Letter to the Editor

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Antigen excess detection by automated assays for free light chains

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To the Editor,

Free light chains (FLCs) are useful markers for the diagnosis, prognosis and follow-up of patients with monoclonal gammopathy. At present, reagents from two manufacturers are available for FLC quantification: Freelite κ and λ reagents (The Binding Site) and N Latex κ and λ reagents (Siemens). Freelite reagents are based on polyclonal antibodies, whereas N Latex reagents are based on monoclonal antibodies [1, 2].

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FLC determinations may suffer from analytical problems. Non-linearity and antigen excess in the high concentration range have been reported for the Freelite assay applied on analyzers from Siemens or Beckman Coulter [3–5]. Antigen excess has been arbitrarily defined as a result ≥ 4 times higher at a higher serum dilution than at a lower dilution and non-linearity as a 2- to <4 -fold difference between dilutions. To overcome these problems, The Binding Site developed an antigen excess detection system on their SPA Plus and Optilite analytical platforms. Also, Siemens developed an antigen excess detection system on their BN platforms. Optilite Freelite antigen excess protection is based on the rate of antibody/antigen reaction, measured at multiple time points during the reaction time. The calculation to determine antigen excess integrates the delta absorption data and calculates if the data falls outside of reference limits. A minimum delta absorbance check limit is also employed to suppress the flag in instances where samples have low levels of FLC (information provided by The Binding Site). The N Latex FLC κ and N Latex FLC λ assays use a built-in prereaction protocol on the BN systems to ensure detection of antigen excess. During the prereaction, a fraction of the sample is prepared with the full amount of reagents. If the measurement after a defined time does not exceed the predefined prereaction threshold, the rest of the sample is added and the main reaction is carried out. If the measurement signal exceeds the prereaction threshold, the sample is autodiluted according to the defined method parameter and the measurement is repeated. A prereaction is also performed on the new dilution. This process is repeated until the prereaction signal is below the threshold (information provided by Siemens).

Most previous studies investigated antigen excess on Siemens or Beckman analyzers without antigen excess detection for Freelite reagents [3–5]. Only few data are available on the performance of antigen excess detection using Freelite on SPA Plus [6] or Optilite [7] [Vercammen, Optilite Seed Site Meeting, Birmingham March 2016] and on BN systems using N Latex [6, 8, 9]. In the current study, we evaluated whether and to what extent the Freelite FLC assay applied on automated Optilite instrument and the

N Latex assay applied on a BN system suffer from antigen excess. Therefore, we systematically analyzed samples from patients with monoclonal gammopathy of unknown significance (MGUS) and with multiple myeloma (MM) on the standard dilution and on a high dilution. In case of failure of antigen excess detection, discrepant results between the two dilutions are observed.

Samples from 205 MM patients (131 males, 74 females) (median age 66 years; age range: 34–88) (88 IgG κ , 26 IgG λ , 28 IgA κ , 19 IgA λ , 1 IgM κ , 1 IgM λ , 22 κ , 19 λ and 1 no clear identification) were included: 191 were from the University Hospitals Leuven and 14 from the University Hospital Brussel. For 159 patients, the sample was obtained close to the diagnosis (<3 months). In addition, 525 samples from 255 different MGUS patients were included (141 males, 114 females) (median age 67 years; age range 34–93) (103 IgG κ , 74 IgG λ , 16 IgA κ , 15 IgA λ , 1 IgA type unknown, 10 IgM κ , 16 IgM λ , 5 λ , 11 a combination of monoclonal proteins, 4 no clear identification): 240 were from University Hospitals Leuven and 15 from University Hospital Brussel VUB. Several of these patients had follow-up determinations (after ~2, 4 and 6 years); 97, 78, 48 and 32 patients had, respectively, 1, 2, 3 and 4 determinations.

The study was approved by the local Ethics Committee (Leuven: ML10187/Brussels: B. U. N.143201421779).

All samples were analyzed for FLC by Freelite and N Latex by the manufacturer according to their instructions. For each sample, an additional analysis was performed on a diluted sample. N Latex tests were run on a BN ProSpec system with Software version 3.1. The reagent lot numbers were 473113, 473114, 473116 for FLC κ , 473213, 473214, 473217 for FLC λ and 473415, 473416 for the standard. Freelite tests were run on an Optilite system with software version 5.2. The reagent lot numbers were 352772 for FLC κ and 352777,

401360-1 for FLC λ . Four samples for which Optilite failed to detect antigen excess were retested at the University Hospitals Leuven using software version 5.3 and reagent lot number 409730 for FLC κ and 409728 for FLC λ .

The final serum dilutions used by BN ProSpec and Optilite to generate FLC results in patients with MGUS or MM are shown in Table 1. The standard dilution was 1:100 for N Latex κ FLC, 1:20 for N Latex λ FLC, 1:10 for Freelite κ FLC and 1:8 for Freelite λ FLC. In patients with MGUS and MM, also higher or lower dilutions were used. The number of dilutions in patients with monoclonal gammopathy was comparable between both assays. For N Latex κ FLC, 623 samples were measured with one dilution, for Freelite κ FLC 613, for λ N Latex 630 and for λ Freelite 605. In MM, reanalysis of the sample at a higher dilution was needed for 37% (N Latex)–39% (Freelite) of the patients for κ FLC and in 16% (Freelite)–23% (N Latex) of the patients for λ FLC. In MGUS, reanalysis of the sample at a higher dilution was needed for 5% (N Latex)–6% (Freelite) of the patients for κ FLC and in 3% (Freelite)–9% (N Latex) of the patients for λ FLC.

We checked for failure of antigen excess detection by analyzing (in parallel) all samples at the standard dilution and at a high dilution [1:2000 for N Latex κ FLC, 1:400 for N Latex λ FLC, 1:100 for Freelite κ FLC and 1:80 for Freelite λ FLC]. The ratio of the result obtained at the high dilution to the result obtained with the standard procedure was calculated. It has been suggested that ratios ≥ 4 indicate antigen excess. Samples with ratios ≥ 4 are summarized in Table 2. High ratios (>20) were found for Freelite in three samples for λ FLC (ratio ≥ 50) and in one sample for κ FLC (ratio: 22). In these samples, there clearly was a failure of antigen excess detection. Ratios between 4 and 7 were found in six samples for κ FLC by N Latex, in two samples for κ FLC by Freelite and in two samples for λ FLC by Freelite. The cutoff of

Table 1: Dilutions used.

	κ Dilution	MGUS (n=525)	MM (n=205)	λ Dilution	MGUS (n=525)	MM (n=205)
N Latex	20		4	5	1	2
	100	498	125	20	475	155
	400	20	50	100	37	26
	2000	7	15	400	11	10
	8000		8	2000	1	9
	32,000		3	8000		2
Freelite	2		2	2	33	38
	10	491	122	8	473	132
	100	33	68	80	18	19
	1000	1	11	800	1	13
	10,000		2	8000		1

Final dilution of the instrument (Optilite for Freelite and BNII for N Latex) that generated the final result.

4 to indicate antigen excess is arbitrary. Ratios slightly above this cutoff could probably also indicate non-linearity or dilution effects. Higher results at higher dilution could be induced by an altered matrix and, for example, altered accessibility of FLC for antibody-binding (which can be patient dependent). All six samples for which we observed non-linearity by N Latex had an IgA type κ monoclonal protein, with IgA concentrations ranging from 7.2 to 115 g/L. In two of these samples, non-linearity/antigen excess was also observed with Freelite. The eight samples for which we observed non-linearity or antigen excess by Freelite had various types of M-proteins with concentration of intact M-protein ranging from 3.6 to 61.8 g/L). Of note, some of the samples with non-linearity or antigen excess had a normal result in the standard dilution, demonstrating that non-linearity/antigen excess was not restricted to samples that are positive at the standard dilution. Other samples had final concentrations <700 mg/L and only three samples had FLC values >1000 mg/L.

Based on these results, The Binding Site optimized the antigen excess algorithm, and four samples could be rerun (18 months later) with this optimized algorithm, available for all reagents from April 2016. In three of the four samples, Optilite gave a correct value (see Table 2).

By systematically performing two dilutions for all MGUS and MM patients, we examined the efficacy of

the antigen excess detection systems on Optilite and on BN ProsPec without bias. To our knowledge, such systematic analysis has not been done before, neither for Freelite on SPA Plus or Optilite nor for N Latex on Siemens analyzers. Previous studies only reported on antigen excess detection by Freelite on Optilite or SPA Plus and by N Latex on a BN system in a limited set of selected samples, i.e. samples with previously demonstrated antigen excess when assayed by Freelite on BNII [6–9]. In studies performed at UZ Brussel and Pitié-Salpêtrière, Optilite failed to detect antigen excess in 1/8 and 0/3 [7] κ and 1/1 and 1/3 [7] λ cases of antigen excess with Freelite on BNII, respectively. In previous studies, N Latex Siemens antigen detection systems did not fail to detect antigen excess in 19 κ (13 [6], 4 [8], 2 [9]) and 16 λ [6] selected samples with antigen excess when assayed by Freelite on a BN system [6, 8, 9]. Jacobs et al. [10] suggested that in some patients, non-linearity/antigen-excess of serum FLC is linked to interference of a highly concentrated intact M-protein. We found that a majority, but not all, of the samples in which non-linearity/antigen excess were observed had a high level of intact M-protein.

In our study, we observed failure of antigen excess detection for κ FLC with N Latex on BN ProsPec and for κ and λ FLC with Freelite on Optilite. The underestimation was more extreme with Freelite on Optilite than with

Table 2: Failure of antigen excess detection.

Disease	Assay	Standard dilution, mg/L	High dilution, mg/L	Ratio	Rerun at standard dilution, mg/L	Rerun at high dilution, mg/L	Ratio rerun	M-protein type	IgA, g/L	IgG, g/L	IgM, g/L
MGUS	Freelite λ	123	489	4	27	498	18	IgA λ	22.7	6.29	1.78
	N Latex κ	17.5	120.8	7				IgA κ , IgG κ	7.21	9.96	0.28
MM	Freelite λ	12.5	71	6				IgG κ	0.26	26.2	0.53
		74	3727	50	6050			IgA λ	61.8	4.76	0.13
		141	11,292	80	13,500			λ	0.12	5.24	0.22
		26	1391	53				IgA λ	3.65 ^a	14.3 ^a	0.25 ^a
	Freelite κ	22.3	93	4				IgA κ	40.2	7.48	0.29
		27	612	23	624			IgA κ	61.1	1.48	<0.04
		44.5	195	4				IgG λ	0.37	18	0.08
		20.8	86	4				IgA κ	40.2	7.48	0.29
	N Latex κ	40	271	7				IgA κ	61.1	1.48	<0.04
		75.6	297	4				IgA κ	38.6	9.60	0.41
		68.6	281	4				IgA κ	115	2.99	<0.04
		29	175	6				IgA κ	74.6	3.05	0.08

Five hundred and twenty-five samples from MGUS patients and 205 samples from MM patients were run for FLC on Optilite and BN ProsPec using standard conditions [starting at the standard dilution with automatic further dilution] and, in parallel, using a high starting dilution [1:2000 for N Latex κ FLC, 1:400 for N Latex λ FLC, 1:100 for Freelite κ FLC and 1:80 for Freelite λ FLC]. Results of samples for which the ratio of results obtained at the high dilution to results obtained at the standard dilution was ≥ 4 are shown. Based on the results obtained, The Binding Site further optimized antigen excess detection. Some samples were rerun (18 months after the first run) using the optimized algorithm. These results are given under 'Rerun'. ^aDetermination of immunoglobulins on a sample obtained 3 days after the sample on which FLC was determined.

N Latex on BN ProsPec. Most pronounced failures were observed for Freelite FLC λ measurements on Optilite, but improvements have been observed during the study period.

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