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Abstract

Objectives: To verify the analytical performance of a new mass spectrometry—based method, termed MASS-FIX, when screening for plasma cell disorders in a routine clinical laboratory.

Patients and Methods: Results from 19,523 unique patients tested for an M-protein between July 24, 2018, and March 6, 2020, by a combination serum protein electrophoresis (SPEP) and MASS-FIX were examined for consistency with pretest implementation performance. MASS-FIX’s ability to verify abnormal results from SPEP and free light chain measurements was then compared with that of immunofixation electrophoresis (IFE) using a separate cohort of 52,586 patients tested by SPEP/IFE during the same period.

Results: Overall, 62.4% of our cohort was negative for an M-protein. Importantly, 7.3% of all specimens had an M spike on SPEP (0.1 to 8.5 g/dL) and MASS-FIX detected an M-protein in all these samples. Of all samples, 30.3% had M-proteins that were detected by MASS-FIX but the SPEP finding was too small for quantification. Of the positive samples, 5.7% contained a therapeutic monoclonal antibody. Of the positive samples, 4.1% had an N-glycosylated light chain (biomarker of high-risk plasma cell disorders). MASS-FIX confirmed a higher percentage of SPEP abnormalities than IFE. MASS-FIX was slightly more sensitive than IFE when confirming an M-protein in samples with an abnormal free light chain ratio. MASS-FIX had a very low sample repeat rate (1.5%). MASS-FIX was highly automatable resulting in a higher number of samples/technologist/day than IFE (~30% more).

Conclusion: Overall, MASS-FIX was successful in maintaining validation characteristics. MASS-FIX was more sensitive in confirming SPEP abnormalities when compared with IFE. Ability to detect therapeutic monoclonal antibodies and glycosylated light chains was distinctly advantageous.

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Plasma cell disorders (PCDs) are a group of disorders that are centrally characterized by an abnormal expansion of clonal plasma cells. These abnormal clones often overproduce a monoclonal immunoglobulin protein (M-protein). Accurately detecting, quantifying, and determining the isotype of this M-protein is important for establishing a clinical diagnosis (multiple myeloma, Waldenstrom macroglobulinemia, heavy chain disease, etc) and subsequent care. For more than 40 years, agarose gel protein electrophoresis and immunofixation electrophoresis (IFE) have been the mainstay for detecting and characterizing the M-proteins in our laboratory.1 In recent years, we2 and others3,4 have proposed using mass spectrometry (MS) as a more sensitive tool for detecting M-protein, isotyping, and tracking disease burden.

Starting in 2014, our laboratory developed a MS-based method that uses immunoglobulin (Ig) light chain (LC) mass to charge
(m/z) distributions to detect and track M-proteins. This technique was later adapted to matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) MS instrumentation for detection and isotyping of M-proteins (assay termed MASS-FIX). A previous study showed that MASS-FIX detected M-protein in 100% of the samples that are positive by both serum protein electrophoresis (SPEP) and IFE; M-proteins were detected in 97% of the samples that are negative by SPEP but positive by IFE. MASS-FIX has also shown increased analytical sensitivity when compared with IFE for detecting M-proteins. In addition, MASS-FIX was able to detect new features of M-proteins such as LC glycosylation, which was found to be a risk factor for light chain amyloidosis (AL), cold agglutinin disease (CAD), and other PCDs. MASS-FIX was automated and validated for clinical use and its clinical performance was verified using patients with PCDs. A subcommittee of the International Myeloma Working Group (IMWG) reviewed the published data on MS-based methods for detecting M-proteins and recommended MASS-FIX as an adequate replacement for IFE. Hence, MASS-FIX replaced traditional IFE as part of routine clinical care of the PCD patients seen at Mayo Clinic starting in July 2018. In parallel, we continued to use SPEP and IFE to perform M-protein analysis of non-Mayo Clinic PCD patients.

As with any new assay, verification of the assay performance after implementation is a prudent exercise. Section 522 of the Federal Food, Drug, and Cosmetic Act provides the US Food and Drug Administration with the authority to require manufacturers to conduct post-market surveillance of certain class II or class III devices. Although the MASS-FIX assay was validated as a lab-developed test using Clinical Laboratory Improvement Amendments guidelines, a post-test launch surveillance is important to assure patient safety. This study documents the 20-month performance characteristics of using a combination of SPEP/MASS-FIX in routine clinical care using 19,523 unique patient samples. In addition, results from a separate cohort of 52,586 unique patients tested by the traditional SPEP/IFE testing during the same period were compiled and compared with those obtained by the SPEP/MASS-FIX combination.

**PATIENTS AND METHODS**

**Subjects and Study Design**

This retrospective study was approved by the Mayo Clinic Institutional Review Board and conducted in accordance to the Declaration of Helsinki. A total of 19,523 unique patients who were assessed for various PCDs by SPEP/MASS-FIX between July 24, 2018, and March 6, 2020, were included in the study. A total of 52,586 unique patients who were assessed using the traditional SPEP/IFE during the same period were also included in the study. If a patient was tested more than once during the study period, only the first result was included in the analysis. There was no overlap in the patients between the SPEP/MASS-FIX and SPEP/IFE cohorts.

**Serum Protein Electrophoresis and Immunofixation Electrophoresis**

Serum protein electrophoresis was performed on the SPIFE 3000 electrophoresis analyzer (Helena Laboratories, Beaumont, TX) following manufacturer’s recommendations. Each SPEP test had both quantitative and qualitative impressions assigned by a lab technician. Qualitative impressions fell into the following categories based on strict criteria defined below:

1. M-spike in the gamma/beta region was assigned to the impression after ruling out the presence of fibrinogen when a significant spike was present. This qualitative impression was the only impression that was assigned an M-spike value.
2. Small abnormality was assigned when a possible low-level monoclonal protein was present but conformation by either IFE or MASS-FIX is needed.
3. Hypogammaglobulinemia was assigned when no spike or abnormality was present, and the gamma region was less than 0.6 g/dL.
4. Hypergammaglobulinemia was assigned to SPEPs with no abnormalities with a gamma region but with the gamma region greater than 1.6 g/dL.

5. No monoclonal protein was assigned when none of the above aspects were present.

M-spikes on SPEP were manually gated and quantified using the Helena QuickScan Touch system via the perpendicular drop method. Serum IFE was performed using Hydrasys 9IF gels (Sebia, Paris, France) following manufacturer’s recommendations.

**Serum Free Light Chain Measurements**

The FLC assay was performed using Freelite Reagent (The Binding Site, Birmingham, UK) on an automated nephelometer (the BNII System, Siemens Diagnostics, Germany) following an established laboratory procedure. Following independent measurements of κ and λ FLC

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**FIGURE 1.** Example MASS-FIX data showing normal and monoclonal protein patterns. A, A patient with no monoclonal protein (i.e., “normal”) was selected and the +2 charge state spectra were shown. The “G” or “A” or “M” notation within the graph signifies either total immunoglobulin G (IgG) or IgA or IgM spectra overlaid with total Ig-kappa and Ig-lambda spectra. B, A patient with IgG-kappa monoclonal protein (M-protein) was chosen and their +2 charge state spectra are shown. The peaks observed in the “normal” patient spectra are typically smooth, denoting a polyclonal pattern. Presence of an M-protein shifts the pattern towards a narrow peak. Overlapping peaks observed in a heavy chain spectrum and a light chain spectrum denotes the isotype of the M-protein (note the narrow peaks in IgG and Ig-kappa spectra in B). Figure continued on next page.
concentrations by nephelometry, the k/\(\lambda\) FLC ratio was calculated. Monoclonal \(\kappa\) LCs were assumed to be present when both the k/\(\lambda\) FLC ratio and the k concentration were both elevated (>1.65 and >1.94 mg/dL, respectively). Similarly, monoclonal \(\lambda\) LCs were assumed to be present when the k/\(\lambda\) FLC ratio was decreased and the l concentration was elevated (<0.26 and >2.63 mg/dL, respectively).

**MASS-FIX Assay**

The method was outlined in detail elsewhere. Brieﬂy, a total of 50 \(\mu\)L of serum from each patient was immuno-enriched using five separate (IgA, IgG, IgM, Kappa, and Lambda) CaptureSelect nanobody resins (Thermo-Fisher Scientific, Waltham, MA). The pre-analytical processing of immuno-enrichment, washing, reduction, and elution were performed on a Microlab StarLet liquid handler (Hamilton Company, Reno, NV). The resulting purified eluates were mixed with \(\alpha\)-cyano-4-hydroxycinnamic acid matrix and spotted onto a Bruker MALDI-TOF plate using a Mosquito nanoliter pipettor (sptlabtech, Hertfordshire, UK). Positive controls, negative controls, and mass calibrants were also spotted on the same MALDI plates. Spotted plates were loaded into a microFlex

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**FIGURE 1.** (Continued).
MALDI-TOF MS (Bruker Daltronics, Germany). Before data acquisition of patient samples, mass calibrant was analyzed to calibrate the mass spectrometer and positive and negative controls were acquired to ensure method fidelity. A laboratory technician inspected the quality control data before proceeding to patient sample data acquisition. For this, the signal intensity of quality controls was evaluated at the maximum signal for all spectra collected. A minimum threshold for a positive control was 3000 cps and 1500 cps for the normal control. Patient sample data was collected automatically, and spectra were displayed using in-house developed software. Results were interpreted by both the laboratory technician and a laboratory director.

**Testing Algorithms**

We paired MASS-FIX with SPEP, which allowed the laboratory and physicians to become accustomed to the new testing format and have the added assurance that traditional SPEP would detect all large M-spikes. We adopted two different testing algorithms (described below) to standardize the testing of patients based on the diagnostic question. The goal of these algorithms was to follow the recommendations of the IMWG for ruling out a monoclonal gammopathy while efficiently using the testing resources.\(^{16}\)

The monoclonal gammopathy screening algorithm was intended to screen patients for an M-protein and consists of three tests that were in accordance with the IMWG recommendations\(^ {14}\): the SPEP, FLC quantitation, and MASS-FIX/IFE.

**TABLE 1. The Types of M-Proteins Detected by SPEP/MASS-FIX in 19,523 Patient Samples**\(^ a \)

<table>
<thead>
<tr>
<th>Overall cohort demographics</th>
<th>MASS-FIX</th>
<th>IFE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, years(^ b )</td>
<td>Sex (F/M/U)</td>
<td>Age, Sex (F/M/U)</td>
</tr>
<tr>
<td>Negative (normal)</td>
<td>60.4±15.4</td>
<td>5897/6572/2</td>
</tr>
<tr>
<td>Positive</td>
<td>67.9±12.3</td>
<td>2972/4051/29</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Relative frequency(^ d )</th>
<th>n</th>
<th>Percent Overall</th>
<th>Percent Positive</th>
<th>n</th>
<th>Percent Overall</th>
<th>Percent Positive</th>
<th>P(^ e )</th>
</tr>
</thead>
<tbody>
<tr>
<td>No monoclonal (normal)</td>
<td>12,471</td>
<td>62.4</td>
<td>NA</td>
<td>36,139</td>
<td>68.5</td>
<td>NA</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Monoclonal - IgG</td>
<td>3094</td>
<td>15.5</td>
<td>55.3</td>
<td>8113</td>
<td>15.4</td>
<td>56.7</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Monoclonal - IgA</td>
<td>817</td>
<td>4.1</td>
<td>14.6</td>
<td>1831</td>
<td>3.5</td>
<td>12.8</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Monoclonal - IgM</td>
<td>760</td>
<td>3.8</td>
<td>13.6</td>
<td>2584</td>
<td>4.9</td>
<td>18.1</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Cannot rule out M-protein</td>
<td>1503</td>
<td>7.5</td>
<td>NA</td>
<td>2322</td>
<td>4.4</td>
<td>NA</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Light chain glycosylation</td>
<td>292</td>
<td>1.5</td>
<td>5.2</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Biclonal</td>
<td>287</td>
<td>1.4</td>
<td>5.1</td>
<td>1048</td>
<td>2.0</td>
<td>7.3</td>
<td>&lt;0.00001</td>
</tr>
<tr>
<td>Free light chain — lambda</td>
<td>188</td>
<td>0.9</td>
<td>3.4</td>
<td>395</td>
<td>0.8</td>
<td>2.8</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Free light chain — kappa</td>
<td>114</td>
<td>0.6</td>
<td>2.0</td>
<td>235</td>
<td>0.4</td>
<td>1.6</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Monoclonal IgD or E</td>
<td>25</td>
<td>0.1</td>
<td>0.5</td>
<td>41</td>
<td>0.1</td>
<td>0.3</td>
<td></td>
</tr>
<tr>
<td>Heavy chain</td>
<td>10</td>
<td>0.1</td>
<td>0.2</td>
<td>24</td>
<td>0.1</td>
<td>0.2</td>
<td></td>
</tr>
<tr>
<td>Triclonal</td>
<td>9</td>
<td>0.1</td>
<td>0.2</td>
<td>27</td>
<td>0.1</td>
<td>0.2</td>
<td></td>
</tr>
</tbody>
</table>

\( ^{a} \) F, female; IFE, immunofixation electrophoresis; Ig, immunoglobulin; M, male; NA, not available; SPEP, serum protein electrophoresis; U, unknown.

\( ^{b} \) Age is represented as a mean ± SD.

\( ^{c} \) P for age computed using Student t test, and sex distribution differences were compared using a 2×2 \( \chi^2 \) test.

\( ^{d} \) A single patient can have more than one result, such as an M-protein and a free light chain or an M-protein and a therapeutic monoclonal antibody.

\( ^{e} \) A 2×2 \( \chi^2 \) test was used to compare frequency difference of each result type between MASS-FIX and IFE.

The monoclonal gammopathy screening algorithm was intended to screen patients for an M-protein and consists of three tests that were in accordance with the IMWG recommendations\(^ {14}\): the SPEP, FLC quantitation, and MASS-FIX/IFE.

The monoclonal gammopathy monitoring algorithm was intended to monitor patients with an established diagnosis. An SPEP was performed and if a quantifiable M-protein was present, no further testing was performed;
for samples without a quantifiable M-protein, MASS-FIX/IFE was used to detect the presence of low-level M-proteins.

Statistical Methods
All statistical analyses were performed using SPSS software (version 25). Continuous variables were summarized using mean and standard deviation. The Student t test was used to compare continuous variables and 2×2 c² tests were used to compare frequency data between any two groups. A Kolmogorov–Smirnov test was used to compare the distributions of involved FLC concentrations detected in samples by MASS-FIX and IFE. A P value less than or equal to .05 was considered statistically significant in all analyses.

RESULTS
Frequency and Types of M-Proteins
Figure 1 shows basic overlaid spectra for detecting an M-protein using example Ig spectral profiles of a “normal” (ie, negative) patient (Figure 1A) and a patient with an M-protein (Figure 1B). The smooth unimodal, bimodal, and trimodal Gaussian peaks observed in normal sample reflect the healthy polyclonal Ig LC mass distributions observed for different heavy chain (IgG, IgA, and IgM) and total LC (IgK and IgL) isotypes. In contrast, patient with M-protein shows a Lorentzian-shaped spike, signaling the presence of a monoclonal Ig. The spikes detected in the IgG and IgK spectra align with each other indicative of an IgG kappa M-protein. This process of detecting abnormal spikes in the intact mass profile of measured LCs enabled efficient detection of various types of M-proteins. Table 1 summarizes the types of M-proteins detected by SPEP/MASS-FIX combination in 19,523 unique patient samples. We also analyzed 52,586 unique patients using the SPEP/IFE method during the same period; these results are also summarized in Table 1. A patient can have more than one reportable
finding in the MASS-FIX assay, such as an M-protein plus a therapeutic monoclonal antibody (t-mAb) or M-protein plus a glycosylated LC, et cetera. In the MASS-FIX cohort, 62.4% of patients had no M-protein whereas 23.4% of patients had an M-protein. The rest of the patients had various types of abnormalities (Table 1). When compared with SPEP/IFE, the MASS-FIX cohort had higher percentages of the following M-protein types: IgA and FLCs (Table 1). In contrast, the MASS-FIX cohort had lower numbers of IgM and biclonal M-proteins (Table 1).

**MASS-FIX Detection of Therapeutic Monoclonal Antibodies**

Therapeutic monoclonal antibodies are increasingly being used to treat PCDs.\(^{17-19}\)
MASS-FIX uses the unique masses of t-mAb LC to detect their possible presence in patient samples. Figure 2 shows example spectral profiles of patients with confirmed t-mAb administration. MASS-FIX was initially set up for detecting four different t-mAbs (shown in Figure 2). In several cases, MASS-FIX could distinguish between patient’s M-protein and a t-mAb (example in Figure 2C showing rituximab peaks distinct from the patient’s M-protein). We implemented this method by enabling the MASS-FIX software to place markers on patient spectra where the m/z of the t-mAb kappa LC was expected to appear. If a pathologist detected an M-protein within a t-mAb marker, the history of the patient was assessed to verify the presence or absence of the t-mAb. Using this approach, we identified the following t-mAbs in a total of 402 patients (5% of positive cases): daratumumab (n=346, 86.1%), elotuzumab (n=42, 10.5%), rituximab (n=11, 2.7%), and isatuximab (n=3, 0.8%).

### MASS-FIX Detection of Light Chain Glycosylation

N-linked glycosylation of Ig LC is increasingly being linked to higher risk of AL, CAD, and progression of monoclonal gammopathy of undetermined significance (MGUS). Glycosylation of the M-protein LC manifests as distinctly broad and jagged peaks on MASS-FIX (example spectral profiles shown in Figure 3). We augmented MASS-FIX software to mark areas of glycosylated LCs and a pathologist can make a call when these distinct peaks are present in a sample. Following this method, we detected LC glycosylation in a total of 292 patients (5.2% of positive patients). Table 2 shows different M-protein types with glycosylated LCs detected in this cohort wherein a majority of them are of IgG kappa isotype. These high-risk markers are not amenable for detection by IFE.

### Confirming SPEP Abnormalities

Table 1 showed that MASS-FIX had a higher positivity rate when compared with IFE. However, the MASS-FIX cohort consisted of PCD patients seen at Mayo Clinic and the IFE cohort were non-Mayo Clinic patients tested for PCDs. We wanted to compare MASS-FIX and IFE by using the two tests that are common to both cohorts: SPEP and FLC. The SPEP results of both MASS-FIX and IFE cohorts were compared using the different qualitative impressions: no monoclonal protein, M-protein, small abnormality, hypogammaglobulinemia, and hypergammaglobulinemia (details in

### Table 2. M-Protein Types With Glycosylated LCs Detected

<table>
<thead>
<tr>
<th>Glycosylated LC</th>
<th>Kappa</th>
<th>Lambda</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG</td>
<td>132 (74.2)</td>
<td>46 (25.8)</td>
</tr>
<tr>
<td>IgA</td>
<td>16 (66.7)</td>
<td>8 (33.3)</td>
</tr>
<tr>
<td>IgM</td>
<td>68 (91.9)</td>
<td>6 (8.1)</td>
</tr>
<tr>
<td>Free</td>
<td>8 (50.0)</td>
<td>8 (50.0)</td>
</tr>
</tbody>
</table>

*IG, immunoglobulin; LC, light chain.

### Table 3. The Detection Rate of a Monoclonal Protein by MASS-FIX and Traditional IFE

<table>
<thead>
<tr>
<th>SPEP interpretation</th>
<th>MASS-FIX</th>
<th>IFE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Normal</td>
<td>Positive</td>
<td>%Positive</td>
</tr>
<tr>
<td>Normal</td>
<td>10,567</td>
<td>2,376</td>
<td>18.4</td>
</tr>
<tr>
<td>M-spike</td>
<td>0</td>
<td>1,625</td>
<td>100.0</td>
</tr>
<tr>
<td>Small Abnormality</td>
<td>169</td>
<td>1,579</td>
<td>90.3</td>
</tr>
<tr>
<td>Hypo</td>
<td>464</td>
<td>6,601</td>
<td>58.7</td>
</tr>
<tr>
<td>Hyper</td>
<td>992</td>
<td>1,972</td>
<td>16.6</td>
</tr>
</tbody>
</table>

*IFE, immunofixation electrophoresis; SPEP, serum protein electrophoresis.

A total of 1343 patients in the MASS-FIX cohort did not have SPEP interpretation available and were excluded from comparison. A 2×2 χ² was used to compare frequency difference of each protein electrophoresis interpretation between MASS-FIX and IFE.
Methods section above). Table 3 displays the detection rate of a monoclonal protein by MASS-FIX and traditional IFE for each of the aforementioned SPEP categories. Excluding the cases with a quantifiable M-protein where both MASS-FIX and IFE detected an M-protein in all cases, MASS-FIX detected a statistically significant higher percentage of M-protein-positive cases in the rest of the categories when compared with IFE. Of note, MASS-FIX detected M-protein in a higher percentage of cases with small SPEP abnormalities (ie, suspicious of M-protein presence). These data suggest that MASS-FIX is more sensitive in detecting Ig abnormalities observed on SPEP.

**Confirming an Abnormal FLC Result**

An abnormal FLC ratio accompanied with a concentration of k-FLC or l-FLC above the reference level is accepted as a clinical standard to detect the presence of an FLC M-protein.\(^2\)

**FIGURE 4.** MASS-FIX versus immunofixation electrophoresis (IFE) detection of M-protein in free light chain (FLC) abnormal samples. Bence Jones (BJ) proteinemia patients with an abnormal FLC test (either abnormally low FLC κ/λ ratio and abnormally high λ-FLC quant or abnormally high FLC κ/λ ratio and abnormally high κ-FLC quant) were taken from MASS-FIX and IFE cohorts for this analysis. Quantitative involved FLC (FLC) values were shown. Red lines represent median values for the groups. Equality of distributions was tested using a Kolmogorov-Smirnov test. An asterisk (*) indicates \(P<.05\), and four asterisks (****) indicate \(P<.00001\).

**TABLE 4.** The Number of Samples Repeated (N=20,000)

<table>
<thead>
<tr>
<th>Repeat category</th>
<th>n (%)</th>
<th>Repeat type</th>
<th>n (%) of repeat type</th>
</tr>
</thead>
<tbody>
<tr>
<td>Re-spot</td>
<td>69 (20.1)</td>
<td>Poor signal(^1), resolved by re-spot</td>
<td>69 (100.0)</td>
</tr>
<tr>
<td>Reprocess</td>
<td>274 (79.9)</td>
<td>Confirm initial spectra</td>
<td>33 (12.2)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poor signal, re-spot attempted</td>
<td>10 (3.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Poor signal, straight to repeat</td>
<td>88 (32.5)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nonspecific binding(^2), resolved with 1:10 dilution</td>
<td>87 (31.8)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Nonspecific binding, not resolved with 1:10 dilution</td>
<td>7 (2.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Interference</td>
<td>7 (2.6)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Liquid handler error</td>
<td>41 (15.1)</td>
</tr>
</tbody>
</table>

\(^1\)Poor signal is defined as noisy spectra lacking signal for light chains.

\(^2\)Nonspecific binding refers to samples with quantitatively large M-spikes that result in immunoglobulin protein binding to all five types of immunopurification beads irrespective of its heavy chain and light chain isotype. Prediluting and repeating the isolation resolved the nonspecific binding in 93% of cases.
We wanted to compare the performance of MASS-FIX and IFE when detecting an M-protein in patients with an abnormal FLC. For this, we assembled a subcohort of 13,150 MASS-FIX and 10,166 IFE patients who had the FLC test ordered. To exclude the effect of quantitatively large M-proteins (ie, >1 g/dL) on the analysis, we only included patients whose M-proteins were categorized as FLC only or Bence Jones (BJ) proteinemia by IFE or MASS-FIX. This resulted in a total of 147 and 112 BJ-κ and BJ-λ protein-positive cases tested by MASS-FIX whereas the IFE subcohort contained 116 and 97 positive cases, respectively. The FLC result was classified into two categories: abnormally low FLC k/l ratio and an abnormally high I-FLC quantitative value (ie, indicates presence of an abnormal I-FLC) and abnormally high FLC k/l ratio and an abnormally high k-FLC quantitative value (ie, indicates presence of an abnormal kFLC). Figure 4 summarizes the FLC k and l quantitative data observed for each M-protein type (ie, BJ-I and BJ-k) in MASS-FIX and IFE cohorts.

The distribution of I-FLC quantitation for MASS-FIX BJ-I cases was positively skewed (ie, more data points in lower quartiles) when compared with that of IFE BJ-I cases (Kolmogorov-Smirnov test, $P=0.047$). We observed the same phenomenon for the distribution of k-FLC quantitation in MASS-FIX versus IFE BJ-k cases (Kolmogorov-Smirnov test, $P=4.265E-7$). These data indicate that the MASS-FIX test was superior in detecting lower concentrations of M-proteins when compared with IFE.

**MASS-FIX Had Low Sample Repeat Rate**

Sample repeat rates are an important metric to assess the feasibility of a test for clinical implementation. The MASS-FIX test development and verification process suggested that the sample repeat rate would be below 5%. To assess this, we tracked the sample repeats and reasons for repeat since test implementation. Table 4 shows the number of samples repeated and reasons for repeat for the first 20,000 consecutive specimens analyzed by MASS-FIX. Overall, the sample repeat rate was 1.7%. However, 20.1% of the repeats were due to the presence of the low signal on MALDI-TOF analysis (Table 4). In such cases, the first step in repeating was to re-spot the prepared sample on the MALDI-TOF plate. A complete reprocessing of sample was performed in 79.9% of repeated samples when re-spotting did not improve the MALDI-TOF signal. When re-spot repeats were excluded, true percentage of sample repeats was 1.4%, which is well within the acceptable range for an assay with large sample volumes.

**MASS-FIX Has Higher Testing Efficiency Than IFE**

We implemented MASS-FIX in a reference laboratory alongside IFE. Our clinical implementation of MASS-FIX was highly automated and supported by specialized software, as described previously. One laboratory technician was able to process up to 320 samples per 8-hour shift using single setup of MASS-FIX instrumentation. When using four IFE instrumentation setups described in the methods (to maximize efficiency), one laboratory technician was able to process 224 samples per 8-hour shift. This 30% gain in testing efficiency for MASS-FIX is appreciable for a test with large sample volumes.

**DISCUSSION**

For more than 40 years, gel electrophoresis has been the primary platform for detecting M-protein in patients with PCDs. Technological advances in intact mass measurement of proteins have enabled the development of a nanobody—MALDI-TOF—MS-based method (tMASS-FIX) for assaying M-protein. MASS-FIX has definitive advantages over the traditional IFE method used in routine laboratories. A prior study showed that MASS-FIX can track an M-protein to a level that is an order of magnitude lower than IFE. Direct mass measurement of a M-protein’s LC in conjunction with improved resolution confers increased specificity to track disease-related M-proteins and distinguish them from clones arising due to bone marrow repopulation and t-mAbs. The use of mass measurements also allowed us to detect a previously underrecognized aspect of
M-proteins, LC glycosylation that has been shown to be a risk factor for development of a PCD. These distinctive analytical advantages of MASS-FIX have led us to implement the method for clinical use in a reference laboratory at Mayo Clinic.

The results of our pretest implementation validation were verified in this retrospective study. Overall, MASS-FIX had a higher positivity rate than IFE (Table 1). This could be potentially attributed to the patient population differences between MASS-FIX and IFE cohorts. Previous studies have shown that the Mayo Clinic PCD patient cohort is enriched for rare PCDs such as AL, which could be underrepresented in the non–Mayo Clinic patient-based IFE cohort. However, samples from both patient cohorts started with SPEP before reflexing to either MASS-FIX or IFE. The laboratory staff members were blind to the reflex method (MASS-FIX or IFE) to be used for a patient sample. This allowed us to compare the performance of MASS-FIX and IFE with SPEP impression as the common factor (Table 3). Both MASS-FIX and IFE detected an M-protein in 100% of SPEP samples with an M-spike (Table 3). However, MASS-FIX confirmed a higher percentage of non–M-spike abnormalities than IFE (ie, small abnormality, hypogammaglobulinemia, and hypergammaglobulinemia) (Table 3). In addition, the distribution of FLC concentrations detected in MASS-FIX–positive BJ proteinemia cases was lower than that of IFE-positive BJ proteinemia cases (Figure 4), which was consistent with a previous study. Both data suggest that clinical MASS-FIX was more sensitive, if not equivalent, in detecting M-proteins when compared with IFE.

MASS-FIX detected a higher percentage of IgA M-proteins when compared with IFE (Table 1). This could be attributable to the differences in patient cohorts. However, when we compared the percentage of IgA M-proteins detected by our laboratory from 1960 to 2017 (before MASS-FIX implementation), the percentage of IgA M-proteins detected by MASS-FIX was still higher than the historical data, which agreed with the IFE cohort (Supplemental Table 1, available online at http://www.mayoclinicproceedings.org). We observed that IgA M-proteins tend to have relatively broader migration patterns on IFE requiring higher levels before detection. This could be linked to the highly variable glycosylation of the IgA heavy chains. Because MASS-FIX disassociates heavy chains from LCs before to analyzing the mass profile of the LCs, heavy chain glycosylation does not affect the detectability of IgA M-proteins by MASS-FIX. This could explain the increased detection of IgA M-proteins by MASS-FIX when compared with IFE. Studying this phenomenon further, albeit out of scope in this study, is important as previous studies have shown that IgA MGUS patients are at a higher risk for developing PCDs. If MASS-FIX is detecting lower levels of IgA M-proteins, this could potentially result in earlier detection of high-risk MGUS PCD patients with IgA M-proteins.

Therapeutic monoclonal antibodies are increasingly being used for treating PCDs. A t-mAb in a treated patient sample could be falsely attributed as an M-protein by electrophoresis. Confirming t-mAbs via IFE requires additional testing and is currently limited to one t-mAb. This process is time consuming for a large-volume reference laboratory. At the same time, t-mAbs are increasingly being used for treating PCDs, with new drugs coming on the market every year. Because MASS-FIX measures the mass of M-protein LC, it can indicate the presence of a t-mAb in a sample (Figure 2). Using this approach, we detected four different types of t-mAbs present in ~5% of the positive cases. A recent study showed that MASS-FIX can directly detect t-mAbs in 87% of the candidate cases containing a t-mAb. In the remaining cases, the low-resolution MALDI-TOF of MASS-FIX was unable to resolve the mass of a suspected t-mAb spike; these cases were resolved by analyzing the isolates on a higher-resolution mass spectrometer. Because MASS-FIX uses LC masses to detect t-mAbs, the method is easily extensible to detect new t-mAbs. This ability to directly detect t-mAbs is distinctly advantageous for both patient care and clinical trial use.
The presence of glycosylated LCs in patient serum is increasingly being linked to a wide variety of aggressive PCDs (ie, diseases such as primary CAD, etc). A recent study also linked the presence of monoclonal LC glycosylation in patients with MGUS to the risk of progression to AL, myeloma, and other PCDs. Hence, detecting these high-risk markers is beneficial for better prognostic assessment and personalized therapy of high-risk MGUS patients. MASS-FIX can readily identify these markers via a distinctive, high-mass, irregular, broad peak profile they produce when analyzed by MASS-FIX (Figure 3). Using this technique, we detected LC glycosylation in ~4% of the positive patients. A recent study established the profile of different PCDs detected in patients with glycosylated LCs.

MASS-FIX has more analytical steps than IFE, making it arguably more cumbersome to implement in a clinical laboratory. However, MASS-FIX is highly automatable, which produced a 30% gain in testing efficiency over traditional IFE (measured as number of tests/tech/8-hour shift). Automation also resulted in very low sample repeat rates (<1.5%). Besides automation, another crucial factor for successful implementation is the ability of laboratory staff to assimilate interpretative knowledge of MASS-FIX spectra. The core, high-frequency, patterns observed by MASS-FIX are easier to grasp (Figures 1-3). We implemented a continuous training process for the laboratory staff and clinical pathologists wherein a library of MASS-FIX patterns and their corresponding interpretation(s) was created. Staff members were trained with this library before they were tasked with interpreting MASS-FIX cases.

As myeloma therapies are becoming increasingly effective, there is an increasing interest in a blood-based assay to assess minimal residual disease (MRD). MASS-FIX was neither intended nor specifically designed for MRD assessment. Researchers have developed MS-based methods that look for clonotypic peptides of the M-protein for MRD assessment. These targeted methods require the knowledge of, or the ability to directly infer, the M-protein's amino acid sequence that is idiosyncratic to each patient to configure the assays. This creates challenges when implementing clonotypic peptide-based MRD assays in a high sample volume reference laboratory. Nevertheless, clonotypic peptide-based MRD assays provide the most sensitive blood-based platforms to date for detecting MRD with MASS-FIX serving as a viable alternative (with higher levels of limit of detection).

There are few additional limitations for this study. An ideal study would have been to analyze the same set of patient samples using MASS-FIX and IFE. However, logistical, and fiscal issues made it unfeasible to conduct such a study on tens of thousands of samples. Another possible critique could be that MASS-FIX may be overly sensitive in detecting clinically significant M-proteins when compared with IFE (as potentially evidenced by higher MASS-FIX positivity rates in hypergammaglobulinemia and hypogammaglobulinemia patients when compared with IFE). However, these additional positives detected by MASS-FIX have never been found before when using IFE. Hence, we sought to establish clinical significance of these cases. Of 857 hyper/hypo cases with a positive MASS-FIX (Table 3), a total of 410 cases had clinical workup within ±6 months of MASS-FIX positivity. Supplemental Table 2 (available online at http://www.mayoclinicproceedings.org) shows the list of diagnoses made for these cases. All but one case had a PCD diagnosis. A total of 175 patients (of 410 cases; 42.7%) had a clinically significant diagnosis (ie, not normal or MGUS). This stands in contrast to an IFE-based study wherein 8.3% of
hypogammaglobulinemia patients with IFE M-spike had a clinically significant diagnosis. These data suggest that higher rates of MASS-FIX positivity observed in this study is associated with making clinically significant diagnosis. A similar analysis for IFE-positive hyper/hypo cases was unavailable because those were not Mayo Clinic patients (a limitation). Despite these limitations, the data presented in this study indicate that MASS-FIX is an effective replacement for IFE when assessing M-proteins in clinical practice.

CONCLUSION
MASS-FIX, as described here, is an MS-based method for detecting and isotyping serum M-proteins. This method was implemented in a high sample volume reference laboratory for routine clinical use. MASS-FIX was highly effective in detecting M-proteins when compared with IFE. In addition, MASS-FIX showed inherent ability to detect t-mAbs and pathogenic glycosylation of LCs in patient samples. Automatability and ease of interpretation enabled MASS-FIX to replace serum IFE in a reference laboratory. Hence, we conclude that MASS-FIX can optimally support the diagnosis and care of PCD patients in a routine clinical practice.

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SUPPLEMENTAL ONLINE MATERIAL
Supplemental material can be found online at http://www.mayoclinicproceedings.org. Supplemental material attached to journal articles has not been edited, and the authors take responsibility for the accuracy of all data.

Abbreviations and Acronyms: AL, light chain amyloidosis; BJ, Bence Jones protein; CAD, cold agglutinin disease; FLC, free light chain; IFE, immunofixation electrophoresis; IMWG, International Myeloma Working Group; LC, immunoglobulin light chain; MALDI-TOF, matrix-assisted laser desorption/ionization time of flight; MGUS, monoclonal gammopathy of uncertain significance; PCD, plasma cell disorder; SPEP, serum protein electrophoresis; t-mAb, therapeutic monoclonal antibody

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REFERENCES
M-PROTEIN DETECTION BY MASS SPECTROMETRY


