less sweat is needed for a correct analysis and the succes rate will be higher. However, the cost of this system will increase the cost of the sweattest.
- Sweat testing can be performed by the age of two weeks in newborns weighing 3 kg or more.[280] Neonates must be normally hydrated and with no significant signs of systemic illness. When clinically indicated, it can be performed in term infants after 7 days of age, but with a higher probability of insufficient sweat collection.

- Collecting sweat from two sites is preferable as this reduces the number of insufficient samples and provides internal validity. Sweat should be collected for not more than 30 min and not less than 20 min. Insufficient sweat collections should not be pooled; the test should be repeated.
- Sweat sodium is also elevated in CF but is less discriminatory when compared to chloride, and therefore should not to be used for the diagnosis of CF.[156]
- Sweat conductivity should not be used to confirm a diagnosis of CF.
- Some *CFTR* mutations that are clearly CF causing (in particular, 3849 + 10 kb C > T) can be associated with normal or equivocal sweat electrolyte values.
- Following sweat collection, chloride analysis should be undertaken promptly (preferably immediately) in order to reduce the waiting period for the family.
- NBS programmes use a sweat test cut-off value of > or = 60 mmol/L for a CF diagnosis.[5] Lower values for sweat tests have however been reported in CF newborns. Therefore infants with an intermediate sweat test (30-59 mmol/L) and 1 mutation detected should proceed to sequencing. Some reports advise an even lower cut-off for intermediate values of 24 mmol/L.[281]

The sweat test thus remains a key component in establishing a diagnosis of CF in infants with a positive NBS result. Sweat collection in infants is challenging, and must be performed according to specific guidelines.

6.3.2 Inconclusive diagnosis

The majority of affected infants recognised through NBS have a clear diagnosis of CF (i.e. two CF-causing mutations in *trans* or one recognised CF-causing mutation and a sweat chloride level above 60 mmol/L).[5] However, in a small but significant number of cases the diagnosis is equivocal, specifically when *CFTR* mutations with unclear phenotypic consequence are detected or when one mutation is found and sweat chloride levels are in the intermediate range (i.e. between 30 and 60 mmol/L).

The ECFS CF NBS Working Group has produced a consensus with regard to the evaluation and management of infants with an equivocal diagnosis following CF NBS.[147] Initial statements were agreed based upon a systematic review of the literature, and formed the basis of a modified Delphi methodology[282] to achieve a Europe-wide consensus. Contributing clinicians, biochemists and geneticists were asked to express their opinions on the statement. Their options were either to agree, disagree or state that they felt unable to comment. If they disagreed they were asked for comments or alternative suggestions. Consensus was established if more than 80% of respondents agreed with a particular statement.

Figure 8 illustrates the pathway developed through the consensus process. Highlighted (shaded) boxes are discussed below.

Repeat sweat test

Sweat collection and analysis should be repeated in an experienced laboratory.

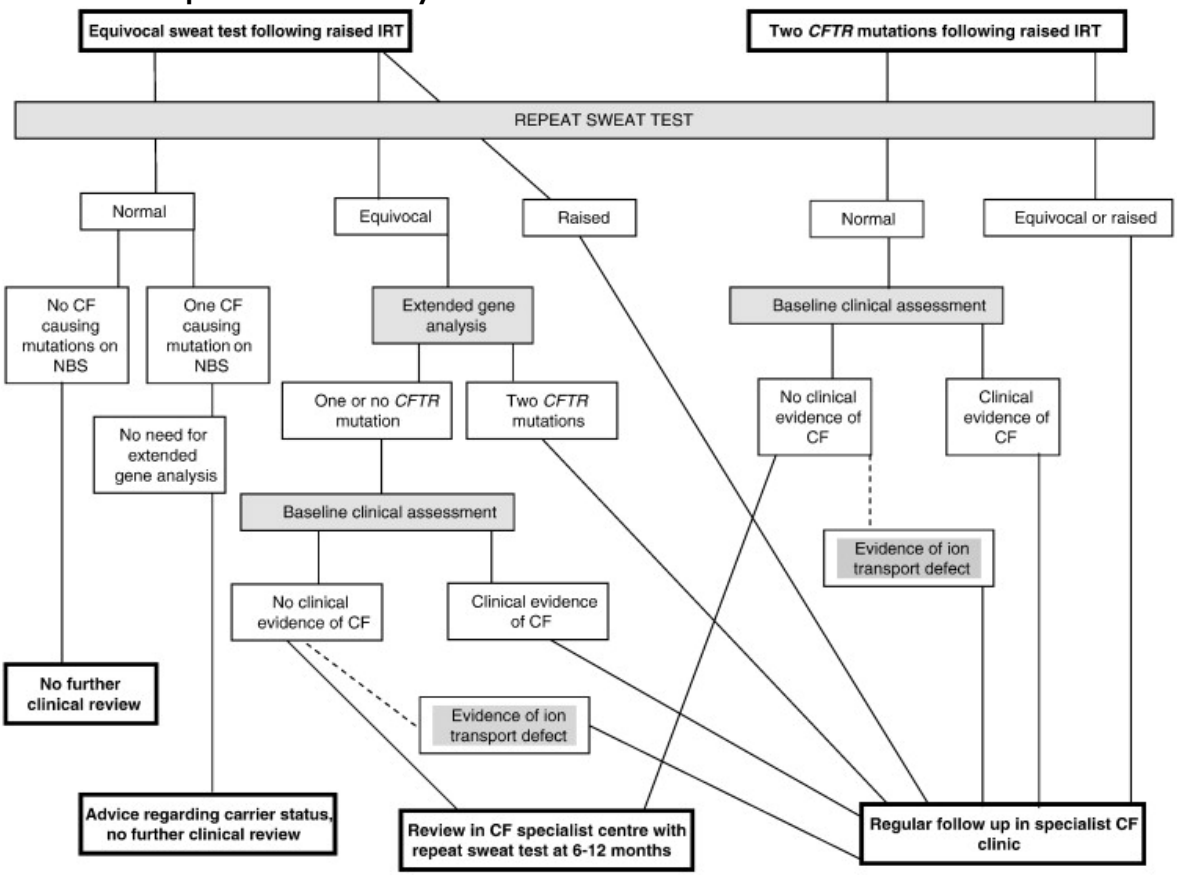
Extended DNA analysis

Further DNA analysis of the CFTR gene should be guided by the type of screening protocol (i.e. protocols that initially only examine a limited panel of CFTR mutations would continue promptly further DNA analysis with an extended panel of population specific mutations). The extent of DNA analysis should reflect the level of clinical suspicion. Care should be taken to avoid a situation where mutations are recognized with an unclear pathogenic potential (e.g. missense mutations identified by sequencing of the CFTR gene). Infants recognized to be compound heterozygotes for R117H should have further characterization of the poly T variant region for the presence or absence of the 5T background.[144, 261]

Baseline clinical assessment

Although infants with CF may have little or no symptoms in the first months of life, it is essential to carefully search for clinical features associated with the diagnosis of CF. Clinical evaluation should be done in a CF clinic, and include assessment of non-respiratory (e.g. fecal elastase examination) and respiratory disease (airways culture and chest radiograph) signs of the disease. Further investigations may be indicated as determined by the clinical symptoms.

Figure 8. Diagnostic algorithm for inconclusive diagnoses following CF NBS. Reproduced from Mayell.[147]



Evidence of ion transport defect

A number of electrophysiological techniques are available to demonstrate the salt transport defect that characterizes CF. Some of these tests are technically challenging in neonates/infants, and they are currently undertaken only in few specialist centers worldwide. Thus far, none have the validity of sweat testing or CFTR genotype analysis, but may provide useful additional information in equivocal cases (Table 13). Intestinal current measurements do not detect mild or atypical CF disease.

Table 14. Electrophysiological diagnostic techniques.

Test	Technical details	What it involves for the infant	Availability
Nasal Potential Difference (PD)	Ion transport across airway epithelium can be assessed by measuring the baseline PD. The impact on the PD of perfusing different solutions and drugs provides further information to differentiate CF from non-CF recordings.	The exploring electrode is placed in the nose. A reference electrode is placed either subcutaneously or over abraded skin on the forearm. Solutions are perfused into the nose and can be swallowed.	Very few centres are able to undertake this measurement in infants although it is more widely available in older children and adults.
Intestinal Current Measurements (ICM)	A biopsy is mounted in the laboratory in a device (Ussing chamber) that enables measurement of transepithelial ion transport. Various aspects of ion transport can be examined.	Biopsy of rectal mucosa. This procedure is painless and well tolerated by young infants. Does not require general anaesthesia or sedation.	This technique requires a dedicated laboratory service with highly skilled technicians. Available in limited number of centres in Europe.
Small bowel biopsy	Similar measures of transepithelial transport processes can be undertaken in the laboratory on upper gastro-intestinal (GI) mucosal biopsies.	Upper GI biopsy; requires general anaesthesia in most cases.	Limited (only currently available in Sheffield, UK; contact Prof Chris Taylor).

Reproduced from [147].

6.4 INTERNATIONAL RECOMMENDATIONS

6.4.1 Australasian paediatric respiratory group (2005)

The Australasian paediatric respiratory group established a consensus statement.[148] The diagnosis of CF can be made in an infant who has been identified by newborn screening with an IRT >99th percentile and has:

Two confirmed CFTR mutations that are known to be associated with CF, or one mutation and a sweat Cl- of 60 mmol/l, or one mutation, a sweat Cl- 30–59 mmol/l, and recognized clinical features.

6.4.2 Cystic Fibrosis Foundation Consensus Report (2008)

This document[5] is a practical guide intended to facilitate the implementation of CF NBS, with a specific chapter about the algorithms. The choice between IRT/IRT and IRT/DNA should be done in function of the goals of the program, the means, the demography, and the clinical care programs.

6.4.3 European best practice guidelines (2009)

This working document[43] is a true state-of-the-art democratic consensus among experts in Europe with respect to guidelines for cystic fibrosis neonatal screening. For the experts, there is little evidence to support the use of IRT alone as a second tier, without involving DNA mutation analysis. However, if IRT/DNA testing does not lead to the desired specificity/sensitivity ratio in a population, a screening programme based on IRT/IRT may be used.

6.4.4 Newborn screening of cystic fibrosis in France (2009)

Several recommendations are included in the Haute Autorité de Santé (HAS) report on CF NBS,[45] obtained after five years of screening with a IRT/DNA/IRT algorithm. They include: evaluation of the interest for the patient because the potential identification of CF heterozygotes; reevaluation of the panel of the screened mutations; evaluation of a strategy involving a PAP step.

6.4.5 Laboratory Guide to Newborn Screening in the UK for Cystic Fibrosis (2009)

This document[117] is a detailed guide for the laboratories which provide a newborn blood spot screening service for CF in UK. The pre-analytical, analytical and post-analytical steps are described. The chosen protocol is a IRT/DNA/IRT algorithm.

7 BELGIAN SITUATION

All screening programs are intended to maximise diagnosis of CF, to minimize second heel pricks, unnecessary sweat tests, diagnostic delay, detection of unaffected CF heterozygotes and diagnosis of very mild form of CFTR defect, and finally to allow for the fact that a clear diagnosis is not always possible. The choice of strategy will depend upon population genetics, costs, and the weight placed on the different aims: maximal sensitivity, minimal or no resampling needed, rate of unwanted CF carrier detection, and reduced numbers of sweat tests.

7.1 CURRENT SCREENING INFRASTRUCTURES

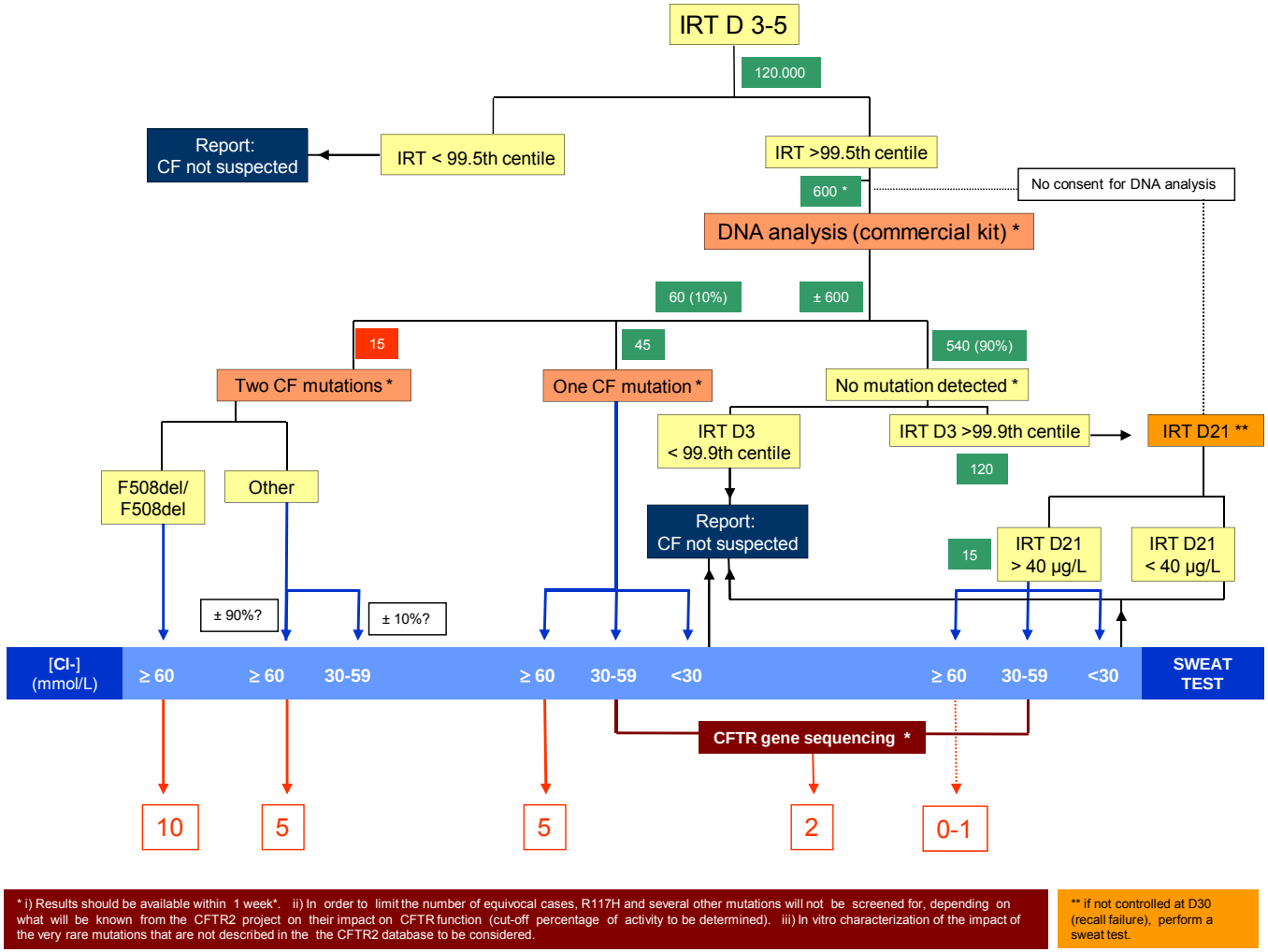
There are approximately 120 000 births per year in Belgium. The screening programs are under the authority of the regional communities. There are 3 newborn screening centres authorized by the French community; and 3 by the Flemish community. Dried capillary or venous blood samples (on Whatman 903 filter paper) are obtained from newborns at the age of 3 to 5 days. These are sent to one of the screening laboratories where the various screening analyses are performed. CF screening should be fully integrated within the existing blood spot screening programs and based on the same screening laboratory populations. Local studies for CF NBS are ongoing.[277]

7.2 CHOICE OF A STRATEGY

The proposed CF NBS algorithm (Figure 9) and the expected patient numbers as shown below is mainly based on the French algorithm, taking into account the neonatal screening situation in Belgium and the fact that appropriate CF DNA kits are available. The aim is to limit as much as possible the number of 'equivocal' cases detected. Recent data from the Netherlands suggest that IRT-PAP based screening may be an alternative, even further reducing the number of CF DNA analyses and the number of 'equivocal' cases, as presented in Figure 11.

Given the fact that the number of IRT-positive (high) newborns who are found to carry no CF-causing *CFTR* mutation is still considerable, the easier and cheaper rIRT-test is preferred over the sweat test.

Figure 9. Proposed Belgian CF NBS algoritm



7.2.1 IRT measurement

The same sample used for the current neonatal blood sampling taken at 3-5 days of life will be used for the first tier IRT.

We propose a cut-off of 99.5th percentile that gives a good sensitivity, and the use of a policy of replication in duplicate for all samples with IRT values above a preliminary threshold, set 10 ng/ml below the final cut-off as recommended.[43] However, the amount of IRT measured will vary from lab to lab, and therefore the cutoff should be set accordingly to reach the 99.5th percentile cutoff of the respective labs. It means that approximately 600 newborns will be positive for IRT and will need CFTR mutation testing each year.

The screening laboratories have to participate to an external quality assessment scheme.

When using the IRT-IRT protocol, a second blood sample is asked to all newborns with a elevated IRT (≥ 70 ng/ml). This is done by sending a letter to the pediatrician of the maternity where the child was born. It is their responsibility to contact the parents and to make sure a second sample is being taken. If the parents do not respond, a second reminder is send, before the file will be closed.

When looking at the data from the Brussels region in 2009,[277] a second sample was obtained in 85% of the cases which means that 15% of the newborns with an elevated IRT are not followed up and might be at risk to develop symptoms of CF. Moreover, when looking at the age of sampling of the control sample, the mean age is 36 days, ranging from 11 days to 167 days, with 27 days as median. These data confirm that CF screening protocols where the algorithm requires a second sample have limitations as obtaining the sample may lead to a considerable delay or no sample may be obtained.

7.2.2 Belgian genetics

With respect to the second tier *CFTR* mutation test, it is generally advised to screen for all mutations that are found at an incidence of at least 0.5% in the mutant *CFTR* genes from which the tested individual originates.[43, 121, 257] However, the ethnic origin of a newborn is not always known. It is therefore advised to test a European panel of mutations, i.e. the most common mutations found in Europe.

Table 15. CFTR mutation spectrum in Belgium.

Mutation	N	Fraction	Cumulative Fraction
F508del	1367	0,695	0,695
G542X	53	0,027	0,722
N1303K	50	0,025	0,748
1717-1G>A	28	0,014	0,762
3272-26A>G	22	0,011	0,773
R117H	22	0,011	0,784
S1251N	22	0,011	0,796
2789+5G>A	17	0,009	0,804
R553X	14	0,007	0,811
W1282X	14	0,007	0,818
A455E	13	0,007	0,825
R1162X	11	0,006	0,831
I507del	11	0,006	0,836
2183AA>G	10	0,005	0,841
L927P	10	0,005	0,846
3849+10kbC>T	7	0,004	0,850

394delTT	7	0,004	0,854
E60X	7	0,004	0,857
W401X	6	0,003	0,860
poly-T-tract	6	0,003	0,863
3659delC	5	0,003	0,866
D1152H	5	0,003	0,868
G920R	5	0,003	0,871
R334W	5	0,003	0,873
3120+1G>A	4	0,002	0,875
G85E	4	0,002	0,877
Y913C	4	0,002	0,879
1078delT	3	0,002	0,881
4218insT	3	0,002	0,883
711+1G>T	3	0,002	0,884
I336K	2	0,001	0,885
1497delGG	2	0,001	0,886
2184delA	2	0,001	0,887
3272-1G>A	2	0,001	0,888
2750delAG	2	0,001	0,889
E656X	2	0,001	0,890
G458V	2	0,001	0,891
G551D	2	0,001	0,892
L165S	2	0,001	0,893
L227R	2	0,001	0,894
Q1313X	2	0,001	0,895
R1070W	2	0,001	0,896
R347P	2	0,001	0,897
S1255P	2	0,001	0,898
Y1092X	2	0,001	0,899
other mutations	33	0,017	0,916
total characterized *CFTR* genes	1801	0,916	
characterized *CFTR* genes, but mutation not known to BMR	2	0,001	
CFTR genes without identified mutation	165	0,084	
missing *CFTR* genes	104	0,053	
total *CFTR* genes	2072		
Total *CFTR* genes - CFTR genes without identified mutation or missing *CFTR* genes	1966	1,000	

Taken from the annual report 2006 of the 'Belgisch Mucoviscidose Register – Registre Belge de la Mucoviscidose (BMR-RBM)' database, which is managed by the 'Wetenschappelijk Instituut Volksgezondheid – Institut Scientifique de Santé Publique'.[169]

Table 15 shows the mutations found in CFTR genes of Belgian CF patients, as taken from the annual Belgisch Mucoviscidose Register – Registre Belge de la Mucoviscidose (BMR-RBM) 2006 report. It should be noted that even within a small country such as Belgium, and even within regions in the Flemish speaking region, and possibly also French speaking region, certain mutations will be more frequent compared to the other regions. A total of 2072 CFTR genes of Belgian CF patients are included in this database. However, a CF-causing mutation has not been identified yet in 165 CFTR genes. Indeed, CFTR mutation testing is performed in different genetic centers in Belgium, and some of these laboratories only screen for the presence of a set of the more common known CF-causing mutations (which may even vary from lab to lab), while other laboratories continue complete scanning or sequencing of the CFTR gene for the presence of a mutation. A total of 104 *CFTR* genes were never referred for genetic testing and or missing. Without doubt, the latter two groups of mutant *CFTR* genes still carry a mutation that is found at a frequency of more than 0.5% in Belgian CF patients. In a total of 91.6 % (1801/1966) of the *CFTR* genes derived from Belgian CF patients, a CF-causing mutation has thus been identified according to this report. If one would screen for all mutations having a frequency of more than 0.5% in the Belgian CF population according to this report, one would need to screen for only 15 mutations and one would obtain a sensitivity of only 84.6%. The incomplete database thus underestimates the number of mutations that need to be tested and the sensitivity that would be obtained. Indeed, in Belgian studies in which the complete *CFTR* gene was studied in CF patients, a higher number of mutations were found to have an incidence of more than 0.5% and a higher cumulative sensitivity of 90-92% was obtained.[283-285] All the CFTR mutations that are of relevance in Belgian CF patients can be detected when commercial available mutation-specific *CFTR* tests are used (Appendix 7).

Innogenetics provides two RDB INNO-LiPA assays. For *CFTR* mutation testing they are usually used in a serial format. When a negative result is obtained with the first RDB INNO-LiPA CFTR17+Tn assay which tests for the most common mutations, the second RDB INNO-LiPA CFTR19 assay is performed. However, it should be noted that the second assay also contains mutations that are found at an incidence of more than 0.5% in the Belgian mutant *CFTR* genes, so that both tests should be used on IRT-positive samples rather than serial analysis. Indeed, many false-positive IRT samples are frequently CF carriers and found to carry a mutation in the first assay, so that the second assay will need to be performed anyway.

Luminex provides two assays. The first xTAG® Cystic Fibrosis 71 kit v2 screens for 39 mutations, while the second xTAG® Cystic Fibrosis 71 kit v2 screens for 71 mutations. All mutations included in the first assay are also included in the second assay. They are thus not used in a serial format, but one of these assays is selected for a given needed sensitivity or spectrum of mutations. The majority of the additional mutations in the second assay are very rare or not found in the Belgian population, but again a few of these mutations have an incidence of more than 0.5% in Belgian mutant *CFTR* genes.

Abbott Molecular provides one assay which screens for 32 mutations.

Elucigene provides 4 different assays. The selection of the assay is again based on the needed sensitivity, spectrum of mutations, but also on the activity (i.e. neonatal screening program).

Elucigene CF29 has been developed to provide laboratories with a simple and accurate means of routinely testing 29 of the most prevalent mutations in individuals of European Caucasian descent.

Elucigene™ CF-EU1 has been specifically developed and approved for *in vitro* diagnostic testing within Europe. Elucigene CF-EU1 identifies 32 of the most frequently observed *CFTR* gene mutations within populations of European origin.

Elucigene CF30 has been developed and approved for routine use in the French national neonatal cystic fibrosis screening program. The panel of mutations detected by Elucigene CF30 are identified as the thirty most frequent within the French population.

Elucigene CF30 is also relevant for screening other European populations, particularly those of Spanish and Scandinavian descent, and might be of interest in the French-speaking region of Belgium.

Elucigene CF7 is particularly useful for cystic fibrosis screening in the Ashkenazi Jewish community.

The obtained sentivitiy will depend on the commercial test which is used. For all, except the Tepnel diagnostics CF7 molecular kit, a sensitivity of 91 to 94% is obtained, which should be sufficient for CF NBS in Belgium (Table 15). It should be however noted, that even these CE-marked commercial *CFTR* tests, may have their pitfalls.[286] In contrast to other *in vitro* diagnostics (eg tests for screening of blood for viral agents), the CE label regulations do not specify minimal performance characteristics for genetic tests. It is therefore advised that these tests are only performed by laboratories which are well aware and have experience with these pitfalls. In practice, this experience is mostly only obtained if the laboratories perform a considerable number of these tests annually. Moreover, the genetic testing laboratories should participate in external annual quality protocols[121, 257].

About 3% of all mutant *CFTR* genes carry a large deletion or insertion, of which the majority or not included in these tests. The CF-causing nature of such large deletions/insertions can, however, be accurately predicted. Specific single tests, such as the MLPA® (Multiplex Ligation-dependent Probe Amplification) assay (MRC-Holland) tests for all possible large deletions or insertions. Although each of these deletions/insertions are rare and found at an incidence below 0.5% and would therefore be not tested, their combined incidence exceeds 0.5% which makes it still worthwhile to include a single MLPA assay in the screening program, since it improves the obtained sensitivity.

Since these DNA tests thus have no 100% sensitivity, not in all IRT-positive individuals who will turn out to be true CF patients, a CF-causing mutation will be identified on both *CFTR* genes. Table 15 shows the fraction of CF patients in whom 2 CF-disease-causing mutations, 1 CF-disease-causing mutation, or no CF-disease-causing mutations can be identified in function of the sensitivity of the *CFTR* mutation test. Of course, if 2 CF-causing disease mutations are found, a CF diagnosis can be made. If no CF-disease-causing mutation at all is found, a CF diagnosis becomes very unlikely. However, if only one CF-disease-causing mutation is found it will be not clear if the newborn carries another CF-causing mutation that is not included in the *CFTR* mutation test, or is truly a CF carrier. A further third tier test is advised in order to try to discriminate newborns who have CF from true CF carriers. A functional test, such as a new IRT test on a new sample or a sweat test in the baby, can be used as a third tier test. It should be noted that in the false-IRT-positive newborns, a higher proportion of CF carriers will be found to carry one CF-causing mutation than in the general population (CF carrier of 1/30). These individuals simple have hypertrypsinaemia but will not develop (severe) CF.[287] It has also been observed that neonates who are heterozygous for a CF-disease-causing *CFTR* mutation with transient hypertrypsinaemia may even carry a second mild *CFTR* mutation, which mostly does not result in (severe) CF.[247]

Table 16. CFTR genotypes, with respect of finding a CF-causing CFTR mutation, or not, in CF patients depending on the sensitivity of the CFTR mutation test.

Sensitivity	MUT / MUT	MUT / No MUT	No MUT / No MUT
90 %	81.0 %	18.0 %	1.0 %
91 %	82.8 %	16.4 %	0.8 %
92 %	84.6 %	14.7 %	0.6 %
93 %	86.5 %	13.0 %	0.5 %
94 %	88.4 %	11.3 %	0.4 %
95 %	90.3 %	9.5 %	0.3 %
96 %	92.2 %	7.7 %	0.2 %
97 %	94.1 %	5.8 %	0.1 %
98 %	96.0 %	3.9 %	0.04 %
99 %	98.0 %	2.0 %	0.01 %
100 %	100 %	0 %	0 %

MUT = CF disease-causing *CFTR* mutation

Given the technological progress in sequencing, and thereby reduction in sequencing cost, complete sequencing of the CFTR gene in IRT-positive newborns may be an option in the near future. Even then, it will be of utmost importance that only mutations of functional relevance are screened/reported. The clinical and functional consequences of 98% of the CF-causing CFTR mutations that are found worldwide in CF patients will be available from the CFTR2 database, and therefore the finding of such a mutation will allow a correct diagnosis. About a quarter of the remainder 2% mutations are nonsense and frame-shift mutations of which the deleterious consequences can be accurately predicted. Only for the remainder 1.5% mutations (missense mutations, small inframe deletions/insertions), the CF-causing defect may not be clear. However, less than 10 mutations are expected in IRT-positive newborns in Belgium annually. More extensive in vitro functional characterization of these mutations may then be an option in order to establish the diagnosis. Here, it should then be investigated if CFTR carrying such a mutation does mature or not, and whether it can function as a chloride channel.[288-290]

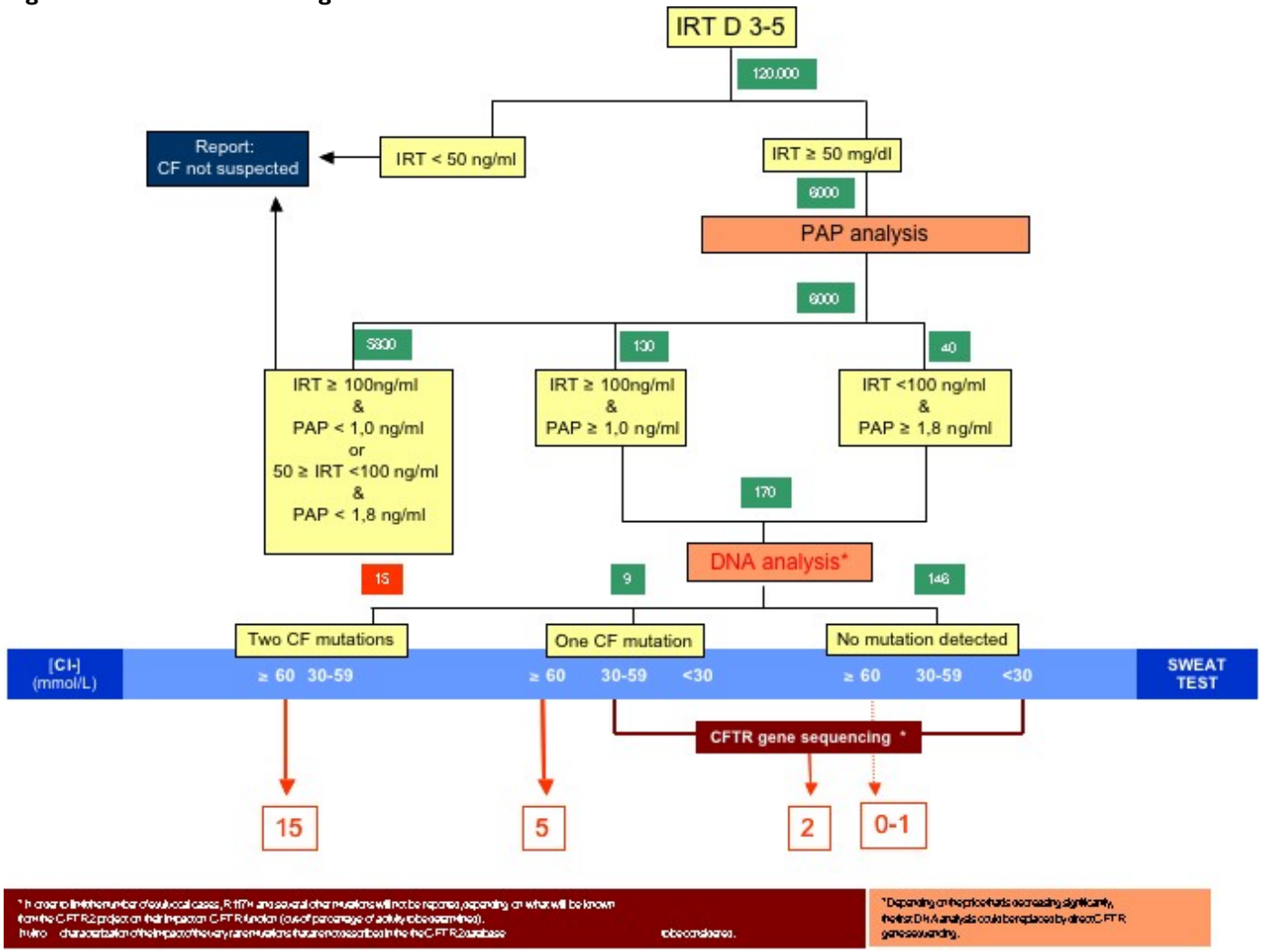
Given the needed expertise, which is even more relevant given the more limited number of samples involved, centralization of complete sequencing of the CFTR gene and in vitro functional characterization of CFTR carrying such mutations/variations in a single center or a few centers should be considered.

In this way, complete sequencing of the CFTR gene thus indeed becomes a valuable option in newborn screening programs. Moreover, it may establish a diagnosis, or absence thereof, in newborns having borderline sweat values. Attention should be given to the turn around time for DNA analysis as this should fit into the overall screening timeline.

7.2.3 Consideration of PAP measurement

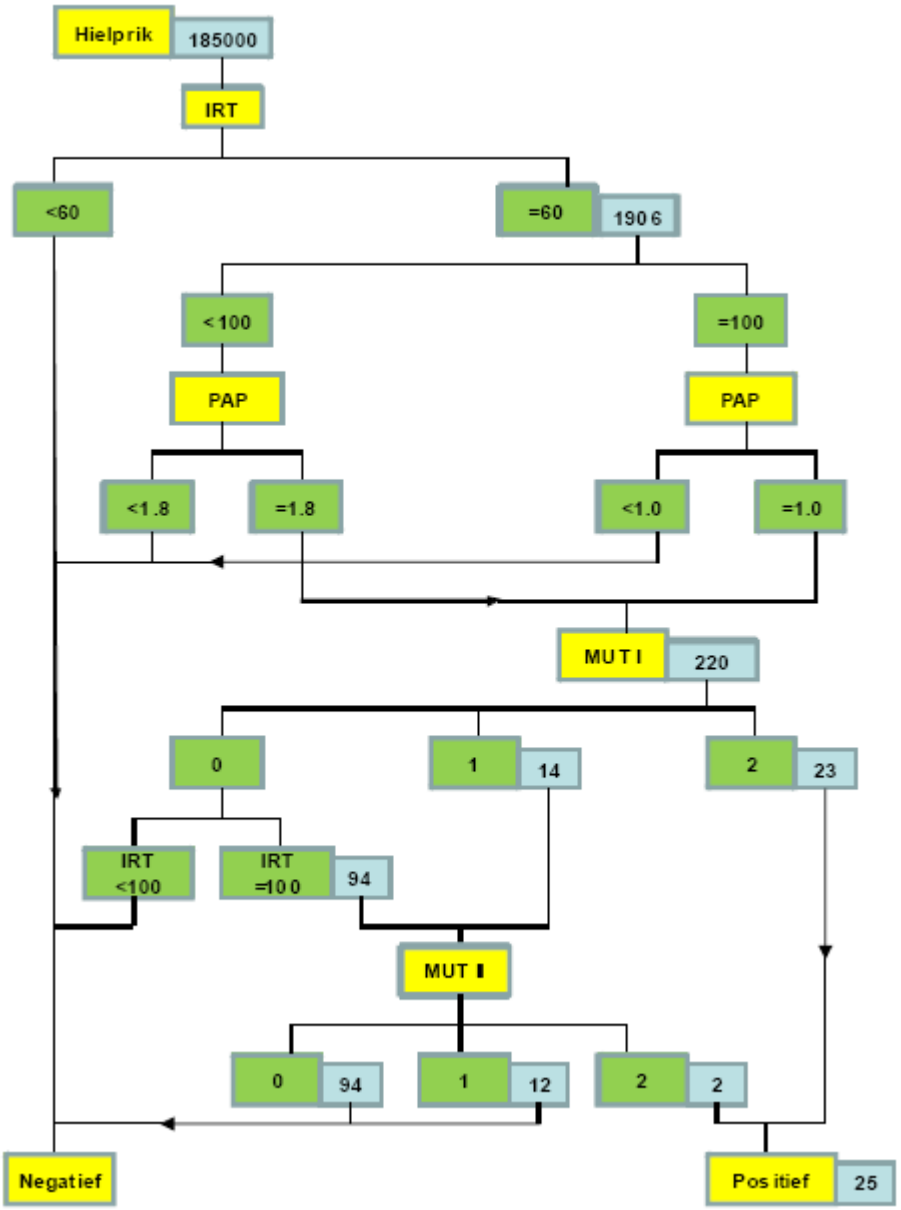
As discussed above, the concentration of pancreatitis associated protein (PAP), a stress protein synthesized by the diseased pancreas, is elevated in the blood of newborns with CF. PAP elevation is not strictly specific for CF and PAP alone as a biological marker would not perform better than IRT alone. However, newborns with CF have a raised level of both IRT and PAP while non-CF newborns may have an increased value for one but rarely for both markers. This combination of markers (IRT and PAP) may overcome the need for genetic testing in the content of a NBS programme. To date, the number of published studies using PAP is however limited. A Belgian study is ongoing[277] with preliminary but yet unpublished data. Additional results from France will become available soon (Munck, Personal communication) and may impact the final choice of the algorithm. The algorithm suggested by A. Meulemans and coworkers (ULB and CHU-Liège) is shown in Figure 10.

Figure 10. IRT-PAP-DNA algorithm



Results of a pilot trial recently resulted in a recommendation of the Health Council of the Netherlands to start CF NBS using the combination of IRT and PAP as illustrated in Figure 11. (http://www.gezondheidsraad.nl/en/publications/neonatal-screening-cystic-fibrosis),

Figure 11. IRT-PAP based algorithms as recommended recently by the Health Council in the Netherlands.



MUT1: test for limited number of mutations using CF kits; MUTII: sequencing; Hielprik: blood sampling from heel prick

KEY POINTS

- **The decision about which method to use for CF NBS involves a compromise between sensitivity and specificity, and between cost and uptake. We need to make sure that the methodology achieves an equitable distribution of both the benefits (early diagnosis) and harms (false-positive and false negative results), particularly within racial and ethnic minorities.**
- **Based on current study data, a NBS protocol based on IRT/DNA seems to be most appropriate for the Belgian situations. From a methodological point of view, the main issue remains the number and type of mutations to be reported. Different commercial CFTR genetic tests are available that screen for the most common mutations found in up to 92% of the CF patients. Given the establishment of the CFTR2 database and advances in sequencing, complete sequencing of the CFTR in IRT-positive newborns becomes a valuable option in the near future. In that case, in vitro characterisation of a small number of mutant/variant CFTR proteins will be also needed.**
- **DNA testing involves certain ethical and legal issues. The drawback of this genetic analysis may be solved by combining IRT with a second biological marker like PAP. At present published data on the performance of NBS algorithms based on IRT/PAP are limited when taking numbers of screened infants into concern. Until clinical utility and validity of the IRT-PAP-DNA protocol have been established, the IRT-DNA-IRT may be the preferred solution. Recent pilot study results from the Netherlands support the IRT-PAP-DNA approach.**
- **It is important, that once a screening programme is started, close monitoring and regular review of data is needed. Based on data of performance of the initial algorithm, the screening procedure can be adapted where needed and according to new available techniques and/or knowledge.**

8 STUDY OF ETHICAL AND LEGAL ASPECTS

8.1 INTRODUCTION

For this project the explicit question is to formulate advice on a newborn screening program for CF. We do therefore not include in our text considerations regarding the challenges that occur in the case of screening programs for several diseases and the implications for informing (information overload) the participants in case of multiplex testing.

For CF-NBS different screening algorithms can be used. Most algorithms currently in use consist of a multistep approach including DNA analysis to improve the sensitivity and specificity. Including genetic testing in a new born screening raises several ethical and legal issues. Therefore some programmes study the used of PAP (pancreatic associated protein) as second step to avoid these additional complexities.

The last section of this chapter provides a tabular overview of the legislation applicable to CF NBS (legislation applicable to CF NBS

Table 17).

8.2 FREE CONSENT AND INFORMATION BEFORE AND AFTER SCREENING

8.2.1 Free consent

First of all it should be noted that when a newborn screening test is offered there will be always a form of pressure because there is an invitation of health authorities towards families who were not asking a test.[291]

According to the report of the American Task Force on Newborn Screening,[292] there are only three States in the USA that require consent for newborn screening (not involving genetic testing). In many other states however there is a right of 'parental refusal', mostly on religious grounds. Also the recent report of the President's Commission[293] recommends that mandatory newborn screening be recommended for those disorders that clearly meet the classical screening criteria. The Dutch Health Council recommends that parents should consent to the performance of screening in their child.[294] The Health Council understands the notion of parental consent in the context of newborn screening as primarily founded in the principle of best interest for the child.

In Belgium, there is no specific legislation concerning newborn screening. The general rules governing the practice of any medical activity and the rights of patients have to be respected. The Belgian law on patients' rights should in this respect be interpreted as: consent of one of the parents has to be given. In principle, express verbal consent should suffice. A written informed consent is only required if a specific legal rule exists in this respect. This is for instance the case for termination of pregnancy and organ removal. As such a specific legal rule does not exist for screening that involves DNA testing, written consent is not required. Opting out is only justified when a legal basis allows for this. This is for instance the case for removal of organs from deceased persons. There is no legal basis for an opting out system in case of screening. According to the law on the rights of patients an express, beit oral consent, is required. In Flanders there is since December 12 2008 the '*Besluit van de Vlaamse Regering betreffende bevolkingsonderzoek in het kader van ziektepreventie'*. But as the concrete interpretation and implementation of this *Besluit* is being studied by a Working Group (*Vlaamse Werkgroep Bevolkingsonderzoek*) it is too early to present here its modalities, forms, and scope.For the American ethicists Faden et al. the parental consent is not necessary in the case of early screening for treatable diseases: "if the principle consideration is the welfare of children, their welfare is best served in this case by a program of compulsory and exception less screening".[295]

The Institute of Medicine[296] on the other hand states that there are no indications that asking consent would lead to lower uptake rates and so would damage the interest of the children.

The Health Council points out that the free participation and informed consent are important, because it commits the parents in the success of the newborn screening.

De Wert does not exclude the possibility of impulsion: in the context of newborn screening for treatable diseases, the caregivers have in his opinion the moral duty to persuade parents to give their parental consent for the screening if applicable.[297] The Health Council agrees with this view, but concludes as follows:" After an intensive dialogue of this type, however, the Committee feels that the decision ultimately reached by the parents will have to be respected, even if this entails a refusal to participate."

8.2.2 Information before the screening

Information will have to be provided to the parents, even in the case of mandatory screening. The Health Council recommends that the information to be provided will need to include answers to the following questions:

- What is the infant being screened for?
- What does the screening entail?
- What are the anticipated benefits and disadvantages?
- How important is it that parents should have their child screened?
- Is it possible that results have implications for us and for family members or previous children?
- Are the data produced by heel prick testing confidential?
- What happens to the blood spot cards once the testing has been completed?
- What happens in the event of non-participation in the screening?
- Who can we turn to with any additional questions?

More specifically, this information could encompass the following issues: information about the nature, prevalence and severity of the disorders for which screening is to be carried out; the importance of early diagnosis; the possibilities for treatment and/or other benefits for the person concerned; the fact that some test results require confirmation or give rise to false alarm the fact that cases can be missed and that not all diseases are detectable; and further information about use of residual material from the heel prick for medical research and about protection of privacy. It is also relevant for the parents to know whether a test method may provide information about the implications of being a carrier. Last, but by no means least, it is important that parents should understand, based on the information provided, that they do, in fact, have a choice.

Regarding the timing of providing the information the Health Council states that the first few days after the birth are unlikely to be a suitable time for the parents to digest information about neonatal screening. Interviews with 115 parents in 13 focus groups in Chicago revealed that only a tiny minority could remember postnatal information that was provided about neonatal screening and that in around one-third of the focus groups not a single parent could remember anything about neonatal screening.[298] It makes more sense to discuss neonatal screening during antenatal check-ups. There is then still time to answer questions and, if necessary, to supply more information. In their systematic review, Hayeems et al.[111] note that parents recommend about newborn screening during the prenatal period as well as during their hospital stay. Also others recommend that all state newborn screening programs should work proactively to ensure that all women receiving prenatal care are educated about newborn screening.[299] As part of this educational process the distributed educational information should also be culturally appropriate.[300] It implies that the educational material should be made available in the languages of the respective language groups/minorities.[294]

8.2.3 Information after the first screening test

Health professionals are involved in providing parents with information at two main stages after the screening/diagnostic process. First, the information they give to parents following an abnormal screening test result, and second, the information they provide to carrier and affected families following sweat testing and DNA results. Tluczek et al.[279] described parents' preferences for counselling content at the time of the infant's sweat test appointment. Preferred content includes: factual information (i.e. probability of CF diagnosis, CF disease facts, CF genetics, sweat test procedure itself) and social support (i.e. providing choice about timing and amount of CF information, empathy, hope, person-centred counselling, and hospitality). Counselling strategies that matched parents' preferences reduced distress whereas strategies that did not match parents' preferences increased parental worry about their infant.

Effective communication of abnormal results is important because psychosocial complications may be related to misunderstanding the information (see also further, the paragraph on carrier information). Tluczek et al.[301] found that parents of newborn CF carriers were distressed when they were informed: (1) by telephone, (2) by an answering machine message, (3) before their infant was old enough to complete a sweat test, or (4) in a setting where there was insufficient time to discuss the information. Parents preferred learning results face-to-face from an experienced professional and understood the information better this way.

The review of Hayeems et al.[111] found evidence suggesting that in the context of cystic fibrosis, uptake of genetic counselling services is higher if offered on the same day as confirmatory sweat testing. In practice, this may be a good timing for counselling in case on negative sweat test. However, if parents just received the news their child has CF genetic counselling is probably best postponed to a later time point. Challenges to follow-up relate to the fact that primary care physicians lack perceived competence to: discuss the meaning of positive screening results, determine which confirmatory test to order, access a convenient laboratory, interpret the meaning of the confirmatory test result, and make the necessary sub-specialty referrals. Their evidence suggests that face-to-face counselling and the provision of simple, positive, and non-threatening language are associated with less distress among parents whose infants were identified as CF carriers. Also Parsons and Bradley[107] conclude that in both the USA and UK, recommendations have been made all carrier families should receive genetic counselling to ensure that they understand the meaning of their carrier status.

8.3 PRIVACY, STORING AND RESEARCH ON DNA

8.3.1 Privacy

Regarding the question "who should be informed about the test result", the Belgian law is clear. Article 7 of the law on the rights of patients provides that a physician has to provide to his/ her patient all information regarding his/her health status and its prognosis. When the patient is minor, this information has to be provided to the parents of the minor. For the ethical aspects of confidentiality regarding blood-spot cards in the Belgian context we refer to the opinion of the Belgian Advisory Committee on Bioethics nr 25 (only in Dutch and French): "Advies nr. 25 van 17 november 2003 betreffende de bewaartijd van de bloedkaartjes en het vertrouwelijk karakter van de gegevens voor het opsporen van aangeboren metabolische afwijkingen".

In Western countries the vast majority of newborns have their blood taken for screening. This blood is kept on blood-spot cards for various lengths of time, depending on the facility that stores them.[302] As shown by Lysaught et al. it is possible to extract DNA from these samples.[303] As such, collections of blood spot cards can be considered as 'inchoate DNA banks'[302]: they form a possibly huge potential resource for genetic research, although they were originally gathered for diagnostic purposes. For example, Klotz[304] describes how these samples can be used for DNA extraction, to study cancer susceptibility genes. Other uses include genetic research (e.g. Sudden Infant Death Syndrome), environmental studies and epidemiological studies of a variety of diseases that are now frequently associated with childhood, such as allergies, asthma, autism.[305]

8.3.2 Stocking the newborn's tissue for research

There are specific ethical issues associated with collections of human biological materials. The issue most discussed is the one of consent.[306, 307] In most countries, such as Canada, USA and UK, consent for newborn screening is presumed: often no written or oral consent is sought and many parents are unaware that they could refuse screening of their newborn.[308] Authors agree that screening for treatable disorders without consent can be defended because the benefits to the child are obvious. However, the same authors make a strong distinction between the diagnostic use of the samples, for which consent may not be needed as this is considered part of routine care, and further storage of these samples and their use for non-therapeutic research. For the latter, they do think explicit consent is needed [303, 308-315]. Hence they suggest that, should blood spot cards be used for something other than diagnostic purposes, consent should be sought, a recommendation supported by empirical research.[316] As an illustration of the importance of such consent, in Holland people were shocked to find that, in the aftermath of a disaster with fireworks blood spot cards were stored and used for identification purposes without consent. As a result, the policy of retention of blood spot cards was changed.[317]

Another ethical issue associated with stored tissue samples is the issue of confidentiality.[318] Such samples, including blood spot cards, are a potential source for genetic information. If insurers and employers could get access to this information this could lead to discrimination of the donors. It is imperative that proper privacy protections are in place and that the researchers only work with an anonymized version of a sample. Also, there should be policies what should happen if researchers find potentially relevant information about the donors: should such incidental research findings be returned to donors or not?[319]

Blood on blood spot cards is de facto from children, which further complicates the issue. On the one hand, as the blood is stored when the children are infants, consent is given by the parents. However, the same blood may be stored until they are 16 or 18 years old. Should the children be recontacted to give renewed consent? Who is responsible for making them aware of the research done on their blood? On the other hand, a major concern in paediatric research is that such research should not burden children[320]. Especially venepunctures are quoted as something children dislike very much.[321] Using blood spot cards, which have been taken in a diagnostics context, would reduce the burden of non-therapeutic research. In how far does this weigh up against the more formal ethical requirements of consent and autonomy?

Blood spot cards can form a good resource for research. This option however would implicate the need for written informed consent, which is less feasble in the context of population screening. In case of research studies, the use of such existing blood spot samples could even be preferred over taking new blood samples from children. Certain conditions should be fulfilled. First, parents should consent to any non-diagnostic uses of the cards. Midwifes should be trained to discuss these issues with mothers and, if possible, also fathers. Second, research on blood spot cards that were stored must be for the benefit of children. In the Flemish community the storage time has been defined at 5 years after which the blood spot cards have to be destroyed. Third, collections should be under scrutiny of external ethics committee to ensure adequate privacy protections. Researchers themselves should never be allowed to recontact donors directly or to have access to their identities. Also, an ethics committee should decide whether certain uses of the blood spot cards require reconsent of parents and/or children when they reach a certain age. Finally, we believe that, if a policy to reuse blood spot cards for research, is adopted, this should become common knowledge to the general public. Children have the right to be made aware of such use and be given the opportunity to opt out. This step is probably the hardest to implement. It is however a very important one to establish and maintain this and further generations' trust in science and the medical profession.

8.4 UNAVOIDABLE DETECTION OF CARRIERS

The results of neonatal screening for CF when DNA testing is used can be expressed in one of three ways: not affected-no carrier, not affected-carrier –affected. The two last possibilities mean that one or both parents are also carriers and therefore these diagnosis have implications with regard to information provision and further investigation involving the parents and their relatives.[294] This part will present what the implications of detection of carrier status are for the child, and the parents.

8.4.1 Implications for children

The provision of information about CF carrier status has raised concerns about potential stigmatization and discrimination.[322] To date, no studies have reported the long-term implications for individuals identified as carriers via newborn screening.[41] There is also little evidence that carriers feel stigmatized in their personal relationships.[323, 324] As with true-positive cases, there is concern that the temporary distress caused by the abnormal test result might disrupt early maternal bonding.[107] However Parsons report that there was no evidence of any difference between the carrier group and a control group drawn from the general population, 6 months after sweat testing.[107] In another study there was no evidence of any of the 57 parents had experienced withdrawal or distancing from their baby.[325]

8.4.2 Implications for parents

Regarding the communication of carrier results of the newborn to the parents, a consensus governs that carrier status information generated through newborn screening results cannot be withheld from parents.[326] This would be an infringement of the rights of parents to manage important reproductive information about themselves and their children.

Tluczek et al.[279] found that prior to the confirmatory CF sweat test, parents of infants who had an abnormal newborn screen were more distressed than parents of infants who had a normal newborn screen. However, after the sweat test, parents whose infants were found to be CF carriers scored comparably with parents whose infants had normal newborn screens. While persistent concern about the carrier infant does not seem to apply to the majority, one to six years after identification, as many as 28-29% of parents worry about the physical health of their carrier child as well as the potential difficulties that being a carrier might have for future relationships.

Evidence from quantitative survey data endorses newborn screening for CF and disclosure of carrier results; 68% of parents agreed that they felt better informed knowing their child's CF carrier status. Evidence from qualitative interview studies corroborates this finding.[111] Reasons parents wanted to have carrier testing include an interest in knowing their own carrier status and whether other family members might be at risk to be carriers. In the same review Hayeems et al. state also that "while it is suggested that the frequency of identifying non paternity by disclosing carrier results generated through newborn screening might be as low as 1.8%, there is no evidence specific to the context of newborn screening that reflects the harm(s) that this revelation is thought to cause".

8.4.3 Use of carrier status for parental reproductive decision-making

In the literature, there is at the moment a vivid debate on the use of carrier status ascertained through newborn screening for CF to guide reproductive decision-making.[327] Lewis et al[328] found that for 82-87%, their infant's carrier status "made no difference" to their reproductive plans whereas 12-13% had decided to have "no more children" and 6% decided to have "fewer children". Mischler et al.[329] suggest in their study that "if the use of prenatal diagnosis is a surrogate marker for the value of genetic information obtained through newborn screening, then this is a benefit for only a minority of the individuals".

Ciske et al.[330] observed in their retrospective study that 78.7% of the parents said that the knowledge of their child's carrier status had not caused them to change their reproductive plans.

More fundamentally one should ask whether such (and other) benefit should play a role in the evaluation of the acceptability of newborn screening for CF. In clinical genetics the traditional stance is that carrier information belongs to the tested individuals who are positioned to make reproductive choices. This position is reflected in the guidelines that recommend not to proceed with carrier testing of asymptomatic children where such testing is not serving the health of the person involved.[331] The traditional criterion of benefit for the child in newborn screening situations has been challenged by recent publications that describe this position as outdated. In their opinion newborn screening can be justified on the basis of secondary benefits (like the reproductive benefit).[332, 333] Others however do not agree and state that there should be a clear benefit to the newborn[109, 296]: in the reproductive benefit scenario (as primary goal) the child is being used as a means for the interests of others. In a recent article Bombard et al.[334] argue that reproductive benefit could remain a secondary goal of newborn screening (including an additional choice to receiving this information or not) but "that pursuing reproductive benefit through newborn screening programs is not appropriate and may be best achieved through traditional antenatal screening programs".

8.5 THE RISK FOR A LEGAL PURSUIT IN CASE OF FALSE POSITIVE/FALSE NEGATIVE TEST RESULTS

8.5.1 Introductory remark

When discussing the legal consequences of false positive or false negative test results in Belgian law one should be aware of the general features of medical liability law in general in Belgium. This is not without difficulties as this law is actually in a phase of transition. The Act of 15th May 2007 on the compensation of damages resulting from health care has introduced a complete new system of compensation that is not based anymore on fault and liability but on solidarity. Since many years the Scandinavian countries have elaborated so called no-fault or no-blame insurance schemes to compensate victims in a medical context. Such a system has also been adopted in the Act of 15th May 2007. This Act had to come into force on 1st January 2009. Because of the fear that this scheme would not be financially viable, the Government decided to replace the Act of 15th of May 2007 by a new Act. A proposal has already been approved by the Government recently but still has to be approved in Parliament. This could be managed end 2009/begin 2010. The inspiration of the newly proposed system comes from French law: a combination of the classic liability compensation system based on fault and a system of compensation based on solidarity of severe damages not attributable to a fault.

8.5.2 Liability for false positive/false negative test results: an exercise in the dark.

At the moment of writing, Belgian medical liability law is still based on fault. This means that the victim of a pretended medical fault has to prove: a fault, the damages and the causal relationship between fault and damages. Regarding the medical fault, a distinction has to be made between the so called technical fault (typically called: medical malpractice) and not respecting the rights of the patients, notably the right to give informed consent. When a CF screening test has been applied according to all the professional standards at stake, it nonetheless can give a misleading, false positive or false negative result. In such a case the patient cannot sue the physician because he has made a technical fault. The only way for the patient to hold the physician liable, is to convince the judges that the physician was under an obligation to inform him, the patient of the possibility of false positive or false negative results of the test. This is information that should be given in the context of the consent (cfr first part). Whether the judges will accept the existence of such an obligation is not easy to predict. There are no legal precedents in Belgium in this regard.

The question that judges will have to answer is the following: would a prudent and careful physician (often referred to as a bonus medicus) placed in the same circumstances have given this kind of information. Judges cannot answer this question without asking the opinion of medical experts and paying attention to practice guidelines, professional standards and protocols, provided they exist. Even if judges accept the existence of an obligation for the physician to inform the patient of the possibility of false positive or false negative results of the test, the patient is faced with other serious difficulties. Under Belgian law the burden of proof that the information has not been given to him rests upon the patient (for example, in France and other countries the burden of proof rests upon the physician: he has to demonstrate that he has informed the patient in a satisfactory way). The proof of not being informed is an extremely difficult one because it relates to a negative fact. The chances of success are therefore very limited. And even if the patient would succeed in this proof, he still has to face another difficult hurdle. Indeed, even if the physician had to inform the patient and the patient demonstrates that he has not been informed, the question arises what the damages are. Damages have to be evaluated not in abstracto but in concreto. This means: what are the damages if parents of a neonate have been told that their baby has CF while afterwards it appears that this is not the case? And vice versa: what are the damages if parents have been told that their baby has no CF while afterwards it appears this information was wrong? This way clinical diagnosis will be postponed which may lead to irreversible damage (see deliverable 1a, late diagnosis being related to worse prognosis. Even if the parents can make their damages in either case concrete they will have to prove the existence of a causal relationship between the improper information and their damages. Again, this is not easy. In case parents have been told that their baby has no CF, while afterwards it appears that this information was wrong, there is clearly no causal relationship between the improper information and the CF status of the child because this status is the result of nature. And what could parents have done if they had received immediately the correct information? Interruption of pregnancy is not possible anymore and "euthanasia" would be inacceptable both legally and ethically. In other words : parents confronted with a false positive or a false negative result of a CF screening test, face enormous difficulties when they want to sue the physicians responsible for the screening.

8.6 LEGISLATION APPLICABLE TO CF NBS

Table 17. Tabular overview of legislation applicable to CF NBS

Patients' Rights		
Law	**Article**	**Application to CF newborn screening**
Wet van 22 augustus 2002 betreffende de rechten van de patiënt, B.S. 26 september 2002	Art. 8 §1 informed consent + art. 12 parental authority Art. 373-374 Civil Code	Informed oral consent of one parent is sufficient. The law defines a non-exhaustive and general list of information-elements Information has to be provided in advance, timely and in clear language If the physician presumes/knows that the parents are separated and that possibly the other parent would not consent, he/she should ask for consent of both parents.
	Art. 8 § 4: right to refuse	The parent(s) has/have the right to refuse CF newborn screening
	Art. 7 § 1: right to information	The parent(s) has/have the right to information of the test result and all information on the health status and probable evolution Persons responsible for the education of the newborn, not being the (legal) parents (e.g. step parent) can receive medical information regarding the minor's health status if they are assigned as confident by the parents having the parental authority or by the guardian (Advies inzake de mededeling van information over de gezondheidstoestand van mindeerjarigen van 18 september 2009 van de Federale Commissie van de Rechten van de Patiënt)
	Art. 7 § 3: right not to know	The parent(s) has/have the right not to know the test result at their explicit request, unless the non-notification of information causes damage to the child or a third party and on condition that the physician consulted an other health care professional
	Art. 7 § 4: Therapeutic exception	If the communication of the test result would cause obviously serious damage to the health condition of the child (e.g. because the parents get anxious and depressed) and on condition that the physician consulted an other health care professional, the physician can decide to not to communicate the negative test result to the parents.

	Art. 9: right to access to the patient file and a copy	The parent(s) has/have direct (implying without the assistance of a physician) access to the patient file and the right to have a copy of it
Professional Secrecy and confidentiality		
Criminal Code, 8 juni 1867, B.S. 9 juni 1867	Art. 458: professional secrecy	The treating physician, the nurses and paramedics taking part in the care process of CF screening are not allowed to disclose patient data unless specific exceptions described in law (see art. 458 Crim. Code); if the parent(s) consented to do so or when data are shared with colleagues (also bound by professional secrecy) of the medical team treating the patient (=shared professional secrecy).
Wet van 8 december 1992 tot bescherming van de persoonlijke levensfeer ten opzichte van de verwerking van persoonsgegevens, B.S. 18 maart 1993. (Data Protection Act- DPA) + Koninklijk Besluit van 13 februari 2001. - Koninklijk besluit ter uitvoering van de wet van 8 december 1992 tot bescherming van de persoonlijke levenssfeer ten opzichte van de verwerking van persoonsgegevens, B.S. 13 maart 2001 (Royal Decree Data Protection Act- RD DPA)	Art. 7, § 4, section 3 DPA Art. 25, 3° RD DPA art. 16 §2 DPA	The health care practitioner, his appointees and authorized representatives are bound by secrecy concerning the health data. The hospital is obliged to assure that the persons processing the health data respect the confidential character of the data by contractual clauses, or legal or statutory provisions The hospital needs to make sure that access to the data and possibilities of processing for the persons who are acting under his authority are limited to what is necessary for the exercise of their duties or for the requirement of the service.
Privacy		
Wet van 8 december 1992 tot bescherming van de persoonlijke levensfeer ten opzichte van de verwerking van persoonsgegevens, B.S. 18 maart 1993. (Data Protection Act, DPA) + Koninklijk Besluit van 13 februari 2001. - Koninklijk besluit ter uitvoering van de wet van 8 december 1992 tot bescherming van de persoonlijke levenssfeer ten opzichte van de verwerking van persoonsgegevens, B.S. 13 maart 2001 (Royal Decree Data Protection Act, RD DPA)	Art. 1 DPA Art. 7, § 2 j) DPA Art. 9 DPA + art. 25 RD DPA Art. 10 DPA Art. 12 DPA Art. 4 § 1, 2° DPA + Chapter II RD DPA	All information resulting from the CF screening is personal health data. DNA as such is not considered to be personal health data. The processing of the test results (e.g. integration in the patient file, invoicing, accounting,…) is subject to the dispositions of the Privacy Law Primary processing: Processing of the CF test results is allowed if this is required for the purposes of preventive medicine, medical diagnosis, the provision of care or treatment to the data subject or a relative, the management of health care services and the data are processed under the supervision of a health care professional. Right to information with regard to the processed data Right to access to the patient file Right to correct, erase data or object to processing Secondary processing If health data are further processed for scientific purposes that are incompatible with the original

		purposes (= unforeseeable by the patient or not considered as compatible by law), extra privacy measures (anonymising , encoding of data, unless consent of the patient, extra information etc.) must be taken into account. For details see Chapter II RD DPA
Genetic testing and the relation employer-employee or the relation insurer-insured		
Wet van 28 januari 2003 betreffende de medische onderzoeken die binnen het kader van de arbeidsverhoudingen worden uitgevoerd, B.S. 9 april 2003. + Wet van 25 juni 1992 op de landverzekeringsovereenkomst, B.S. 20 augustus 1992	Art. 3 § 1 Art. 95	Predictive genetic testing by employers is prohibited Medical certificates needed for the contracting or executing of contracts delivered by the physician of an insured individual, may not contain predictive medical information. Genetic testing must be excluded from the medical examination for the contracting or the execution of the contract.
Information exchange networks		
Decreet van 16 juni 2006 betreffende het gezondheidsinformatiesysteem., B.S. 7 september 2006	Art. 36	The epidemiological information system enables the exchange of health data among care providers and other institutions involved in the provision of health care for purposes of organizing the public health system. The data in this system are allowed to be further processed in the scope of population screening.
Wet van 21 augustus 2008 houdende oprichting en organisatie van het eHealth-platform, B.S. 13 oktober 2008		The eHealth Platform is a government institution, managed by representatives of several actors in the healthcare sector creating and managing a cooperation platform for secure electronic exchange of information about patients, provided care and the results of the provided care, and for the exchange of electronic care prescriptions between all relevant actors in the health care sector. Besides providing a network and basic services, it will also coordinate the development of functional and technical interoperability standards. The eHealth Platform can be useful in the scope of data processing in population screening
Liability for false positive/false negative test results		
Wet van 31 maart 2010 betreffende de vergoeding van schade als gevolg van gezondheidszorg, B.S. 2 april 2010 (not yet came into force!)		The parent(s) (or the patient), victims of damage above a certain degree of gravity that was caused by an act of healthcare do no longer need to prove the existence of fault by the health care professional and can be reimbursed by a Fund. It is doubtful if the damage that was caused by the effect of a false positive or a false negative result can reach the degrees of gravity specified in law. Damage linked to false positive tests will be mostly moral. In case of a false negative result the damage will consist of the loss of a chance to improve the health status of the child because of earlier treatment (resulting from the early detection).

Civil Code - Tort Liability or contractual liabilty	Art. 1382 Civil Code	The parent(s) need(s) to prove the fault of the physician and a causal link between the damage and the fault. Solely if the parent(s) can prove that he/she/they would not have consented to CF screening if the physician informed them on the possibility of a false positive/false negative result, the physician can be held liable
Liability for defective products		
Civil Code - Tort Liability or contractual liability, Wet van 25 februari 1991 betreffende de aansprakelijkheid voor produkten met gebreken, B.S. 23 maart 1991	1384 section 1, 1604, 1641 Civil Code	If damage (e.g. a false test result) was caused because of defective material or equipment for CF screening the parent(s) can claim liability of different parties based on different legislations. The easiest way to get compensation is the application of the Product Liability law because the solely causal link between the damage and the defective product needs to be proven. No fault has to be established.
Scientific research		
Wet van 19 december 2008 inzake het verkrijgen en het gebruik van menselijk lichaamsmateriaal met het oog op de geneeskundige toepassing op de mens of het wetenschappelijk onderzoek, B.S. 30 december 2008 (see also executing Royal Decrees of 28 september 2009, B.S. 23 oktober 2009). Did not yet come into force!	Art. 3§3 b) Art. 10 §3, § 5 Art. 11 Art. 20 § 1 Art. 20 § 2 Art. 21 Art. 22 Art. 17	The law is not applicable to the (sole) primary use of DNA or sweat for the CF diagnostic test (or screening). Primary use of DNA (except if exclusively for diagnosis): written informed consent of the parent(s) or of the minor (according to art. 12 Patients' rights act) is needed. Right to information if analyses result in relevant medical information for the donor. Secondary use of DNA (e.g. for further scientific research): the patient (or parent(s) according to art. 12 Patients' rights act) needs to be informed + written informed consent of the patient (or parent(s) according to art. 12 Patients' rights act) needs to be obtained by the physician-guardian of the DNA, unless this is impossible (e.g. patient deceased) or not appropriate. In the latter case the positive advise of an Ethical Committee is needed Secondary use with residual DNA samples: consent is presumed as far as the parent(s) or the patient (≥18 years old or allowed to consent according to art. 12 § 2 Patients' rights act) did not refuse before the scientific research has started. The patient or the parent(s) has/have to be notified, preferably in written of the option to refuse The secondary use of the DNA sample as well as the specific purpose of the research necessitate the positive advice of an ethical committee (cfr. also art. 2, 4°, van de wet van 7 mei 2004 betreffende de experimenten op de menselijke persoon). The purposes and activities of biobanks (banks storing and providing human tissue for scientific research), as well as any provisions of blood spot cards need to be subject to a positive advice of an ethical committee. Biobanks are obliged to keep a register regarding the character, the source and the destination of the blood spot cards. Several obligations are foreseen in this article for entities storing, providing, analysing etc. the DNA.
Wet van 7 mei 2004 inzake experimenten op de menselijke persoon, B.S. 18 mei 2004.		Clinical trials on minors affected with CF are solely allowed

	Art. 7	With the written informed consent of the parent(s) or the guardian, expressing the wish of the minor. The researcher has to ask for and respect the minor's wish as far as the minor is capable to evaluate the information and the consequence of participation to the experiment; the clinical trial needs to be directly related to the health status of the neonatal or solely possible with minors; the trial is essential to confirm the results of trials with consenting individuals or other research methods and the trial grants a direct benefit for the patient group The risks are proportionate to the (expected) advantages Pain, fear, discomfort or any other foreseeable risk is minimised; A positive advice is given by an ethical committee, composed of at least 2 paediatrics or an ethical committee that consulted 2 paediatrics; The minor or the representatives do not get any financial incentives other than a remuneration; The scientific guidelines of the "Europees Bureau" were taken into account.
Advies van 17 juli 2004 van de Nationale Raad van de Orde der Geneesheren betreffende het DNA onderzoek en farmacogenetica		Processing of genetic data is highly confidential and the use needs to be limited to the protocol to which the person consented. The use of these data for other purposes needs to be approved by the same ethical committee and an informed consent of the person concerned is needed.
Population screening		
Besluit van 12 december 2008 de Vlaamse Regering betreffende bevolkingsonderzoek in het kader van ziektepreventie, B.S. 19 februari 2009 + Protocolakkoord van 28 september 2009 tussen de federale overheid en de overheden bedoeld in artikelen 128, 130 en 135 van de Grondwet inzake preventie, B.S. 29 oktober 2009	Art. 4 and 5	The Flemish government can organise population screening if The expected health gain in the target population and efficacy is evidence based; Participants will probably have more gain than disadvantage The screening programs guarantees the possibility to participate to the entire target group The working group Population screening advices on the proposition of the Flemish government. Modalities for the organisational elaboration of a population screening program are identified in law.

Disclaimer: The information in the table is a summary of the respective legislation. We limited ourselves to highlight the dispositions that are obviously applicable to the CF newborn screening. Details regarding the specific legislations mentioned were not elaborated.

9 REVIEW OF THE COST-EFFECTIVENESS LITERATURE

9.1 METHODS

9.1.1 Literature search strategy

The search for the economic literature on newborn screening for cystic fibrosis was performed by consulting electronic databases up to mid December 2009. The HTA(CRD) database and websites of Health Technology Assessment (HTA) institutes listed on the INAHTA (International Network of Agencies for Health Technology Assessment) website were consulted to retrieve HTA reports on this topic. The NHS EED(CRD), Medline(OVID), and EMBASE databases were searched to retrieve full economic evaluations and reviews of full economic evaluations on this topic. No restrictions on the time period and language were imposed. At this stage, 160 potentially relevant references were identified. See Appendix 8 for an overview of the search strategies and results.

9.1.2 Selection criteria

All retrieved references were assessed against pre-defined selection criteria (in terms of population, intervention, comparator and design - Table 18) in a two-step procedure: initial assessment of the title, abstract and keywords; followed by full-text assessment of the selected references. When no abstract was available and the citation was unclear or ambiguous, the citation was assessed on the basis of keywords and/or full-text assessments. Reference lists of the selected studies were checked for additional relevant citations. The selected full economic evaluations (i.e. studies comparing at least two alternative treatments in terms of costs and outcomes - Figure 12) were critically assessed.

Table 18: Economic evaluation selection criteria

	Inclusion criteria	Exclusion criteria
Population	newborn babies	others
Intervention	screening for cystic fibrosis	others
Comparator	non-screening alternative (traditional symptomatic diagnosis)	Analyses only looking at other screening protocols without including the no-screening alternative
Design	full economic evaluations	no full economic evaluations

Figure 12: Classification of economic studies

		Are both costs (inputs) and consequences (outputs) of the alternatives examined?		
		No		Yes
		Examines consequences only	Examines costs only	
Is there a comparison of at least two alternatives?	No	Partial evaluation		Partial evaluation
		Outcome description	Cost description	Cost-outcome description
	Yes	Partial evaluation		Full economic evaluation
		Efficacy or effectiveness evaluation	Cost comparison	Cost-minimisation analysis (CMA) Cost-effectiveness analysis (CEA) Cost-utility analysis (CUA) Cost-benefit analysis (CBA)

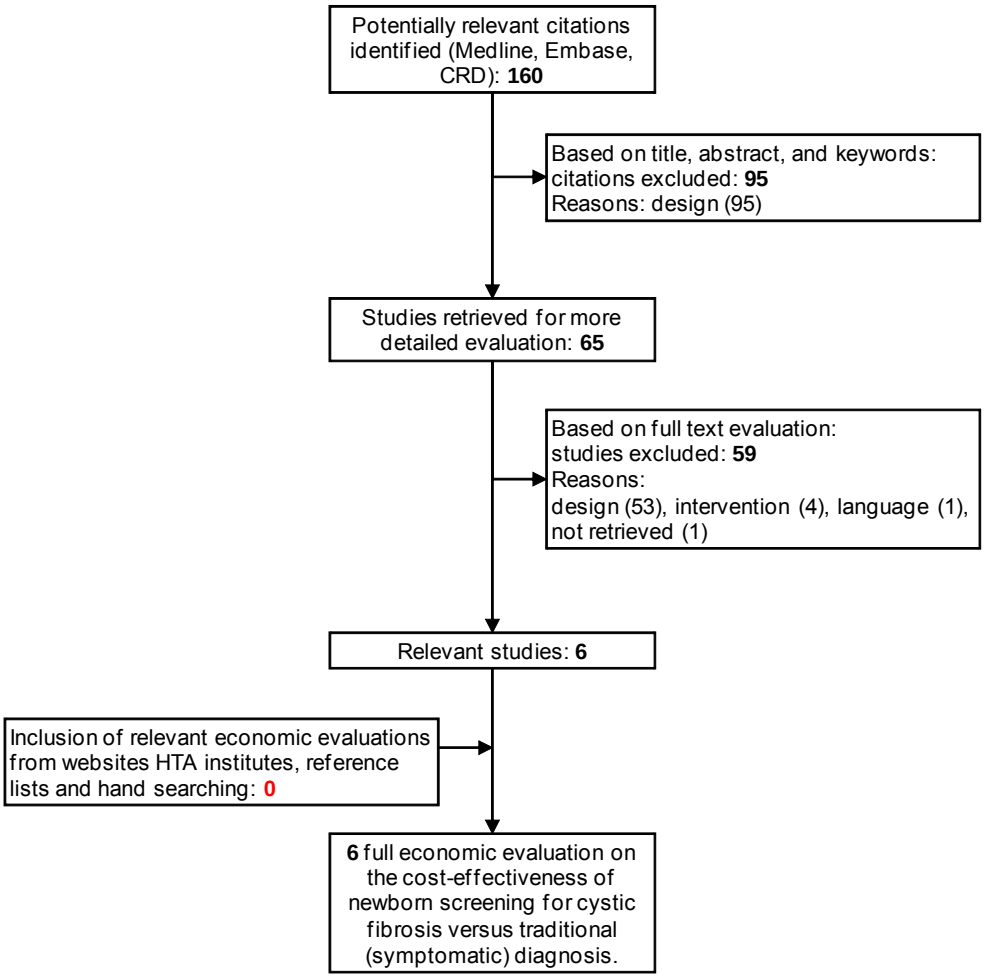
Adapted from Drummond et al., 2005[335]

9.1.3 Selection process

The searches of the HTA websites and databases returned 202 citations. After exclusion of 42 duplicates, 160 unique citations were left. Based on title, abstract and keywords (if available), 95 citations were excluded since they were no full economic evaluations. The 65 remaining citations were retained for full-text assessment. Six evaluations were selected and are reviewed in this manuscript.

Further exploration of articles' references did not bring additional citations.

Figure 13. Flow chart of the literature selection process



Several studies were excluded because they were cost analyses not comparing screening with a no-screening situation, e.g. Farrell et al., 2007[336] is a cost comparison of three different procedures for sweat testing in Ireland; Gregg et al., 1993[337] is a cost comparison of two protocols (IRT and IRT/DNA) for newborn screening for cystic fibrosis; Rosenberg et al., 2005[338] compares the costs of single CF mutation and CF multi-mutation test. The latter study[338] mentions to compare with the traditional means to diagnose CF. However, no such results are presented in the manuscript and therefore it was withdrawn from our review.

Several studies only mention the cost per diagnosed case.[325, 339, 340] These results are not taken into account since they are merely cost analysis and are not considered being full economic evaluations. Such studies were included in the overview if there is a comparison with a no screening alternative and when the analysis shows that costs with screening are lower than without. These studies do not explicitly compare effects expressed as life-years gained or QALYs gained. However, we consider them since results can be interpreted as being located in the second quadrant of the cost-effectiveness plane, i.e. being cheaper and better/not worse.

The following studies were selected and are summarized in this report:

- Dauphinais RM. A cost analysis of blood-spot screening newborns for cystic fibrosis. Journal of Clinical Immunoassay 1992; 15(2):121-125.[341]
- Farrell PM, Mischler EH. Newborn screening for cystic fibrosis. The Cystic Fibrosis Neonatal Screening Study Group. Advances in Pediatrics 1992; 39:35-70.[342]
- Lee DS, Rosenberg MA, Peterson A, Makholm L, Hoffman G, Laessig RH, Farrell PM. Analysis of the costs of diagnosing cystic fibrosis with a newborn screening program. Journal of Pediatrics 2003; 142(6):617-623.[343]
- Simpson N, Anderson R, Sassi F, Pitman A, Lewis P, Tu K, et al. The cost-effectiveness of neonatal screening for cystic fibrosis: An analysis of alternative scenarios using a decision model. Cost Effectiveness and Resource Allocation. 2005;3(8).[344]
- Sims EJ, Mugford M, Clark A, Aitken D, McCormick J, Mehta G, et al. Economic implications of newborn screening for cystic fibrosis: a cost of illness retrospective cohort study.[see comment][erratum appears in Lancet. 2007 Jul 7;370(9581):28.]. Lancet. 2007;369(9568):1187-95.[345]
- Van Den Akker-van Marle ME, Dankert HM, Verkerk PH, Dankert-Roelse JE. Cost-effectiveness of 4 neonatal screening strategies for cystic fibrosis. Pediatrics. 2006;118(3):896-905.[346]

9.2 OVERVIEW AND DISCUSSION OF ECONOMIC EVALUATIONS

9.2.1 Dauphinais, 1992[341]

This US (Connecticut) cost analysis questions whether it is more cost-effective to screen for CF or continue to rely on conventional methods of diagnosis by signs and symptoms. The authors mention that in Connecticut, roughly one half of the newborn population is screened for CF because of the voluntary nature of hospital participation. The average costs were compared between IRT screened and non-screened CF patients seen at their CF center. Only patients diagnosed between 1982 and 1990, both screened and non-screened, were included in this study. Costs of 16 non-screened patients with a delayed diagnosis of 1.5 months to 21 years were compared to IRT screening costs based on an incidence rate of 1/2700.[341]

For non-screened patients, CF-related costs generated prior to diagnosis were evaluated. Hospital, physician (office visits, medications, and special needs such as air purifiers), and sweat test costs were included. These costs were obtained from physicians' offices, hospital billing and medical records, and by direct communication with families of affected children. Costs of sweat-testing non-CF children between 1982 and 1990 because of non-specific signs and symptoms were added to non-screened CF patient costs.[341] Screening costs were based on a detailed analysis of labour and materials used in the Connecticut program. Screening costs included those for kits, special collection blotters, disposables, materials used in generating and reporting results, and technical and non-technical labour costs. Costs were included from a health care payer's perspective. Sweat-test charges were based on the 1988 average charge of three regional hospitals included in the Connecticut program, i.e. $119.60 (range: $93.40 – $138.11).[341]

In this cost analysis, total costs (1988 prices) per year per CF patient diagnosed by screening and non-screening were $6,243.00 and $14,497.00, respectively. The authors conclude that the cost of diagnosing CF by IRT screening is more cost-effective than relying on signs and symptoms.[341]

9.2.2 Farrell et al., 1992[342]

The cost analysis of Farrell et al. analyzed the costs of the Wisconsin screening program. Two potential approaches are considered, i.e. a one-tiered testing strategy (IRT analysis only) and a two-tiered method involving the IRT/DNA approach. This is compared with how much money is being spent to identify CF patients by the standard diagnostic approach. This includes performing a sweat test because of either a positive family history or the occurrence of signs/symptoms suggesting CF.[342]

If laboratory supplies plus personnel (technician) budgets per year were combined and sweat tests included, one-tier testing (with an IRT cut-off 180 ng/mL or the 99.8 percentile) would cost $130,400 to screen 75,000 newborns. IRT/DNA testing (using an IRT 100 ng/mL cut-off or the 99 percentile plus mutation analysis for the SF508 allele) would cost $126,770. This assumes 150 people would have a sweat test using the one-tier screening strategy and about 50 people would need a sweat test using the two-tier method. In both groups, the researchers expected to identify 18 individuals with CF. Without screening, a total cost of $182,030 was revealed. This was based on a total number of 1,670 sweat tests, applying an average sweat test charge of $56, and assuming that the equivalent of at least one extra "sick visit" occurred (per sweat test) in the primary care physician's office at a usual and customary fee of $53.[342]

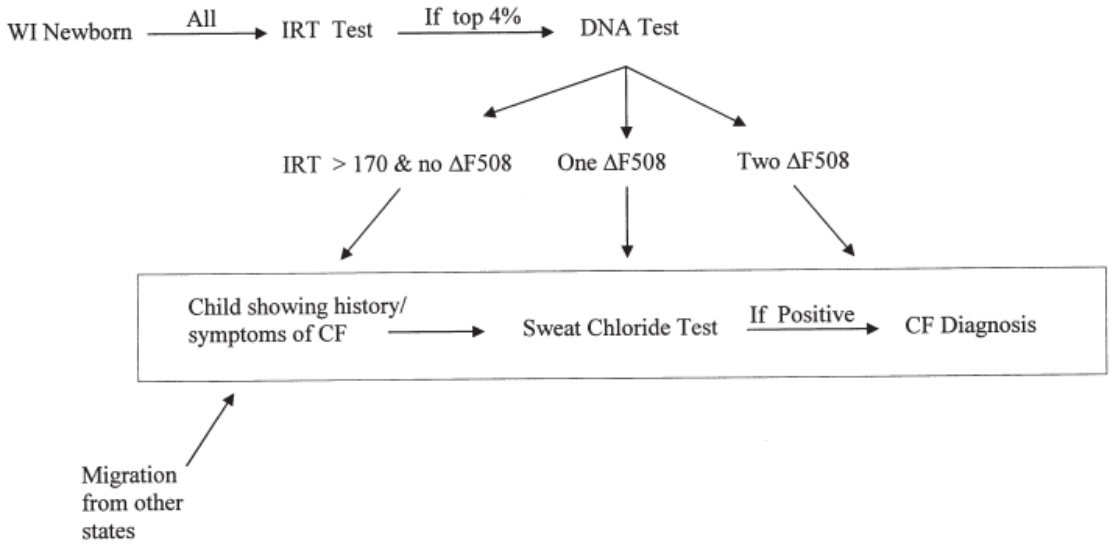
Furthermore, delays in diagnosis can increase medical costs significantly because of unnecessary laboratory tests and hospitalizations. Farrell et al. mention that advocates of screening suggest that neonatal diagnosis would prevent these unnecessary costs and decrease the probability of hospitalization. On the other hand, they remark there is good reason to expect intuitively that diagnosing CF early will lead to an increased likelihood of more (rather than less) treatment and that physicians will be more likely to admit known CF patients to the hospital for parenteral antibiotic treatment and other types of inpatient management. Therefore, this is a difficult issue to address.[342]

The authors conclude that if better treatments are developed that would prevent the need for hospitalizations in CF patients diagnosed through neonatal screening, the cost of care could be significantly reduced. In comparison to cumulative direct medical costs for patients requiring frequent or prolonged hospitalization, the expenses for screening are relatively small. However, at that time, the authors also mentioned that they cannot offer a treatment known to prevent hospitalization. They also did not know at that time if CF children diagnosed because of screening will be more healthy than those identified because of their symptoms.[342] Early identification of children with cystic fibrosis may lead to increased cost in the short term because of a greater probability that children will be hospitalized for management of respiratory illnesses. On the other hand, it is possible that screening would decrease the total cost of the diagnostic process. This may be true if therapy in early childhood prevents progressive lung disease requiring fewer hospitalizations. [342] The authors conclude that the major gap in knowledge that must be closed before CF neonatal screening can be recommended generally in the US concerns the potential long-term medical benefits of initiating treatment in early infancy. In their opinion, in 1992, it would be premature to implement mass population screening of newborns for CF until the benefits and risks have been fully defined, and an adequate and logistically feasible testing system developed and/or highly effective therapy for CF lung disease becomes available.[342]

9.2.3 Lee et al., 2003[343]

This US (Wisconsin) study compares the cost of diagnosing CF through a newborn screening program with the traditional method. Total costs for a national newborn screening program were also calculated but are not discussed in this report. The following figure shows the pathways to CF diagnosis for both approaches.

Figure 14. Pathways to CF diagnosis in Wisconsin by neonatal screening during 2000 or by traditional methods (shown in the box).[343]



Source: Lee et al., 2003 [343]

Since 1994, the state of Wisconsin performs CF neonatal screening with the use of both IRT and DNA analysis tests. Newborn infants with IRT levels below the daily 96th percentile are reported as normal, whereas newborn infants with IRT levels above the daily 96th percentile are analyzed for the ΔF508 mutation. Unless two CF mutations are demonstrated, however, the sweat chloride test is still used to confirm the diagnosis of CF. Newborn infants with IRT levels >170 ng/mL and no ΔF508 mutation are reported as possibly having CF. In this case, physicians are advised to refer the newborn for a sweat chloride test if any signs or symptoms of CF develop or if there is a positive family history.[343]

The annual cost of a CF newborn screening program was calculated as a function of the cost per test for the IRT/DNA screening, the cost per test for the sweat chloride testing, and the annual numbers of newborn infants who undergo the IRT/DNA and sweat chloride tests. The costs for the tests were $1.50 for IRT, $18 for DNA analysis, and on average $161.40 for a sweat chloride test. The numbers of annual sweat tests performed in Wisconsin were determined by surveying hospital and clinic laboratories with a questionnaire. Two surveys were performed with similar methods during 1991 (before routine CF neonatal screening) and for the year 2000.[343]

In this study, the total cost of CF newborn screening was estimated to be $180,491, or $9025 per newly diagnosed patient with CF (n=20), or $2.66 per screened baby. However, the hospital survey in 2000 revealed that next to 134 follow-up evaluations in the screening program, another 804 sweat test were performed. Adding another $129,766 for these sweat tests to the total cost of screening and diagnosis results in an estimated annual costs in Wisconsin in 2000 of $310,257, or $15,513 per CF diagnosis or $4.58 per newborn infant.[343]

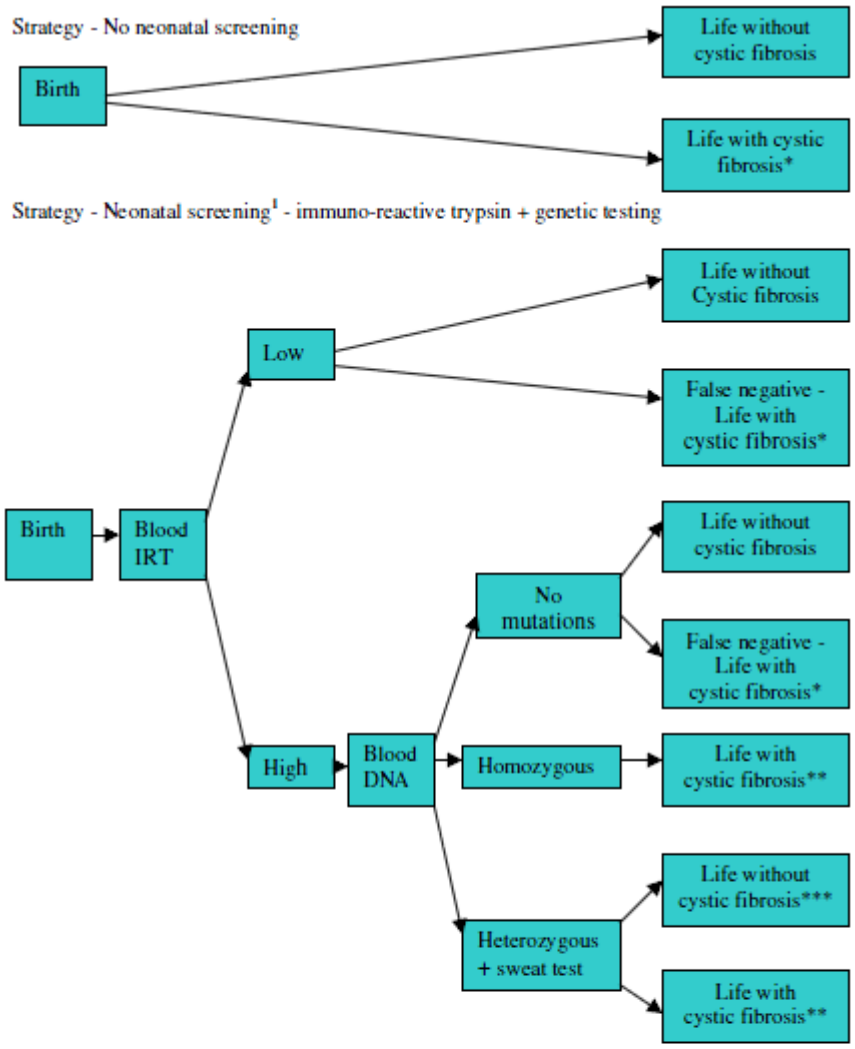
The 1991 hospital survey (before routine CF NBS) determined that 1670 sweat tests were performed. Assuming a sweat chloride test charge of $161.40 and 20 newly diagnosed patients with CF, an estimated total cost for the traditional method was $336,923, or $16,846 per newly diagnosed CF child, or $4.97 per newborn infant.[343]

The authors conclude that a CF newborn screening program provides a potentially cost-saving alternative to the traditional method of diagnosis of CF.[343]

9.2.4 Simpson et al., 2005[344]

This paper explores the cost-effectiveness of adding CF screening to an existing routine neonatal screening programme. The analysis was performed from the perspective of a hypothetical UK Health Authority that has an existing routine neonatal screening programme for congenital hypothyroidism and phenylketonuria but not for cystic fibrosis.[344]

Figure 15. Decision tree for no screening and neonatal screening strategies for cystic fibrosis.[344]



* = life with cystic fibrosis diagnosed "late" by presentation of symptoms

** = life with cystic fibrosis diagnosed "early" by screening

*** = represent screening false positives

[1] For simplicity in our model confirmatory sweat tests were included as part of the genetic analysis stage, and with assumed test sensitivity and specificity of 100%.

Source: Simpson et al., 2005[344]

The study is based on a decision model (Figure 15) comparing lifetime cost-effectiveness of neonatal screening to no screening. Future treatment costs and QALYs were discounted at 6% and 2% per year, respectively. The screening programme used a two-stage IRT combined with genetic testing strategy. A decision tree, incorporating Markov processes, was set up to model lifetime costs and quality of life. The health states included were ('born' into) the pre-symptomatic health state, the symptomatic disease state, the severe irreversible lung disease-state, and finally death. Probabilities of moving into the last two states changed over time.[344] Under the "no screening" strategy infants would be diagnosed with CF symptomatically (late diagnosis). Under the screening strategy most CF cases would be detected by screening (early diagnosis), with the remainder, i.e. the false negatives, experiencing the disease under late diagnosis assumptions. The model excluded those cases (15% in the base case scenario) diagnosed at or shortly after birth, for example by meconium ileus or family history, since these infants would have received the same prognosis and treatment under both strategies.[344]

The following screening costs were included in the model: counselling time required by midwives to obtain consent for testing, IRT test, DNA analysis and sweat chloride test. Other costs related to obtaining the blood spot and feedback of results by health visitors, were assumed to be sunk in the existing neonatal screening programmes for phenylketonuria and congenital hypothyroidism. Time for genetic counselling for carriers identified by the screening programme were excluded from the model because of difficulties to quantify the benefits of such counselling and because this cost would only be small in relation to total screening costs.[344]

The following table provides an overview of all model parameters and values used in their model. The pre-diagnosis healthcare costs for children with late diagnosis (no-screening) was estimated via an audit of the clinical notes of 25 children with cystic fibrosis. Disease state-specific costs of treatment were derived from the cost of care of 161 patients at a large UK cystic fibrosis unit during 1996 which were based on annual medical costs for patients at different age groups and at different disease stages.[344]

Quality of life values, based on Quality of Well-Being scores, were assigned to each Markov state (Table 19) and multiplied by survival time in the same state to produce quality-adjusted life expectancy.[344]

Table 19. Model parameters and values used in the model of Simpson et al.[344]

Model parameters		Base case value	Range used in sensitivity analysis
Probabilities			
- Incidence of cystic fibrosis		0.0004	0.00067 – 0.00029
- % diagnosed at birth (MI & family history)		0.15	0.10 – 0.40
- IRT test sensitivity		0.9	0.99
- IRT test specificity		0.995	0.999
- DNA test: % of mutations detected		0.88	0.85 – 0.95
- DNA test sensitivity		0.9856	0.9975
- DNA test specificity[a]		1	
- Increased annual transition probability of remaining without symptoms (in early-diagnosed cases)[b]		0.1	10 – 40%
Costs (all inflated to reference year 1998)			
Costs of screening			
- Additional time to explain test		£0.40 (2.1 mins)	£0 – £1.44 (0 – 7.6 mins)
- Obtaining and transport of blood sample[c]		£0	
- IRT test		£0.97	£0.50 – £1.50
- DNA test		£79.48	£40.00 – £120.00
- Sweat test		£29.40	£15.00 – £45.00
- Administration and feedback of results[c]		£0	
Cost of pre-diagnosis care in unscreened group			
- Presumed GP visits (mean number of visits)		£14.77 (1.27)	£11.63 – £46.52 (1 – 4)
- Outpatient attendances (mean number)		£129.07 (1.47)	£0 – £263.40 (0 – 3)
- Inpatient admissions (mean number of admissions and days per admission)		£792.55 (0.87) (3.0 days)	£0 – £1821.96 (0–2)
Costs of treatment per year in health state by age group			
- Presymptomatic	0–5	£2,950	
	6–10	£3,995	
	11–15	£4,570	
	> 16	£4,275	
- Symptomatic	0–5	£15,241	
	6–10	£15,704	
	11–15	£19,247	
	> 16	£19,291	
- Severe irreversible symptoms	0–5	£28,722	
	6–10	£30,692	
	11–15	£37,224	
	> 16	£37,388	
Utility values of symptom states			
- Asymptomatic – late[d]		0.95	0.9
- Asymptomatic – early[e]		0.95	0.9
- Symptoms (FEV1 – 60%, range 40%–80%)		0.75	0.65 – 0.90
- Severe irreversible symptoms (FEV1 – 30%, range 20–40%)		0.68	0.58 – 0.78

a The authors assumed that there are no false positives (from the combined DNA and sweat tests), because all test results that are either homozygous or heterozygous for cystic fibrosis mutations, are confirmed using sweat tests; b Assumed effect of early diagnosis; c Assumed to be a shared cost with existing screening programmes; d utility value to reflect "taking regular medication or staying on a prescribed health diet for health reasons" derived from community surveys; e arbitrary utility weight to reflect the probable repeated visits to GP with undiagnosed CF.

Source: Simpson et al., 2005344 The sources of all parameter values are in the original manuscript.

Survival estimates were derived from the UK National Cystic Fibrosis Survey. The transition probabilities in the Markov model were estimated to achieve age-specific survival rates and other estimated parameters. Three alternative scenarios were modelled: conservative, balanced and optimistic. The authors mention the annual transition probabilities that best predicted these calibration data for the balanced scenario, 'late diagnosis': from asymptomatic to symptomatic, 0.491 per year (with the remainder all staying asymptomatic); symptomatic to severe irreversible lung disease, 0.0064 increasing exponentially according the accumulated years with symptoms (with hazard rates derived from Dodge et al., 1997[347]); severe irreversible lung disease to death, 0.09 increasing according to the number of years spent in the severe irreversible disease stage.[344]

Sensitivity analysis was restricted to one-way and two-way sensitivity analysis (due to absence of published data on the uncertainty surrounding the mean parameter estimates). A threshold analysis was also performed to calculate the needed delay in the emergence of symptoms to make the screening scenario less costly and better than no screening.[344] Scenario analysis on whether or not explaining CF screening can be incorporated in the existing process (and how much time this takes of this is not the case) and the impact of reducing birth prevalence of CF due to introducing routine antenatal screening are also considered.[344] For results of the latter, we refer to the original manuscript.

Some of the assumptions made in the analysis are the following:

- Coverage: The addition of CF screening does not increase refusals or insufficient blood samples and 100% of the neonatal population would be covered by the programme.[344]
- Specificity: There are no false positives from the combined DNA and sweat tests because all test results that are either homozygous or heterozygous for CF mutations, are confirmed using sweat tests.[344]
- Treatment effect: There is an increased annual transition probability of remaining without symptoms (in early-diagnosed cases) of 10% (range 10-40%). This hypothetical benefit of early diagnosis through neonatal screening was modelled as a difference in the annual transition probability of remaining pre-symptomatic. In the initial model this probability was 69% for those diagnosed through screening (compared with 59% for those diagnosed symptomatically) resulting in a delay of the emergence of symptoms of 6 months.[344]

Under initial assumptions, the IRT/DNA two tier screening protocol costs £5,387 per infant diagnosed, or £1.83 per infant screened (1998 costs). 75% of these costs are due to performing the IRT test and the explanation of the test by midwives, i.e. the components of the screening process that are carried out for every infant screened. Based on these findings, the authors think it is unlikely that substantially different results would be obtained with other screening protocols because all employ an IRT test as the initial screening stage for all neonates.[344] Screening resulted in an average of 0.36 QALYs gained per life with CF at an additional cost of £2,895. The incremental cost-effectiveness ratio (ICER) for CF NBS versus no screening was £6,864 per QALY gained assuming a 6 month delay in the emergence of symptoms. Under the conservative and optimistic survival scenario, this would become £7,474 and £6,532 per QALY gained, respectively. If the delay in the emergence of symptoms would be 11 months or more, than CF NBS would be less costly and better than no screening.[344] Results were most sensitive to the incidence of the disease, and the proportion of cases detected at birth by family history of MI.[344]

The authors conclude that neonatal screening is expensive as a method of diagnosis and may be a cost-effective intervention if the hypothesised delays in the onset of symptoms are confirmed. Furthermore, they do not see good reasons to discontinue existing neonatal screening programmes on cost-effectiveness grounds since evidence of health benefits of early diagnosis is beginning to emerge.

9.2.5 Sims et al., 2007[345]

This cross sectional UK cost analysis estimates the potential savings in treatment costs attributable to newborn screening. The cost of illness was based on a retrospective snapshot cohort study based on the UK Cystic Fibrosis Database (year 2002). Patients aged 1–9 years were divided into two groups: newborn screening, i.e. 184 patients identified by newborn screening within 2 months of birth; and clinically diagnosed, i.e. 950 patients presented at any time by clinical diagnosis. Patients presenting with MI or with a CF family history were excluded. Yearly costs (no discounting) of long-term therapies and intravenous antibiotics were estimated. Assumptions were made on the used type of antibiotics. Subgroup analyses were performed according to age (3 age groups) and for homozygous ΔF508 newborn screening. It was also tested whether possible cost savings could off set the known costs of a national newborn screening programme (as used in Scotland), when scaled to the UK as a whole. Finally, a separate analysis was made restricting the cost estimates to patients diagnosed after the age of 2 months (since newborn screening can only benefit patients who would otherwise have presented after 2 months of age), and to those diagnosable with a 31 CF transmembrane regulator (CFTR) mutation assay (as used in the Scottish Newborn Screening Laboratory).[345]

Incremental cost estimates included staff costs (actual yearly salary costs adjusted for the proportion of time each member of staff devoted to CF screening), overheads (including equipment maintenance and capital charges), and consumables used in adding CF NBS to an established NBS programme (for phenylketonuria and congenital hypothyroidism) in Scotland. The costs of midwife or health visitor counselling, collection of blood spots, and health promotion are not included. Indirect costs (economic effects on carers, patients' productivity, etc) were also not included.[345] The perspective of the analysis was not explicitly mentioned.

The main results were: 1) The cost of therapy for patients diagnosed by newborn screening was significantly lower than equivalent therapies for clinically diagnosed patients: mean $7228 vs $12 008 (95% CI of difference: −6736 to −2028, p<0·0001). Depending on the subgroup, this difference remained significant. And 2) When the clinically diagnosed group was limited to only those diagnosable with a 31 CFTR mutation assay and similar disease progression in the clinically diagnosed group as in the newborn screening group was assumed, the mean ($3 397 344) drug cost savings could have off set the estimated cost of adding CF to a UK national newborn screening service ($2 971 551). In other scenarios, these cost savings did not outweigh the CF NBS costs. The authors remark that CF NBS might have greater financial benefits to society than their estimate shows if indirect costs savings are included.[345]

The authors conclude that newborn screening is associated with lower estimated treatment costs and reduced hospital admissions for invasive therapy than for clinically diagnosed patients. This also suggests that indirect costs and disruption to family life will also be less. According to these authors, universal newborn screening programmes for CF should be adopted internationally.[345]

9.2.6 Van den Akker et al., 2006[346]

This economic evaluation, performed for the Netherlands, compares the cost and effects of 4 CF neonatal screening strategies and a no screening strategy. The four screening strategies are: IRT/IRT, IRT/DNA, IRT/DNA/IRT, and IRT/DNA/DGGE (denaturing gradient gel electrophoresis). Positive results and referral for sweat test are considered if:[346]

- IRT/IRT: concentration of IRT from the second IRT measurement is above the cut-off level.
- IRT/DNA: one or two CF mutations are detected.
- IRT/DNA/IRT: two CF mutations are found or when the second IRT test is above the cut-off level (IRT performed if one mutation based on DNA).
- IRT/DNA/DGGE: two CF mutations are identified, either by multiple mutation analysis or by the extensive gene analysis (DGGE if one mutation based on DNA).

Results of the economic modelling exercise are expressed in costs per life-year gained. No CUA was performed since the authors did not find adequate estimates for health-related quality of life in CF patients identified by screening compared with clinically detected patients. The model assessed the costs and number of life-years gained as a result of CF NBS for a hypothetical cohort of 200 000 neonates, i.e. the approximate number of children born annually in the Netherlands. The analysis was performed from a societal perspective. A lifetime time horizon was taken into account and all costs and effects were discounted at a 3% rate.[346]

The following table provides an overview of the parameters used in their modeling exercise.

Table 20. Model parameters and values used in the model of Van den Akker et al.[346]

Model parameters	Base case value	Range used in sensitivity analysis
Probabilities		
- Incidence of CF	0.0003	0.0002
- % newborns with meconium ileus	17	10 - 20
- Participation neonatal screening, %	99.5	95
- Sensitivity first IRT test, %	98	86 - 100
- Cutoff level first IRT test, daily percentile	99th	
- Sensitivity second IRT test, %	92	88.5 - 95
- Specificity second IRT test, %	94	88.3 - 98
- % infants with positive IRT test who are carriers	6	
- % CF patients with 1 mutation detected by DNA test	25	
- % CF patients not detected by DGGE test	1	
- CF mortality in childhood, %	6	3 - 10
- Reduction in childhood CF mortality because of screening, %	25	50
- % parents opting for genetic counseling	90	
- % parents opting for carrier status testing after genetic counseling	50	
- Savings in lifetime costs of treatment because of screening, %	0	10
- % decrease in CF patients because of neonatal screening	10	
- % decrease in CF patients in a situation with screening because of prenatal diagnosis	50	
- % pregnancies terminated when fetus affected with CF	69	
Costs, € (2004 values)		
- Adding CF screening to neonatal screening program	133116	75000 - 150000
- First IRT	4.79	
- Second IRT	18.47	
- DNA test	100	50
- DGGE test	500	300
- Sweat test	109.56	
- Testing for carrier status	663.62	
- Genetic counselling	449.24	
- Clinical diagnosis CF	9096	7500 - 11 000
- Lifetime of treatment for clinically diagnosed patient	406266	812531
- Costs of prenatal diagnosis	1648	
- Costs of terminating pregnancy	690	
Others		
- Annual No. of neonates	200000	
- No. of life-years gained per prevented death because of screening (3% discounting)	40 (20.5)	35 (19.4) - 45 (21.8)
- No. of sweat tests for diagnosis of non-CF patients per clinically diagnosed CF patient	100	

Source: Van den Akker et al., 2006[346]
The model parameters were retrieved form literature, based on expert opinions or on primary data collection. We refer to the original manuscript for original sources of parameter values.

For the situation without screening, information about the number of hospital days and the medical procedures performed in the diagnostic process was reviewed in 36 patients. Multiplying the numbers of hospital days (not mentioned) with the hospitalization cost (not mentioned) and adding the costs of medical procedures resulted in an average diagnostic cost of €9096 per patient diagnosed with CF. Furthermore, the non-screening scenario included the cost of 100 sweat tests for each clinical CF patient diagnosed.[346]

The costs of including neonatal screening for CF in the existing neonatal screening program was based on the costs of adding screening for congenital adrenal hyperplasia to the neonatal screening program in the Netherlands (€133 116 per year). These costs include the costs of a medical consultant, administration costs, and evaluation costs. No costs were included for changing the information leaflet since it is updated regularly. For the second IRT test (€18.47), it was assumed that a new blood spot has to be collected by the district nurse at the child health clinic. This figure includes the cost of the laboratory, the cost of the district nurse (15 minutes), and travel and time (30 minutes) for one of the parents. The costs of the sweat test (€109.56) include pediatric consultation, laboratory costs, and parents' travelling expenses and time (60 minutes). Also the costs of genetic counselling (€449 per couple) and testing parents for carrier status (€664) include parents' travelling expenses and time (60 minutes). The costs of the multiple mutation tests (€100) and DGGE tests (€500) include the cost of DNA extraction, DNA analysis, laboratory space, equipment, reagents, supplies, licenses, technical and administrative personnel, and new technical advances. Costs incurred during life-years gained because of neonatal CF screening are not included in the analysis.[346]

One of the main assumptions was a gain of 40 life-years per CF death prevented by neonatal screening (excluding benefits for CF patients with MI which do not benefit from neonatal screening). Furthermore, a 6% CF mortality (range 3% – 10%) in childhood and a 25% reduction (changed to 50% in sensitivity analysis) in childhood CF mortality because of screening were assumed.[346]

Both univariate and multivariate sensitivity analyses were performed. In the probabilistic multivariate sensitivity analysis, a uniform distribution was assumed for the parameters between the lower and upper values. In a scenario analysis, the costs and effects of changes in reproductive decisions because of neonatal screening were also assessed.[346]

The CEA finds the most favorable ICER for the IRT/IRT protocol: €24 800 per life-year gained. Compared to no screening the ICER for the other strategies is as follows: IRT-DNA: €38 300/LYG; IRT-DNA-IRT: €39 800/LYG; and IRT-DNA-DGGE: €33 000/LYG. Working on the efficiency frontier (i.e. comparing with the previous most cost-effective alternative) IRT-DNA-IRT is excluded by extended dominance. The ICER of IRT-DNA-DGGE (compared with IRT-IRT) becomes €130 700/LYG and for IRT-DNA (compared with IRT-DNA-DGGE) becomes €2 154 300/LYG.[346]

Results were most sensitive to the uncertainty about whether screening for CF will lead to savings in lifelong costs of treatment, the lifelong costs of treatment of clinically diagnosed patients, mortality in early childhood because of CF in a situation without screening, and the reduction in childhood mortality as a result of screening for CF. Changing the CF mortality in childhood to 3% would result in a less favourable ICER of €49 600/LYG. Also changing the IRT threshold has a large impact on results. Changing the IRT threshold to the 95th daily percentile would result in an ICER of €40 400/LYG. Finally, in the scenario where changes in reproductive decisions as a result of neonatal screening were also taken into account, this study finds that CF neonatal screening may lead to financial savings (~€1.8 million annually, depending on the screening strategy used).[346]

The authors conclude that NBS for CF is economically beneficial, and positive health effects are expected as a result of the screening. IRT/IRT and IRT/DNA/DGGE are the most cost-effective strategies.

9.2.7 Discussion

In our overview, only studies comparing with the no-screening alternative were included. Exclusion of the no-screening alternative seems inappropriate, as the type and scale of the benefits of CF screening remain uncertain.[344] The lack of data on CF screening benefits is a major problem to calculate the intervention's cost effectiveness. Several studies solved this problem by only looking at the costs to diagnose CF cases without including the impact of (possible) benefits. We looked at studies that concluded that screening reduced the costs per diagnosed case. If this would be the case, then screening could be supported, i.e. an intervention that is cheaper, has potential benefits and risks or harms are limited.

The first study of Dauphinais et al., 1992[341] compared the costs of 16 non-screened patients with a delayed diagnosis of 1.5 months to 21 years versus IRT screening costs. A major problem with this study is the difference in time horizon between the two groups. The costs of the non-screened group of CF patients were accrued over a period of 1.5 to 253 months. In contrast, the time window is only a couple of months for the screening group. A comparison of costs over very different time periods is not appropriate and therefore, results are not reliable.[341] Furthermore, the comparability of populations in the non-screened and screening group is questionable.

The cost analysis of Farrell et al., 1992[342] also finds that screening might be cheaper: a one-tier protocol (IRT) would cost $130,400 to screen 75,000 newborns, a two tier IRT/DNA protocol would cost $126,770 and without screening their analysis revealed a total cost of $182,030. The number of sweat tests was assumed to be 150 and 50 for the IRT and IRT/DNA protocol, respectively. Without screening this was 1,670. The latter was determined by a hospital survey in 1991. The costs of the IRT and IRT/DNA analysis are however underestimated. The 2000 hospital survey[343] revealed that in the screening program, next to follow-up evaluations, still another 804 sweat tests were performed. Only looking at the laboratory costs, e.g. excluding the extra costs of genetic counselling, this would already make the screening protocols more expensive than no screening. This illustrates the difficulty in answering the question of whether or not neonatal screening for CF will reduce (direct) medical costs and that the in- or exclusion of certain cost data may completely alter the results.

A more elaborated cost analysis of the same research group performed about 10 years later included the extra costs of these sweat tests.[343] The previous analysis[342] applied an average sweat test charge of $56 and assuming an extra "sick visit" per sweat test in the primary care physician's office ($53). In the more recent analysis, these costs increased to $161.40 for a sweat chloride test. The analysis indicated that a CF newborn screening program could be a potentially cost-saving alternative to the traditional method of diagnosis of CF. However, as for the other analysis, a lot of important cost factors are not included. For example, almost all studies did not include the expenses for genetic counselling.[343-345] The same remark is true for a lot of other aspects such as the influence of false-positives and false-negatives with screening, the delay in diagnosis and expenses for other tests without screening, etc.

The cost analysis of Sims et al.[345] included in this review illustrates the problem of comparability between population. Children identified by use of newborn screening are not comparable with those identified by clinical presentation because the former probably includes more patients with mild disease.[348] The same criticism applies to the other cost and cost-effectiveness studies. The models explicitly or implicitly assume that the CF diagnosed patients are a relatively homogeneous group. However, as mentioned by Simpson et al.[344] the spectrum of cases ranges from neonates that are severely affected, to cases who live a normal life undiagnosed until adulthood. It is possible that the more severe but asymptomatic cases would benefit most from early diagnosis. Those with milder forms of the disease would be diagnosed later under the no-screening strategy and would have their age at diagnosis advanced most under screening.[344] The authors could not investigate this issue with the available data and ignored this issue. However, this may bias the outcome of analyses. Results and conclusions of analyses comparing costs of two possibly incomparable patient groups should be interpreted cautiously.

Only two analyses[344, 346] performed a full economic evaluation including both costs and effects of one or more screening protocols versus the no-screening alternative. Only Simpson et al.[344] include QoL for several health states. However, for the asymptomatic states, utility weights are arbitrary or derived from non-CF specific health surveys. For the symptomatic disease state and the severe irreversible lung disease-state, reference is made to two publications published about two decades ago.[349, 350] The analysis of Van den Akker et al. did not perform a CUA since they did not find adequate estimates for health-related quality of life in CF patients identified by screening compared with clinically detected patients.[346] Good information on the QoL impact of screening versus no screening is currently lacking.

Similar to the other analysis, Simpson et al. do not include all relevant items that have an influence on costs and effects, mainly because information is still lacking. For example, they also excluded time for genetic counselling because of difficulties to quantify the impact and because they argue that this cost would only be small in relation to total screening costs. However, if the number of false positives is relatively large, this costs should not be underestimated. Furthermore, in their analysis they assume that there are no false positives. This is a rather optimistic scenario, probably not reflecting reality. Another very important assumption is the modelled treatment effect: an increased annual transition probability of remaining without symptoms (in early-diagnosed cases) of 10% (range 10-40%). This results in a 69% probability of remaining pre-symptomatic with screening versus 59% in the symptomatically diagnosed CF population. As the authors conclude, the intervention may be cost effective if the hypothesised delays are confirmed. In other words, the analysis has to be interpreted as a hypothetical analysis.

The problem of lack of data is reflected in the absence of state-of-the art probabilistic sensitivity analysis. Simpson et al.[344] mention they have not performed such analysis due to the lack of data reflecting the uncertainty around the mean estimates for input variables. Furthermore, the used ranges of values for some key variables may be unrealistic. For example, Simpson et al. only increased the hypothetical base case treatment effect up to 40% (no lower treatment effect was modelled). With a 59% transition probability for those diagnosed symptomatically, this results in a 99% transition probability of remaining without symptoms for the CF population diagnosed through screening.[344]

The study of Van den Akker et al.[346] has the same merits and shortcomings of the previous discussed study: it tries to make an estimation of CF screening's cost effectiveness but it remains a hypothetical analysis. The main assumptions are: 1) there is a 25% reduction in childhood CF mortality because of screening; and 2) the number of life-years gained per prevented death because of screening is 40 years. With current available evidence, the impact on mortality is uncertain. Furthermore, the baseline risk for CF mortality in childhood was set at 6% in this analysis, ranging from 3% to 10%. Current treatments of CF are probably better than a decade ago. Lowering the mortality risk of the comparator lowers the possible gains by introducing screening. The ICER for the IRT/IRT protocol increased from €24 800/LYG to €49 600/LYG applying a 6% or 3% CF mortality risk in childhood, respectively (note: 'childhood' is not defined). It would be interesting to know the real-world CF mortality risk (for the Belgian population) with current available treatment options and optimal/timely referral of patients to specialised centres. Currently, the estimated 10-year mortality would be about 2% with a very small percentage also being identified through screening. Reducing the baseline mortality risk without screening deteriorates the intervention's cost effectiveness. Finally, the problem of reaching patients for a second IRT test was also not taken into account. Up to 20% of patients may be lost for this examination.[250] In France this number was reduced to 3.2%. In the local CF screening initiative in Brussels this may be as high as 15% (personal communication, A. Meulemans). This will also have a negative impact on the intervention's cost effectiveness. On the other hand, other possible positive effects are also not included in the analyses (e.g. the costs of the diagnostic odysseys).

The report of the Dutch Health Council (http://www.gezondheidsraad.nl/en/publications/neonatal-screening-cystic-fibrosis) mentions that some aspects of the cost calculation are uncertain. If screening results in a decrease in treatment costs, it might lead to costs savings. A related (but not-yet-published) article assesses the costs of three neonatal screening strategies (IRT-PAP, IRT-DNA-EGA, IRT-PAP-DNA-EGA) for CF in relation to health effects (personal communication). It concludes that neonatal screening for CF leads to financial savings in comparison to a situation without screening. Similar as for the previous studies, the outcomes mainly depend on the assumptions that are made, based on expert opinion. For example, in this Dutch study, it is assumed that costs for about 3000 sweat tests without screening would disappear if screening is introduced. This can be questioned, especially because screening is not 100% sensitive eg cases with bronchitis suspected of CF, will still exist after screening is introduced. It is also assumed that CF treatment costs would decrease with about 0% - 5% due to screening. Nevertheless, CF treatment costs may both increase or decrease, depending on whether or not mild and/or severe cases are detected, how much morbidity actually decreases because of early referral to optimal treatment, etc. In short, there are many uncertain arguments/assumptions that may make the outcome of the cost-effectiveness calculation positive or negative.

The published results on CF screening may look promising. However, results of the costs analyses depend on assumptions, the cost items included and their valuation. Furthermore, comparability of the screening and no-screening group remains a challenge and should be interpreted with caution. For the economic evaluations, results mainly depend on the assumed treatment effects. The models rather reflect hypothetical situations, not supported by reliable evidence. This lack of reliable data remains a major problem to calculate the intervention's cost effectiveness. In the following chapter, several uncertain elements that may determine the intervention's cost effectiveness are listed.

Key points

- **Cost analyses face the problem of possible incomparability of CF patients diagnosed through screening versus symptomatic diagnosis.**
- **Results of cost analysis depend on the included cost items and their valuation.**
- **There is a lack of reliable evidence to feed the economic models. Especially the effect of screening without interference of an improved treatment over time is unclear.**
- **The published economic evaluations should be interpreted as hypothetical analyses since the values of most important parameters (e.g. treatment effect) are based on assumptions.**

10 ECONOMIC ASPECTS OF NEWBORN SCREENING FOR CYSTIC FIBROSIS IN BELGIUM

10.1 COST EFFECTIVENESS

The review of the economic literature has shown that there is a lack of reliable evidence to feed the economic models. Therefore, the published economic evaluations should be interpreted as hypothetical analyses since the values of most important parameters (e.g. treatment effect) are based on assumptions. There are both items that will/can increase or decrease costs and effects if CF NBS would be implemented. However, for most of these items it is difficult to quantify their exact impact in monetary terms or their influence on (quality-adjusted) life-years. Instead of making a hypothetical calculation of the incremental cost-effectiveness ratio for which most input parameters (and thus results) can be questioned, a non-exhaustive overview is given of elements that might have an influence on costs and/or effects (Table 21). A positive sign indicates the influence would be positive (i.e. decreases costs or increases effects) and vice versa.

Table 21. Items which might have a positive or negative effect on costs and/or effects if screening is introduced

Item	Impact on costs	Impact on effects
cost/effect neutral		
education of midwives/nurses	no incremental cost	
information leaflet	no incremental cost	
Guthrie cards	no incremental cost	
informed consent	no incremental cost	
taking blood sample	no incremental cost	
follow-up of patients in registry	no incremental cost if already present	
etc.		
negative impact on costs and/or effects		
tests in screening protocol	-: incremental cost	
false positives	-: retesting, counselling	-: psychosocial effects
false negatives	-: diagnostic odysseus	-: relatively late diagnosis
counselling	-: incremental cost	
genetic counselling (e.g. IRT/DNA protocol)	-: incremental cost	-: psychosocial effects
recall (e.g. IRT/IRT protocol)	-: incremental cost	
contacting physician for patients with abnormal test result	-: incremental cost	
coordination of screening programme	-: incremental cost	
supervision of screening programme	-: incremental cost	
set up an audit/control system	-: incremental cost	
etc.		
positive impact on costs and/or effects		
tests for undiagnosed patients with symptoms	+: less sweat tests if (most) patients already identified through screening	
avoiding diagnostic odysseus	+: avoidance of other (expensive) diagnostic tests and/or treatments	+: psychosocial effects
future pregnancies	+: decrease in CF prevalence (and thus treatment costs)	+/-: psychosocial effects
burden of care	+: could be lower if symptoms are more under control	+: less severe treatment
	-: could be higher if 'overdiagnosis' of cases (that otherwise would never be identified)	-: overly aggressive treatment (medicalisation of society)
hospitalisations	+: could be reduced	+: positive impact on QoL if less hospitalisations
	-: could increase due to more planned hospitalisations (e.g. for administration intravenous antibiotics)	-: negative impact on QoL if more hospitalisations
pulmonary status, nutritional benefits, cognitive development	+: possibly less treatment costs	+: possibly positive impact on QoL if improved situation
mortality		+: possibility to gain life years
etc.		
Determining factors		
Incidence	Lower incidence will result in a lower possible impact on a population level of	
Part of MI or mild CF	No/less benefit of screening	
Current situation without screening	The better the identification of patients (based on e.g. family history or symptoms) ,	
Screening protocol	Tests included, cut-off levels, etc. will influence both costs and effects.	
(timely) referral identified patients to treatment centres	Necessary to have a positive impact of identifying patients.	
Cost of tests	Importance of low cost, scale and scope effects	
Heterogeneity of population	Possibility of overshooting with screening	
Carrier screening (on a large scale)	Less benefit for neonatal screening if parents already screened.	
etc.		

Some of these items would very probably not be an incremental cost, such as: education of midwives/nurses if this is included in the training sessions for other screened diseases, adaptation and distribution of the existing information leaflet if it is made up-to-date and reprinted on a regular basis, purchase of the Guthrie cards, providing informed consent and taking the blood sample since this already happens in the currently running screening programme. It is clear that the presence of an existing screening programme reduces the incremental costs of an extra test. Of course the pro's and con's, both from a medical and economic point of view, should be regarded. Explicitly doing so is currently not possible due to a lack of reliable data for most of these elements.

10.2 BUDGET IMPACT

In this part, the budget impact of NBS CF is calculated. This exercise is performed for the proposed Belgian CF NBS algorithm (Figure 9) and the IRT-PAP-DNA algorithm (Figure 10). Cost data are gathered for the tests that occur in these protocols. Cost estimates are based on data received from two laboratories (AZ Brugge-Oostende and ULB). We would like to acknowledge Dr. Dirk Bernard and Dr. Ann Meulemans for their willingness, time and efforts to gather relevant cost data.

We would like to remark that this exercise is a simplified costing exercise. Data for each test is based on input data from one laboratory. Certain data are based on invoices, and thus reflect real-world situation. However, other data are based on expert opinion (e.g. time needed for certain acts), while better and more reliable but also more time-consuming approaches exist (e.g. timekeeping of the necessary time to perform a certain activity). Some costs are based on information received from the producer, while for other items included costs reflect negotiated prices. For some of these items, (further) price negotiations may be possible if implemented on a larger scale.

Uncertainty around cost parameters, estimated equipment service life, and other parameters was left out of consideration. Once there is more certainty on whether or not to introduce NBS CF and the preferred protocol, more detailed cost data (from several laboratories) may be gathered and an improved costing exercise including parameter uncertainty could be performed. Nevertheless, this budget impact exercise provides a first general idea of the possible budget impact.

10.2.1 Costs for individual tests

Costs were expressed in 2010 (April) values using the Health Index (Table 22).

Table 22. CPI and Health Index

Year	CPI	Health Index
1996	100.00	99.97
1997	101.63	101.29
1998	102.60	102.58
1999	103.75	103.54
2000	106.39	105.49
2001	109.02	108.38
2002	110.81	110.32
2003	112.57	111.92
2004	114.93	113.74
2005	118.13	116.21
2006	120.25	118.26
2007	122.44	120.35
2008	127.94	125.44
2009	127.87	126.18
2010*	130.25	127.81

*: April 2010; CPI: Consumer Price Index
Source: http://statbel.fgov.be/

The tests that occur in (one of) the two screening protocols are the following: IRT, DNA, PAP and sweat test.

For the IRT test, both a calculation of a manual and automatic test are provided. For the manual test, it is assumed that the equipment was used for a total of 10100 tests per year (AZ Brugge-Oostende laboratory). For the automatic test, 75740 tests were performed (ULB laboratory: T4: 2585 patients, 17a-OH: 18828 patients, TSH: 30001 patients and IRT: 24326)

The estimated cost for a 'manual' IRT test is €2.55 if the equipment costs are not included (i.e. if they are not interpreted as an incremental cost), and otherwise €3.95: i.e. €1.28 (reagents), €1.27 personnel, and €1.40 equipment ((€8 761 + €5 127 + €234)/10100) (Table 23). For an 'automatic' IRT test the cost is €2.00 if the equipment costs are not included (i.e. if they are not interpreted as an incremental cost), and otherwise €2.19: i.e. €1.33 (reagents and consumables), €0.40 (software), €0.27 personnel, and €0.19 equipment ((€7 829 + €6 455 + €234)/75740) (Table 24).

Whether or not the equipment is already used for (several) other tests and whether or not these costs are interpreted as incremental costs clearly has an impact on the calculated costs per test. The costs for reagents represent a relatively large part in the calculated incremental costs. There was some disagreement about the time needed for validation and communication of results which might be underestimated. More refined costing approaches might provide more reliable data. Furthermore, other approaches to perform this test are also possible, e.g. Elisa Classic, but not regarded in our calculations.

Table 23. IRT test (manual)

Cost items	Cost	Year of information	Indexation to 2010 (April)	Estimated longevity	Source
Equipment					
Investment cost & depreciation					***€ 8761/year***
Puncher	€ 20 651	2007	€ 21 931	5 years	invoice
Diskwasher & remover	€ 7 556	2008	€ 7 699	5 years	invoice
Shaker	€ 1 805	2003	€ 2 061	10 years	invoice
Victor D fluorometer	€ 22 688	2002	€ 26 284	10 years	invoice
Maintenance of equipment					***€ 5127/year***
Puncher	€ 2 275	2010	€ 2 275	yearly	invoice
Diskwasher & remover	/				
Shaker	/				
Victor D fluorometer	€ 2 853	2010	€ 2 853	yearly	invoice
Disposables substances					***€ 234/year***
Head of puncher	€ 1 171	2010	€ 1 171	5 years	inquiry
reagents	€ 1.28[a]	2010	***€ 1.28***	per test	invoice and expert opinion

Personnel	Estimated time	Time on yearly basis	Time/test	Cost/hour	Source
MLT	~70 minutes/76 samples[b]		1 minute/test	€ 37.5	expert opinion
Clinical biologist[c]	1h/week	52 hours	0.31 minute/test	€ 125.0	expert opinion
					€ 1.27/test

Personnel cost	Yearly cost	Hours/year[d]	
MLT	€ 60 000	1600 hours	estimate HR
Clinical biologist	€ 200 000	1600 hours	department[e]

MLT: Medical laboratory technologist
a: (€995 x 13kits)/10100 patients = €1.28 per patient. The cost per IRT kit is €995 (incl. 21%TAV). 13 kits (960 tests per kit) would be used for 10100 patients (based on the number of TSH kits used for this number of patients). The surplus of tests (960 x 13 = 12480) is used for calibration and checks.

b: The time needed by the MLT was measured for 76 samples (starting the test, punching the cards, adding reagents, etc.). The MLT needed about 70 minutes, i.e. on average less than one minute per sample.
c: One hour per week, 52 weeks, for 10100 patients (Validating results, checking protocol, communicating results, staff meeting, etc.).
d: One full-time equivalent equals about 1650 hours per year. Taking into account sickness, absence, reduction of working hours, seniority, etc. this becomes about 1600 hours.
e: Personnel costs are a rough estimate. Data can vary widely across hospitals.

Table 24. IRT test (automatic)

Cost items	Cost	Year of information	Indexation to 2010 (April)	Estimated longevity	Source
Equipment					
Investment cost & depreciation					***€ 7829/year***
AutoDelfia and puncher	€ 76 835	2008	€ 78 287	10 years	invoice
Maintenance of equipment					***€ 6455/year***
AutoDelfia and puncher	€ 6 373	2009	€ 6 455	yearly	invoice
Disposables substances					***€ 234/year***
Head of puncher	€ 1 171	2010	€ 1 171	5 years	inquiry
					€ 1.33/test
neonaltal IRT kit, Whatman 904	€ 1 119	2010	€ 1 119	for 1008 tests[a,b]	invoice
Eppendorf pipette tips	€ 143	2010	€ 143	for 1008 tests[c]	invoice
autodelfia wash concentrate	€ 519	2010	€ 519	for 1008 tests[d]	invoice
autodelfia enhancement solution	€ 1 198	2010	€ 1 198	for 1008 tests[e]	invoice
new dilution vessels (100/package)	€ 114	2010	€ 114	for 1008 tests[f]	invoice
					€ 0.40/test
Others: software[g]	€ 30 000			yearly	rough estimate

Personnel	Estimated time	Time on yearly basis	Time/test	Cost/hour	Source
MLT	~4 hours/1008 samples[h]		0.24 minute/test	€ 37.5	expert opinion
Clinical biologist	~1 hour/1008 samples[i]		0.06 minute/test	€ 125.0	expert opinion
					€ 0.27/test

Personnel cost	Yearly cost	Hours/year[j]	
MLT	€ 60 000	1600 hours	estimate HR
Clinical biologist	€ 200 000	1600 hours	department[k]

MLT: Medical laboratory technologist
a: 168 samples per run, i.e. 96 samples per plate, two plates each time, 12 for checking and 12 for calibration. Six runs (i.e. 12 plates per kit), i.e.168 x 6 = 1008 samples per kit.
b: €1.11/test, i.e. €1119.25/1008.
c: €0.01/test, i.e. ((€142.78/960)x48)/1008. €142.78 is the price for 960 tips, 8 tips needed for two plates (or 48 tips needed for 1008 samples).
d: €0.05/test, i.e. ((€519.09x7)/75.1)/1008. €519.09 per kit and 7 kits needed for four tests (T4, 17a-OH, TSH and IRT). For these four tests, 75.1 kits are needed (T4: 2.6 kits (2585 patients/1008), 17a-OH: 18.7 kits (18828 patients/1008), TSH: 29.8 kits (30001 patients/1008) and IRT: 24.1 kits (24326 patients/1008).
e: €0.16/test, i.e. ((€1197.90x10)/75.1)/1008. €1197.90 per kit and 10 kits needed for four tests (T4, 17a-OH, TSH and IRT). For these four tests, 75.1 kits are needed (see previous remark).
f: €0.01/test, i.e. ((€113.74/100)x6)/1008. €142.78 is the price for 100 vessels, 6 vessels needed for 6 runs (i.e. for 1008 samples).
g: €0.40 per test, i.e. €30000/75740. 75740 tests are performed (T4: 2585 patients, 17a-OH: 18828 patients, TSH: 30001 patients and IRT: 24326).
h: ~40 minutes for 168 samples (punching, starting PC, reporting abnormal results, etc.)
i: 10 minutes for 168 samples (Validating results, checking protocol, communicating results, staff meeting, etc.).
j: One full-time equivalent equals about 1650 hours per year. Taking into account sickness, absence, reduction of working hours, seniority, etc. this becomes about 1600 hours.
k: Personnel costs are a rough estimate. Data can vary widely across hospitals.

For DNA, the costs items are provided in Table 25. The cost per test will, among others, depend on the total number of tests and the number of tests per batch

The latter depends on how many centres will perform this test and if this happens e.g. weekly or every two weeks. For example, if about 600 tests are needed on a yearly basis, one centre can handle this and the costs per test will be somewhere between the costs calculated in scenario 2 and 3 (in which on average 650 tests are provided). This results in an average estimated cost per DNA test of €150 or €196, depending on whether or not the equipment costs are interpreted as incremental costs. This would be on average €150 or €242 if two centres perform about half of these tests (the average cost of scenario 6 and 7 which also provides an average of 650 tests if two centres are performing the test). This has an influence on the average costs if the equipment costs are included (i.e. being interpreted as incremental costs). In other words, whether or not the equipment is already available will have a relatively large impact on costs per test and thus total costs.

If 170 DNA tests are needed, and taking into account a small overcapacity, one centre could handle this. The average of scenario 5 and 6 results in 195 yearly samples with an average cost of €176 or €342, again depending on ex- or inclusion of equipment costs.

Table 25. DNA test (part A)

Cost items		Cost	Year of information	Indexation to 2010 (April)	Estimated longevity	Source
Equipment						
Investment cost & depreciation						***€ 16588/year***
qiasymphony		€ 75 988	2009	€ 76 970	7 years	invoice
incubator		€ 12 100	2010	€ 12 100	10 years	expert opinion
ABI9700		€ 6 733	2010	€ 6 733	5 years	invoice
autolipa		€ 30 353	2010	€ 30 353	10 years	invoice
Maintenance of equipment						***€ 12079/year***
qiasymphony		10%		€ 7 697	yearly	expert opinion
incubator		/		/	/	expert opinion
ABI9700		20%		€ 1 347	yearly	expert opinion
autolipa		10%		€ 3 035	yearly	expert opinion
Disposables substances						***€ 85.89/test***
qiasymphony[a]		€ 9.44	2010	***€ 9.44***	per test	expert opinion
incubator		/		/	/	expert opinion
ABI9700		€ 1.00	2010	***€ 1.00***	per test	expert opinion
autolipa	CFTR19 test[b]	€ 60.25	2010	***€ 60.25***	per test	invoice
	trays[c]	€ 10.20	2010	***€ 10.20***	per test	invoice
	template strips[d]	€ 3.00	2010	***€ 3.00***	per test	invoice
	TAQ-polymerase	€ 2.00	2010	***€ 2.00***	per test	expert opinion
Personnel		**Estimated time**		**Cost/hour**	**total cost**	**Source**
MLT	sampling	2.5h/batch[e]		€ 37.5	€ 93.8	expert opinion
	amplification	1.5h/batch		€ 37.5	€ 56.3	expert opinion
	autolipa	1.5h/batch		€ 37.5	€ 56.3	expert opinion
Clinical biologist		1.5h/week		€ 125.0	€ 187.5	expert opinion
Molecular biologist		15minutes/sample		€ 125.0	€ 31.3	expert opinion

Personnel cost	Yearly cost	Hours/year[f]	
MLT	€ 60 000	1600 hours	estimate HR
Clinical/molecular biologist	€ 200 000	1600 hours	department[g]

MLT: Medical laboratory technologist
a: €9.44/test, i.e. (€7.5x1.21)x1.04 (i.e. €7.5 excl. 21%TAV and another 4% for checking).
b: €60.25/test, i.e. €1205 (incl. TAV) for 20 tests (19 mutations).
c: €10.20/test, i.e. €255 (incl. TAV) for 25 trays.
d: €3/test, i.e. 75€ (incl. TAV) for 25 strips.
e: Scale effect: it is assumed that 5 to 20 samples can be incorporated per batch and processed in about the same time.
f: One full-time equivalent equals about 1650 hours per year. Taking into account sickness, absence, reduction of working hours, seniority, etc. this becomes about 1600 hours.

Table 26. DNA test (part B)

Cost per analysis	samples/ batch	batches/ year	samples/ year	equipment [a]	disposables [b]	personnel [c]	Total (incl. eq.)	Total (excl. eq.)
scenario 1	5	52	260	€ 110	€ 86	€ 110	€ 306	€ 196
scenario 2	10	52	520	€ 55	€ 86	€ 71	€ 212	€ 157
scenario 3	15	52	780	€ 37	€ 86	€ 58	€ 180	€ 143
scenario 4	20	52	1040	€ 28	€ 86	€ 51	€ 164	€ 137
scenario 5	5	26	130	€ 221	€ 86	€ 110	€ 416	€ 196
scenario 6	10	26	260	€ 110	€ 86	€ 71	€ 267	€ 157
scenario 7	15	26	390	€ 74	€ 86	€ 58	€ 217	€ 143
scenario 8	20	26	520	€ 55	€ 86	€ 51	€ 192	€ 137

a: Total equipment cost is €28667 (€16588 + €12079). This amount is divided by the number of yearly test (e.g. €28667/260=€110)
b: see previous table
c: personnel costs: (((cost per batch x batches per year) + (cost per week x batches per year))/samples per year) + cost per sample (see cost items in previous table). E.g. €110 = ((€93.8+€56.3+€56.3+€187.5)x52)/260 + €15.6

The costs for the PAP test exclude equipment costs (both investment and maintenance), again not being interpreted as incremental costs. As such, total costs per test would be about €7.59 per test. The test kit represent the largest part of this cost.

Table 27. PAP test

Cost items	Cost	Year of information	Indexation to 2010 (April)	Estimated longevity	Source
Equipment					
Investment cost & depreciation	no incremental cost				expert opinion
Maintenance of equipment	no incremental cost				expert opinion
Disposables substances					***€ 6.08/test***
MucoPAP ELISA kits	€ 12 015	2010	€ 12 015	for 1975 tests[a]	invoice and expert opinion

Personnel	Estimated time	Time/test	Cost/hour	Source
MLT	2h38minutes/79 samples[b]	2 minutes/test	€ 37.5	expert opinion
Clinical biologist	10minutes/79 samples	0.13 minute/test	€ 125.0	expert opinion
				€ 1.51/test

Personnel cost	Yearly cost	Hours/year[c]	Source
MLT	€ 60 000	1600 hours	estimate HR
Clinical biologist	€ 200 000	1600 hours	department[d]

MLT: Medical laboratory technologist
a: €6.08/test, i.e. €12015/1975. The total cost for 25 kits was €12015 (€9930 excl. 21% TAV). Each kit contains 96 samples of which 79 are used (i.e. excluding controls).
c: One full-time equivalent equals about 1650 hours per year. Taking into account sickness, absence, reduction of working hours, seniority, etc. this becomes about 1600 hours.
d: Personnel costs are a rough estimate. Data can vary widely across hospitals.

For the sweat test, the costs are calculated relying on the assumption that the number of yearly analyses would be about 400, i.e. 50 weeks of testing, four patients per week and two tests per patient. Accounting the cost of €29 336 (€1 759 + €3 147 + €7 139 + 18 333 (Table 28)) to these 400 test results in an estimated cost of €76.94. Adding another €1 for small material like recipient and gauze (rough estimate, expert opinion) and providing two tests per patient results in €204.30 per patient. Excluding the equipment costs this would become €125.85. In contrast, the nomenclature provides the following codes: 545753-545764 (€15.55 per act, 2 times), 593154 (€32.87) and 593176 (€7.68), or a total fee of €71.65 per patient. Note that the wording of the nomenclature code 545753-545764 is not clear with regard to the testing of two independent samples, as is recommended by international guidelines. Health insurers however commonly accept to reimburse two such acts.

Table 28. Cost sweat test

Cost items		Cost	Year of information	Indexation to 2010 (April)	Estimated longevity	Source
Equipment						
Investment cost & depreciation						***€ 1759/year***
Flame photometer		€ 6 749	2001	€ 7 959	10 years	invoice
Cl-titrator		€ 6 000	2010	€ 6 000	10 years	inquiry
Collector[a]		€ 3 630	2010	€ 3 630	10 years	inquiry
Maintenance of equipment						***€ 3147/year***
Flame photometer	- contract	€ 1 321	2009	€ 1 338	yearly	invoice
	- work	€ 834	2009	€ 845	yearly	invoice
Cl-titrator		10%		€ 600	yearly	expert opinion
Collector		10%		€ 363	yearly	expert opinion
Disposables substances						***€ 7139/year***
Flame photometer		€ 363	2009	€ 368	yearly	invoice
		€ 261	2009	€ 265	yearly	invoice
		€ 162	2009	€ 164	yearly	invoice
Cl-titrator		€ 55	2009	€ 56	yearly	invoice
		€ 2 723	2009	€ 2 758	yearly	invoice
		€ 69	2009	€ 70	yearly	invoice
		€ 2 723	2009	€ 2 758	yearly	invoice
Collector[b]		€ 50.42	2010	***€ 50.42***	per patient	inquiry
Others: Checking		€ 1 384	2009	€ 1 402	2 years	invoice
Personnel		**Estimated time**	**Time on yearly basis**	**Cost/hour**		**Source**
MLT	sampling	4h/week	200 hours	€ 37.5		expert opinion
	measurements	4h/week	200 hours	€ 37.5		expert opinion
	other[c]	40min/week	33 hours	€ 37.5		expert opinion
Clinical biologist[d]		20min/week	17 hours	€ 125.0		expert opinion
						€ 18333/year
Personnel cost		**Yearly cost**	**Hours/year[e]**			
MLT		€ 60 000	1600 hours			estimate HR
Clinical biologist		€ 200 000	1600 hours			department[f]

MLT: Medical laboratory technologist; a: Macroduct sweat collection system
b: Macroduct supply kit: € 125 (excl. 21% taxes) for 6 samples and 2 samples are needed per patient.
c: Weighing samples, ordering reagents, writing/adjusting protocol, etc.
d: Validating results, checking protocol, communicating results, staff meeting, etc.
e: One full-time equivalent equals about 1650 hours per year. Taking into account sickness, absence, reduction of working hours, seniority, etc. this becomes about 1600 hours.
f: Personnel costs are a rough estimate. Data can vary widely across hospitals.

10.2.2 Costs for NBS CF protocols

The total costs to perform several tests are shown in Table 29. Only the calculated costs to perform the tests are included in these tables. Ex- or inclusion of equipment costs (i.e. interpreting them as incremental costs or not) clearly has an important impact on total costs. Relying on existing infrastructure might limit extra costs. The alternative screening algorithm with PAP testing might lower total costs if this results in less DNA and sweat tests.

Table 29. Budget impact

Proposed Belgian CF NBS algorithm (see also Figure 9)

Type of test		Number of tests	Cost per test[a]		Budget impact	
			excl. eq.	*incl. eq.*	*excl. eq.*	*incl. eq.*
IRT	manual	120000	€ 2.55	€ 3.95	€ 305 852	€ 473 642
	automatic		€ 2.00	€ 2.19	€ 240 283	€ 262 914
			excl. eq.	*incl. eq.*	*excl. eq.*	*incl. eq.*
DNA	1 centre, once a week	600	€ 150	€ 196	€ 89 970	€ 117 534
	2 centres, every 2 weeks		€ 150	€ 242	€ 89 970	€ 145 098
			excl. eq.	*incl. eq.*	*excl. eq.*	*incl. eq.*
IRT	manual	120	€ 2.55	€ 3.95	€ 306	€ 474
	automatic		€ 2.00	€ 2.19	€ 240	€ 263
			excl. eq.	*incl. eq.*	*excl. eq.*	*incl. eq.*
sweat test		75	€ 126	€ 204	€ 9 439	€ 15 323
					min	*max*
					Total	**€ 339 932**	**€ 634 536**

IRT-PAP-DNA algorithm

Type of test		Number of tests	Cost per test		Budget impact	
			excl. eq.	*incl. eq.*	*excl. eq.*	*incl. eq.*
IRT	manual	120000	€ 2.55	€ 3.95	€ 305 852	€ 473 642
	automatic		€ 2.00	€ 2.19	€ 240 283	€ 262 914
PAP		6000	€ 7.60		€ 45 584	
			excl. eq.	*incl. eq.*	*excl. eq.*	*incl. eq.*
DNA	1 centre, every 2 weeks	170	€ 176	€ 342	€ 29 954	€ 58 069
			excl. eq.	*incl. eq.*	*excl. eq.*	*incl. eq.*
sweat test		24	€ 126	€ 204	€ 3 021	€ 4 903
					min	*max*
					Total	**€ 318 842**	**€ 582 198**

a: excl. eq.: equipment is already present (and also used for other purposes) and not considered as incremental cost; incl. eq.: equipment cost is considered as incremental cost.

The costs for the Guthrie cards (€9414,25 incl. 21% TAV for 16 042 cards (in 2009)) are not included since these are not interpreted as incremental costs if another test is added to the current screening programme. Other costs, however, should also be taken into account and might increase or decrease these costs (see Table 21). For example, from the patient's perspective, transport and time/productivity costs of patient and family might be important. From the payer's perspective, there are also costs for the coordination and supervision of the programme, costs for genetic counselling, etc. Price negotiations and scale effects might also further influence total budget impact.

Therefore, these cost estimates can only be considered as a rough estimate of real costs. This can provide an idea of screening programme costs, depending on the suggested protocol (which tests are included, what are the used cut-off values, who will perform these tests (centralised or not), etc.). If the decision would be taken to introduce a certain screening protocol, or if new evidence becomes available, more detailed cost estimations could be considered.

An important remark concerns the reimbursed amount for tests with a billing code (CF DNA and sweat test), which does not reflect the current real cost. In Belgium, DNA tests in general are reimbursed at about €320, which is about double the real cost. In France, CF DNA tests are reimbursed at €128.1. In Belgium, a sweat test conducted in duplo costs about €126 while it is only reimbursed at €72. Of note is also the high volume of 15 000 CF DNA tests (see KCE report no 65) and 6400 single sweat tests currently reimbursed (RIZIV/INAMI) per year in Belgium.

Key points:

- **There is not enough reliable data to calculate incremental cost and benefits of NBS CF, nor to conclude on the possibility that NBS CF is cost saving. Therefore, the incremental cost-effectiveness ratio of NBS CF compared to no screening can only be calculated hypothetically (i.e. largely depends on assumptions).**
- **The budgetary impact of NBS CF in Belgium was roughly estimated to be about €340 000 – €635 000, including only costs to perform IRT, DNA, 2nd IRT and sweat tests. A lot of other items (e.g. costs for coordination and supervision, including costs for genetic counselling, price negotiations, etc.) might increase/decrease this budget impact.**
- **Real costs of tests and nomenclature values may differ largely.**

11 APPENDIXES

APPENDIX 1: SEARCH STRATEGY

We attempted to identify all potential sources of data about CF newborn screening recorded in MEDLINE and EMBASE database from 1985 till end of June 2009. We used MESH (MEDLINE) or EMTREE terms (EMBASE) and general keywords to retrieve the maximum number of references. EMBASE, Ovid Medline and Pubmed Medline were searched. Major terms used were "cystic fibrosis" in combination with "neonatal screening" OR "screening" OR "newborn" OR "newborn screening".

Table 1. First EMBASE search strategy details

Date	24/06/2009
Database	EMBASE .com
Search Strategy	Session Results..... No. Query Results Date #1. cystic AND fibrosis 37,685 24 Jun 2009 #2. cystic AND ('fibrosis'/exp OR 'fibrosis') 37,701 24 Jun 2009 **#3. 'cystic fibrosis'/exp 32,250 24 Jun 2009** #4. mucoviscidosis 1,460 24 Jun 2009 #5. #4 NOT #3 110 24 Jun 2009 #6. #4 NOT #1 103 24 Jun 2009 **#7. 'newborn screening'/exp 7,169 24 Jun 2009** #8. 'genetic screening'/exp 25,175 24 Jun 2009 #9. 'newborn'/exp 392,103 24 Jun 2009 #10. #8 AND #9 847 24 Jun 2009 #11. #7 OR #10 7,792 24 Jun 2009 #12. #1 OR #3 OR #4 OR #7 OR #10 44,798 24 Jun 2009 #13. #1 OR #3 OR #4 37,788 24 Jun 2009 #14. #11 AND #13 782 24 Jun 2009 #15. #11 AND #13 AND [1985-2009]/py 740 24 Jun 2009 #16. #11 AND #13 AND ([cochrane review]/lim OR [control led clinical trial]/lim OR [meta analysis]/lim OR [randomized controlled trial]/lim OR [systematic r eview]/lim) AND [review]/lim AND [1985-2009]/py 18 24 Jun 2009 #17. #3 OR #4 32,360 24 Jun 2009 #18. #3 AND #4 AND #11 AND #17 13 24 Jun 2009 #19. #11 AND #17 750 24 Jun 2009 #20. #11 AND #17 AND [embase]/lim 610 24 Jun 2009 #21. #7 AND #17 709 24 Jun 2009 #22. #7 AND #17 AND [embase]/lim 599 24 Jun 2009 #23. #3 AND #7 709 24 Jun 2009 #24. #3 AND #7 AND [embase]/lim 599 24 Jun 2009 **#25. #3 AND #7 AND [embase]/lim AND [1985-2009]/py 565 24 Jun 2009**

Note that only searches in bold typeface were used. Other searches were used to verify the completeness of the search strategy.

Table 2. Ovid MEDLINE search strategy details

Date	24/06/2009
Database	Ovid MEDLINE(R) 1950 to Present with Daily Update
Search Strategy	No. Query — Results 1 Cystic Fibrosis/ (23442) 2 Neonatal Screening/ (4827) 3 Mass Screening/ (63762) 4 exp Infant, Newborn/ (423905) 5 4 and 3 (4169) 6 2 or 5 (8828) 7 6 and 1 (572) 8 **limit 7 to yr="1985 -Current"** **(463)** 9 mucoviscidosis.mp. (1362) 10 9 not 1 (92)

Table 3. Second EMBASE search strategy details

Date	23/07/2009
Database	EMBASE .com
Search Strategy	..... No. Query — Results — Date **#1. 'cystic fibrosis'/exp — 32,372 — 23 Jul 2009** **#2. 'newborn screening'/exp — 7,207 — 23 Jul 2009** #3. #1 AND #2 — 711 — 23 Jul 2009 #4. 'evaluation and follow up'/exp — 1,902 — 23 Jul 2009 **#5. #1 AND #2 AND [1985-2009]/py — 678 — 23 Jul 2009** #6. #4 AND #5 — 117 — 23 Jul 2009 #7. 'quality of life'/exp — 134,749 — 23 Jul 2009 #8. #1 AND #7 — 666 — 23 Jul 2009 #9. #1 AND #7 AND [1985-2009]/py — 666 — 23 Jul 2009 #10. #2 AND #8 — 14 — 23 Jul 2009 #11. #5 AND #7 — 14 — 23 Jul 2009 #12. 'mental development'/exp — 18,305 — 23 Jul 2009 #13. #1 AND #12 — 11 — 23 Jul 2009 #14. #3 AND #12 — 1 — 23 Jul 2009 #15. 'cognitive development'/exp — 2,390 — 23 Jul 2009 #16. #5 AND #15 — 1 — 23 Jul 2009 #17. 'outcome assessment'/exp — 70,273 — 23 Jul 2009 #18. #5 AND #17 — 18 — 23 Jul 2009 #19. #4 AND #5 AND [1985-2009]/py — 117 — 23 Jul 2009 #20. #1 AND #17 — 194 — 23 Jul 2009

Note that the search in bold was used. Other search were used to verify the completeness of the search strategy.

Table 4. PUBMED Medline search strategy details

Date	07/08/2009
Database	MEDLINE via PUBMED
Search Strategy (details)	("cystic fibrosis"[MeSH Terms] OR ("cystic"[All Fields] AND "fibrosis"[All Fields]) OR "cystic fibrosis"[All Fields]) AND ("neonatal screening"[MeSH Terms] OR ("neonatal"[All Fields] AND "screening"[All Fields]) OR "neonatal screening"[All Fields] OR ("newborn"[All Fields] AND "screening"[All Fields]) OR "newborn screening"[All Fields]) →862 articles
Note	Electronic research performed again for last updating of database in order to get all articles theoretically available by end of June 2009 according to selection criteria. Cross-checking of database manually performed.

Note that this search was only used to check for recent publications. However, no additional publications were identified.

847 abstracts or title of publications were identified by the search strategy as described when combining Medline results with Embase results in Endnote library. Table 5 provides the inclusion criteria raised accordingly to HTA guidelines.

Table 5. Inclusion criteria

	Inclusion criteria
Population	Cystic fibrosis newborn infants screened by NBS program
Intervention	Follow-up of screened CF patients
Outcome	Effect of early diagnosis on nutritional parameters, on respiratory outcomes, on mortality and possible harms(Pseudomonas acquisition), psychological harm effects
Design	Large population based observational studies, review studies, randomized, control-randomized or not
Language	Data published in English, except French or Dutch if about very large scale population (case by case discussion)
'PICO' definition	

Based on the large amount of data available from 1985 to end of June 2009, it was decided to mainly focus on the recent data available and outstanding older data. A cut-off date of 2004 was decided after agreement between both major authors and any new relevant article published after end of June 2009 was to be taken into account till the redaction of the report. The Table 6 presents the exclusion criteria defined.

Table 6. Exclusion criteria

	Exclusion criteria
Population	Not fulfilling inclusion criteria
Intervention	Not fulfilling inclusion criteria
Outcome	Not fulfilling inclusion criteria
Design	Not fulfilling inclusion criteria
Language	Not fulfilling inclusion criteria
Other	Articles published in review with impact factor < 1
Other	Published before 2004 (except if keystone study)
Other	General statement about NBS
Other	Redundant data
Other	True duplicated data

Table 7: Reasons for exclusion expressed by number

Reason for exclusion	**n =**
Population	41
Intervention	8
Outcome	106
Design	2
Language	0
Impact factor <1	32
Published before 2004	478
Generalities about topic	62
Redundant data	38
Duplicated data missed by endnote program	30
Total number:	**792**

Full publications were retrieved based on abstracts or title (when abstract not available) if at least one reviewer suspected they were relevant to this report. Exclusion of a reference was decided after agreement between both major authors. From the 847 references identified, 792 references were excluded; remaining 55 articles.

These 55 abstracts were selected and the full texts were read. In the references of these articles 17 additional references were found as possibly relevant. For these 55+17=72 references (read as full text) the following selection criteria were used: studies comparing outcome parameters of a screened versus a non screened group, RCT (1), non-RCT (2), and reports form CF registries (3). Additionally, systematic reviews were kept together with articles on guidelines after screening. Were not selected: non-systematic reviews, letters and comments, studies not specific on screening, studies not comparing a screened versus an non screened group.

With this selection criteria 29 articles were selected for the chapter on effectiveness. After a quality appraisal (see text and tables chapter 2), 4 articles were rejected leaving 25 articles. In a later phase 3 articles were selected after the initial search (see tables below).

Table 8. Inclusion and exclusion of references read as full text.

	Type of study	**number**
1	Reports from RCT's comparing outcome of screened versus non screened	10
2	Reports from retrospective cohort studies comparing outcome of screened versus non screened	9: 4 rejected (see text)
3	Reports from CF registries comparing outcome from screened versus non screened	5
4	Systematic reviews	2
5	Guidelines on NBS and CF, FU after screening	3
	TOTAL	29 (-4) = 25

Three articles were added to the 25 articles after the search was performed:

	reason	**number**
Castellani et al 2009 JAMA	Association between carrier screening and incidence of cystic fibrosis. This publications appeared months after the initial search and was considered as important in the context of benefits after screening	1
Grosse	references of Grosse after contacting the author on the systematic review on mortality	2

Remarks:

- non systematic review were read to check for eventual new references but not quoted
- publications on possible harms, psychological and other were not selected and discussed in the chapter on benefits
- the other references in this chapter (n=17) are quoted in order to give background information on the topic (meaning of early diagnosis, data on the development of early disease in CF, background on vitamin deficiency in CF etc)
- quality appraisal was performed on the non-RCT data (5+5) by two reviewers; for the RCTs the Cochrane quality appraisal was taken

APPENDIX 2: FIVE KEY REFERENCES

Grosse S, Boyle C, Botkin J et al. Newborn Screening for Cystic Fibrosis. Evaluation of Benefits and Risks and recommendations for State Newborn Screening Programs. MMWR 2004; 53: 1-36.

This exhaustive document (156 references) summarizes a workshop cosponsored in November 2003 by the CDC and the US Cystic Fibrosis Foundation. Peer-reviewed evidence is presented. The CDC concludes that CF NBS is justified. In 2004, CF NBS was implemented in 5 out of the 50 US states. At the end of 2009, all but one have developed a national CF NBS program.

Southern K, Mérelle M, Dankert-Roelse J, Nagelkerke A. Newborn screening for cystic fibrosis. Cochrane Database Syst Rev. 2009 Jan 21;(1):CD001402. Review.

This recent paper comprehensively analyses current evidence for the effectiveness of CF NBS from randomised controlled trials (RCTs) in CF, and could be used as the basis for the effectiveness chapter. Table 1 summarizes detailed data derived from this work. In contrast to many Cochrane reviews, the author's conclusions about implications for practice, are so clear and positive that we found it useful to reproduce them below: "Newborn screening for CF benefits growth and prevents malnutrition in people with CF. Malnutrition may adversely influence cognitive function. From the available RCTs at this time, pulmonary benefits from CF newborn screening are likely in early childhood. Results on long-term pulmonary prognosis were biased by confounding factors such as infection and pancreatic status. The expense of CF newborn screening is similar to the costs for other screening tests such as phenylketonuria and is less expensive than when diagnosed clinically."

Castellani C, Southern K, Brownlee K et al. European best practice guidelines for cystic fibrosis neonatal screening. J Cyst Fibros. 2009; 8: 153-173

This third major paper (126 references) is a summary of the consensus achieved by 31 international experts having attended a meeting which took place on March 2008 in Italy. This meeting was organized by the European Cystic Fibrosis Society, the European Coordination Action for research in Cystic Fibrosis (Eurocare CF) in partnership with The International Society for Neonatal Screening, the EU EuroGentest Network of Excellence and the European Molecular Genetics Quality Network. This text comprehensively addresses the following issues: 1) The rationale for CF neonatal screening (benefits, hazards and costs) 2) Protocols and technical issues 3) Diagnosis through CF neonatal screening (including inconclusive cases) 4) Information to families.

Comeau A, Accurso F, White T et al. Guidelines for Implementation of Cystic Fibrosis Newborn Screening Programs: Cystic fibrosis Foundation Workshop Report. Pediatrics 2007; 119: e495-e518.

This document (96 references) details step by step the components of a successful CF NBS program. Difficult situations (mild forms of the disease and diagnostic dilemmas) are also discussed.

Haute Autorité de Santé. Le dépistage néonatal systématique de la Mucoviscidose en France: état des lieux et perspectives après 5 ans de fonctionnement, 2009. http://www.has-sante.fr/portail/jcms/c_765713/le-depistage-neonatal-systematique-de-la-mucovsicidose-en-france-etat-des-lieux-et-perspectives-apres-5-ans-de-fonctionnement

This report (174 pages - 156 references) carefully analyses the results of the French CF NBS program 5 years after its nationwide implementation. It includes an updated review of the medical literature on this topic, specifically addresses problems encountered in relation to the current strategy and provides recommendations to improve it.

APPENDIX 3: EFFICACY AND EFFECTIVENESS

Table 1: Randomized controlled studies

Author	Study Protocol	Number CF SG/CG	Aim	Follow-up	Results	Time period NBS test
{Chatfield, 1991 #526} UK	464,586 babies screened on alternate weeks MI excluded	230,076 screened →58 CF 234,510 control →44 CF	clinical benefit of NBS : growth, hospitalisation survival,	5 years n assessed at age of : 1y 34+22 2y 33+21 3y 23+13 4y 12+7	* mean age at diagnosis 9.1 (3.1) weeks for SG and 50.5 (60.5) for CG (p< 0.001) * H en W sds: no difference * 3 non MI deaths in CG, no deaths in SG * Chrispin-Norman and Shwachman scores: no difference * mean n (sd) of admission in first year: 1.3 (1.9) for SG and 3.3 (2.7) for CG (p< 0.01)	1985-1989 IRT
[56] UK	same cohort as above source data Pediatrician UK CF Survey	176 CF registered	NBS and death rate < 5 years	5 years	CF deaths reported in first 5 years: *7 CF children died -3 MI (before NBS diagnosis possibility) -4 non MI related in the CG compare to 0 in the SG (p< 0.05)	1985-1989 IRT
{Farrell, 1997 #431} USA	650,341 babies Randomized , MI excluded SG →325,171 → 74 CF (5 false-negative) CG : →325,170 → 40 CF	SG 56 CG 40	clinical benefit of NBS: weight/ height, head circumference	10 years at 1year : 52 / 26 at 10years : 14 / 9	*90% PI in SG and 72% in CG (p=0.04); F508del homozygous 58% and 50% (p=0.001) *HC at diagnosis better in SG (p= 0.003) *W and H better in SG: at diagnosis (W z score p= 0.008 L z score p < 0.001; at 1y: W z score p = 0.001 H z score p = 0.002; at 10y: W z score P=0.04+ H p= 0.02 *Shwachman-Kulczycki score: no difference	1985-1995 IRT after 1995: IRT/DNA
{Farrell, 1997 #732} USA	same as above + 1 year	SG 67 CG 56	*Pseudomonas aeruginosa* acquisition	10 years	* overall no difference in *Pa* acquisition between SG and CG * in 1 centre median time to chronic Pa lower for the SG: 52 weeks versus 234 weeks; p = 0,0003 * other centre median time to *Pa* acquisition 130 weeks in the CG versus 289 weeks (NS)	1985-1996 IRT after 1995: IRT/DNA

Author	Study Protocol	Number CF SG/CG	Aim	Follow-up	Results	
Farrell, 2001[54] USA	same cohort as above	SG 56 CG 48	clinical outcome after NBS : weight height	13 years assessed at : 1y 52+26 5y 45+32 10y 17+13 13y 2+5	* OR for W + H < P10 in the CG compared to SG: For W 4.12 (95%CI 1.64-10.38) and for H 4.62 (1.70-12.61) * Repeated measurement analysis until age 13y:W z score better in SG but ns (p= 0.06) H z score better in SG (P=0.009)	1985-1994
Farrell, 2003[78] USA	same cohort as above	SG 56 CG 47	pulmonary outcome after NBS FEV_1, chest Xray.	16 years assessed at 7- 16y	* at diagnosis (14.3 SG weeks compared with 108 weeks CG (p<0.001), better WCXR and BCXR mean scores for SG * LF (FVC,FEV1) no difference : at 7 y, 88% of SG and 75% of CG LF had FEV1 of ≥ 89% * Wisconsin X-ray score progressively worse in the SG after 10 years	1985-1995
Farrell, 2005[60] USA	same cohort as above	SG 49 CG 31	clinical outcome after NBS : weight/ height chest Xray	16 years assessed at : 10y 32+19, 13y 11+11, 16y 3+5	* advantage for H and W for the SG persist long term * Wisconsin and Brasfield X-ray score better in SG at diagnosis but declining after age 10y (ns)	1985-1995
Koscik, 2004[72] USA	same cohort as above	SG 42 CG 47	effect of NBS on cognitive function and Vit E	17 years CSI at 7.3-17 y	* Cognitive skill index: 104.4 (14.4) SG versus 99.8 (18.5) CG (p=0.24) * lower CSI correlate with growth deficit: height <P10 at 6 months: CSI 86.1(18.5) vs. 99.9(16.9) p=0.02 * CSI lower in patients with Vit E deficiency at diagnosis (p< 0.05)	1985-1995
Koscik, 2005[73] USA	same cohort as above	SG 37 CG 34	effect of NBS on cognitive function Head CF and Vit E	16 years head PC at 0-3y CSI at 7-16y	* HC z score significantly lower in CG up to age 3 p=0.019 * HC at diagnosis correlate with <300E r= 0.328 p = 0.025 * higher % of low CSI score < 84 in CG<300 E at diagnosis (p=0.003)	1985-1994

Table 2: Retrospective cohort studies comparing outcome of CF patients after clinical diagnosed and NBS

Author	Study protocol	Number assessed	Follow-up	Aim	Results	Time period
Waters, 1999[65] Australia	historical controls	SG 60 CG 57	10 years	clinical outcome growth respiratory	* W and H sds better in the SG at all ages *10y : W sds 0.4 (95%CI -0.1-0.8) and H sds 0.3 (95%CI -0.1-0.7)(1.7 kg and H 2.7 cm) * FVC, FEV1 % significantly better in SG: at 10 years mean diff 9.4% (95%CI 0.8-17.9) * X rays score not different * Shwachman score at 10y was 5.3 points higher in SG (95%CI 1.2-9.4)	1978-81 CG 1981-84 SG
Siret, 2003{Siret, 2003 #303} France	contem-porary controls	SG 77 CG 36 At 5y 27+17 At 10y 9+7	10 years	growth lung age at diagnosis	* age at diagnosis 38 days SG vs 472 in CG (p < 0.005) * W z score better in SG age 1 and 8y (p<0.005); H z score better in SG at age 1, 3 + 5 (p<0.005) * LF no diff * Brasfield score (p<0.001) and Shwachman score (p<0.05) higher for SG * *Pa* infection : no diff * % hospitalization: 86% CG vs. 49% SG (p< 0.001)	1989-1998
McKay, 2005{McKay, 2005 #197} Australia	historical controls	SG 52 CG 48	15 years	clinical outcome growth respiratory death	outcome at 15y * 7 deaths in the NSG, out of 57 children enrolled (12.3%) compare to 4 deaths in the SG, out of 60 enrolled (6.7%). * significant difference in cumulative mortality at age 10 (0 in the SG and 9.1% in CG)7 deaths in CG vs. 4 in SG (ns): deaths at age 10 higher in CG * age at death in SG significantly older: mean difference of 48 months; p < .05 * W and H: not different * Shwachman-Kulczycki score better in SG: X-ray score (diff 2.3 (95%CI 0.3-4.2) composite score 7.0 (0.2-13.8) (p< 0.05) * FEV1 better in SG: 12.3 % (95%CI 2.9-21.7%)(p< 0.001)	1978-1981 CG 1981-1984 SG
Baussano, 2006[97] Italy	historicalcontrols	SG 52 NS 32	untill 2005	risk of *Pa* infection	* overall annual *Pa* infection rate similar for the 2 groups 21.% (95%CI 15.4-30.1) * survival estimates: no difference in risk for *Pa* infection * median time between diagnosis and first *Pa* shorter in SG 183 (range 35-951) vs. 448 (range 53-2170) days * *Pa* within 60 days after diagnosis excluded from analysis	1997-2000 CG 2000-2004 SG
Collins, 2008[68] USA	Contemporary controls from same CF centre	SG 48 →34 CG 50 → 21	untill 2005	BMI *Pa* infection rate FEV1	* BMI higher in SG from age 3 , diff increasing with age (p=0.01) * Brasfield score: not different * *Pa* acquisition: not different * LF not different by univariate analysis except at age 15y (Fev1 90% SG vs. 74% CG p=0.08) * by multivariate analysis FEV1 better in SG at all ages * between age 6-15 years: CG FEV1 decline of 14% , SG FEV1 increase of 4% (p=0.01)	1983-1997

Table 3: Registry data comparing outcome of CF patients after clinical diagnosed and NBS

Author, reference	Time period	Number studied	Length of Follow-up	Aim	Results
Sims, 2005[85] UK registry	diagnosed 1994 aged 1-9 years in 2002	SG 184 CG 950	untill 2002	evaluate if NBS is associated with a higher treatment intensity	SG *versus* CG: * H sds (-0. 41 vs. -0.81) p= 0.05 * W: comparable * LF: no difference * Northern and Shwachman score: better in SG * N° of long term therapies: 2 vs. 3 (p=0.005) * % receiving low intensity treatment: 65% vs. 47% (p<0.005) * % without neb therapy: 65% vs. 47 (p< 0.005) * % with ≥2 neb therapies: 9 vs. 19 (p< 0.005)
Sims, 2005[70] UK registry	diagnosed 1994, aged 1-9 years in 2002	SG 183 CG 950	untill 2002	evaluate outcome after NBS in the context of modern CF treatment	SG versus CG * better H z scores * W: no difference * Northern X-ray score and Shwachman score: better in SG at all ages * LF : no difference * *Pa* infection: % with ≥Pa positive infection lower in SG between 1-3 years
Accurso, 2005 [69] US registry	* diagnosed 2000 through 2002 < age 1 * all patients in data base up to age 20y * MI excluded	1. CG 1760, SG 256 2.14647	NA	complications and hospitalizations by mode of diagnosis	SG versus CG * less stunting: 9% vs. 26% L < P3 (OR 3.7 CI 2.3-5.9) * less wasting: 11% vs. 33% W < P3 (OR 4.0 (2.6-4.2) * less *Pa*: mucoid *Pa* 0.46% vs. 3% (OR5.9 (0.8-43.8) * less complications: 29% vs. 70% OR 5.6 (4.1-7.7) * days in hospital: no difference
Lai, 2005[87] US registry	patients diagnosed > 1986	n=13687	NA	Impact of age and type of diagnosis on mortality	% of deaths (p< 0.05) * SG 1.4% * family history 0.9% * MI 3.9% * CG 3.5%

Table 4: Summary of the Wisconsin trial- Demographic data

Study		Wisconsin CF NBS		P value/ Adjusted P value	Comment
Number of babies		650 341 randomized			Parents in cohort group aware about CF disease and CF screening. Only 0.03% of parents requested screening test result.
		Screened 325 171	Unscreened 325 170		
NBS positive		501	Not mentioned		
CF diagnosis		74 (15 MI) (5 false- negatives) (- 3 not enrolled)	67 (18 MI) *(9 positive NBS)* (9 not enrolled)		At 4 years, unblinding of NBS result in cohort group revealed 9 undiagnosed CF on the 40 previously clinically diagnosed, apart the 18 MI.
DF508/DF508 homozygote: Farrell, 1997[53] Farrell, 2001[54] Farrell, 2005[60] **Pancreatic insufficiency:** Farrell, 1997[53] Farrell, 2001[54]		**Characteristic of patients:**			
		32 (58%) 41 (53%) 32 (63%) 46 (90%) 61 (79%)	19 (50%) 35 (43%) 22 (71%) 26 (72%) 47 (58%)	0.001 0.031/0.323 0.12 0.04 0.012 /0.164	Screened group more severe than cohort group based on DF508 homozygote and PI in 1997. Not confirmed with P value adjusted in 2001.
		Sample size (Meconium ileus excluded)			
Farrell, 1997[53]		56	40		
Farrell, 2001[54]		75	76		
Farrell, 2003[78]		56	47		
Farrell, 2005[60]		49	31	0.82	
Mean age of diagnosis (Weeks) **Median (range)**	Farrell, 1997[53]	12 +/- 37 7 (4–281)	72+/- 106 23 (3–372)	< 0,001	Mean age of diagnosis do not differ in cohort group(72weeks compared to 73 weeks) but median age is reduced compared to 'usual' time before randomization(median age 23 weeks compared to 36 weeks)
		Before the screening trial: Mean age diagnosis was 73 weeks; Median, 36 weeks			
	Farrell, 2001[54]	13.6 +/- 37 7 (4–281)	100.6+/- 117 28 (3–372)	< 0.001	
	Farrell, 2003[78]	12.4 weeks	95.8 weeks		
	Farrell, 2005[60]	7.4 ± 2.5 6.9 (3.6-14.4)	60.1 ± 89.8 22 (5.4- 329.9)	0.0001	
Mean age diagnosis for false-negative of screening		7, 8, 21, 84, and 287 weeks Farrell, 1997[53] 7,19, 21, 124, and 281 weeks Farrell, 2001[54]			

Table 5: Summary of the Wisconsin trial- Anthropometric data and pulmonary outcomes

Outcome	Study	NBS	Control	P value	Comment
Weight/ Height % of < 10 th Percentile(OR)	Farrell, 2001 [54]	W< 10th: 4.12 (95% CI: 1.64 –10.38) H<10th : 4.62 (95% CI: 1.70 –12.61)		0.007 0.004	Significantly worse in the control group
Height z-score **No.%< 10th Per**	Farrell, 1997 [53] Farrell, 2005 [60]	- 0.2 +/- 1 -0.40 ± 0.16 5 (10%)	- 1.2 +/- 1.1 -1.35 ± 0.21 14 (45%)	0.001 <0.001 <0.001	A reduced growth is always the sign of severe disease, energy intake always going first in growth at the price of a reduced weight gain as observed in these groups.
Weight z-score **No.%< 10th Per**		- 0.5 +/-1.1 -0.96 ± 0.18 20 (41%)	- 1.2 +/- 1.1 -1.84 ± 0.23 19 (61%)	0.008 0.003 0.074	
Head circumference Z-score **No% <10th Per**		-0.55 ± 0.12 7 (17%)	-1.07 ± 0.19 10 (40%)	0.021 0.039	Severe reduction in head size in late diagnosed group
Head circumference Percentile	Farrell, 1997 [53]	52+/- 28	32+/- 24	0.003	
Lung function Farrell, 2003[78]	FEV1	94 +/- 1.8%	95 +/-1.3%	0.54	But very little deterioration with age in both groups despite PA acquisition
	% > at 89% FEV1 predicted	88%	75%	ns	13% more in SG despite PA
	FEV1/ FVC; FEV1; FEF25−75; RV/TLC			ns	Ns despite PA
Shwachman–Kulczycki score	Farrell, 1997 [53]	92+/- 1	87+/- 2	0.006	Activity, Physical examination, Growth, nutrition, Chest film
Brasfield X ray score **No. (%) with Brasfield score**	Farrell, 2003 [78] Farrell, 2005 [60]	20.1 (1.4) 21.8 ± 0.3 (38) 8 (21%)	18.9 (1.8) 20.5 ± 0.6 (25) 10 (40%)	0.01 0.038 0.103	Trend of better chest x-ray score in SG despite more PA acquisition in one

< 21						group
Wisconsin X ray score **No. (%) with Wisconsin CXR score > 5**	Farrell, 2003 [78] Farrell, 2005 [60]	8.3 (3.3) 4.1 ± 0.6 (38) 11 (29%)		12.3 (8.3) 7.4 ± 1.5 (25) 12 (48%)	0.01 0.022 0.124	
Pseudomonas acquisition	Farrell, 1997 [84]	n CF	Center A	Center B	P value	Median pseudomonas-free survival period was 52 weeks contrasted with 289 weeks in the other center Center A better for PA than control group
	n Screened G, % Prevalence PA % Control% Incidence rate/ person _year Median PA_ free survival period(Weeks)	67 (54.5%) 62.7 % 53,6% 0.240 SG CG	34 (53.1%) 41.2% 56,7% 0.138 289 130	33 (55.9%) 84.8% 50,0% 0.389 52 234	NS 0.001 NS 0.004 <0.001	
	Farrell, 2003 [78]	45 (80%)		28(60%)	0.021	Same cohort as 1997
	No other lung function assessment since 2003 except Lai,2009 (responders vs non- responders)					

Table 6: Summary of the Wisconsin trial- Cognitive assessment[72, 73]

Author	Sample size	NBS		Control cohort		P value	Comment
Koscik, 2004 [72]	89 children non-meconium ileus→ 67 without missing data	The screened group had a 5-point higher mean CSI score relative to the control group, which is equivalent to one third SD, controlling for confounding variables. No association was identified with any anthropometric indicator, including head circumference, height, and weight [41]				NS	
Koscik, 2005[73]	71 children non-meconium ileus	n = 37		n = 34			False-negative not diagnosed later than clinically diagnosed[53]. 1 false-negative not in calculations; CF diagnosis done at 287 weeks
	CF with Pancreatic insufficiency	n = 33		n= 21			
	Mean age at diagnosis(Weeks)	8 (2-21)		54 (5-373)		<0.001	
Patients categorized into 4 groups	1) control patients with an a-T level<300 mg/dL at diagnosis (C<300E); 2) control patients with an a-T level >300 mg/dL (C>300E); 3) screened patients with an a-T level <300 mg/dL (S<300E); 4) screened patients with an a-T level >300 mg/dL (S>300E)						Alphatocopherol (a-T)
Outcomes	**Results**						Verbal skills, short-term memory function, and the ability to perceive relationships. Differences in cognitive ability of 1SD are perceptible to patients; a reduction of 1 SD in cognitive scores is associated with a decrement in reported HRQoL
Cognitive skill test (CSI)	n = 49	CSI was not significantly correlated with HC z-scores obtained at diagnosis (r = .119)				0.42	
	n = 71	Or at the time of cognitive assessment (r = .191)				0.11	
	Scoring < 84 for patients <300E	0		7 (41.2%)		0.003	
	CSI (mean = 100; SD = 16)	CG had adjusted CSI scores 12.5 points lower than scores for the 17 screened subjects who also had vitamin E deficiency at diagnosis (- 1 SD)				< 0.05	
Head circumference HC z-score	n = 49 (30 S+19 C) From 6 weeks to 3 years of age (time of cognitive assessment)	**S<300E**	**>300E**	**C<300E**	**>300E**		
		n=17	n=20	n=17	n=14		
		- 0.7 (0.7)	- 0.2 (0.7)	- 1.2 (1.1)	- 0.2 (0.9)	0.014	
		0.7 (0.8)	0.4 (0.9)	- 0.2 (1.0)	0.6 (1.2)	0.047	
Vitamin E	At diagnosis, <300E	17 (46%)		17 (55%)		0.47	
	CF with PI, <300E	16 (48%)		16 (76%)		0.04	
	C<300E group were diagnosed an average of 29.4 weeks later than their peers in the S<300E group. Thus, the duration of deficiency, is significantly longer for the control group.					0.049	

Table 7: Impact of Weight z score recovery on Lung function Lai, 2009[351]

Design	80 CF patients with pancreatic insufficiency Sample size: 80→ 63→ 51 for final analysis				**P value**	**Comment**
	Responders n= 41 28 NBS diagnosed		Non responders n= 22 14 NBS diagnosed			From 80 patients, 41 were defined responders. > 30 % excluded from analysis- 10% because no FEV record validated- More responders in CF diagnosed by NBS (Trend?).
Mean age diagnosis (Month)	4.0+/-6.2		2.6+/3.2		0.39	Non responders trend to be diagnosed earlier than responders- more severe if earlier clinically involved?
Weight z	0.28+/-0.93		_1.23+/-0.81		< 0.0001	
Height z	0.06+/-0.89		_1.03+/-0.79		< 0.0001	
BMI z	0.43+/-0.92		_0.38+/-0.90		0.001	
FEV1	99.5% +/- 13.9%		88.3% +/-18.5%		0.015	Improvement in lung function results from improvement in growth. The nutrition had no impact on infections rate for instance
Shwachman-Kulczycki scores	95.7+/- 3.8		90.7+/- 7.3		0.001	
Clinical signs	10% daytime cough; 7% nighttime cough 5 % wheezing		27% daytime cough; 36% nighttime cough 9% wheezing		0.25 0.09 0.61	
Nutritional energy Intake over age 2--6	Confirmed	Lessened	Improved	Unchanged	0.009	Higher energy intake correlates with improved or maintain growth in group. In non responders, no response despite high energy intake
	124+/-26	108+/-22	148+/-17	136+/-29		
Linoleic acid (% total plasma fatty acids) over ages 2–6	27.4+/-3.1	26.7+/-5.0	27.6+/-4.4	27.0+/-3.4	0.9	Higher energy intake but same dosage Fat level- Non responders are more severe?
Brasfield X ray score **Wisconsin X ray score**	21.1+/- 1.5 5.9+/- 3.5		20.5+/- 2.2 8.0+/_ 5.8		0.16 0.08	
Pseudomonas acquisition	6 (15%)		6 (27%)		0.24	Trend of more PA and SA in non responders. Role of infection in 'non-responding' state?
Staphylococcus Aureus	7 (18%)		6 (27%)		0.37	

Table 8: Summary of the UK trials 1991[55, 56]

Study		UK CF NBS		P value	Comment
Number of babies		464 586 screened on alternate week			Practical organisation? Administrative management of follow-up of screened patients? Meconium ileus(MI) excluded because CF diagnosis made within first days in both group.
		Screened 230 076	Unscreened 234 510		
CF diagnosis **MI excluded**	Chatfield, 1991[55]	58	44		
	Doull, 2001[56] [42]	86 (16 false negative) thus, 70 identified by screening	90		"Because no screening was performed in the control group, an unknown number of undiagnosed cases of CF in this group were not ascertained"[41]
Mean age of diagnosis (SD)		9,1weeks [55] 8 weeks [42] (3 to 22 weeks)	50,5 weeks (6 to 22 months)	< 0,001	Median ages were not reported[42]
Sample size of babies assessed	1 year old	34	22		
	2 years old	33	21		
	3 years old	23	13		
	4 years old	12	7		
Mean number of hospitalisation at one year		1.3	3.3	< 0,01	
Weight/ Height		NS	NS		No support CF care prescribed after NBS
Chrispin-Norman/ Shwachman scores		NS	NS		
Death in first 5 years after birth	3 death related on meconium ileus on 7 deaths reported on 176 CF registered.	0	4 on?	< 0,05	"A review of registry data revealed previously unknown CF-related deaths among children in the unscreened group" [41, 56]

APPENDIX 4: QUALITY ASSESSMENT OF THE NON RCT TRIALS DISCUSSED

Waters et al 1999[65], McKay 2005[66]

1. Groups compared: In this retrospective cohort study clinical outcome is compared for a group of CF patients clinically diagnosed from '78-81 with a group of CF –NBS diagnosed between '81 and '84. MI patients are included in the 2 cohorts. Clinical outcome parameters are compared at fixed ages.
2. Evaluation of selection bias: Age at diagnosis was lower in the SG group as expected. The 2 groups were comparable in other disease aspects like PI, presentation with MI etc. This excludes a large bias due to a higher proportion of less severe CF in the SG.
3. Length of follow-up: Until age 10 (15 years for McKay)
4. Loss to follow-up: Comparable in the 2 groups. For the analysis reported in Mc Kay et al: 2 out of 57 patients in the CG and 4 out of 60 in the SG.
5. Possible confounders: The mean possible confounder in this study is the use of an historical comparison group. The authors are aware of this possible confounder and clearly state the therapeutic changes that occurred over the time course of the study.
6. General evaluation: The results of the study have to be interpreted with caution mainly because of the comparison on historical cohorts. Because on all other points the quality of the study was evaluated as good, and the follow-up was long-term, the study results are included in this report.

Siret et al 2003[67]

1. Groups compared: In this retrospective cohort study clinical outcome is compared for a group of CF clinically diagnosed versus a group diagnosed after NBS. Patients are born in the same period (1989-1998). Patients with MI are excluded for both groups. Clinical outcome data are cross-sectional.
2. Evaluation of selection bias: Age at diagnosis was lower in the SG group as expected. In other disease aspects like PI, frequency F508del etc groups were comparable. Also the incidence of CF was similar for the 2 regions. This excludes a large bias based on a higher representation of less severe CF in the SG. False negative diagnoses in the SG are excluded from the analysis (5 cases with 77 positive screening cases) which could be criticized in the context of an intention to treat analysis.
3. Length of follow-up: Follow-up until 1999 which is age 10 for the oldest patients only. Therefore the number of evaluable patients at later ages is limited.
4. Loss to follow-up: 15 children in the SG (excluded).
5. Possible confounders: Patient treated in 2 different CF centers although with similar treatment protocol.
6. General evaluation: Patients born in the same time period are compared. Similar treatment protocols are used. Therefore this study is evaluated as valid. Care has to be taken in interpreting data for the older children since numbers are very limited. Exclusion of false negative screening cases may be a confounder.

Collins 2008[68]

1. Groups compared: In this retrospective cohort study clinical outcome is compared for a group of CF clinically diagnosed versus a group diagnosed after NBS. Patients are born in the same period (1983-1997) in Connecticut. In this period some hospital in the region offer NBS while others do not. Patients with MI are excluded for both groups. Clinical outcome data are cross-sectional.
2. Evaluation of selection bias: Only patients with FU in the CF center until 2005 and if consent was obtained were included in this analysis. Of the 48 CF patients of the SG and 50 of the CG only data from 34 and 21 were analyzed. Insufficient data are provided to exclude a possible selection bias here. No data are given on the data of false negative screening patients.
3. Length of follow-up: Until 2005 or age 15 for the oldest patients. Therefore the number of evaluable patients at later ages is limited.
4. Loss to follow-up: 8 of 63 patients (proportion of SG versus CG not stated)

5. Possible confounders: Patients attending the screening hospitals reflected the racial diversity of the Connecticut area and also represented patients from similar socio-economic background compared to patients attending the hospitals were no NBS was offered. Age at diagnosis was lower in the SG group as expected. In other disease aspects like PI, F508del frequency etc groups were comparable. This excludes a large bias based on a higher representation of less severe CF in the SG.

6. General evaluation: Care has to be taken in interpreting data because of a possible selection bias. No information is given on analysis of data of false negative screening cases. However, patients of both groups born in the same time period and are and are treated in the same CF center. Therefore this study is evaluated as valid.

Baussano 2006[97]

1. Groups compared: In this retrospective cohort study clinical outcome is compared for a group of CF clinically diagnosed between 1997-2000 versus a group diagnosed after NS (2000-2004) in Piemonte Italy and followed in the same CF center. Patients with MI are included for both groups.

2. Evaluation of selection bias: SG and CG were comparable for pancreatic status, F 508 del frequency and gender. This excludes a large bias based on a higher representation of less severe CF in the SG. False negative diagnoses in the SG are excluded from the analysis (5 cases with 47 positive screening cases).

3. Length of follow-up: Follow-up until 1999 which is age 10 for the oldest patients only. Therefore the number of evaluable patients at later ages is limited.

4. Loss to follow-up: 4 children in the SG (excluded)

5. Possible confounders: Patients with PA positive culture within 60 days from clinical diagnosis were excluded from the analysis. This may be in favor of the data on Pa acquisition in the CG.

6. General evaluation: Patients from both groups are born in the same region and treated in the same CF center. Care has to be taken in interpreting data since the CG groups are born earlier. However, no major changes in therapy have been reported for this time period. Major confounders may be exclusion of false negative screening cases in the SG and exclusion of Pa positive patients in the SG within 60 days after diagnosis. Despite these limitations, the study was included in the analysis because it studies the important topic of Pa acquisition.

Sims 2005 J Pediatrics, Sims (bis) J Pediatrics 2005[70, 85]

1. Groups compared: Comparison of UK CF registry data of CF patients diagnosed after NBS (12% of UK CF patients) versus clinical diagnosis. Registry data of 2002 were analyzed in a cross-sectional way of all patients aged 1-9 years if data of annual review were available.

2. Evaluation of selection bias: Of all registered UK CF patients, data of 73% of patients was available for analysis. This group was comparable to the whole group when looking at sex distribution, age at diagnosis for the CG and occurrence of F508del mutation. Important selection bias at this stage can be excluded. No information is given on the number of non registered CF patients in the UK.

The definition of NBS group in the text is: diagnosed within 3 months of age after NBS. It is unclear where the false negative screen CF patients are analyzed

3. Length of follow-up: NA

4. Loss to follow-up: NA

5. Possible confounders: All patients in studied in this report are diagnosed after 1994. They all received CF center care and were treated with acid resistant enzymes.

As discussed in this article the % of NBS patients was different for the CF centers in the UK. Outcome after NBS may thus be influenced by center quality and this could not be measured. Some geographical regions like Scotland and Wales have no screening programs. It cannot be excluded that these geographical (remote) areas also differ in quality of CF care. The only evaluation reported was that mean age at diagnosis for the CG was not influenced by the % NBS patients in the respective center. Since outcome difference where comparable to the Farrell RCT, the authors suggest that important bias based on center quality differences is unlikely.

In the second report by Sims et al, the potential geographical confounding was addressed by a patient matching procedure. Social deprivation as confounder was excluded by measuring an 'Index of multiple deprivation score' for the different groups.

6. General evaluation: Possible bias and confounders cannot be excluded but are unlikely (see above). Therefore these UK registry data were included in this report. Possible confounders were addressed in the second report. However, the exact matching procedure not fully clear.

Accurso J Pediatr 2005[69]

1. Groups compared: Two different patient group data were analyzed.
A. All new CF diagnosis (age 0-20) reported in the 2000, 2001 and 2002 registry (n = 2566) split up in diagnostic categories (prenatal, MI, NBS, symptoms). The % of homozygous F 508del patients was comparable for the symptomatic and NBS group.
B. A cross sectional analysis of complications was performed for all patients registered in the 2002 data base. Patient were split up in diagnostic and age categories.

2. Evaluation of selection bias: It is estimated that data of 90% of US CF patients are registered. Since the cross sectional analysis concerns 14647 patients, this large sample covers a wide sample of CF patients.

3. Length of follow-up: NA

4. Loss to follow-up: NA

5. Possible confounders: No detailed data are given on deaths in each group over the years, making it difficult to evaluate a possible survival effect in certain subgroups. The only information given is that neither the age at death nor the proportion of deaths in each diagnostic category and age group were statistically different.

6. General evaluation: Cross sectional analysis of very large data set; no major bias or confounder suspected.

Lai 2004, 2005, J Pediatrics[82, 87]

1. Groups compared: Analysis of all patient data reported during 1986-2000. Patients with insufficient data or born before 1960 were excluded. Patients are categorized in 4 groups (Screen, family history, MI and symptoms). Groups are well defined. MI, screen and symptom group are further classified according to type of symptoms at time of presentation.

2. Evaluation of selection bias: After exclusion (see above) 27 692 patients from the initial 32 229 are available for analysis. No data on genotype or other parameters are given for the group excluded. Selective selection bias in unlikely based on the reported exclusion criteria.
Analyses are performed for all patients and for the subgroup of patients diagnosed after 1986.

3. Length of follow-up: NA

4. Loss to follow-up: not specifically reported

5. Possible confounders: To reduce a potential cohort effect (advances in CF treatment) a subgroup analysis was reported, only including the patients diagnosed after 1986. The age range of patients in each diagnostic category is however not explicitly reported. In case median age would be different in subgroups, treatment advances could bias outcome.

6. General evaluation: The value of this study is to analysis the effect of diagnostic categories and age at diagnosis separately. Age distribution bias cannot be excluded. However large differences in age and year of diagnosis in this large data set are unlikely.

APPENDIX 5: TABLES AND FIGURE FOR CHAPTER 3

Table 1- Summary of findings regarding knowledge and information from studying parents after neonatal screening results for cystic fibrosis

Authors	Location	Findings	Note
Mischler et al., 1998 [329]	USA	False-positive: 95% understood that their child definitely did not have CF (92% at 1 year). True positives: at 3 months, 90% could correctly identify recurrence risk (97% at 1 year). Most parents of carriers, but not all, retained and understood this information.	Recall risks
Tluczek et al., 1992[352]	USA	The majority of families (73%) knew about neonatal screening. There is a significant relationship between knowledge and the level of education. Telephone communication leads to more misunderstanding than face-to-face communication.	Phone call
Tluczek et al., 1991[353]	USA	Parents of children in early disclosure group were much more knowledgeable than those told 4years later. Parents informed of negative sweat test result by telephone had significantly lower understanding than those informed face-to-face.	Time delay Phone call

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[a] Mischler EH et al. Cystic fibrosis newborn screening: Impact on reproductive behavior and implications for genetic counseling. Pediatrics 1998; 102: 44-52.

[b] Tluczek A et al. Parents' knowledge of neonatal screening and response to false-positive cystic fibrosis testing. J Dev Behav Pediatr 1992; 13: 181-186.

[c] Tluczek A et al. l. Psychological impact of false-positive results when screening for cystic fibrosis. Pediatric Pulmonol 1991; Supplement 7: 29-37.

Table 2 - Parents attitudes toward true positive test compared to parents with clinically diagnosed CF child

Study	Location	Findings	Notes
Baroni, 1997[354]	USA Wisconsin	Parents of children diagnosed through screening were more likely to have 'at risk' scores (Severe anxiety).	
Boland and Thomp-son, 1990[355]	New South Wales UK	Mothers in screened symptomatic group scored significantly lower on 'fostering dependency' scale. Mothers in screened asymptomatic group scored significantly higher on intrusiveness scale. Absence of observable symptoms at diagnosis did not appear to increase mothers' protectiveness as indicated by anxiety scale. In unscreened infants, mothers desire to foster dependency decreased with length of delay in diagnosis.	Three groups of children with CF: Symptomatic SG Asymptomatic SG and Unscreened clinically diagnosed
Al-Jader, 1990[356]	Wales UK	A total of 11 out of 29 parents experienced diagnostic delays resulting in 'extreme anxiety' – nine were in the screened group.	
Helton, 1991[357]	Colorado USA	No significant differences between groups on their report of depression and anxiety at the time of diagnosis, most rated it a time of high anxiety whenever it occurred.	

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[a] Baroni MA et al. Cystic fibrosis newborn screening: impact of early screening results on parenting stress. Pediatr Nurs 1997; 23: 143-151.

[b] Boland C et al. Effects of newborn screening of cystic fibrosis on reported maternal behaviour. Arch Dis Child. 1990; 65: 1240-1244.

[c] Al-Jader LN et al. Attitudes of parents of cystic fibrosis children towards neonatal screening and antenatal diagnosis. Clin Genet 1990; 38: 460-465.

[d] Helton JL et al. Parental attitudes toward newborn screening for cystic fibrosis. Pediatric Pulmonol. 1991; Supplement 7: 23-28.

Table 3 : Summary of Psychological impacts reported at true-positive NBS results and comparison to parents with clinically CF diagnosis

Psychological impact	NBS	Clinical Diagnosis	Difference between groups		Comment
			All studies	Cochrane 2009-OR	
Depression	++	+	NS	1.73 [0.54, 5.51]	No lasting depression reported
Strong emotional shock	++	+	Trend	14.42 [4.99, 41.68]	Reported in high IRT level as well
Anxiety	+++	+	NS	161.45 [21.57, 1208.60]	Much stronger in NBS group but no lasting (Peak of anxiety)-Young mothers at risk.
Stress	+++	+	NS	/	Diagnosis never expected
Anger	0	++		/	No anger reported in NBS
Disruption in mother-baby relationship	0	0		/	No stigmatization or excessive maternal behavior reported
Failure in medical confidence	0	+		/	Only Observed in delayed diagnosis
'Fostering' to dependency	0	+	/	/	However, this feeling decrease while waiting period increased – Reflect loss of confidence on reliability of health care?
Feeling of 'Intrusiveness'	+	Not reported		NA	Only for mother with child totally asymptomatic
'Relief'	0	+		NA	We hope that no other mother will have to say that...

Mischler EH eta l. Cystic fibrosis newborn screening: Impact on reproductive behavior and implications for genetic counseling. Pediatrics 1998; 102: 44-52.
Tluczek A et al. Parents' knowledge of neonatal screening and response to false-positive cystic fibrosis testing. J Dev Behav Pediatr 1992; 13: 181-186.
Tluczek A et al. Psychological impact of false-positive results when screening for cystic fibrosis. Pediatr Pulmonol. 1991; Supplement 7: 29-37.
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Boland C et al. Effects of newborn screening of cystic fibrosis on reported maternal behaviour. Arch Dis Childh. 1990; 65: 1240-1244.
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Lewis SL et al. Parental attitudes to the identification of their infants as carriers of cystic fibrosis by newborn screening. J Paediatr Child Health 2006; 42: 533-537.
Hayeems RZ et al. A systematic review of the effects of disclosing carrier results generated through newborn screening. J Genet Couns 2008; 17: 538-549.

Table 4 - Summary of parental affects while awaiting sweat test results

Author	Sample Size	Study	Harm effect			After normal sweat test
			Distress	Anxiety	Mother-baby Relationship	
Tluczek et al. (2005) [301]	14 (IRT), 29 (IRT/DNA)	Wisconsin, USA Mixed methods	43%			All Relieved
		Interview				
Lewis et al. (2006) [328]	31 (IRT) 35 (IRT-DNA)	Victoria, Australia Cross-sectional		74%		64% Relieved
		Interview		76%		71% Relieved
Baroni et al. (1997) [354]	14 14a	Wisconsin, USA Case-control		+		Other
		Questionnaire				
Mischler et al. (1998) [329]	206;109b 106;63c	Wisconsin, USA, Case-control		+		Other
		Questionnaire				
Parsons et al. (2003) [358]	99	UK, Mixed-Methods			No effect	Other

Adapted from Hayeems RZ (30)

[a] Tluczek A et al . Psychosocial risk associated with newborn screening for cystic fibrosis: Parents' experience while awaiting the sweat-test appointment. Pediatrics 2005; 115: 1692-1703.

[b] Lewis S et al. Parental attitudes to the identification of their infants as carriers of cystic fibrosis by newborn screening. J Paediatr Child Health 2006; 42: 533-537.

[c] Baroni M et al. Cystic fibrosis newborn screening: impact of early screening results on parenting stress. Pediatr Nurs 1997; 23: 143-151.

[d] Mischler EH et al. Cystic fibrosis newborn screening: Impact on reproductive behavior and implications for genetic counseling. Pediatrics 1998; 102: 44-52.

[e]Parsons EP et al. Psychosocial issues in newborn screening for cystic fibrosis. Paediatr Resp Rev 2003; 4: 285-292.

Table 5 - Psychological effects of disclosing carrier status: Long-term follow-up

Author	Long term follow-up (to 6 months to 6 years after disclosing result)
Lewis et al. (2006) [328]	28–29% of parents worry about the physical health of their carrier child and about potential difficulties that being a carrier might have for future relationships. 12–17% of parents worry more about the health of their carrier child than that child's siblings
Baroni et al.(1997) [354]	Stress score one year post-disclosure higher among parents who received CF carrier results compared to parents' who received negative results.
Mischler et al.(1998)) [329]	10% of parents of carrier infants thought about the results once a week or more after 1 year
Parsons et al.(2003) [358]	6 months post-disclosure, there was no evidence that the mother–baby relationship had been affected by carrier identification or that carrier status was seen by parents as a problem in terms of spoiled identity.

Adapted from Hayeems RZ (30)

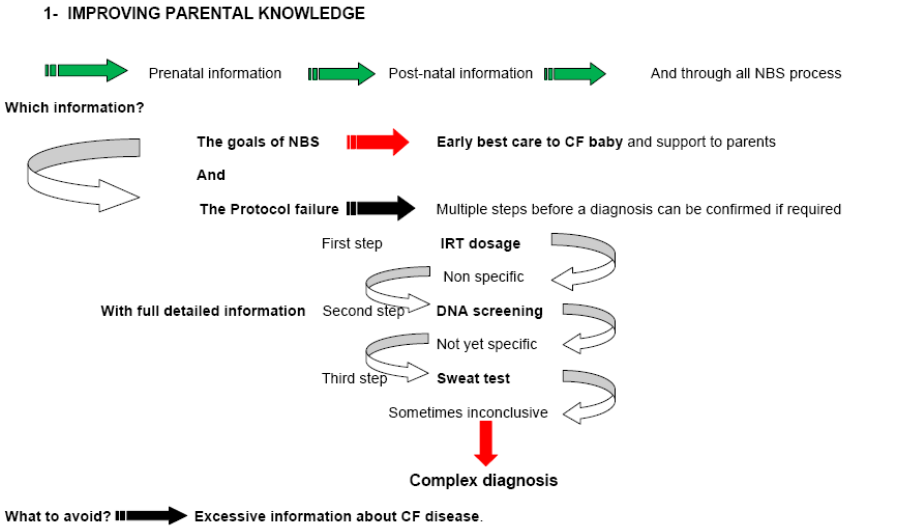
[a] Lewis S et al. Parental attitudes to the identification of their infants as carriers of cystic fibrosis by newborn screening. J Paediatr Child Health 2006; 42: 533-537.

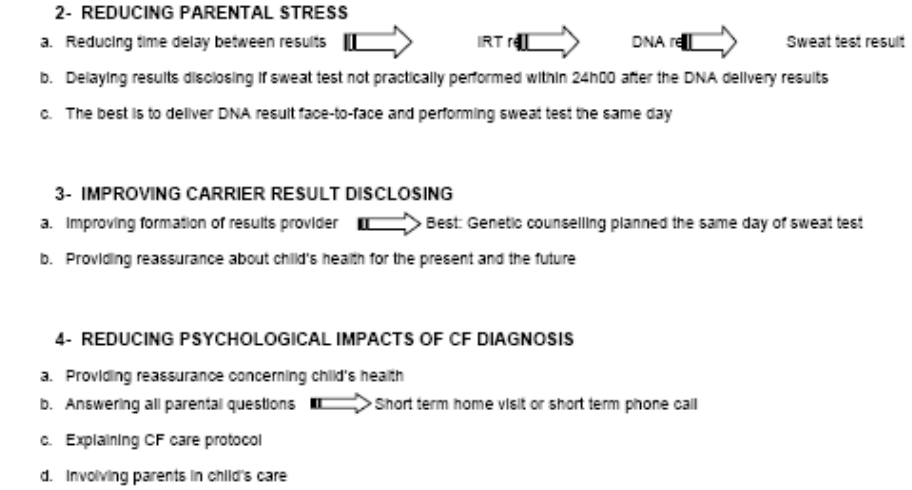
[b] Baroni MA et al. Cystic fibrosis newborn screening: impact of early screening results on parenting stress. Pediatr Nurs 1997; 23: 143-151.

[c] Mischler EH et al. Cystic fibrosis newborn screening: Impact on reproductive behavior and implications for genetic counseling. Pediatrics 1998; 102: 44-52.

[d] Parsons EP et al. Psychosocial issues in newborn screening for cystic fibrosis. Paediatr Resp Rev 2003; 4: 285-292.

Figure 1 – Suggested approach to sensitive communication at different steps of the CF NBS process





Adapted from Hayeems RZ (30)

APPENDIX 6. IMPLEMENTATION OF SCREENING

Table 1: The Australasian experience

Country/continent	Australasia
Source	Australasian Paediatric Respiratory Group
Methodology	Consensus – Working group (n=52)
Consensus/publication	2003/2005
Pages	7
Number of references	40
Experience	
Duration	>20 y (≥1981)
Screened NB	5.301.040
Algorithm	IRT (D3) / DNA
IRT cutoff (centile)	P99
DNA	- 83% of newborns screened for a few class I,II or III mutations only (1 to 5 class I, II or III mutations in 5 out the 6 territories; 8 mutations including R117H in the Queensland). - Extended analysis if one mutation + positive or borderline sweat chloride value
False positive rate	(-)
False negative rate	± 5%
Excess detection of carriers	x 1.5-1.8
Topics covered	Practical points
- recall process	- identify a CF screening coordinator for each screening centre
- clinical features	- ± 50 % asymptomatic at the time of diagnosis - 25% of infants with Meconium ileus have an IRT value below the cutoff level
- sweat testing	- reliable after 1 week of age in a term infant - sodium measured too - [Cl]<30 : nl, 30-59: borderline - quality-assurance programs - conductivity not accepted
- equivocal cases	- 1-2% (1 CFTR mutation after extensive analysis and intermediate sweat chloride values) - Repeat assessment at 3-6 months of age (including sweat test)
- genetic counselling	- CF + carriers - carrier testing of parents, estimation of recurrence risk, offer of a cascade family testing
- older siblings of infants detected with CF	- at least sweat test
- Consensus statement on diagnosis of CF after NBS in infants	1) 2 CFTR CF-causing mutations 2) or 1 CFTR mutation + positive sweat test 3) or 1 CFTR mutation + intermediate sweat chloride values + recognized clinical features

Massie J, Clements B; Australian Paediatric Respiratory Group. Diagnosis of cystic fibrosis after newborn screening: the Australasian experience--twenty years and five million babies later: a consensus statement from the Australasian Paediatric Respiratory Group. Pediatr Pulmonol 2005; 39: 440-446..

Table 2: The French experience

Contents	1) *Review of the literature* * • CF NBS : the evidence towards benefits • international recommendations • CFNBS programs outside France 2) *Analysis of the French data* • results • identification of practical problems ** • suggestions for improvement
Methodology	* Literature: search strategy, ** Questionnaires, National Data analysis, Working group (n=17) + reading committee (n=38)
Pages	174
Number of references	151
Experience	
Duration	5 ans (2002 -2006)
Screened NB	3.527.353
Identified CF	808
Carriers	1.763
Algorithm	IRT D3 (± D21) / DNA
IRT cutoffs	D3: 60 µg/l → 65 (2003); D21 for infants with ↑ IRT D3 and without DNA testing: 30 µg/l → 40 (2003) D21 for infants with ↑ IRT D3 and negative DNA testing: 30 µg/l → 100 µg/l.
DNA	20 mutations → 30 (2004)
False positive rate	"± 0.6"
False negative rate	± 3.4 %
Excess detection of carriers	x 3.1
Median age at diagnosis	35 days
Identified practical problems	- ↑IRT (D3): lost at follow-up (%): 1.4% - ↑IRT (D3) & no available consent for DNA testing (%): 2.2% **- equivocal cases: ± 15% (!),**inconsistencies in the management, lack of assessment of psychological impact
Suggestions for improvement	- consider the withdrawal of R117H mutation from the initial panel - consider including only Class I, II or III mutations in the initial panel - further study the PAP analysis mainly in order to reduce the ret of carrier detection - identify a screening coordinator for each maternity - consider prenatal information of parents about CF NBS - clarify the ethical point of obtaining consent for DNA testing from *both* parents - need for (external) quality assessment of CF centres not only for technical aspects (sweat test, IRT dosage, DNA testing) but also in terms of diagnostic process … - track and publish data related to infants lost at follow-up, false-negatives, lack of consent for DNA testing, clinical symptoms at the moment of diagnosis.
Varia	- leaflet redacted by the AFDPHE about all NBS was the single source of information for 32 % of the hospitals (about 40% of nurses and paediatricians did not respond to the questionnaire) and by far the most used information for parents. - total cost of CF NBS/ newborn: 2.24 € - cost of NBD/ detected CF: 9.974 € - a strategy for assessing the CF NBS program should have been defined a priori

Haute Autorité de Santé. Le dépistage néonatal systématique de la Mucoviscidose en France: état des lieux et perspectives après 5 ans de fonctionnement, 2009. http://www.has-sante.fr/portail/jcms/c_765713/le-depistage-neonatal-systematique-de-la-mucovsicidose-en-france-etat-des-lieux-et-perspectives-apres-5-ans-de-fonctionnement

Table 3: US guidelines for implementation of CF NBS programs (table of contents)

Systematic planning for CF NBS

components of successful CF NBS programs

CF NBS workgroups Topics for successful CF NBS programs

Specifications for successful CF NBS programs

Prescreen education

- notice to parents of availability of CF NBS
- notice to pediatric health care providers of CF NBS
- standardized presentations

Blood spots testing algorithms for CF NBS

- IRT analysis: a cautionary note
- IRT/IRT algorithms
- IRT/DNA algorithms
  - reagents availability
  - CF genotype variation
  - Genotype/phenotype relationships
- fail-safe for IRT/DNA protocols

Postscreening report and education

- positive CF NBS Report
- negative CF NS Report

Diagnostic testing of infants after a positive CF NBS Result: the sweat test

- sweat laboratory qualifications
- minimum age and weight of the infant for valid sweat testing

Diagnostic interpretation after the sweat test

- positive sweat test results
- negative sweat test results
- borderline sweat test results

Communicating Diagnostic-evaluation results to the parents

- positive CF diagnosis
- negative CF diagnosis
- insufficient sweat for analysis
- diagnostic dilemmas

Genetic counseling

Quality assurance of CF NBS: tracking and outcome assessments

Financing CF NBS and care

Research opportunities

Special considerations

- community-customized mutation panel
- CFTR mutations and mild disease
- diagnostic dilemmas
  - borderline sweat-test results
  - negative sweat tests results

Comeau AM, Accurso FJ, White TB et al. Guidelines for implementation of cystic fibrosis newborn screening programs: Cystic Fibrosis Foundation workshop report. Pediatrics. 2007; 119: e495-518

Consensus / publication (year)	2004/2007
Organizing Group	Cystic Fibrosis Foundation
Contributors (n)	41
Contributors (country of origin)	US (40) , Australia
Number of references	97

Table 4: US guidelines for diagnosis of cystic fibrosis in newborns through older adults (table of contents)

Phenotypic features consistent with a diagnosis of CF

Methods

- Sweat chloride test
  - test methodology
  - test interpretation
  - sweat chloride values in infancy
  - recommended sweat chloride reference values in infancy
  - sweat chloride values beyond infancy
  - recommended sweat chloride reference values beyond infancy
- Role of DNA analysis in CF diagnosis
  - recommended panel of CF-causing mutations
- Role of ancillary tests in CF diagnosis

Diagnosis of CF : consensus statement

- Recommended CF diagnosis process for screened newborns
- Recommended general process for diagnosing CF

Farrell PM, Rosenstein BJ, White TB et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. J Pediatr. 2008 ; 153: S4-S14

Consensus / publication (year)	2007/2008
Organizing Group	Cystic Fibrosis Foundation
Contributors (n)	18
Contributors (country of origin)	US, Canada (1), Australia (1), Europa (1)
Number of references	80

Table 5: European best guidelines for CF NBS (table of contents)

1. The rationale for CF neonatal screening

1.1 Benefits

- pancreatic disease
- growth
- nutritional deficiencies
- lung involvement
- burden of care
- psychosocial effects
- survival

1.2 Hazards

- anxiety
- knowledge of carrier status
- inconclusive NBS results
- infection with *Pseudomaonas aeruginosa*
- potential for ethnic discrimination

2. Protocols and technical issues

2.1 The first tiers: IRT

- IRT cut offs
- quality control
- age at sampling
- stability of IRT in blood spots
- non CF causes of increased IRT

2.2 Intermediate tiers

- second sample IRT assay
- CFTR mutation analysis
- PAP analysis

3. Diagnosis through CF NBS

3.1 Sweat test

3.2 Inconclusive diagnosis

3.3 Clinical follow-up

4. Information to families

4.1 Before CF NBS

4.2 After a positive NBS test

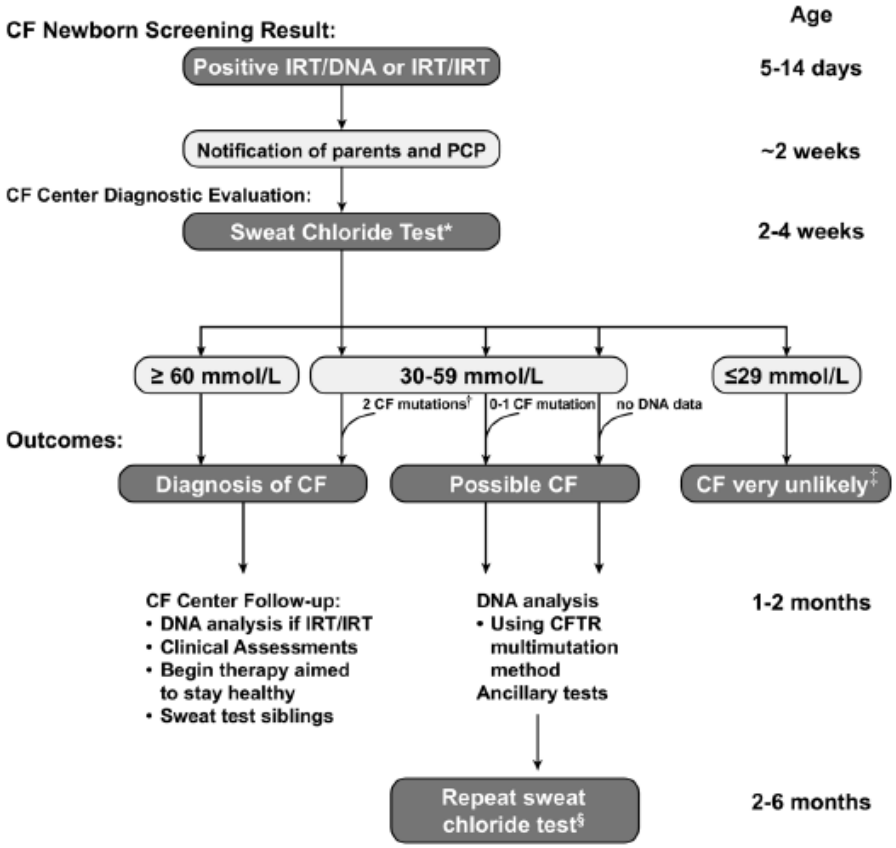
4.3 At diagnosis

4.4 For parents of carrier infants

Castellani C, Southern K, Brownlee K et al. European best practice guidelines for cystic fibrosis neonatal screening.
J Cyst Fibros. 2009; 8: 153-173.

Consensus / publication (year)	2008/2009
Organizing Group	European Cystic Fibrosis Society
Contributors (n)	34
Contributors (country of origin)	Europa, US (2), Australia (2)
Number of references	126

Flowchart 1: The CF diagnostic process for screened newborns



* If the baby is at least 2kg and more than 36 weeks gestation at birth, perform bilateral sweat sampling/analysis with either Gibson-Cooke or Macroduct® method; repeat as soon as possible if sweat quantity is less than 75 mg or 15 µl, respectively.

† CF mutation refers to a CFTR mutant allele known to cause CF disease.

‡ The disease is very unlikely; however, if there are 2 CF mutations in trans, CF may be diagnosed.

§ After a repeat sweat test, further evaluation depends on the results as implied above.

Source : Farrell PM, Rosenstein BJ, White TB et al. Guidelines for diagnosis of cystic fibrosis in newborns through older adults: Cystic Fibrosis Foundation consensus report. J Pediatr. 2008 ; 153: S4-S14

Table 6: Components of successful CF NBS programs

TABLE 1 Components of Successful CF NBS Programs

NBS System	CF NBS Program Specific Component
Prescreen	
Inform family (brochure)	
Prenatal distribution	
Neonatal distribution	
Educate health care providers	Notice of CF NBS inclusion; grand rounds presentation; encourage consideration of CF diagnosis in screened children with symptoms
Quality specimen collected	
Quality specimen transported	
Screen	
Laboratory analysis	Blood spot testing algorithm chosen, considering:
	Spectrum of CF disease to be identified
	Resources available
	Sweat-testing services
	Genetic counseling (when applicable)
Quality control	
Data integration/integrity	
Postscreen contact with PCP	
Every infant: notification	Integration with existing systems
Positive screen result	
Deliver report to PCP	Materials for PCP on risk
Recommend next steps	Materials for PCP on recommendations
Request repeat test; or	Contact information for sweat testing and clinical evaluation; materials to supplement report to subspecialist
Recommend subspecialty referral	
Track to assure next step	
Negative screen result but clinical concern	
Deliver report to inquirer	
Educate inquirer on next steps	Materials for PCP on risk and recommendations
Prediagnostic test contact with parent	
Positive screen result	
Deliver report to parent	
Educate parent	Materials for PCP to use/hand to parent including description of sweat test
Schedule subspecialty referral	Specifics of CF center location; work to ensure rapid referral
Negative screen result but clinical concern	
Educate parent	Materials for PCP to use/hand to parent
Schedule subspecialty referral	
Diagnostic evaluation	
Evaluation	Infection control
Interpretation	Define positive, negative, and borderline sweat-test results for NBS program
	Case definition of CF
Postdiagnostic test	
Report outcome to parent, PCP, and NBS	Materials on positive, false-positive, and ambiguous results and mechanisms for reporting
Positive diagnosis	Infection control and care guidelines
Intake by subspecialty care	
Education	
Equivocal diagnosis	
Follow-up plan	Infection control; follow-up and care
Education	
Negative diagnosis	Genetic or posttest counseling
Education	
Release	
Quality control	Regular meetings between NBS core program and CF care centers
Tracking	
Outcomes assessment	

Source : Comeau AM, Accurso FJ, White TB et al. Guidelines for implementation of cystic fibrosis newborn screening programs: Cystic Fibrosis Foundation workshop report. Pediatrics. 2007; 119: e495-518

Table 7: Suggested CF NBS Workgroups topics for successful CF NBS programs

TABLE 2 CF NBS Workgroup Topics for Successful CF NBS Programs

Component	Action	Considerations
Screening algorithm	Define goals for disease detection	Responsibility of NBS program
	Select CF case definition relative to program goals	Responsibility of CF Workgroup
		Does program aim to identify only infants with classic CF, or those with mild/variable CF also?
	Develop algorithm	Does NBS program collect 1 or 2 specimens?
	IRT/IRT	Projected numbers of true/false-positive results; true/false-negative results; need for sweat tests
	IRT/DNA	Projected numbers of true/false-positive results; true/false-negative results; CF carriers; need for sweat tests, genetic counseling
	ΔF508 only	What are the allele frequencies in population served?
	Mutations associated with severe disease	
	Mutations associated with severe or variable disease	
Prescreen education		
Lay community	Develop resources for families	Responsibility of NBS program
Medical community		Responsibility of NBS program
	Develop resources for PCPs	
	Develop presentation for grand rounds	Presentation by members of CF workgroup
Postscreen reporting		Responsibility of NBS program
Positive result	Develop materials for reports	
	Develop checklists for telephone report and recommendations to PCP	
	Issue reports and recommendations	
	Fax confirmation of details in writing	
Negative result	Develop materials and fact sheets for report	Include written reminders
	Issue reports	Need to sweat test any infant with MI or other clinical signs of CF
	Recommend sweat test for any infant with CF-specific clinical concern	Negative result does not rule out carrier status
Diagnostic evaluation	Sweat test is gold standard	All infants with a positive NBS result need sweat test (regardless of parental carrier testing or NBS result)
		All infants with clinical concern for CF should have sweat test
	Recommended site: CF care center with infection controls	
	Define positive, negative, and borderline values	
	Genetic counseling	
	On same day as sweat test, or	
	As a follow-up appointment	
Quality control: tracking and outcomes assessment	All sweat-test results for NBS-positive infants should be reported to the NBS program	Centralized tracking by NBS program allows for quality improvement, detection of unanticipated risks, and continuing modification and evaluation of the program; CF clinician from care centers recommended to act as liaison with NBS program
	Extended DNA results should be reported to the NBS program	
	Any discordant result should be reported to NBS program (2 severe mutations detected and negative sweat-test result; discordant genotypes)	
	Any positive results of sweat testing resulting from clinical concerns should be reported to NBS program (false-negative results)	

Source : Comeau AM, Accurso FJ, White TB et al. Guidelines for implementation of cystic fibrosis newborn screening programs: Cystic Fibrosis Foundation workshop report.

Table 8: Suggested items to be recorded for quality control of a CF NBS program

TABLE 5 Tracking Needed for Evaluation of Follow-up to CF NBS

Parental compliance with follow-up recommendations

1. Infants/families who complied with follow-up testing
2. Attempts at additional contact
3. Infants who were lost to follow-up
4. Dates of birth, NBS, and sweat test
5. Date of genetic counseling

Diagnostic effectiveness of screen

1. Infants born and infants screened (per month/year)
2. Infants given sweat test (recall rate)
   a. Negative sweat-test results (false-positive results)
   b. Positive sweat-test results (true-positive results)
   c. Borderline sweat-test results (ambiguous diagnosis)
   d. Failed sweat-test results (QNS)
3. Screen-negative children diagnosed with CF later via conventional methods (false-negative results)

CF NBS algorithm sensitivity

1. False-negative results
   a. Determine why the screen failed to detect CF
   b. Determine whether the IRT cutoff and recall rates meet the stated goal for sensitivity
2. *CFTR* genotypes of patients diagnosed with CF
   a. Children diagnosed with CF through NBS
   b. False-negative results
   c. *CFTR* mutation panel used: does it meet the stated goal for sensitivity?

Management of care center protocols

1. Date the infant entered care at CF care center
2. Confirmation of implementation of appropriate care plan for unique NBS population
3. Confirmation that data are provided to CF Foundation's patient registry including anthropometrics, cultures, hospitalizations, and complications
4. Confirm offer and receipt of genetic counseling (when applicable); documentation of obstacles to genetic counseling (time, lack of counselors, reimbursement)

Source : Comeau AM, Accurso FJ, White TB et al. Guidelines for implementation of cystic fibrosis newborn screening programs: Cystic Fibrosis Foundation workshop report.

Table 9: Recommendations for counselling about positive NBS results

Table 5
Recommendations for counselling about positive NBS results.

1) Information given to parents about positive NBS results must be adapted to reflect local protocols and the results' significance. To facilitate this, as an inherent part of the program, health care professionals should be educated on the significance of the NBS result and written reminder materials should be attached to the NBS result.
2) Ideally parents should be informed about the positive NBS result in person. If this is not possible, the person communicating by letter or telephone should be extremely careful about wording and be aware that parents may be prone to misunderstanding and psychological problems.
3) It is important to emphasise that a raised (positive), screening test result is not confirmation of a diagnosis of CF. Further tests will be needed to confirm or exclude the diagnosis of CF. A parent of a child who has a raised IRT and a positive DNA test (in some programmes an elevated IRT alone), will need to be informed of the need for further investigation.
4) Delays should be minimised. Ideally, no more than 48 h should pass between the moment the parent is aware of the result to the time of expert assessment and confirmatory testing.
5) Minimising delays should not be at the expense of intentionally alarming parents about the potential for CF-related complications. Whilst this may be common practice for NBS diseases with a more emergent time course, it is not necessary for CF NBS.
6) The health care professional undertaking the counseling of parents should be knowledgeable about CF NBS and genetics, and have had training in cautious, empathic communication skills.

The following three statements are protocol-specific and their inclusion depends on the relative probability that the child has CF.

7) Ideally, both parents should be present when the diagnosis of CF is first communicated and explained. If only one of the infant's parents can be present then it is strongly advised that a supportive family member or friend should accompany the parent to the appointment.
8) Health care professionals should be aware that parents are more likely to understand information provided if initial counselling focuses on conveying a few high-value messages rather than attempting to fully educate the parent about every aspect of CF and NBS.
9) The panel recommends that the post-NBS, pre-diagnosis conversation and written materials should include a review of points made before NBS, plus some version of the following messages, questions and answers.
   a) *Q:* What does my baby's screening result mean? *A:* The screening results suggest your baby has cystic fibrosis (CF), although further tests will be needed to confirm or exclude this.
   b) *Q:* What happens next? *A:* We need to do a more detailed assessment, but otherwise your baby does not need any urgent treatment or special care from you now. The most important step is to see the doctor about further tests for your baby. You have an appointment to see a doctor who is a specialist in children with CF. They will examine your baby and, if necessary, arrange further tests. They will explain the results to you.
   c) A repeat sample/test may be needed because of an initial inadequate test (e.g. multiple drops, inadequate sample, technical error), a previous false-positive result or a probable diagnosis of CF.
   d) *Q:* What is CF? *A:* CF is a hereditary condition which mainly affects the lungs and digestive system. Children born with it are susceptible to chest infections and may not put on weight like they should.
   e) *Q:* What treatment is available for CF? *A:* Screening means that babies can be treated early with an appropriate diet, medicines and chest physiotherapy. Treatments are improving all the time.
   f) *Q:* How do you feel? *A:* You may feel a sense of shock, disbelief, anger, or fear. These reactions are normal. Remember it is not yet known for certain that your baby has CF. We will make sure that all your questions answered.
   g) [Inform the parent where they may access other sources of information and support (tailored to local resources). Provide some cautionary advice about information that might be found on the Internet].

Source : Castellani C, Southern K, Brownlee K et al. European best practice guidelines for cystic fibrosis neonatal screening. J Cyst Fibros. 2009; 8: 153-173.

Table 10: Recommendations for counselling parents when CF NBS identifies carrier infants

Table 7
Recommendations for counselling parents when CF NBS identifies carrier infants.

What is my baby's screening result?
The screening result suggests that your baby is a carrier of CF. Approximately 1 in every 20 to 37 healthy people are carriers of a mutation in the CF gene.
What does it mean to be a carrier of the CF gene?
Your baby is just like one of his or her parents and has only one copy of a mutated CF gene.
To have CF you need two copies of a mutated CF gene passed from each of the baby's carrier parents.
How will being a carrier affect my child?
Your child will not be affected by the condition and will not need any special treatment. 'Carriers' can pass on the altered gene to their children and you may wish to tell your child this when they are older.
What is CF?
Children with CF are susceptible to chest infections and may not put on weight like they should.
Is it possible that my child does have CF?
The answer to this question is determined to some extent by the CF NBS programme. However, the screening test is not perfect and there is a very small risk that your baby has CF. There are uncommon mutations in the CF gene that are not recognised by the screening test. It is therefore possible that a baby with this result will have a second, uncommon CF gene mutation and will have CF.
If you are worried about the result you should discuss this issue with your family doctor.
If we have children in the future, could they have CF?
Your baby has been recognised to be a carrier of CF, but there is an increased risk that if you have children in the future they may have CF. Accessing genetic counselling is preferable and could be important before planning further pregnancies.
Who else can I talk to about my baby's screening result?
You can discuss this with your health care professional.
Where can I find more information or support?
[INSERT Local CF Association address, phone and website].

Source : Castellani C, Southern K, Brownlee K et al. European best practice guidelines for cystic fibrosis neonatal screening. J Cyst Fibros. 2009; 8: 153-173.

Figure 2. General scheme.

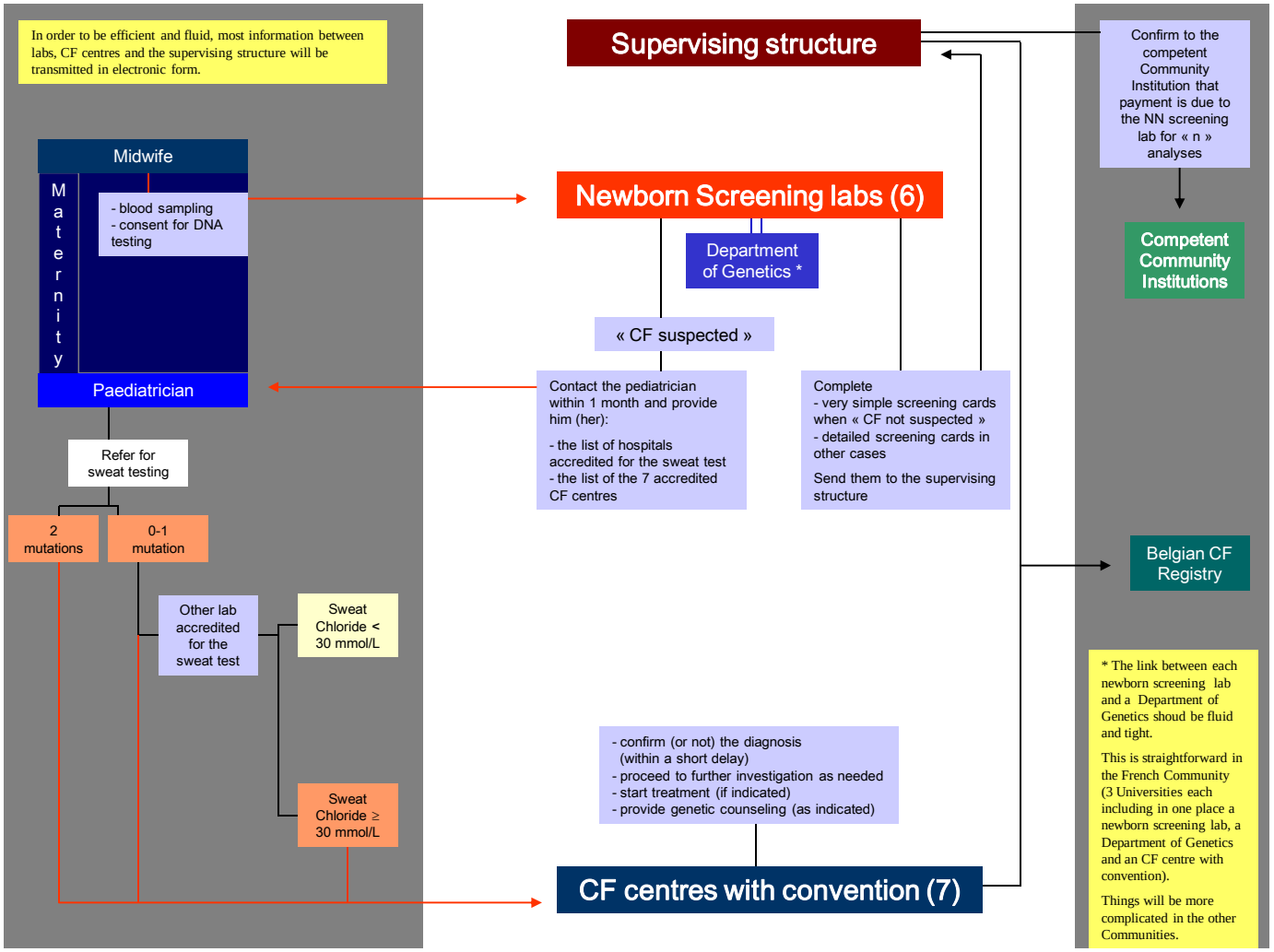


Figure 3. Suggested role of supervising structure.

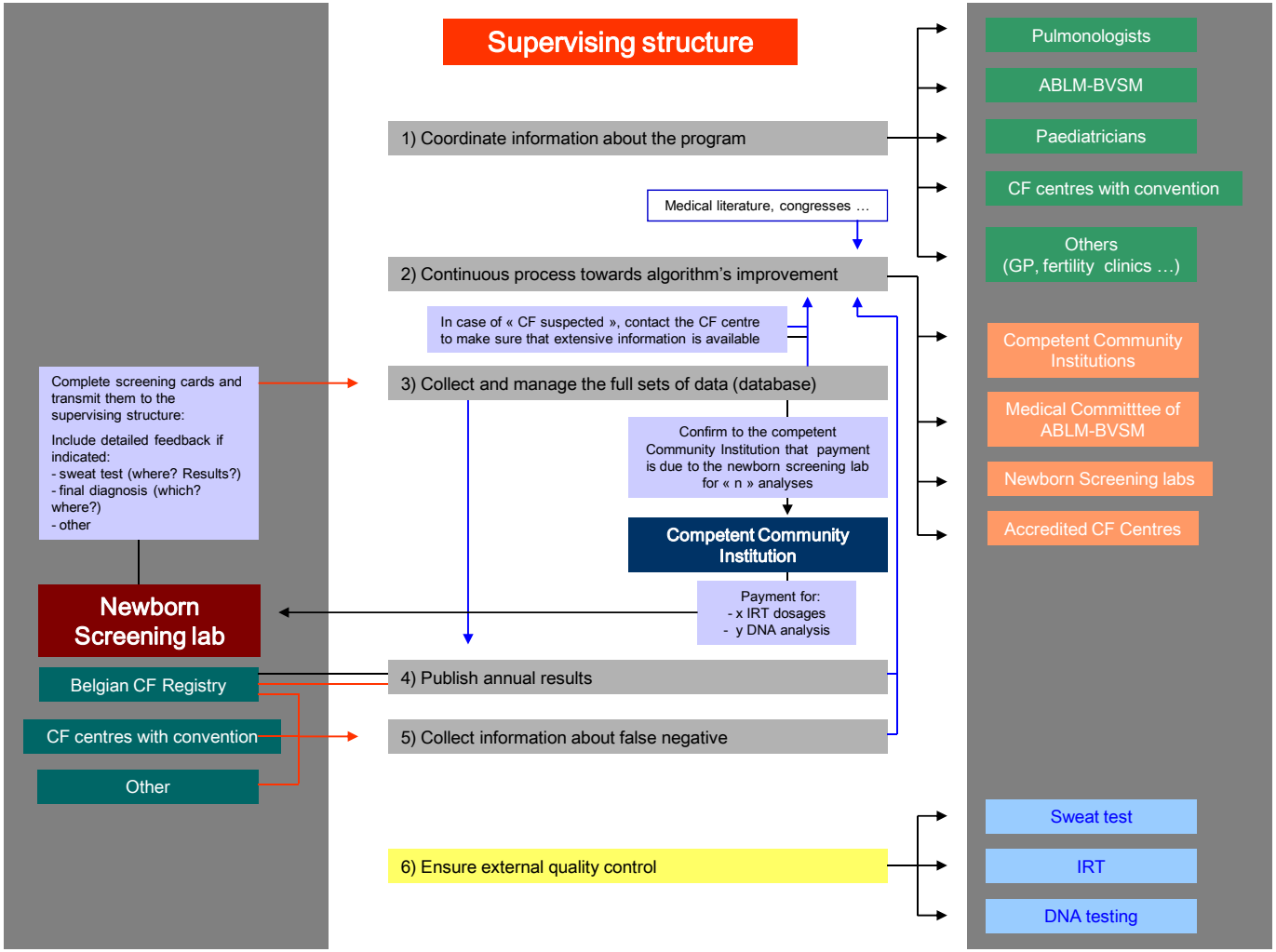


Figure 4. Suggested role of newborn screening laboratories.

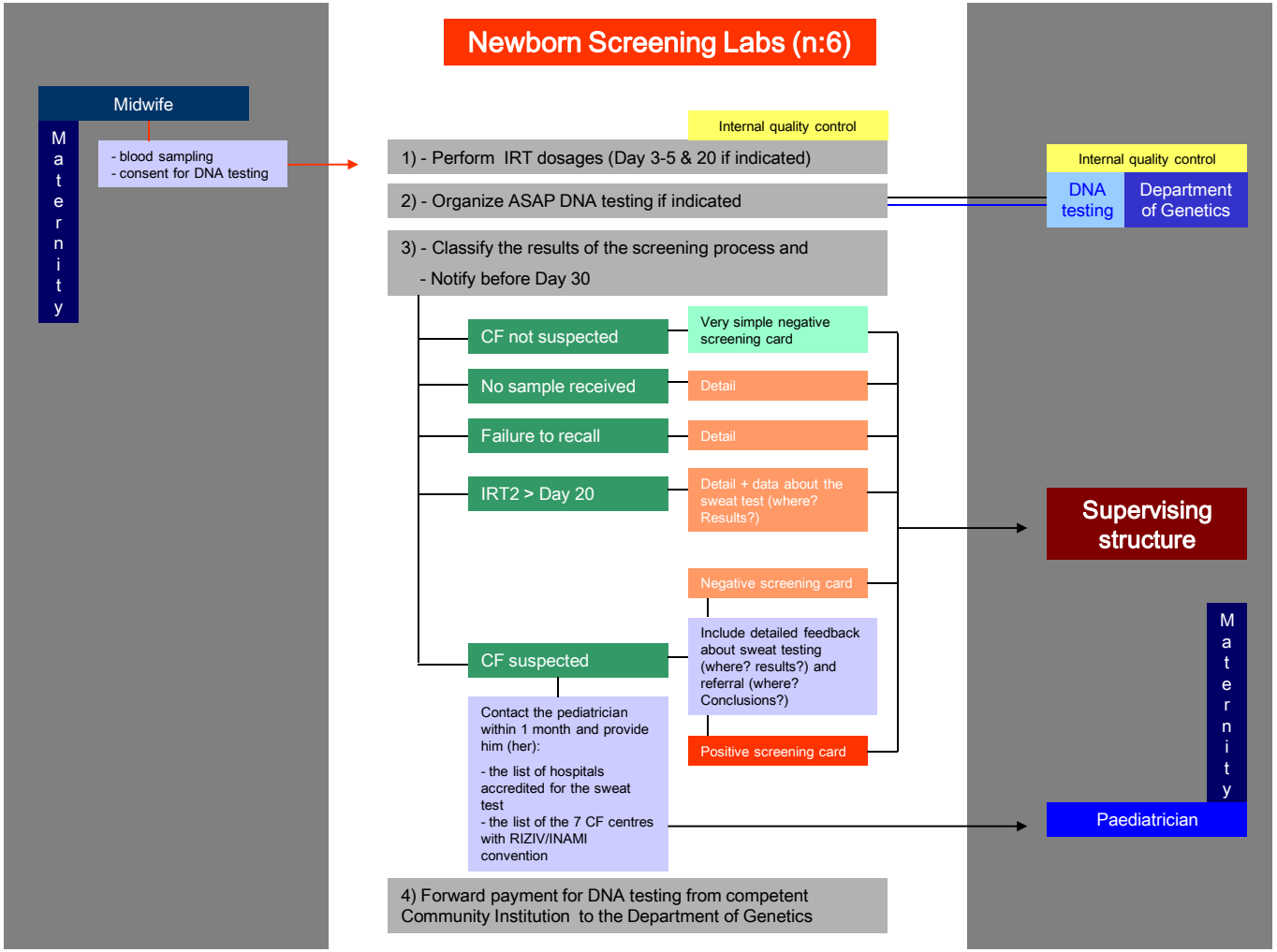
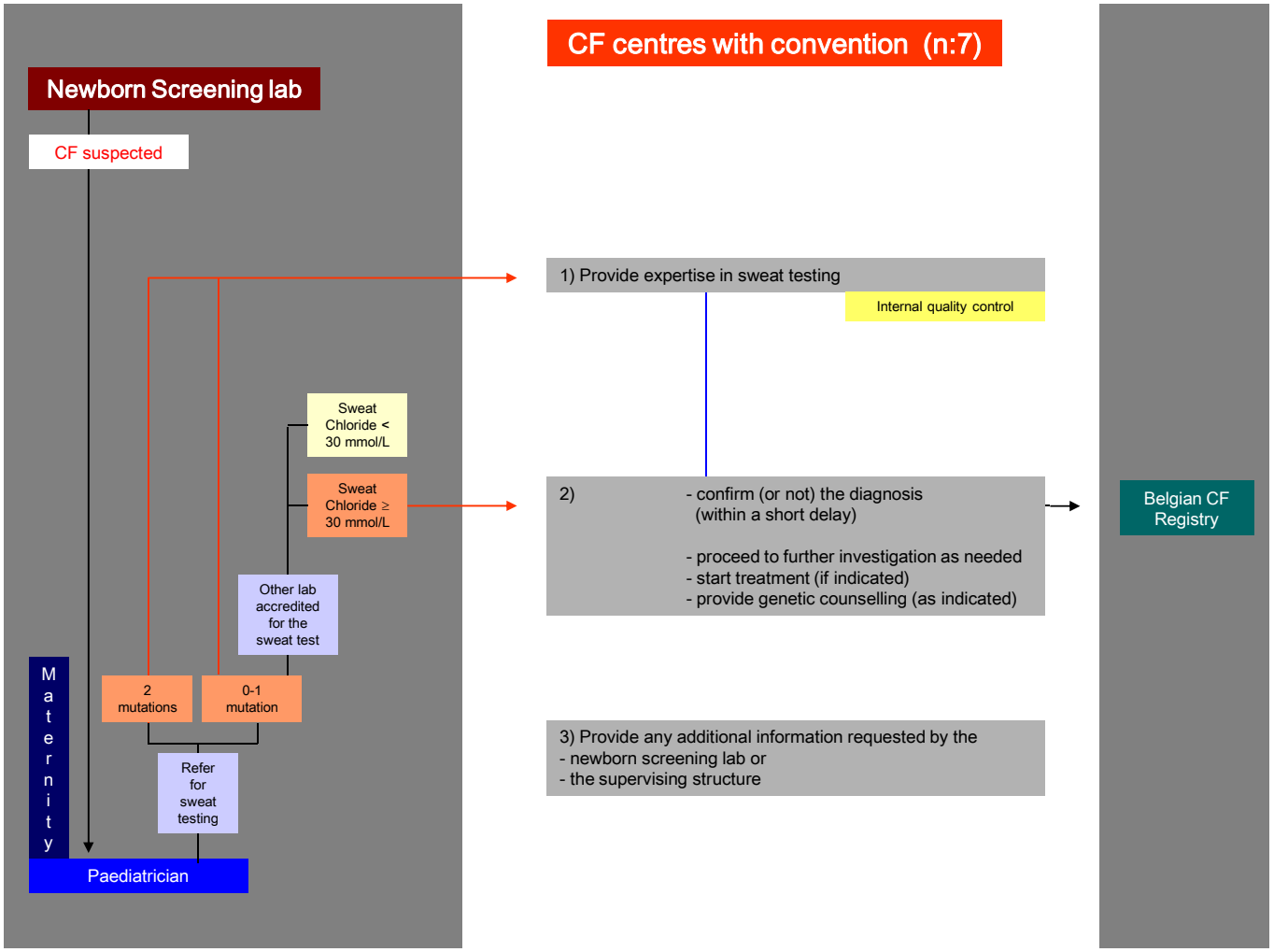


Figure 5. Suggested role of the CF centres.



APPENDIX 7. CFTR MUTATION TEST KITS

Innogenetics		Luminex Corporation	
RDB-INNO LiPA CFTR17+Tn Update	**RDB-INNO LiPA CFTR19**	**xTAG® Cystic Fibrosis 39 kit v2**	**xTAG® Cystic Fibrosis 71 kit v2**
E60X	I148T	F508del	F508del
G85E	711+1G>T	I507del	I507del
394delTT	I507del	G542X	G542X
R117H	F508del	G85E	G85E
621+1G>T	1717-1G>A	R117H	R117H
711+5G>A	G542X	621+1G>T	621+1G>T
1078delT	G551D	711+1G>T	711+1G>T
R334W	Q552X	R334W	R334W
R347P	R553X	R347P	R347P
A455E	R560T	A455E	A455E
2143delT	1898+1G>A	1717-1G>A	1717-1G>A
2183AA>G	3120+1G>A	R560T	R560T
2184delA	S1251N	R553X	R553X
2789+5G>A	3905insT	G551D	G551D
R1162X	W1282X	1898+1G>A	1898+1G>A
3659delC	N1303K	2184delA	2184delA
3849+10kbC>T	3272-26A>G	2789+5G>A	2789+5G>A
5T	CFTRdele2,3	3120+1G>A	3120+1G>A
7T	3199del6	R1162X	R1162X
9T		3659delC	3659delC
		3849+10kbC>T	3849+10kbC>T
		W1282X	W1282X
		N1303K	N1303K
		5T	5T
		7T	7T
		9T	9T
		F508C	F508C
		I507V	I507V
		I506V	I506V
		1078delT	1078delT
		394delTT	394delTT
		Y122X	Y122X
		R347H	R347H
		V520F	V520F
		A559T	A559T
		S549N	S549N
		S549R	S549R
		1898+5G>T	1898+5G>T
		2183AA>G	2183AA>G
		2307insA	2307insA

		Y1092X M1101K S1255X 3876delA 3905insT	Y1092X M1101K S1255X 3876delA 3905insT E60X R352Q 2869insG R75X S364P 3120G>A 405+3A>C G480C 3199del6 406-1G>A Q493X 444delA G622D W1089X R117C 1677delTA D1152H R1158X G178R 1812-1G>A 3791delC L206W 2055del9>A S1196X 935delA 2143delT CFTRdel2,3 F311del K710X R1066C G330X Q890X

Abbott Molecular	Tepnel Molecular Diagnostics - Gen-Probe Life Sciences			
Cystic Fibrosis Genotyping Assay	**CF29v.2**	**CF-EU1**	**CF30**	**CF7**
G85E	E60X	CFTRdele2,3	E60X	F508del
394delTT	G85E	E60X	G85E	1717-1G>A
R117H	R117H	P67L	394delTT	G542X
621+1G>T	621+1G>T	G85E	R117H	D1152H
711+1G>T	711+1G>T	R117H	Y122X	3849+10kbC>T
1078delT	1078delT	621+1G>T	621+1G>T	W1282X
R334W	R334W	711+1G>T	711+1G>T	N1303K
R347H	R347P	1078delT	1078delT	
R347P	A455E	R334W	R334W	
A455E	1507del	R347P	R347P	
1507del	F508del	A455E	A455E	
F508del	1717-1G>A	1507del	1507del	
V520F	G542X	F508del	F508del	
1717-1G>A	G551D	1717-1G>A	1717-1G>A	
G542X	R553X	G542X	G542X	
S549N	2183AA>G	G551D	G551D	
S549R(T>G)	2789+5G>A	R553X	R553X	
G551D	3120+1G>A	R560T	2183AA>G	
R553X	R1162X	1898+1G>A	2789+5G>A	
R560T	3659delC	2184delA	3120+1G>A	
1898+1G>A	3849+10kbC>T	2789+5G>A	R1162X	
2183AA>G	S1251N	3120+1G>A	3659delC	
2184delA	W1282X	M1101K	3849+10kbC>T	
2789+5G>A	N1303K	D1152H	S1251N	
3120+1G>A	2184delA	R1162X	W1282X	
R1162X	R560T	3659delC	N1303K	
3659delC	1898+1G>A	3849+10kbC>T	3272-26A>G	
3849+10kbC>T	D1152H	S1251N	1811+1.6kbA>G	
3905insT	394delTT	3905insT	Y1092X	
W1282X		W1282X	W846X	
N1303K		N1303K		
3876delA		S549RT>G		
		5T (incl. TGm)		
		7T		
		9T		

Mutations shown in italics are polymorphisms. Some of them are included in some assays since these polymorphisms may either result in a false positive signal for a mutation because of cross-reactivity with the corresponding mutant probe and which can be controlled in this way. 1148T was initially assigned as a CF-causing mutation, but later turned out to be a polymorphism (1148T) [359, 360]. Data are provided by the kit instruction manuals or the respective company websites.

APPENDIX 8. REVIEW ECONOMIC LITERATURE

PUBLISHED LITERATURE – SEARCH STRATEGY

Search for cost-effectiveness studies

On December 19, 2009, the websites of HTA institutes (Table) and following databases were searched: Centre for Reviews and Dissemination (CRD) databases (Health Technology Assessments (HTA) and NHS Economic Evaluation Database (NHS EED)) Medline (indexed and non-indexed citations), and Embase. The following four tables (Table to Table) provide an overview of the search strategy.

Table 1: List of INAHTA member websites searched for HTA reports

Agency	Country
AETMIS - Agence d´Évaluation des Technologies et des Modes d´Intervention en Santé	Canada
AETS - Agencia de Evaluación de Tecnologias Sanitarias	Spain
AETSA - Andalusian Agency for Health Technology Assessment	Spain
AGENAS - L'Agenzia nazionale per i servizi sanitari regionali - The Agency for Regional Healthcare	Italy
AHRQ - Agency for Healthcare Research and Quality	USA
AHTA - Adelaide Health Technology Assessment	Australia
AHTAPol - Agency for Health Technology Assessment in Poland	Poland
ASERNIP-S - Australian Safety and Efficacy Register of New Interventional Procedures -Surgical	Australia
AVALIA-T - Galician Agency for Health Technology Assessment	Spain
CADTH - Canadian Agency for Drugs and Technologies in Health	Canada
CAHTA - Catalan Agency for Health Technology Assessment and Research	Spain
CDE - Center for Drug Evaluation	Taiwan
CEDIT - Comité dÉvaluation et de Diffusion des Innovations Technologiques	France
CENETEC - Centro Nacional de Excelencia Tecnológica en Salud Reforma	Mexico
CRD - Centre for Reviews and Dissemination	United Kingdom
CVZ - College voor Zorgverzekeringen	The Netherlands
DACEHTA - Danish Centre for Evaluation and Health Technology Assessment	Denmark
DAHTA @DIMDI - German Agency for HTA at the German Institute for Medical Documentation and Information	Germany
DECIT-CGATS - Secretaria de Ciëncia, Tecnologia e Insumos Estratégicos, Departamento de Ciência e Tecnologia	Brazil
DSI - Danish Institute for Health Services Research	Denmark
ETESA - Department of Quality and Patient Safety of the Ministry Health of Chile	Chile
FinOHTA - Finnish Office for Health Care Technology Assessment	Finland
GÖG - Gesunheit Österreich GmbH	Austria
GR - Gezondheidsraad	The Netherlands
HAS - Haute Autorité de Santé	France
HIQA - Health Information and Quality Authority	Ireland
HSAC - Health Services Assessment Collaboration	New Zealand
ICTAHC - Israel Center for Technology Assessment in Health Care	Israel
IECS - Institute for Clinical Effectiveness and Health Policy	Argentina
IHE - Institute of Health Economics	Canada
IMSS - Mexican Institute of Social Security	Mexico
IQWiG - Institut für Qualität und Wirtschaftlichkeit im Gesundheitswesen	Germany
KCE - Belgian Federal Health Care Knowledge Centre	Belgium

LBI of HTA - Ludwig Boltzmann Institut für Health Technonoly Assessment	Austria
MaHTAS - Health Technology Assessment Section, Ministry of Health Malaysia	Malaysia
MAS - Medical Advisory Secretariat	Canada
MSAC - Medicare Services Advisory Committee	Australia
MTU-SFOPH - Medical Technology Unit - Swiss Federal Office of Public Health	Switzerland
NCCHTA - National Coordinating Centre for Health Technology Assessment	United Kingdom
NHS QIS - Quality Improvement Scotland	United Kingdom
NHSC - National Horizon Scanning Centre	United Kingdom
NOKC - Norwegian Knowledge Centre for Health Services	Norway
OSTEBA - Basque Office for Health Technology Assessment	Spain
SBU - Swedish Council on Technology Assessment in Health Care	Sweden
UETS - Unidad de evaluacíon Technologias Santarias	Spain
UVT - HTA Unit in A.Gemelli University Hospital	Italy
VASPVT - State Health Care Accreditation Agency under the Ministry of Health of the Republic of Lithuania	Lithuania
VATAP - VA Technology Assessment Program	USA
ZonMw - The Medical and Health Research Council of The Netherlands	The Netherlands

Table 2: Search strategy and results for CRD

Date	December 19, 2009		
Database	CRD (HTA & NHS EED)		
Date covered	No restrictions		
Search Strategy	**#**	**Search History**	**Results**
	1	MeSH Cystic Fibrosis EXPLODE	111
	2	MeSH Neonatal Screening EXPLODE	169
	3	#1 and #2	15
Note	15 references were found in CRD, of which 4 were from the HTA database and 9 from the NHS EED database. The other two references were identified in the DARE (Database of Abstracts of Reviews of Effectiveness) database and thus not included in this search for economic literature.		

Table 3: Search strategy and results for Medline (OVID) (part I)

Date	December 19, 2009		
Database	Medline (OVID)		
Date covered	1996 to November Week 3 2009		
Search Strategy	**#**	**Search History**	**Results**
	1	economics/	5197
	2	exp "Costs and Cost Analysis"/	85445
	3	"Value of Life"/ec [Economics]	190
	4	Economics, Dental/	150
	5	exp Economics, Hospital/	7770
	6	Economics, Medical/	690
	7	Economics, Nursing/	437
	8	Economics, Pharmaceutical/	1707
	9	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8	94164
	10	(econom$ or cost$ or pric$ or pharmacoeconomic$).tw.	221337
	11	(expenditure$ not energy).tw.	8042
	12	(value adj1 money).tw.	6
	13	budget$.tw.	7868
	14	10 or 11 or 12 or 13	229073
	15	9 or 14	266657

	16	letter.pt.	358796
	17	editorial.pt.	166047
	18	historical article.pt.	91459
	19	16 or 17 or 18	608734
	20	15 not 19	252634
	21	Animals/	1929502
	22	human/	5235629
	23	21 not (21 and 22)	1280513
	24	20 not 23	231814
	25	(metabolic adj cost).ti,ab,sh.	344
	26	((energy or oxygen) adj cost).ti,ab,sh.	1104
	27	24 not (25 or 26)	230720
	28	exp Cystic Fibrosis/	10886
	29	exp Neonatal Screening/	4067
	30	28 and 29	305
	31	27 and 30	41
Note			

Table 4: Search strategy and results for Medline (OVID) (part II)

Date	December 19, 2009		
Database	Medline (OVID), In-Process & Other Non-Indexed Citations		
Date covered	No restrictions		
Search Strategy	**#**	**Search History**	**Results**
	1	cost$.mp.	18466
	2	economic$.mp	7845
	3	budget$.mp.	930
	4	expenditure$.mp.	1514
	5	1 or 2 or 3 or 4	25865
	6	cystic fibrosis {Including Related Terms}	2520
	7	screening {Including Related Terms}	28775
	8	newborn {Including Related Terms}	7849
	9	neonatal {Including Related Terms}	7265
	10	8 or 9	8015
	11	6 and 7 and 10	24
Note			

Table 5: Search strategy and results for EMBASE

Date	December 19, 2009		
Database	EMBASE		
Date covered	No restrictions		
Search Strategy	**#**	**Search History**	**Results**
	1	socioeconomics'/exp	119571
	2	'cost benefit analysis'/exp	51280
	3	'cost effectiveness analysis'/exp	63016
	4	'cost of illness'/exp	10000
	5	'cost control'/exp	34899
	6	'economic aspect'/exp	832197
	7	'financial management'/exp	215570
	8	'health care cost'/exp	144133
	9	'health care financing'/exp	9756
	10	'health economics'/exp	453519
	11	'hospital cost'/exp	19115
	12	'finance'/exp	8156
	13	'funding'/exp	5714
	14	fiscal	4983
	15	financial	245565
	16	#12 OR #13 OR #14 OR #15	253890

	17	'cost minimization analysis'/exp	1615
	18	estimate*:ti,ab,de,cl	445891
	19	cost*:ti,ab,de,cl	446949
	20	variable*:ti,ab,de,cl	419922
	21	unit:ti,ab,de,cl	277494
	22	'#19 *4 #18' OR '#18 *4 #19'	441333
	23	'#19 *4 #20' OR '#20 *4 #19'	416796
	24	'#19 *4 #21' OR '#21 *4 #19'	32639
	25	#1 OR #2 OR #3 OR #4 OR #5 OR #6 OR #7 OR #8 OR #9 OR #10 OR #11 OR #16 OR #17 OR #22 OR #23 OR #24	1691747
	26	'cystic fibrosis'/exp	33329
	27	'newborn screening'/exp	7359
	28	#26 AND #27	728
	29	#25 AND #28	124
Note			

RESULTS OF SEARCH STRATEGY

A total of 202 manuscripts were identified in several databases: 65 with Medline, 124 with Embase, and 13 with the CRD NHS EED and HTA databases (Table). After removing 42 duplicates, 160 articles were left. These were considered for further inclusion in two rounds; a first round based on title, abstract (if available), and keywords (if available); a second round based on full text evaluation.

Table 6: search for cost-effectiveness studies: summary

Database	**Search date or Years included**	**References identified**
CRD	Dec. 19, 2009	
HTA		4
NHS EED		9
MEDLINE	1996 to Nov. Week 3 2009	41
MEDLINE In-Process & Other Non-Indexed Citations	Dec. 19, 2009	24
EMBASE	Dec. 19, 2009	124
Total references identified		202
Duplicates		42
Total		**160**

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Wettelijk depot : D/2010/10.273/41

KCE reports

1. Effectiviteit en kosten-effectiviteit van behandelingen voor rookstop. D/2004/10.273/1.
2. Studie naar de mogelijke kosten van een eventuele wijziging van de rechtsregels inzake medische aansprakelijkheid (fase 1). D/2004/10.273/2.
3. Antibioticagebruik in ziekenhuizen bij acute pyelonefritis. D/2004/10.273/5.
4. Leukoreductie. Een mogelijke maatregel in het kader van een nationaal beleid voor bloedtransfusieveiligheid. D/2004/10.273/7.
5. Het preoperatief onderzoek. D/2004/10.273/9.
6. Nationale richtlijn prenatale zorg. Een basis voor een klinisch pad voor de opvolging van zwangerschappen. D/2004/10.273/13.
7. Validatie van het rapport van de Onderzoekscommissie over de onderfinanciering van de ziekenhuizen. D/2004/10.273/11.
8. Financieringssystemen van ziekenhuisgeneesmiddelen: een beschrijvende studie van een aantal Europese landen en Canada. D/2004/10.273/15.
9. Feedback: onderzoek naar de impact en barrières bij implementatie – Onderzoeksrapport: deel 1. D/2005/10.273/01.
10. De kost van tandprothesen. D/2005/10.273/03.
11. Borstkankerscreening. D/2005/10.273/05.
12. Studie naar een alternatieve financiering van bloed en labiele bloedderivaten in de ziekenhuizen. D/2005/10.273/07.
13. Endovasculaire behandeling van Carotisstenose. D/2005/10.273/09.
14. Variaties in de ziekenhuispraktijk bij acuut myocardinfarct in België. D/2005/10.273/11.
15. Evolutie van de uitgaven voor gezondheidszorg. D/2005/10.273/13.
16. Studie naar de mogelijke kosten van een eventuele wijziging van de rechtsregels inzake medische aansprakelijkheid. Fase II : ontwikkeling van een actuarieel model en eerste schattingen. D/2005/10.273/15.
17. Evaluatie van de referentiebedragen. D/2005/10.273/17.
18. Prospectief bepalen van de honoraria van ziekenhuisartsen op basis van klinische paden en guidelines: makkelijker gezegd dan gedaan.. D/2005/10.273/19.
19. Evaluatie van forfaitaire persoonlijk bijdrage op het gebruik van spoedgevallendienst. D/2005/10.273/21.
20. HTA Moleculaire Diagnostiek in België. D/2005/10.273/23, D/2005/10.273/25.
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