Development of a rapid and quantitative lateral flow assay for the simultaneous measurement of serum kappa and lambda immunoglobulin free light chains (FLC): inception of a new near-patient FLC screening tool.

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ABSTRACT

BACKGROUND: Serum free light chains (FLC) are sensitive biomarkers used for the diagnosis and management of plasma cell dyscrasias, such as multiple myeloma, and are central to clinical screening algorithms and therapy response criteria. We have developed a portable, near-patient, lateral-flow test (Seralite®) that quantitates serum FLC in 10 minutes, and is designed to eliminate sample processing delays and accelerate decision-making in the clinic.

METHODS: Assay interference, imprecision, lot-to-lot variability, linearity, and the utility of a competitive-inhibition design for the elimination of antigen-excess ('hook-effect') were assessed. Reference ranges were calculated from 91 healthy donor sera. Preliminary clinical validation was conducted by retrospective analysis of sera from 329 patients. Quantitative and diagnostic results were compared to Freelite®.

RESULTS: Seralite® gave a broad competitive-inhibition calibration curve from below 2.5 mg/L to above 200 mg/L, provided good assay linearity (between 1.6 and 208.7 mg/L for κ FLC and between 3.5 and 249.7 mg/L for λ FLC) and sensitivity (1.4 mg/L for κ FLC and 1.7 mg/L for λ FLC), and eliminated anomalous results from antigen-excess. Seralite® gave good diagnostic concordance with Freelite® (Roche Hitachi Cobas C501) identifying an abnormal FLC ratio and FLC difference in 209 patients with newly diagnosed multiple myeloma and differentiating these patients from normal healthy donors with polyclonal FLC.

CONCLUSIONS: Seralite® sensitively quantitates FLC and rapidly identifies clinical conditions where FLC are abnormal, including multiple myeloma.

INTRODUCTION

The measurement of serum kappa (κ) and lambda (λ) immunoglobulin free light chains (FLC) is central to the diagnosis, prognostication and monitoring of patients with plasma cell dyscrasias, such as multiple myeloma (MM). The absolute levels of involved FLC (iFLC) versus uninvolved FLC (or the FLC ratio [κ:λ]) are used routinely to monitor light chain only (LCO) (1) and oligosecretory myeloma (2), as well as other plasma cell dyscrasias such as light chain amyloidosis (3), and light chain monoclonal gammopathy of undetermined significance (MGUS) (4), and their differentiation from healthy donors (5). Additionally, the relatively short half-life of FLC (3-6 hours) in blood compared to intact immunoglobulins (1-3 weeks) permits sensitive monitoring of response to anti-myeloma therapy and can identify early relapse in patients with intact immunoglobulin myeloma (6). Accordingly, the availability of FLC testing over the last fifteen years (7) has resulted in new patient screening algorithms and disease management criteria, and measurement of FLC is incorporated into the International Myeloma Working Group guidelines (8).

In the context of myeloma, rapid diagnosis and early disease intervention remains elusive. In the United Kingdom (UK), 50% of newly presenting patients require three or more visits to the general practitioner (GP) before obtaining a hospital referral – the highest of twenty-four cancers assessed in a recent retrospective study (9). As a result, 34% of 32,236 newly diagnosed myeloma patients presented as emergencies between 2006 and 2013, and 38% of these late presentations suffered mortality within 6 months (10). As a consequence, and by the time of hospital presentation, severe and life-threatening myeloma-associated disease complications have been fostered that include infections from profound immunosuppression, skeletal fractures caused by bone loss, and acute renal failure from nephrotoxic iFLC (11). In such cases, early diagnosis and treatment intervention increases disease-free survival (11, 12), and the early identification and reduction of iFLC in cases of acute kidney injury by prompt therapy is essential for renal recovery (13, 14). Early identification of an abnormal test result and commencement of therapy is thus critical (15, 16). In the weeks, months and years after myeloma diagnosis, FLC monitoring remains vitally important for managing patients, whereby FLC testing can inform, at an early stage, whether early changes to treatment are needed, and can identify refractory myeloma and initiation of new anti-myeloma therapy, particularly in cases of FLC escape (17).

Currently, FLC testing is conducted on either the first generation of FLC assays (Freelite®, The Binding Site, UK) which utilises latex-adsorbed sheep polyclonal anti-human FLC antisera, or a more recently launched assay (N Latex, Siemens, Germany), which uses mouse anti-human FLC monoclonal antibodies (mAbs). These assays require nephelometric or turbidimetric analysers that are limited to large biochemistry departments or specialist centralised laboratories. Thus, FLC testing is often subject to undue delays of days or weeks whilst the sample is processed at these independent sites, meaning that clinical interventions – based on the interpretation of the FLC results – are deferred. In addition to a clinical need for rapid, near-patient testing, any new FLC assay should overcome a number of analytical limitations that are associated with nephelometric and turbidimetric FLC measurements. As outlined by the International Myeloma Working Group (18), these problems include lot-to-lot inconsistency (19), poor sensitivity (i.e., Freelite® 'gaps') (20, 21), and variable specificity (i.e., cross reactivity with whole immunoglobulin-bound light chains) (22, 23), that together gives rise to differences in concordance between assays in the measurement of absolute FLC concentrations (24-29). In the assay described herein, we employ anti-FLC mAbs with excellent specificity,

sensitivity and lot-to-lot consistency, which have been shown to positively identify monoclonal FLC, where present and confirmed by immunofixation electrophoresis (IFE), in over 14,000 consecutive patient samples (21). A significant and undesirable feature of existing FLC assays is the well-described issue of antigen-excess arising from samples with high FLC levels which causes an underestimation of FLC concentration, giving rise to significant non-linearity (27, 30, 31). Indeed, there are reports of Freelite® and N-Latex measuring 'normal' absolute FLC in samples with IFE positive M-protein at initial sample dilution followed by clinically 'abnormal' absolute FLC at higher sample dilution (27, 31-35). Therefore, we have adopted a competitive-inhibition format that is designed to eliminate antigen excess and prevent the phenomenon of false negatives in patients with multiple myeloma – where the iFLC level can range from a few milligrams to tens of grams per litre – and has already demonstrated utility in a prior Luminex-based method (21).

In the present study, we describe the development and preliminary validation of a portable lateral-flow test that can rapidly and simultaneously quantitate serum κ and λ FLC in 10 minutes (Seralite®, Abingdon Health Ltd., UK). The assay incorporates a number of technical advances which are designed to overcome existing analytical problems in FLC testing.

MATERIALS AND METHODS

Test principle and procedure

Seralite® is a lateral flow device (LFD) for the quantification of κ and λ FLC levels in serum based on the principle of competition between FLC in a serum sample and immobilised purified FLC antigens for binding to gold-labelled anti-FLC mAbs; the anti-FLC mAbs utilised in Seralite® (BUCIS 04 [anti-κ] and BUCIS 09 [anti-λ]) were selected based on previous validation, described in detail elsewhere (21). The composition of the LFD is illustrated and described fully in Figure 1. To conduct the test, 100 µL of serum is added to 200 µL of sample buffer containing foetal bovine serum. From this, 100 µL is added to the sample application port of the LFD, which is immediately inserted into a Seralite® LFD reader (ADLxR3, Abingdon Health Ltd, York, UK). After a 10 min incubation period at room temperature, the signal produced from the gold-labelled mAbs bound to the FLC antigens immobilised on the LFD membrane is inversely proportional to the amount of FLC in the sample; the Seralite® LFD reader converts the signal into mg/L of κ and λ FLC, and calculates the FLC ratio.

Assay calibration

Calibrator material was prepared using a pool of human serum samples containing monoclonal FLC (confirmed by IFE, and quantified by Freelite® using a Roche Hitachi Cobas® C501) from the University of Birmingham Clinical Immunology Service. To produce calibration curves the calibration material was diluted (κ at 1 in 2; λ at 1 in 1.5) in sample buffer, followed by subsequent serial 2-fold dilutions in assay buffer. To establish the calibration range of Seralite®, calibration adjustments were made by measuring 91 normal human serum samples (UK National Health Service Blood Transfusion Service) that contained 'normal' polyclonal light chains (confirmed by IFE) on Freelite® and Seralite®. The calibration adjustments were made as follows: separate calibration curves are produced for each manufactured batch of devices using pooled serum containing elevated monoclonal free light chains; separate pools for κ and λ FLC are used to derive the respective calibration curves. In the absence of an international reference material, these pools were initially tested on Freelite® to obtain a starting concentration, and based on these results, the pooled sera are diluted to set concentrations in foetal bovine serum to produce a calibrator set. The calibrator set is run in triplicate, across multiple readers. The mean reader response is calculated and plotted as a 4-parameter logistic against the assigned values of the calibrator set. 91 normal serum samples containing known concentrations of polyclonal free light chains (established by Freelite®) are tested and then used to make minor adjustments to the original assigned concentration of the calibrator set. Following this process and to confirm performance, sets of internal control samples based on polyclonal and monoclonal FLCs that cover the calibration range are tested. Finally when set quality control parameters are met, the curve parameters and batch lot number are converted into a bar code and the batch is released. Test results are read against these stored κ and λ FLC calibration curves.

Assay dynamics

Linearity

Assay linearity was assessed by serially diluting two healthy donor serum samples spiked with purified κ or λ monoclonal FLC two-fold in assay buffer; the purification of κ and λ FLC is described elsewhere (1). A minimum of

9 steps of dilutions were performed; 3 replicates for each dilution were measured and the mean values were analysed against the expected linear results.

Limit of detection

Assay buffer was measured using 20 replicates to obtain a 'blank' concentration and a normal serum sample was serially diluted 9 in 10. The limit of detection was determined by selecting the lowest κ and λ concentration of the normal sample detected above the mean blank value.

Imprecision

Test imprecision was assessed by calculating the mean intra-day and inter-day coefficient of variation percentages (CV%). For these analyses, 3 large volume samples with normal (containing polyclonal FLC), elevated and high levels (containing monoclonal FLC) of κ and λ FLC were obtained from a biorepository at the University of Birmingham Clinical Immunology Service. All samples were analysed a total of 4 times per day: analysis was performed in duplicate, twice per day (with a 2 h gap between runs), for 11 days over a period of 28 days. To determine between reader precision, 3 samples with normal, elevated and high levels of κ and λ FLC were used. Each sample was analysed 20 times on the same day and each LFD was read using 3 different LFD readers; CV% was calculated for each of the 20 replicates across the readers and averaged to provide an inter-reader CV% for each sample. All imprecision analyses were conducted on the same lot of reagents.

Analyte stability

To determine analyte stability using Seralite®, 4 samples with below normal, normal, elevated and high levels κ and λ FLC were used and measured in triplicate at each time point. All samples were measured at baseline (hour/day 0) then aliquots were stored under different conditions. Samples were stored at room temperature (21–24°C) and tested at 1, 8, 24, 48 and 72h. Samples stored in the fridge between 3–5°C were tested across a period of 4 consecutive days, then for an additional day 7 days from baseline. Aliquots of each sample were frozen on day 0 then tested daily for 6 consecutive days providing a total of 6 freeze thaw cycles.

Interference

To assess the susceptibility of Seralite® to interference, known quantities of common interference agents were added to serum samples containing normal κ (12.72 mg/L) and λ (10.88 mg/L) polyclonal FLC levels. Serum samples were individually spiked with purified (all obtained from Sigma Aldrich, UK): bilirubin (0.2 g/L), cholesterol (2.0 g/L), haemoglobin (2.0 g/L) and triglyceride (10.0 g/L); and the following immunoglobulin proteins (purified by the University of Birmingham Monoclonal Antibody Production Service): IgG-κ (0.84 g/L), IgG-λ (0.32 g/L), IgA-κ (0.65 g/L), IgA-λ (0.15 g/L), IgM-κ (0.45 g/L), IgM-λ (0.38 g/L), κ FLC (0.53 g/L) or λ FLC (0.73 g/L); these concentrations represent the final concentration of interference agents in the sample.

Lot-to-lot consistency

Variability between batches of Seralite® was assessed by analysing 65 serum samples exhibiting FLC levels that covered the entire calibration range of the assay, using three consecutive lots of assay kits. The samples were healthy

donor sera containing polyclonal FLCs within the normal FLC reference range, or myeloma patient sera containing monoclonal FLC identified by IFE. Data was analysed using Passing and Bablok regression.

Antigen excess

Seralite® was used to analyse 10 serum samples from multiple myeloma patients, tested previously during routine clinical measurement of FLC, that had been identified to give erroneously low results using Freelite® (Roche Hitachi Cobas® C501) due to antigen excess. Data is presented for each sample at the original standard dilution and with further dilution for both methods.

Reference ranges

95 serum samples were obtained from healthy random donors from the NHS Blood and Transplant service (NHSBT Birmingham, UK). All samples were first analysed for abnormal FLC on Freelite® and creatinine (both on Roche Hitachi C501) to screen for impaired renal function, or monoclonal gammopathies or immune dysregulation: 4 patients were excluded on the basis of this screening. The remaining 91 samples were analysed using Seralite® to generate references ranges for κ and λ FLC, and the κ:λ ratio.

Clinical Specificity

To compare FLC levels between the new near-patient device and a commonly used laboratory-based immuno-turbidimetric method, a range of stored (cryopreserved) patient samples were analysed retrospectively by Seralite® and Freelite®. 120 serum samples from patients with a range of conditions associated with abnormalities in FLC levels (but not monoclonal gammopathy) were analysed: rheumatoid arthritis (n = 18); systemic lupus erythematosus (n = 16); Sjögren's syndrome (n =23); recurrent infections (n = 9); nephrotic syndrome (n =18); vasculitis (n = 11); B-cell non-Hodgkin lymphomas (n = 12) or chronic lymphocytic leukaemia (n =13). All patients were screened to confirm they did not have a monoclonal gammopathy by serum IFE, in conjunction with each patient's clinical history.

Serum samples from 209 multiple myeloma patients at disease presentation were measured for κ and λ FLC using both methods. Patients had been diagnosed with light chain only (n = 122), IgG (n = 49) or IgA (n = 38) myeloma. Clinical results were compared between Seralite® and Freelite® using the κ:λ ratio. Discrepancies between methods were defined as any sample with an abnormal κ:λ ratio on one assay but not the other. Discrepant samples were investigated using serum and urine IFE and patient history.

Statistical Analyses

Passing and Bablok regression and linearity analysis were conducted using the Microsoft Excel add-in Analyse-it software (version 4.60, Method Evaluation, www.analyse-it.com). Wilcoxon signed rank tests were used to compare FLC parameters between methods (IBM SPSS, Version 21). Figures were produced using SigmaPlot version 12.0 (SystatSoftware Inc., USA) and GraphPad Prism (GraphPad Software Inc., USA).

RESULTS

Calibration and assay dynamics

Representative calibration curves are illustrated in Figure 2a, where the assay range was between 1.7 and 213.4 mg/L for κ FLC, and between 1.9 and 239.9 mg/L for λ FLC. A dynamic range of 2.5 to 200 mg/L for κ and λ FLC calibration curves was subsequently programmed into the Seralite® reader to: (i) enable matching curves for κ and λ FLC (ii) provide a fixed consistent range between future lots for absolute κ and λ FLC, and the FLC ratio. The limit of detection was 1.4 mg/L for κ and 1.7 mg/L for λ.

Representative assay linearity is presented in Figure 2B and 2C, where κ FLC was linear between 1.6 and 208.7 mg/L and λ FLC between 3.5 and 249.7 mg/L. The differences between expected linear and observed concentrations were calculated at all dilutions and the maximum difference between results was 7% for κ FLC and 10% for λ FLC.

Precision was assessed by measuring three samples with levels of FLC within, above and highly-elevated above the normal range. Across the three samples, intra-day CV% was between 7.4–9.6% and inter-day CV% was between 3.6–7.1%. Inter-reader precision was also assessed using three samples and CV% between 2.0–5.5% were observed. Precision data for all samples are provided in Supplementary Table 1.

Good stability was observed for FLC measured by Seralite® when samples of various concentrations were stored under different conditions. Supplementary Table 2 describes the median changes from baseline in κ and λ FLC concentrations following storage at room temperature, when refrigerated and after freeze thaw cycles. Generally, median changes from baseline were < 2 mg/L and the highest median change observed across conditions and samples was -7.8 mg/L in samples containing monoclonal FLC concentrations above 120mg/L.

Assay interference tests showed minimal assay cross-reactivity to potential interfering agents, and no more than a median 2.47 mg/L change was observed for κ FLC, and 2.23 mg/L for λ FLC (Supplementary Figure 1).

Assay lot-to-lot variability was assessed by analysing 65 patient samples with varying FLC levels across the full range of the calibration curves (Supplementary Figure 2). Passing and Bablok regression analysis gave the following slopes (95% CI) and intercepts (95% CI), respectively: 1.06 (0.96 to 1.23) and -0.67 (-3.30 to 0.83) for batch A vs B; 1.02 (0.92 to 1.11) and -1.29 (-2.76 to 0.66) for batch A vs C; 0.94 (0.88 to 1.01) and -0.82 (-0.39 to 1.75) for κ FLC; 0.92 (0.83 to 1.03) and 0.53 (-0.52 to 1.41) for batch A vs B; 0.94 (0.88 to 1.02) and 0.28 (-0.37 to 1.05) for batch A vs C; 1.06 (0.99 to 1.14) and -0.69 (-1.56 to 0.28) for λ FLC. The mean absolute differences between batches for κ and λ, respectively were: 5.5 and 4.0 mg/L (batch A vs B), 4.6 and 3.4 mg/L (batch A vs C) and 3.8 and 3.4 mg/L (batch B vs C).

To assess the elimination of antigen excess on Seralite®, samples from myeloma patients previously shown to give erroneously low results by Freelite® due to antigen excess where analysed on Seralite® (Supplementary Table 3).

Seralite® correctly identified all iFLC in these patients using the standard dilution; one sample exhibited a false negative on Freelite and the iFLC in that sample was correctly identified upon higher dilution of the sample.

Reference ranges

The values obtained for Seralite® and Freelite® from 91 donor sera are presented in Figure 3. On Seralite®, the observed median (5-95% range) for κ FLC was 10.75 mg/L (5.25 to 22.66), λ FLC was 10.46 mg/L (3.96 to 25.13), the κ/λ FLC ratio was 1.13 (0.48 to 2.49), and the FLC difference (κ FLC minus λ FLC) was 1.07 mg/L (-6.48 to 6.96) . On Freelite®, κ FLC was 11.24 mg/L (4.52 to 27.40), λ FLC was 11.28 mg/L (6.63 to 17.55), the κ/λ FLC ratio was 0.99 (0.45 to 1.6), and the FLC difference was -0.12 mg/L (-3.60 to 4.74). There were no significant differences in κ FLC, λ FLC, or the FLC difference measured by Seralite® and Freelite®. However, the κ/λ FLC ratio was higher when measured by Seralite® compared to Freelite® ($Z = -2.39$, $p = .017$).

Clinical specificity

Sera from 120 patients with dysregulated polyclonal FLC levels arising from an array of clinical disorders were measured on Seralite® and Freelite®. As illustrated in Figure 4, elevated levels of both κ and λ FLC were found in the majority of patients when measured by both Seralite® and Freelite®. In general, Seralite® produced lower FLC values compared with Freelite®. On Seralite®, the observed median (5-95% range) for κ FLC was 22.75 mg/L (6.44 to 92.72) compared with 25.11 mg/L (4.54 to 144.51) on Freelite® ($Z = -2.24$, $p = .025$). On Seralite®, both the κ/λ FLC ratio (0.96, 0.38 to 2.49) and FLC difference (-0.75, 37.41 to 44.33 mg/L) were lower than the κ/λ FLC ratio (1.22, 0.57 to 3.02) and FLC difference (3.73, -5.31 to 76.87 mg/L) measured by Freelite® ($Z = -3.45$, $p = .001$ and $Z = -4.67$, $p < .001$, respectively). Alternatively, λ FLC levels were higher when measured by Seralite® compared with Freelite®, with values of 23.30 mg/L (5.78 to 86.41) and 18.72 (7.47 to 58.52), respectively ($Z = -3.51$, $p < .001$).

Sera from 209 newly presenting myeloma patients were measured on Seralite® and Freelite®. Illustrated in Figure 5 are the absolute levels of κ FLC, λ FLC, κ:λ FLC ratio and FLC difference (iFLC−uninvolved FLC) in these patients. Seralite®. Levels of FLC parameters were significantly lower on Seralite® compared with Freelite® ($Z = -4.25$ to -8.20, $p < .001$ for all comparisons). The κ:λ FLC ratio 5-95th reference range for Seralite® was applied for diagnostic purposes. In agreement with Freelite®, Seralite® gave an abnormal κ:λ FLC ratio in all patients (Figure 6).

DISCUSSION

This study describes the development and initial validation of a rapid, fully-quantitative, near-patient lateral flow assay for the measurement of serum FLC. Seralite® has been designed to measure absolute κ and λ FLC levels and the FLC ratio in 10 minutes to enable 'on-the-spot' decision-making and eliminate the dependence on sending patient samples to biochemistry departments or specialist laboratories where serum FLC are measured on complex analysers in batches, usually not on a daily basis. This process incurs delays during sample shipment, testing and processing and defers decisions about patient treatment.

As the diagnosis of multiple myeloma necessitates a battery of tests (8), Seralite® may be used to 'red flag' patients with suspected monoclonal gammopathy for prioritisation and accelerated testing of sample specimens at clinical laboratories. Early detection of multiple myeloma – and treatment of the acute clinical complication that initiated the hospitalisation – are highly prognostic for survival (12), and is particularly vital in cases of acute renal failure where early reduction of nephrotoxic iFLC is critical for renal recovery (14). In myeloma patients being monitored, iFLC levels can be used to identify efficacy of anti-myeloma therapy and can be used to exclude relapse until FLC levels increase, in which case, further laboratory testing can be prioritised to confirm active myeloma. FLC testing is especially important in diagnosing cases of serum and urine IFE negative LCO myeloma and amyloidosis where there are few measures of disease activity beyond bone marrow examination, as well as LCO myeloma when serum IFE is negative and no urine has been sent. It has also been established that serum FLC testing is more sensitive than urine, and is particularly important in monitoring LCO patients, patients with low levels of whole M-protein and for light chain escape (36). To overcome the reliance on laboratory analysers for the measurement of FLC, Seralite® fulfils a number of important analytical and performance requirements. Firstly, as reported herein, Seralite® exhibits a broad calibration range from 2.5 mg/L to 200 mg/L for both κ and λ FLC. This extensive range enables accurate quantitation of healthy donor sera and similar enumeration of normal polyclonal FLC as the Freelite® assay (5). We note that the Seralite® reference ranges reported herein are based on 90% ranges, whereas well-cited Freelite ranges are derived from 95% ranges (5); accordingly, this may result in a higher proportion of Seralite® results falling outside its associated references ranges, as compared to Freelite® and its associated reference ranges. As with all clinical tests, it is important that end users establish normal ranges for their own laboratory. In addition to accurate enumeration of polyclonal FLC, the low limit of detection on Seralite® enables sensitive measurement of low levels of FLC indicative of immunosuppression or immunoparesis, and avoids the low-sensitivity 'gaps' that are a feature of Freelite® (20, 21). The 'gaps' are observed when levels of absolute FLC (e.g., <7mg/L) in a patient specimen are below the initial measuring range of Freelite® using the automated dilution (e.g., 1 in 8); a subsequent repeat of the neat sample then reveals that the sample is either >7mg/L or <2mg/L, presumably due to the non-linearity of FLC assays at low FLC levels (31). This is an key limitation of Freelite® when monitoring patients with plasma cell dyscrasias because an 'artefactual' shift from 7 mg/L to 1 mg/L in the uninvolved Freelite® FLC level will cause an 'artefactual' seven fold change in FLC ratio, thus potentiating incorrect clinical interpretation. On Seralite®, the initial upper-range of the assay is designed to enable the identification of large numbers of patients with abnormally high iFLC outside the normal reference range; a 1 in 20 dilution of patient sample extends the assay range to 4000 mg/L, thus quantitating the majority of patients with myeloma.

Although Seralite® provides similar diagnostic concordance with Freelite® in myeloma patients, we note that Seralite® has a broader FLC ratio range than Freelite®, whereas absolute levels of κ and λ FLC measured on Seralite® are lower than Freelite®. Overestimation of Freelite® in samples containing elevated monoclonal FLC has been reported previously (37), and a recent study showed that this may be a feature of nephelometric assays, as both N Latex and Freelite® nephelometric assays substantially overestimated FLC compared to a mass spectrometry method (38). As a result of the over-estimation of Freelite® (and N Latex) nephelometric methods, it is not possible to apply a calibration correction to harmonise inter-assay differences, and as a consequence, it is unlikely that FLC assays (Seralite®, Freelite®, or N Latex) can be used interchangeably in monitoring individual patients (39). Currently, no international standard is recognised or available, thus laboratories and clinical centres seeking to change FLC method should analyse any new sample on the old assay (i.e., the assay being replaced) and the new assay concurrently so that a new FLC benchmark can be established for each individual patient (39, 40). A consequence of the exponential increase in FLC levels on existing FLC assays – as outlined by the International Myeloma Working Group – is the non-linearity of sample dilutions (18). Seralite® results presented herein show good linearity on serial dilutions of sera containing high levels of monoclonal FLC. A related problem that affects existing FLC assays on certain analysers – that Seralite® overcomes – is the problem of antigen-excess. In this study, we report one Freelite® false negative, and numerous occasions where high FLC levels were initially low on Freelite® that subsequently gave higher results when less sample (i.e., more dilute sample) was added. To address this issue, Seralite® has a competitive-inhibition format that eliminates this risk, and any samples with high iFLC above the calibration range of the assay are identified as being above 200 mg/L and should be re-diluted. This is an important development that will ensure, not only that false negatives are reduced which delay diagnosis, but that patients can be monitored accurately throughout therapy without confusion e.g., during efficacious anti-myeloma therapy where the assay should report falling iFLC levels rather than giving erroneously higher iFLC levels (35).

Incorporated into Seralite® are extensively-validated anti-FLC mAbs, which have previously been shown to have excellent specificity, sensitivity, and lot-to-lot consistency (21), and the incorporation of these mAbs into Seralite® yielded similar results with minimal interference and good inter-test consistency found between batches. These archetypal characteristics of mAbs overcome many limitations of polyclonal antibody-based assays (18), but, despite these many advantages, their application and utility in FLC assays has been a contentious issue in recent years (25, 41). This is partly founded on the experience of groups, including ours, where mAbs have been produced that were unsuccessful in detecting FLC from substantially all neoplastic cell clones, or did not analyse performance against a sufficiently high number of diverse patient samples. However, in recent years, we have shown that mAbs – which target a specific site on the constant region domain [that is 'hidden' on light chain that is bound to immunoglobulin] – have desired utility and perform effectively when compared to polyclonal antibody-based assays (21). The mAbs selected for Seralite® were validated in a Luminex multi-plex assay format and found to identify all M-proteins, where present and detected by IFE, in over 14,000 patient specimens, and showed good diagnostic concordance with clinical features, IFE of serum and urine and Freelite®. Incorporation of these mAbs into Seralite® yielded similarly efficacious results. Reported herein, we found that Seralite® detected all cases of LCO myeloma, and intact immunoglobulin IgA and IgG myeloma. These results support the view that the mAbs used in Seralite® are very close

to the ideal of detecting all FLC M-proteins from a wide assortment of patients and future studies independent of vendor-bias will establish Seralite® viability in the clinical setting.

CONCLUSION

Seralite® can be used to simultaneously and sensitively measure κ and λ serum FLC in 10 minutes. The rapid and portable nature of Seralite® can enable rapid FLC screening and may be used to accelerate decision making near-patient in the clinical setting.

AUTHORS DISCLOSURES AND POTENTIAL CONFLICTS OF INTEREST

Stock ownership: JC, MG, RJ, MC, TP, CH and MD have shares in Abingdon Health Ltd.

Employment or Advisory Role: MS, AG, EO, and DB are, or were, employees of Abingdon Health Ltd. MD has, and RJ has previously had, an advisory role with Abingdon Health Ltd.

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