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Laboratory Detection and Initial Diagnosis of Monoclonal Gammopathies

Guideline From the College of American Pathologists in Collaboration With the American Association for Clinical Chemistry and the American Society for Clinical Pathology

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• ***Context.***—**The process for identifying patients with monoclonal gammopathies is complex. Initial detection of a monoclonal immunoglobulin protein (M protein) in the serum or urine often requires compilation of analytical data from several areas of the laboratory. The detection of M proteins depends on adequacy of the sample provided, available clinical information, and the laboratory tests used.**

Objective.—**To develop an evidence-based guideline for the initial laboratory detection of M proteins.**

Design.—**To develop evidence-based recommendations, the College of American Pathologists convened a panel of experts in the diagnosis and treatment of monoclonal gammopathies and the laboratory procedures used for the initial detection of M proteins. The panel conducted a systematic literature review to address key questions. Using the Grading of Recommendations Assessment, Development, and Evaluation approach, recommendations were created based on the available evidence, strength of that evidence, and key judgements as defined in the Grading of Recommendations Assessment, Development, and Evaluation Evidence to Decision framework.**

Results.—**Nine guideline statements were established to optimize sample selection and testing for the initial detection and quantitative measurement of M proteins used to diagnose monoclonal gammopathies.**

Conclusions.—**This guideline was constructed to harmonize and strengthen the initial detection of an M protein in patients displaying symptoms or laboratory features of a monoclonal gammopathy. It endorses more comprehensive initial testing when there is suspicion of amyloid light chain amyloidosis or neuropathies, such as POEMS (polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes) syndrome, associated with an M protein.**

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Monoclonal gammopathies (MGs) represent a spectrum of disorders with varying manifestations ranging from a completely asymptomatic condition picked up on laboratory testing to a catastrophic, life-threatening presentation in the clinic. Monoclonal gammopathy of undetermined significance (MGUS) is the most common category, is asymptomatic, and requires no intervention in the vast majority of cases. On the other hand, multiple myeloma (MM) and light chain amyloidosis represent the 2 most common parts of the spectrum that require clinical intervention. In the last decade of the 20th century, the median overall survival of patients with MM was about 3 years for those who received chemotherapy. When chemo-

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Expert panel member Harold H. Harrison, MD, died June 6, 2018.

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Authors' disclosures of potential conflicts of interest and author contributions are found in the Appendix at the end of this article.

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Table 1. Categories of Monoclonal Gammopathy (N = 39 929)[a]

Plasma cell proliferative disorders
Multiple myeloma (17.5%; n = 6794)
Lymphoproliferative disease (3.3%; n = 1298)
Waldenström macroglobulinemia (2.4%; n = 940)
Plasmacytoma (1.9%; n = 774)
Plasma cell leukemia (0.2%; n = 90)
Heavy chain disease (0.1%; 31)
Protein/low tumor burden diseases
Primary amyloidosis (9.5%; n = 3781)
Cryoglobulinemia (0.9%; n = 379)
POEMS syndrome (0.5%; n = 217)
Light chain deposition disease (0.3%; n = 113)
Cold agglutinin disease (0.2%; n = 74)
Acquired Fanconi syndrome (0.1%; n = 43)
Scleromyxedema (0.1%; n = 31)
Benign hypergammaglobulinemia purpura of Waldenström (0.1%; n = 31)
Capillary leak syndrome (0.1%; n = 29)
Premalignant
Monoclonal gammopathy of undetermined significance (58.0%; n = 23 179)
Smoldering (asymptomatic) multiple myeloma (3.7%; n = 1494)
Bence Jones proteinuria (1.1%; n = 450)

Abbreviation: POEMS, polyneuropathy, organomegaly, endocrinopathy, monoclonal protein, skin changes.

[a] Data derived from Willrich MA, Katzmann JA. Laboratory testing requirements for diagnosis and follow-up of MM and related plasma cell dyscrasias. *Clin Chem Lab Med*. 2016;54(6):907–919.[103]

therapy was combined with autologous stem cell transplantation, median overall survival grew to about 5 years.[1,2] The first decade of the 21st century witnessed dramatic changes in how MGs were categorized (Table 1) and treated. Indeed, the overall approach to these disorders has changed to include evaluation of early intervention in asymptomatic stages (eg, smoldering MM [SMM]) and screening of high-risk populations (eg, African American or those with family history of MM).[3]

From 2001 to 2012, the use of novel therapy (eg, proteasome inhibitors: bortezomib, carfilzomib, ixazomib; immunomodulatory agents: thalidomide, lenalidomide, pomalidomide) together with autologous stem cell transplantation lengthened median overall survival to 8 to 10 years.[1,2] Those studies were published 3 years prior to the advent of therapeutic monoclonal antibodies (eg, daratumumab and elotuzumab) for treatment of refractory MM.[4,5] Ongoing research such as deploying chimeric antigen receptor T cell therapy augurs further improvement in outcomes.[6–9]

Clinical laboratory methods to facilitate screening and diagnosis of patients with MGs advanced during the first year of the 21st century with implementation of the serum free light-chain (sFLC) assay.[10] This was followed by the establishment of a diagnostic interval for the ratio of serum free κ to serum free λ (rFLC) with relatively high specificity by using the highest (1.65) and lowest (0.26) ratio found among the 282 normal control samples rather than using the traditional 2 SD, which would have resulted in a 5% false-positive rate.[11] Combining the sFLC technique with protein electrophoresis, immunofixation electrophoresis (IFE), and immunosubtraction (ISUB) to identify, measure, and characterize monoclonal immunoglobulin protein (M protein) in the serum or monoclonal free light chain (MFLC) in urine effectively stratifies the risks for patients with MGUS or SMM progressing to MM or related malignancy.[12–14] These immunochemical methods, together with bone marrow plasma cell enumeration and new imaging procedures, facilitated the revised International Myeloma Working Group (IMWG) classification of MM, SMM, and MGUS, allowing asymptomatic high-risk patients to benefit from earlier deployment of modern treatment.[13]

A wide variety of laboratory procedures are now available to detect M proteins, including gel and capillary serum and urine protein electrophoresis (SPEP and UPEP), serum and urine IFE (sIFE and uIFE), ISUB, sFLC, mass spectrometry, and heavy/light chain (HLC) isotype quantitative measurement. Lacking an evidence-based guideline from a systematic review, laboratories have developed disparate practices for M-protein detection and quantitative measurement, complicating harmonization of results.

In 2017, Genzen et al[15] reported the results from a survey of 774 laboratories across 38 countries quantifying the various patterns of use of immunochemical methods for detecting M protein. The findings indicated wide variations in practice that, when compared with current IMWG guidelines and recent studies, could interfere with initial detection of individuals at high risk for progression. The wide variety of conditions producing M protein need to be considered when determining how screening for their initial detection is performed (Table 1).

Consequently, the College of American Pathologists (CAP) established an expert panel (EP) to create a contemporary guideline as a first step to harmonizing the initial detection and quantitative measurement of M proteins. The EP included broad representation of individuals who are experts in the diagnosis and treatment of MGs and the laboratory procedures used for their initial detection. For this EP, the CAP collaborated with the American Association for Clinical Chemistry and the American Society for Clinical Pathology, and included representatives from the American Society of Hematology and the International Myeloma Foundation's International Myeloma Workgroup.

METHODS

This evidence-based guideline was developed following the standards endorsed by the National Academy of Medicine. A detailed description of the methods and the systematic review (including the quality assessment and complete analysis of the evidence in Supplemental Tables 1 through 8) used to create this guideline can be found in the supplemental digital content (SDC).

Panel Composition

Each collaborating organization nominated representatives to the EP. The CAP approved the appointment of the members. Detailed information about the panel composition can be found in the SDC.

Conflict of Interest Policy

In accordance with the CAP conflict of interest policy (October 2017), expert and advisory panel members disclosed all financial interests from 24 months prior to appointment through the time of guideline publication. Individuals were instructed to disclose any relationship that could be interpreted as constituting an actual, potential, or apparent conflict. Complete disclosures of the EP members/authors are listed in the Appendix. The majority of EP

members (8 of 12) were assessed as having no relevant conflicts of interest. The CAP provided funding for the administration of the project; no industry funds were used in the development of the guideline. All panel members volunteered their time and were not compensated for their involvement, except for the contracted methodologist. Please see the SDC for full details on the conflict of interest policy.

OBJECTIVES

The EP addressed the overarching question, "What are the specimen requirements and appropriate tests needed for the initial laboratory detection of M proteins?" This led to the following key questions:

1. What specimens are useful in the detection of M proteins?
2. What are the appropriate tests needed to accurately detect M proteins?
3. What are the appropriate tests needed to accurately quantify M proteins?

See Supplemental Table 1 for a detailed description of the key questions.

Outcomes of Interest

The primary outcomes of interest included laboratory performance, clinical outcomes, complication rates, and risk stratification. Laboratory data and test performance include diagnostic test characteristics, sensitivity and specificity of testing methods, and accuracy of detection of M protein. Clinical outcomes included overall survival, disease-free survival, progression-free survival, recurrence-free survival, time to recurrence, and response to therapy (eg, complete or partial response). Complications included the EP's assessment of the clinical impact of a false-negative or a false-positive MG diagnosis. Risk stratification outcomes included assessment of factors that predicted whether a patient with MGs would progress to MM or a B-cell lymphoproliferative disorder. See the SDC for a detailed description of outcomes of interest (Supplemental Table 1).

Literature Search and Collection

Literature search strategies were developed in collaboration with a medical librarian for the concepts of MGs, specimen type, diagnosis, and ancillary testing. In consultation with the EP, the search strategies were created using standardized database terms and text words. Databases searched included Ovid MEDLINE (segment PPEZV) and Embase.com. Additional searches for literature from other sources such as government documents, policy statements, and issue papers (aka gray literature) were run in the Cochrane Library, National Guideline Clearinghouse, ClinicalTrials.gov, and Trip search engine, and on applicable US and international organizational Web sites. Initial searches were completed on January 31, 2018, and refreshed in MEDLINE and Embase on January 29, 2019.

All searches were limited to English and from January 1, 2008, to the date of search. Case reports, commentaries, editorials, and letters were excluded. The Cochrane search filter for humans was applied in PubMed and Embase.com. MEDLINE and conference abstract records were excluded in the Embase search. The detailed search strategy and the Preferred Reporting of Systematic Reviews and Meta-analyses (PRISMA[16]) chart are provided (Supplemental Figures 1 and 2).

Inclusion Criteria

Studies were selected for inclusion in the systematic review of evidence if they met the following criteria: (1) the study population consisted of patients with clinical features raising consideration for MGs, including MGUS, MG of renal significance, light chain MM, nonsecretory MM, SMM, heavy chain disease, amyloid light chain (AL) amyloidosis, Waldenström macroglobulinemia (WM), solitary plasmacytoma, or polyneuropathy, organomegaly, endocrinopathy, M protein, and skin changes (POEMS) syndrome; (2) the study evaluated the use of serum and/or urine for accurate detection of an M protein and the ability of ancillary testing to diagnose and/or stratify the risk of progression for patients with MG; (3) the study included one of the following outcomes: accuracy of diagnosis; risk stratification; rate of appropriate treatment; time to appropriate treatment; diagnostic test accuracy; patient survival outcomes; patient experience, quality of life, or complication rates; concordance between intervention and the standard of care; and appropriate use of samples, correct test selection, testing efficiency, and/or test turnaround time; and (4) the study was a peer-reviewed full-text article.

Exclusion Criteria

Articles were excluded from the systematic review if they were conference abstracts that were not published in peer-reviewed journals; qualitative studies, including editorials, commentaries, case reports, narrative reviews, consensus documents, and letters; included fewer than 30 patients per study arm or fewer than 10 confirmed MG cases; studies in animal models or cell lines; full text articles that were not available in English; or studies that did not report on outcomes of interest. Detailed information about the exclusion criteria is available in the SDC.

Quality Assessment

Each study received a risk of bias assessment and each recommendation an aggregate assessment of the strength of evidence (Table 2). Refer to the SDC for definitions of the strength of evidence (Supplemental Table 2), individual study quality assessment (Supplemental Tables 4 through 7), and aggregate strength of evidence assessment for each guideline statement (Supplemental Table 8).

Assessing the Strength of Recommendations

Development of recommendations required that the panel review the identified evidence and make a series of key judgments using the Grading of Recommendations Assessment, Development and Evaluation[17] approach. See Table 3 for the definitions of strength of recommendation. Supplemental Table 9 found in the SDC provides a summary with total vote tallies of the key judgments the panel considered, including the benefits and harms of each guideline statement, using the Grading of Recommendations Assessment, Development, and Evaluation Evidence to Decision (EtD) framework.[18]

RESULTS

A total of 5199 unique studies met the search term requirements. Based on a screening of titles and review of abstracts from these studies, 232 articles met the inclusion criteria and continued to full-text review. A total of 60 articles were included for qualitative analysis and potential data extraction, and 25 studies provided data that informed

Table 2. Strength of Evidence[a]	
Designation	**Description**
High	There is high confidence that available evidence reflects true effect. Further research is very unlikely to change the confidence in the estimate of effect. Included studies will be of high or intermediate quality
Moderate	There is moderate confidence that available evidence reflects true effect. Further research is likely to have an important impact on the confidence in estimate of effect and may change the estimate. Included studies will be of intermediate or low quality
Low	There is limited confidence in the estimate of effect. The true effect may be substantially different from the estimate of the effect. Included studies will be of low quality
Very low	There is very little confidence in the estimate of effect. The true effect is likely to be substantially different from the estimate of effect. Any estimate of effect is very uncertain. Included studies will be of low or very low quality

[a] Data derived from Grading of Recommendations Assessment, Development and Evaluation (GRADE) working group materials.[17]

the recommendations. Data were not extracted from excluded articles, but these were available as discussion or background references. Additional information about the systematic review is available in the SDC, including a PRISMA[16] table outlining details of the review.

The EP convened 8 times (6 times by teleconference and 2 in-person meetings) to develop the scope, draft recommendations, review and respond to solicited feedback, and assess the quality of evidence that supported the final recommendations. A nominal group technique was used for consensus decision-making to encourage unique input with balanced participation among group members. An open comment period was posted on the CAP Web site (www.cap.org) from January 30, 2019, to February 22, 2019, during which the draft recommendation statements were posted for public feedback. The EP approved the final recommendations with a supermajority vote after review of the feedback from the advisory panel. Refer to the SDC for more details. An independent review panel, masked to the EP and vetted through the conflict of interest process, recommended approval by the CAP Council on Scientific Affairs. The final recommendations are summarized in Table 4.

Guideline Statements

1. Strong Recommendation.—Clinical care providers should order both SPEP and sFLC for the initial detection of M protein in all patients with suspected MG.

The strength of evidence to support this guideline statement is *moderate*.

Serum protein electrophoresis has been a traditional and reliable screening test for M protein detection since its early use with moving-boundary electrophoresis in the 1930s.[19] The advent of zone electrophoresis using filter paper and later cellulose acetate electrophoresis broadened its use worldwide in mid–20th-century clinical laboratories.[20–22] Despite its ubiquitous deployment and later improvements in resolution with agarose gel and capillary electrophoresis,[23,24] traditional SPEP is limited in its ability to detect MFLCs, which are the sole products in about 15% to 20% of patients with MM.[25] If a cryoglobulin is suspected, the serum should be drawn, transported, and processed under conditions to maintain the sample at 37°C. Detection of MFLC had been accomplished early on by the Bence Jones protein test, which used variations of the acidified heat test of urine originally described by Henry Bence Jones.[26] The insensitivity and lack of specificity of that method led to the use of UPEP and uIFE on concentrated urine to detect and characterize MFLC.[27] Unfortunately, urine assays remain underused, at least partially because of the challenge of collecting 24-hour samples, even though an early-morning void could substitute for the initial screen.[28]

The quantitative measurement of sFLC was first introduced by Bradwell et al[10] in 2001, and using the high specificity range for the rFLC (0.26–1.65) established in 2002 by Katzmann et al[11] has revolutionized this field. The free light chain test has been shown to have clinical significance for screening, risk stratification, monitoring, and response assessment.[29]

Katzmann et al[30] demonstrated that the combined use of SPEP and rFLC identified 94.3% (n = 1770 of 1877) of M proteins in 1877 patients (100% [n = 467] for MM, 100% [n = 26] for WM, and 88.7% [n = 465 of 524] for MGUS). McTaggart et al[31] reported that using SPEP only as a screen had a 94.4% sensitivity; this increased to 100% sensitivity by adding rFLC, whereas the addition of UPEP gave a smaller increase in sensitivity to 96.1% (n = 2799). Combined use of SPEP and sFLC is effective in the initial assessment of MG and is recommended in the IMWG guideline for the evaluation of MM and related disorders.[32] In its recommendation, IMWG also includes sIFE. Though some laboratories do not currently perform the sFLC assay, the EP concurred on the importance of this test for the initial detection of M

Table 3. Strength of Recommendation[a]		
Designation	**Recommendation**	**EtD Judgement**
Strong recommendation	Recommend for or against a particular practice (can include "must" or "should")	Supported by assessment with the GRADE EtD framework showing EP consensus of judgements directed to the far right or far left poles of the framework
Conditional recommendation	Recommend for or against a particular practice (can include "should" or "may")	Supported by assessment with the GRADE EtD framework showing EP consensus of judgements directed toward the center of the framework or with a dispersed pattern

Abbreviations: EP, expert panel; EtD, Evidence to Decision; GRADE, Grading of Recommendations Assessment, Development and Evaluation.

[a] Data derived from Grading of Recommendations, Assessment, Development and Evaluation Working Group materials.[17,18]

Table 4. Summary of Guideline Statements	
Guideline Statement	**Strength of Recommendation**
1. Clinical care providers should order both SPEP and sFLC for the initial detection of M protein in all patients with suspected MG	Strong
2. Laboratorians should confirm an SPEP abnormality suspicious for a presence of a M protein with additional testing by sIFE or alternative method with similar sensitivity	Strong
3. Laboratorians and/or clinical care providers should follow up an abnormal sFLC ratio for the presence of a M protein with an sIFE or alternative method with similar sensitivity	Conditional
4. Clinical care providers should order SPEP, sFLC, sIFE, and uIFE for the initial detection of M protein in all patients with suspected AL amyloidosis	Strong
5. Clinical care providers should NOT order HLC for initial detection of M protein in patients with suspected MG	Strong
6. Clinical care providers should NOT use total/intact light chains for the quantitation of M proteins in patients with suspected myeloma	Strong
7. In patients with intact M proteins outside the γ region by SPEP, laboratories should use total immunoglobulin (IgA, IgG, or IgM) for the quantitation of the M proteins; quantitation of a band in the β region by SPEP can be performed if the M protein is distinguished from background normal protein bands	Conditional
8. Laboratorians should report both quantitative levels of free κ and free λ and the rFLC when the sFLC assay is performed	Strong
9. Clinical care providers may use rFLC, IgM isotype, M protein >1.5 g/dL, and immunoparesis as risk factors for progression to MM or a B-cell lymphoproliferative disorder	Conditional

Abbreviations: AL, amyloid light chain; HLC, heavy/light-chain isotype assay; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; M protein, monoclonal immunoglobulin protein; MG, monoclonal gammopathy; MM, multiple myeloma; rFLC, ratio of serum free κ to serum free λ; sFLC, serum free light chain; sIFE, serum immunofixation electrophoresis; SPEP, serum protein electrophoresis; uIFE, urine immunofixation electrophoresis.

proteins. For laboratories that choose not to perform the sFLC assay, it is available from reference laboratories.

Although sFLC provides notable improvements over previous methods in detecting MFLC, it is a complex method and lacks the specificity of uIFE.[28] The fact that it is measuring heterogeneous molecules that differ in charge, amino acid composition, and size makes it vulnerable to issues with consistency, linearity, antigen excess effect, and lot-to-lot variations.[33]

Laboratories need to be aware that complicating the use of sFLC is the heterogeneity of currently available products. Virtually all the studies on sFLC reported between 2001 and 2011 were performed using the assay from the Binding Site (The Binding Site Group, LTD, Birmingham, United Kingdom).[34] However, in the past few years, new manufacturers have produced sFLC assays with different methods, reagents, and reference intervals.[33]

An important concern raised during EP deliberations regarded the cutoff value used for abnormal rFLC. As noted above, the reference range for the Binding Site assay as determined by Katzmann et al[11] was chosen for high specificity and is not a typical 2 SD (central 95% reference interval) cutoff. However, that cutoff has been validated only for the Binding Site assay. There are currently other commercial products available for the sFLC assay. All of the reference ranges used are different. In addition, 2 of these assays use different reference ranges for patients with impaired renal function, whereas the other 2 do not.[35] Laboratories must not use a cutoff and reference ranges from one assay for a result from another. A key factor unclear at the present time is whether the IMWG-established specific criteria based on the Binding Site assay have been shown to apply to other assays.[13] A recent study comparing the 4 currently available methods showed overall good concordance but significant absolute differences, especially among serum with increased concentrations.[35] The EP recommendation is that when following patients, the same method on the same assay and the same laboratory should be used to avoid platform-to-platform variation.

During the public comment period, 91.7% of respondents (n = 155 of 169) agreed with the recommendation statement. Of the 8.2% (n = 14 of 169) in disagreement, most asked for inclusion of sIFE in the screening protocol. An sIFE is needed when polyneuropathies or renal disorders are part of the differential diagnosis, because of the small size of the monoclonal proteins that may be present in these disorders.[36,37] As noted below, sIFE and uIFE are needed when AL amyloidosis is under consideration (recommendation statement 4).[38] In addition, the EP suggests that an sIFE or ISUB be performed if either of the 2 screening tests is positive (recommendation statements 2 and 3). A couple of comments warned of false-positive or false-negative results and detection of transient or clinically irrelevant abnormalities that may increase costs. Comments also included reservations about the overuse of laboratory tests and that insurance companies may decline payment for laboratories unable to perform the assay in house. The EP agreed with the concerns about overuse and that these concerns should be considered when working with clinicians on appropriate stewardship, to ensure that only patients with reasonable risk for an MG are screened. The absence of an established sFLC assay in the local laboratory was not considered a reason not to recommend its use because the sFLC assay can be sent to a reference laboratory.

There was also public comment concern that sFLC did not add value because of the high number of false positives and negatives. It is correct that sFLC ratios may be out of the normal range for some patients, especially among individuals with chronic renal disease.[39] However, renal disease itself may be related to MFLC as part of MG of renal significance.[3,36] One reviewer asked about the use of mass spectrometry, a promising technique that has created enthusiasm for its possibilities in improving detection and quantitative measurement of M proteins.[40] Recommending mass spectrometry for screening did not seem practical to

the EP at this time; it will be important to keep a keen watch on technology to see if mass spectrometry becomes practical for routine laboratory studies.

The evidence for this statement comprises 5 studies[30,31,41–43] that evaluated the diagnostic test characteristics of SPEP and sFLC in the initial detection of M proteins. This included a high-quality systematic review,[41] an intermediate-quality diagnostic accuracy study,[42] 1 low-quality diagnostic accuracy study,[31] and 2 very-low-quality diagnostic accuracy studies.[30,43] The aggregate risk of bias across all 5 studies was serious, but the evidence was not further downgraded for any domain.

Although the EP members believed the associated harms of conducting both assays in the initial detection of M proteins ranged from large to trivial, a majority of the members (87.5%; n = 7 of 8) believed the moderate to large benefits outweighed these harms. Additionally, although most of the EP members (62.5%; n = 5 of 8) agreed that there would be a moderate cost increase when using both assays, all members felt the recommendation would be acceptable to key stakeholders and feasible to implement. Refer to Supplemental Table 8 in the SDC for the strength of evidence assessment for this statement, and Supplemental Table 9 for a complete summary of the EtD framework.

2. Strong Recommendation.—Laboratorians should confirm an SPEP abnormality suspicious for presence of an M protein with additional testing by sIFE or alternative method with similar sensitivity.

The strength of evidence to support this guideline statement is *moderate*.

When an M spike is suspected on an SPEP, the standard is to determine that the abnormality is an M protein. For many years, sIFE has been the gold standard to confirm and characterize the initial detection of an M protein, and, as such, it is used as a comparator for other methods.[44–46] Early comparison studies reported a similar sensitivity of ISUB and sIFE for detecting MFLC or small intact M proteins[47,48] (<0.3 g/dL). However, later Jolliff et al[49] reported complete agreement between sIFE and ISUB on 240 cases with M proteins as low as 0.22 g/dL. Katzmann et al[47] noted that the removal of the polyclonal immunoglobulins by ISUB allowed detection of several small M proteins in the presence of a polyclonal increase where the sIFE required multiple dilutions to achieve this identification. A recent evaluation of small M proteins reported ISUB to be a comparable alternative to sIFE in detecting small M proteins.[50] Each method has benefits and weaknesses. When the identity of a suspicious band is not clarified by sIFE or ISUB, reflex to the other method may be of help. Though not widely available, a new technique involving immunoenrichment followed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MASS-FIX) has been shown to be a highly sensitive, specific, and cost-effective method comparable to sIFE to detect and identify M proteins).[51,52]

Confirmation by sIFE (or a comparable technique such as ISUB or MASS-FIX) of a suspected M protein is needed to rule out nonimmunoglobulin sources of suspicious spikes such as fibrinogen in gel electrophoresis or radiocontrast dyes in capillary electrophoresis.[53] It also establishes that suspected M proteins in patients with immunoglobulin (Ig) G4–related systemic disease are actually polyclonal, not monoclonal, processes.[54] Identification of the M protein's isotype has significance for prognosis and risk-stratification significance for progression of MGUS to MM or other related malignancies.[14,55] Repeat sIFE is needed on follow-up specimens only when the M protein is not demonstrable in the same migration position by SPEP.[56] If the IFE or ISUB discloses a κ or λ M protein with no corresponding M-protein isotype in IgG, IgA, or IgM, studies should be performed to detect possible IgD and IgE M proteins.[57]

During the public comment period, 98.8% of the respondents (n = 166 of 168) agreed with the recommendation statement (83.3% [n = 140] agreed and 15.4% [n = 26] agreed with modifications). This recommendation was initially drafted to include only sIFE as a confirmatory method. The comments received included the suggestion that other methods (ISUB, MASS-FIX) may be equivalent to sIFE and that the statement should not be limiting in the examples included. The EP suggested a minor edit to include specific alternative methods with similar performance characteristics, such as ISUB and MASS-FIX. The final decision was to modify the statement to be inclusive of any method with similar sensitivity that has been appropriately validated against sIFE.

The evidence base supporting this recommendation comprises 5 studies that all used sIFE as a reference standard when determining diagnostic test characteristics of SPEP.[31,58–61] All 5 studies were of a diagnostic-accuracy design and assessed as intermediate,[58] intermediate to low,[59] low,[31,60] and very low quality.[61] The aggregate risk of bias across the included studies was serious. Although the identified evidence did not provide diagnostic test characteristics for sIFE, this is believed to be a consequence of studies assessing the test characteristics of sIFE having been published prior to our search inception. In current practice, sIFE is the gold standard.

During the EtD framework discussion, 1 panel member self-recused based on the aforementioned disclosures. When considering the available evidence and the use of sIFE as usual practice, all voting EP members believed that the large benefits of increased specificity using sIFE for confirmation outweighed the small to trivial harms of an additional test. The EP discussed the use of alternative assays that have demonstrated similar sensitivities when compared with sIFE.[44–50] Again, studies comparing the sensitivity and specificity of sIFE versus other assays, such as ISUB and mass spectrophotometry, were published prior to our inclusion dates. The MASS-FIX technique is relatively new and has shown to have comparable or better sensitivity than sIFE or sFLC.[52] As such, although the systematic review did not provide current evidence for comparable sensitivities for other assays, the EP is comfortable recommending the use of assays with known sensitivity similar to sIFE. All voting EP members felt that this recommendation would be acceptable to key stakeholders and feasible to implement. Refer to Supplemental Table 8 in the SDC for the strength of evidence assessment for this statement, and Supplemental Table 9 for a complete summary of the EtD framework.

3. Conditional Recommendation.—Laboratorians and/or clinical care providers should follow up an abnormal sFLC ratio for the presence of a M protein with an sIFE or alternative method with similar sensitivity.

The strength of evidence to support this guideline statement is *low*.

When an unexpected abnormal rFLC is encountered, sIFE should be used to further investigate the etiology. An abnormal rFLC is encountered in most[62,63] individuals with MM; therefore, sIFE should be used to confirm that the

abnormal rFLC is attributable to an M protein. Abnormal rFLC also may be encountered in nonneoplastic conditions such as chronic kidney disease[39] or chronic inflammation. It may be unclear whether this abnormal ratio truly reflects the presence of an M protein or whether a shift in the rFLC production is related to a nonneoplastic condition. When one is unconvinced that an abnormal rFLC truly indicates the presence of an M protein in the serum, an sIFE sample can provide supporting evidence. Although identification of M protein by sIFE can explain an abnormal rFLC, a negative sIFE does not exclude an M protein when the rFLC is abnormal, because M proteins in some diseases (eg, light chain–only myeloma, AL amyloid, oligosecretory myeloma, or nonsecretory myeloma) may be undetected by sIFE. Clinical experience suggests that uIFE can also be informative for investigating an abnormal rFLC, but data supporting the utility of such an approach were not identified. As noted above, ISUB and MASS-FIX are alternatives to sIFE. Studies reviewed for this guideline evaluated sIFE, which was thought to be more sensitive than ISUB for detecting some M proteins.[47,50,60,64–66]

During the open comment period, there was an 86.1% agreement (n = 143 of 166) among the respondents. There were recurring suggestions related to this recommendation, which included removing sFLC testing from the initial investigation of an MG, performing sIFE irrespective of the sFLC result, adding urine testing to the investigation of an abnormal rFLC, and defining criteria for a significantly abnormal rFLC. Adequate evidence to support these suggestions was not identified. Defining a significantly abnormal rFLC may be possible for a specific assay, but a consistent threshold that reliably distinguishes significant from incidental abnormalities in the rFLC across methods is not presently possible because of lack of assay harmonization. Those rFLCs that are reported slightly outside of the reference interval are commonly associated with nonmalignant conditions and may cause unnecessary patient anxiety, but as the ratio of involved to uninvolved free light chain increases so does the probability of a malignant plasma cell disorder.[67]

Six studies that evaluated rFLC[25,31,68–71] were identified to inform this statement. The evidence base carried a serious risk of bias and was further downgraded for serious inconsistency. The 6 studies included 1 prospective cohort study assessed as intermediate to low quality,[69] 3 retrospective cohort studies assessed as low quality,[25,70,71] and 2 diagnostic cohort studies assessed as intermediate to low[68] and low[31] quality. As was discussed in the previous recommendation, although the identified evidence did not provide diagnostic test characteristics for sIFE, this is believed to be a consequence of the sIFE being historically defined as the gold standard. Based on the available evidence and the use of sIFE as usual practice, a majority of EP members (87.5%; n = 7 of 8) expected a moderate to large benefit from the increased specificity achieved by using sIFE to confirm a M protein suggested by an abnormal rFLC, outweighing the possible harms from an additional test. The remaining EP members (12.5%; n = 1 of 8) felt that there was balance between the benefits and harms of rFLC confirmation by sIFE, with the principal harms being a possible discrepancy between rFLC and sIFE leading to prolonged patient anxiety with additional workup and additional cost. Although there was inconsistency surrounding weighing of the benefits and harms, all EP members still felt that this recommendation would be acceptable to key stakeholders and feasible to implement. However, the recommendation remains conditional based on the low strength of evidence. Refer to Supplemental Table 8 in the SDC for the strength of evidence assessment for this statement, and Supplemental Table 9 for a complete summary of the EtD framework.

4. Strong Recommendation.—Clinical care providers should order SPEP, sFLC, sIFE, and uIFE for the initial detection of M protein in all patients with suspected AL amyloidosis.

The strength of evidence to support this guideline statement is *moderate*.

Amyloid light chain amyloidosis is an incurable systemic MG resulting from the deposition of MFLC and presenting with vague symptoms such as fatigue, edema, and weight loss resulting from variable organ damage including cardiac, renal, neural, liver, and gastrointestinal.[72,73] Diagnosis requires demonstration of the amyloid on biopsy material. However, before a biopsy is performed, a screening test of serum and/or urine is often performed to detect an M protein.

Because the quantity of MFLC produced is small or the concentration in serum and/or urine is low because of deposition in tissue, a combination of several tests is recommended to maximize detection. This recommendation is supported by Palladini et al,[38] who evaluated the diagnostic sensitivities of sFLC and IFE prospectively in 121 patients with biopsy-proven AL amyloidosis. The sIFE had a sensitivity of 80% (95% CI, 72%–87%) and uIFE had a sensitivity of 67% (95% CI, 58%–75%). By combining sIFE and uIFE, a 96% sensitivity was achieved (95% CI, 91%–98%). Alone, sFLC had a 76% sensitivity (95% CI, 68%–84%). The combination of sIFE, uIFE, and sFLC yielded an overall detection of 100% (95% CI, 99.7%–100%). Unfortunately, urine samples often do not always accompany serum samples sent for the sIFE.[15] Without urine, the combination of sIFE and sFLC achieves a sensitivity of 96% (95% CI, 91%–98%).[38] This information is supported by the retrospective study by Katzmann et al[30] of 524 patients with AL amyloid using SPEP, sFLC, sIFE, and uIFE. They reported that SPEP together with free light chain had a sensitivity of 94.3% that increased to 97.4% when sIFE was added. Adding uIFE improved the sensitivity to 98.6%. Although the improvement in detection by including uIFE to the sIFE and sFLC was only 1% to 4%, because of the severity of this condition and subtlety of some symptoms, the EP recommends using the complete panel when AL amyloidosis is suspected.

Because therapy is more effective in earlier disease,[38] decreasing the interval between initial symptoms and conclusive diagnosis by providing urine for IFE with the initial sample is recommended. Potential harms of this broader strategy for AL amyloidosis are a modest increase in cost, the inconvenience of providing a urine (early-morning void or 24-hour) sample, and the risk of detecting minor abnormalities that could be misleading in some cases. However, the latter is more likely to occur from the sFLC assay, which has a lower specificity than a uIFE.

During the open comment period, 94% (n = 154 of 164) of respondents agreed with this recommendation. One comment suggested that the initial investigation should be urine and serum, with sFLC used as a follow-up test. Another commented that he or she only performed uIFE after a suspicious band was seen on a UPEP and questioned the utility of information where an MFLC was detected only by

sIFE and not quantifiable if it was not seen by UPEP. However, even if not quantifiable, an MFLC seen on uIFE is supportive evidence for AL amyloid.[38,50] One comment suggested that the SPEP could be eliminated because sIFE is more sensitive than SPEP in detecting a M protein. This is consistent with current information. However, because SPEP is part of the initial screen for all conditions, its isolated exclusion for this instance would not alter the need for the blood sample and would have minimal economic or practical effect. Another comment suggested that ISUB on capillary electrophoresis could be used in these cases. That suggestion is supported by a study of 50 patients with AL amyloid showing equivalence or superiority of ISUB to sIFE by Miyazaki and Suzuki.[50] Other reviewers suggested a staggered approach beginning with sIFE, if that is negative following it with sFLC, and if that is still negative a uIFE, or beginning with serum studies and using urine only later. Although a staggered approach is reasonable if good follow-up and coordination among the laboratory, clinician, and patient exists, this must be balanced with the convenience of collecting the relevant samples at one time and providing the test results expeditiously. A couple of comments suggested that we specify a 24-hour urine sample. Unfortunately, the complexity and inconvenience of requiring a 24-hour urine sample is a deterrent to obtaining any urine sample. For the initial detection, an early-morning void would frequently be sufficient.[74]

The evidence supporting this statement comprises 2 diagnostic accuracy studies[30,38] that carried an aggregate serious risk of bias, and consistently reported increased diagnostic sensitivity in AL amyloidosis patients when SPEP, sFLC, and both sIFE and uIFE were conducted. Based on the available evidence, the EP believed the benefits of using all 4 assays ranged from small to large and the harms ranged from trivial to moderate. However, all members still believed that the benefits outweighed the harms, and that the recommendation was feasible to implement. There was disagreement among the EP in relation to clinician and patient values and preferences, with 12.5% (n = 1 of 8) believing there would be variability in how patients and clinicians valued the main study reported outcomes, 25% (n = 2 of 8) feeling there would probably be variability, 12.5% (n = 1 of 8) believing there would be neutrality, 25% (n = 2 of 8) feeling there would probably be no variability, and the final 25% (n = 2 of 8) believing there would be no variability. This was further reflected in a minority of EP members (12.5%; n = 1 of 8) feeling this recommendation would probably not be acceptable to all key stakeholders and would probably result in reduced health equity. Refer to Supplemental Table 8 in the SDC for the strength of evidence assessment for this statement, and Supplemental Table 9 for a complete summary of the EtD framework.

5. Strong Recommendation.—Clinical care providers should NOT order HLC isotype assay for initial detection of M protein in patients with suspected MG.

The strength of evidence to support this guideline statement is *low*.

There is limited evidence indicating a use for HLC in the initial detection of M protein. Identified studies reported a lower diagnostic sensitivity for HLC when compared with sIFE,[75,76] sFLC,[75] or SPEP.[77] A prospective cohort study that enrolled patients with AL amyloidosis reported a lower clonal disease diagnostic sensitivity for HLC when compared with sIFE/uIFE and sFLC alone and in combination.[75] However, a utility for HLC in measuring M-protein production in patients with normal sFLC was demonstrated by the study[75] and may indicate a supplemental benefit for HLC. A 2015 study from Katzmann and colleagues[77] validated the performance of HLC in both IgG MM and IgA MM patients. This study reported no additional benefit for HLC over SPEP in IgG MM patients. In IgA MM patients, HLC demonstrated superior estimation of the spike of IgA M proteins that migrated in the β region; however, detection by HLC was still inferior to that of IFE.[77] An additional study not included in the systematic review based on its small sample size showed a limited value for HLC in quantifying γ heavy chain diseases after initial detection of IFE.[78]

The evidence base informing this statement includes 1 intermediate- to low-quality study[76] and 2 low-quality studies,[75,77] all of which compared HLC with IFE or SPEP. In addition to a very serious risk of bias across the evidence base, the evidence was further downgraded for serious inconsistency of results. Based on the identified evidence, the EP decided to draft a strong recommendation statement against the use of HLC. The EP believed that harms of using the assay outweighed any benefit that may be incurred with its use. The EP identified a lack of value with inclusion of HLC in an MG screening context. Further, the EP highlighted the risk of missing clinically important conditions (eg, light chain and biclonal gammopathy) if HLC was used in isolation. The majority of the EP (87.5%; n = 7 of 8) felt that this recommendation would be acceptable to key stakeholders, and all members felt it would be feasible to implement. Most of the open comment period responses agreed with the recommendation. Refer to Supplemental Table 8 in the SDC for the strength of evidence assessment for this statement, and Supplemental Table 9 for a complete summary of the EtD framework.

6. Strong Recommendation.—Clinical care providers should NOT use total/intact light chains for the quantitation of M proteins in patients with suspected myeloma.

The strength of evidence to support this guideline statement is *low*.

The sFLC assay is a sensitive test for the detection of MGs and is recommended as part of the initial evaluation for patients suspected of having MGs (see recommendation statement 1) and for the initial evaluation of patients with MM according to the IMWG.[13] A similarly named test, the total light-chain assay, lacks the sensitivity of the sFLC assay and should not be used in the evaluation of patients with suspected MGs. The sFLC assay uses an antibody that specifically recognizes κ and λ immunoglobulins in the light-chain region when not bound to a heavy-chain partner, thereby measuring the very small concentration of only free light chains. The total light-chain assay quantifies the concentration of all antibodies of a particular class, both intact and free light chains. This results in decreased sensitivity of the assay because the polyclonal background can obscure a small clonal population. The EP, therefore, recommends against the use of total/intact light-chain quantitation for patients with suspected MM.

During the public comment period, 96.5% (n = 137 of 142) of respondents agreed (agree as written/agree with suggested modifications) with the recommendation statement and that the benefits outweighed potential harms. With this recommendation, institutions will benefit from the avoidance of using an outdated test that can be misinterpreted, providing a better and more accurate identification of

disease by using the appropriate test (eg, sFLC), decreasing the false-negative rates, and reduction of cost spent on an inadequate test.

The evidence base supporting this statement includes 1 retrospective cohort study that evaluated the utility of quantitation using intact and total light chains.[70] This study was assessed as low quality based on a moderate risk of detection bias, a critical risk of selection bias, and no reporting of study funding.[70] Although the strength of evidence supporting this statement is low, the EP proposed a strong recommendation against the use of total/intact light chains based on substantial harms to patients when the assay is used. All EP members felt this guidance would be acceptable to key stakeholders and feasible to implement. Refer to Supplemental Table 8 in the SDC for the strength of evidence assessment for this statement, and Supplemental Table 9 for a complete summary of the EtD framework.

7. Conditional Recommendation.—In patients with intact M proteins outside the γ region by SPEP, laboratories should use total immunoglobulin (IgA, IgG, or IgM) for the quantitation of the M proteins; quantitation of a band in the β region by SPEP can be performed if the M protein is distinguished from background normal protein bands.

The strength of evidence to support this guideline statement is *very low*.

Most of the M proteins that migrate outside of the γ region reside in the β region, only rarely more anodal. Transferrin (β1) and C3 (β2) are the most prominent proteins found in the β region that may obscure a β-migrating M protein, especially in electrophoretic systems that do not provide a crisp separation of transferrin and C3.[79] Because of this, some laboratories have adopted a protocol of performing an sIFE when the β region is 1.6 g/dL or greater.[77] Although some studies[10,82] using a reference range derived from 2 SD (central 95% reference interval) advocated the use of the HLC assay to identify β-migrating IgA monoclonal cases (56%; n = 83 of 149), data from Katzmann et al[77] found that this practice was 5% inferior to using sIFE (61%; n = 86 of 141) and achieved only a 6% improvement from using the less costly and commonly available total IgA quantitative measurements (50%; n = 74 of 149). Because of the interference by nonimmunoglobulin proteins in non–γ-migrating M proteins, in 2014, the IMWG recommended that IgA M proteins found in the β region be measured by nephelometry (or equivalent techniques such as turbidimetry).[80] They specifically noted IgA because of its prominence among M proteins in this region, but also because of less robust performance of nephelometric measurements of IgG and IgM.[80,81] The EP endorses the nephelometric measurement of total immunoglobulins in non–γ-migrating IgG or IgM M proteins because alternatives, such as MASS-FIX, are not readily available.

The public comment period results for this statement had the lowest levels of endorsement compared with the other statements. The total agreement was 83% agree and agree with modifications (n = 128 of 154), with 16% of the respondents disagreeing with the drafted statement (n = 26 of 154). Initially, the draft statement recommended only including total IgA for the quantitation of the M proteins outside the γ region. Because the testing of IgM and IgG was not included in the recommendation statement, there was confusion in the public comments received resulting in the lower percentage of agreement. The IMWG specified only IgA quantitative measurements in its guideline but did not precisely provide guidance on what to do with IgM or IgG.[81] Although the IgA accounts for about half of the M proteins in the β region, this recommendation did not provide consistent and harmonized guidance on how to measure total immunoglobulins. Therefore, the EP decided to include the other major protein quantitative measurements by nephelometry in this recommendation.

The evidence base supporting this statement comprises 3 retrospective cohort studies that evaluated quantitation using total immunoglobulin.[77,82,83] In addition to being limited by an aggregate very serious risk of bias, evidence was downgraded for serious inconsistency and indirectness. All EP members considered the issue of quantitation of M proteins outside the γ region to be a priority. Although the benefits were considered to range from small to large, all EP members believed the benefits outweighed the small to trivial harms. When discussing resource use, most of the EP members (75%; n = 6 of 8) believed the costs to be negligible, whereas the minority (25%; n = 2 of 8) felt quantitation using total immunoglobulin would carry a moderate cost. Irrespective of the potential increase in resources, all EP members felt this statement would be acceptable to key stakeholders and feasible to implement. Refer to Supplemental Table 8 in the SDC for the strength of evidence assessment for this statement, and Supplemental Table 9 for a complete summary of the EtD framework.

8. Strong Recommendation.—Laboratorians should report both quantitative levels of free κ and free λ and the rFLC when the sFLC assay is performed.

The strength of evidence to support this guideline statement is *very low*.

Reporting the serum rFLC together with the quantitative measurements of serum free κ and free λ was deemed essential because 5% of the time, free κ, free λ, or both will be above or below the 2 SD (central 95% reference interval) most commonly used when measuring these analytes in the general population. As discussed above, recognizing this dilemma, Katzmann et al[11] expanded the cutoff limits for rFLC to the range of 0.26 to 1.65, boundaries that included all 282 control individuals, vastly improving the specificity of the rFLC. Conditions other than MGs increase sFLC levels; polyclonal increases in the γ region or renal impairment may increase the levels of both free light chains.[13,84,85] Although the rFLC is often normal in these conditions, it may be increased, especially in kidney disease.[86]

During the public comment period, most respondents (98.8%; n = 166 of 168) agreed with the statement as written. The strength of recommendation of the statement was initially drafted as conditional based on the limited evidence base supporting it. Some comments suggested that the statement should be a strong recommendation because the interpretation should only be done when all the elements are reviewed with the sFLC. In contrast, a few of the respondents that disagreed commented that sFLC on its own is not a very useful test, because it does not necessarily exclude a specific condition.

The evidence base supporting this statement includes 1 low-quality retrospective cohort study.[25] This study was limited by a moderate risk of performance and detection bias, a serious risk of reporting bias, and a critical risk of selection bias. Although the strength of evidence supporting this statement is very low, the EP proposed a strong recommendation for reporting of both quantitative levels

Table 5. Risk of Progression: Immunoglobulin (Ig) M Versus Non-IgM Monoclonal Gammopathy of Undetermined Significance (n = 1384)[a]

	IgM Progression, No.	IgM Relative Risk (95% CI)	Non-IgM Progression, No.	IgG Relative Risk (95% CI)
Any progression	34	10.8 (7.5–15.0)	107	5.7 (4.7–6.9)
Multiple myeloma	0	0.0 (0.0–6.5)	93	27.5 (22.2–33.7)
NHL	17	10.6 (6.2–17.0)	2	0.2 (0.0–0.7)
AL amyloidosis	3	13.1 (2.7–38.1)	11	8.3 (4.2–14.9)
Waldenström macroglobulinemia	11	287.7 (143.6–514.7)	0	0.0 (0.0–16.2)
CLL	3	4.3 (0.9–12.6)	0	0.0 (0.0–0.9)
Plasmacytoma	0	0.0 (0.0–342.6)	1	15.0 (0.4–83.7)

Abbreviations: AL, amyloid light chain; CLL, chronic lymphocytic lymphoma; NHL, non-Hodgkin lymphoma.

[a] Data derived from Kyle RA, Larson DR, Therneau TM, et al. Long-term follow-up of monoclonal gammopathy of undetermined significance. *N Engl J Med*. 2018;378(3):241–249. doi:10.1056/NEJMoa1709974.[14]

and κ:λ ratio when sFLC is performed based on substantial harms to patients if only one element is reported. The EP discussed the necessity of the ratio for diagnosis and the quantitation level for monitoring response to therapy and relapse. The EP's strong recommendation is based on the need to normalize sFLCs when the total immunoglobulin concentration is abnormal, such as with immunosuppression, polyclonal gammopathy, and renal disease. In these conditions, the ratio provides additional information. Further, rFLCs are reported to be an independent risk factor for progression of MGUS.[55] All EP members agreed this recommendation would be acceptable to key stakeholders, with 75% (n = 6 of 8) of members believing it be acceptable and 25% (n = 2 of 8) believing it to be probably acceptable. Similarly, 87.5% (n = 7 of 8) of members felt this statement to be feasible to implement and 12.5% (n = 1 of 8) felt it would probably be feasible. Refer to Supplemental Table 8 in the SDC for the strength of evidence assessment for this statement, and Supplemental Table 9 for a complete summary of the EtD framework.

9. Conditional Recommendation.—Clinical care providers may use rFLC, IgM isotype, M protein higher than 1.5 g/dL, and immunoparesis as risk factors for progression to MM or a B-cell lymphoproliferative disorder.

The strength of evidence to support this guideline statement is *low*.

The current standard of care for MGUS and SMM is to monitor the patient regularly to enable earlier detection of progression to MM or B-cell lymphoproliferative disorders and institute appropriate intervention.[87] Risk of progression of MGUS is known to vary among patients, and clinicians caring for MGUS patients can choose to perform further studies or vary the frequency of follow-up based on the patient's risk of progression.

The EP recommends using abnormal rFLC, IgM isotype, M-protein concentrations equal to or above 1.5 g/dL, and immunoparesis[14] as factors that increase the risk of progression.

An abnormal rFLC, as well as the degree of increase or decrease in that ratio, predict progression in virtually all MGs: MGUS, SMM, MM, and AL.[13,55,62]

Based on isotypes, there are 2 major arms of progression for patients with MGUS: IgM and non-IgM.[14] A prospective study by Kyle et al[14] following 1384 MGUS patients with readily visible M proteins for a median of 34.1 years determined that the overall risk of progression for individuals with MGUS was 1% per year, 6.5 times higher than the controls (age and sex matched) (95% CI, 5.5–7.7) (Table 5). Cases with IgM MGUS had a 10.8-fold risk of progression (mainly to WM, none to MM), whereas cases with IgG MGUS had a 5.7-fold risk of progression (mainly to MM, none to WM) as noted in Table 5. Turesson et al[88] evaluated risk factors for progression from MGUS to MM in 728 patients. Of the 53 patients who progressed to MM, none had an IgM MGUS, and of the 14 who progressed to WM, all originated from an IgM MGUS.

Whereas Kyle et al[14] evaluated MGUS spikes that were readily seen by SPEP, Murray et al[89] reported the persistence and progression of sIFE MGUS (n = 437) that presented with M spikes that were too small to quantify, but that were confirmed as M protein by sIFE. Overall, the risk of progression of these small sIFE MGUS cases was 0.8% per year, similar to the 1.0% overall MGUS progression in the Kyle et al[14] study. Because of the relatively small numbers, the significance of the relative risk of a particular isotype was marginal.

A serum M-protein concentration of 1.5 g/dL or higher is a significant risk factor for increased risk of progression.[14,62,88]

In addition, studies have also pointed to immunoparesis, defined as having a non–M-protein immunoglobulin concentration below the reference range, as a significant risk factor.[88,90] Laboratories choosing to report this risk factor will need to have quantitative immunoglobulin levels available at the time of reporting risk assessment.

For the clinician, MGUS patients without any risk factors can be considered to be at low risk for progression, patients with all risk factors present can be considered at high risk, and patients with 1 to 2 factors can be considered at intermediate risk.[14,25,55,90,91]

The evidence base supporting these risk factors comprises 5 studies.[14,62,88,89,91] Three studies evaluated the association between isotype and risk of progression.[14,88,89] Four studies evaluated both abnormal sFLC ratios and intact M-protein concentrations,[14,62,88,91] and 1 evaluated risk associated with immunoparesis.[88] The 5 studies included 1 prospective cohort study assessed as intermediate to low quality,[14] 3 retrospective cohort studies assessed as low quality,[62,88,89] and 1 retrospective cohort study assessed as very low quality.[91] The aggregate risk of bias across the studies was very serious, and for the non-IgM isotype risk factor, evidence was further downgraded for serious inconsistency of results. In brief, all EP members believed that defining these risk factors for progression was a priority; however,

Table 6. Good Practice Statements
1. To ensure completeness of the reporting of the M protein, the EP recommends that laboratories report test results for M protein using the template in Table 7, which details recommended reporting elements
2. To promote test sequence standardization in initial analysis of suspected MGs, the EP recommends laboratories consider the test algorithm in the Figure
3. To promote the harmonization of the nomenclature used for the diagnosis of MGs, the EP recommends the use of the term *M protein* when pertaining to monoclonal immunoglobulin proteins (aka, paraproteins, M components, monoclonal protein)
4. To promote harmonization, the EP recommends the use of the term *immunosubtraction* when pertaining to immunotyping and immunodisplacement

Abbreviations: EP, expert panel; M protein, monoclonal immunoglobulin protein; MGs, monoclonal gammopathies.

there was variability in responses related to the balance of benefits and harms, as well as the acceptability and feasibility of the guidance. When discussing abnormal sFLC ratios and intact M-protein concentrations, a majority of the EP believed the benefits of these risk factors outweighed any harms, and all EP members felt the guidance would be acceptable for stakeholders and feasible to implement. Based on the available evidence, the EP believes that the use of the non-IgM isotype as a risk factor for progression carries small to large benefits and small to trivial harms. Because of this range in perceived benefit, the EP was divided on whether the benefits outweighed the harms, with 25.0% (n = 2 of 8) believing there was a balance and 75.0% (n = 6 of 8) believing the benefits to outweigh the harms. However, all EP members believed the statement was both acceptable to key stakeholders and feasible to implement. Finally, although the majority of EP members (75.0%; n = 6 of 8) believed inclusion of immunoparesis as a risk factor was valid, a minority of the EP members felt that there was only a balance between the benefits and harms (25%; n = 2 of 8) and that the guidance probably would not be acceptable to stakeholders or feasible to implement (12.5%; n = 1 of 8). Refer to Supplemental Table 8 in the SDC for the strength of evidence assessment for this statement, and Supplemental Table 9 for a complete summary of the EtD framework.

Good Practice Statements

According to the Grading of Recommendations Assessment, Development, and Evaluation approach, good practice statements are recommendations panels may consider important but are not appropriate to be formally rated for quality of evidence.[92] In addition to the set of key questions formulated a priori, the EP decided to write good practice statements (Table 6), which reflect expert consensus opinions supported by a limited number of studies and data that were not formally included in the evidence base nor systematically rated. The EP wanted to address the following questions: (1) What reporting elements should be included in the pathology report? (2) Is there an optimal testing sequence, strategy, or approach for the initial detection of M proteins?

A targeted literature search was performed based on these questions. The EP cochairs reviewed the available literature and incorporated data collected in a preguideline development practice survey to arrive at the good practice statements. A detailed process for the literature review for the good practice statements is included in the SDC (Supplemental Figure 3).

1. To ensure completeness of the reporting of the M protein, the EP recommends that laboratories report test results for M protein using the template in Table 7, which details recommended reporting elements.
2. To promote test sequence standardization in initial analysis of suspected MGs, the EP recommends laboratories consider the test algorithm in the Figure.
3. To promote the harmonization of the nomenclature used for the diagnosis of MGs, the EP recommends the use of the term *M protein* when pertaining to monoclonal immunoglobulin proteins (aka paraproteins, M components, monoclonal protein).[93–95]
4. To promote harmonization, the EP recommends the use of the term *ISUB* when pertaining to immunotyping and immunodisplacement.

Quantitative Measurement of M Spike

In patients with intact M proteins within the γ region detected by SPEP, laboratorians should use quantitative measurement of the M spike. For patients with light-chain–only M proteins, the involved serum free light chain and quantitative measurement of the M spike in a 24-hour urine (if present) should be performed.[13]

This statement was initially included as an evidence-based conditional statement. The EP agreed that although there are benefits of providing a recommendation about M-spike quantitation, the designation of a conditional recommendation because of a very low aggregate quality of evidence may diminish its importance in practice.

Overall, it was agreed that the benefits of this recommendation include harmonization of M-protein quantitative measurement and that it would allow providers a means to monitor patients during the course of their disease by assessing the progression or decline of the M protein(s). In addition, initial quantitation is critical for patient management decisions, and a standardized approach would allow for improved and accurate quantitative measurement of the relevant M protein(s). The 2 factors that had the strongest impact on intralaboratory precision were low M-protein concentration and high polyclonal background.[96,97] The harm of deploying quantitative measurements includes the need to educate laboratories on the different techniques used, such as perpendicular drop and tangent skimming. Because results of quantitative measurements vary between laboratories, even those using the same technique, it is recommended that quantitative measurements be performed in the same laboratory for subsequent samples. This historic recommendation was emphasized recently by an international study involving 16 unique institutions representing Australia, Canada, Estonia, Italy, the Netherlands, New Zealand, the United Kingdom, and the United States compared the quantitative measurement of serum with known quantities of spiked M protein in normal, hypogammaglobulinemic, and hypergammaglobulinemic serum by perpendicular drop and tangent skimming.[96,97] Although they found satisfactory intralaboratory precision, the coefficient of variation was higher for laboratories using tangent skimming than for those using perpendicular drop. Whichever method was used, all laboratories performed well on calculating percentage reduction, a key measure for IMWG response criteria. However, the differences both within measurement techniques and among manufacturers

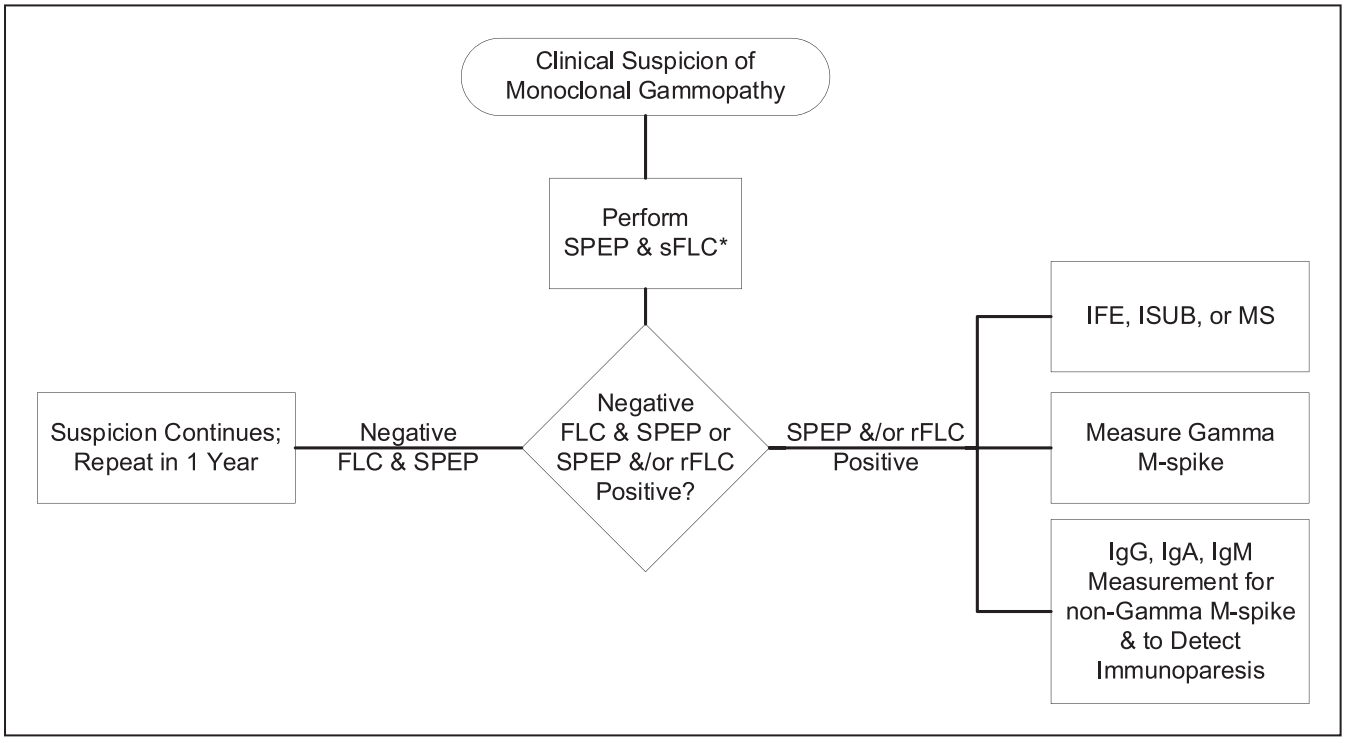
Table 7. Key Reporting Elements for M Proteins[a]		
	Results	**Notes**
SPEP/UPEP		
Abnormal band(s) presence	Yes/no/equivocal	
Migration pattern	α, β, γ	
Quantitation of abnormal band(s)	_____g/dL (g/L)	
	Limitations (if any)	For example: comigration of multiple bands, background globulins, interferences
Previous history	Yes/no	
	Isotype(s)	
	Describe change in quantity or migration from previous	For example, change in pattern could indicate the presence of a therapeutic monoclonal antibody or development of a new clone (possibly reactive)
Hypogammaglobulinemia	Yes/no	
Recommendation	Follow-up testing	For example, immunofixation recommended (if site does not reflex)
Interpreter		
sIFE/ISUB		
M protein	Yes/no/equivocal	
Migration pattern (ISUB)	α, β, γ	To align with quantitation
Isotype(s)		IgG κ, IgA λ, etc
Recommendation	Follow-up testing	For example, recommend ordering a urine sample, or IgD/IgE by uIFE
Interpreter		
Intact Immunoglobulin Measurement		
IgA, total, immunoparesis	Yes/no	With reference interval
IgG, total, immunoparesis	Yes/no	With reference interval
IgM, total, immunoparesis	Yes/no	With reference interval
Interpreter		
Serum free light chains		
κ	_____ units	With reference interval
λ	_____ units	With reference interval
Ratio		With reference interval
Methodology	Optional	Different manufacturers may require different reference intervals
κ-λ difference	Optional	For trending purposes after diagnosis
Interpretation	Optional	For example, renal disease or high immunoglobulin background contributing to elevations in both κ and λ versus clinically significant disease

Abbreviations: IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; ISUB, immunosubtraction; sIFE, serum immunofixation electrophoresis; SPEP, serum protein electrophoresis; UPEP, urine protein electrophoresis.

[a] Data derived from McCudden CR, Booth RA, Lin DCC, McCurdy A, Rupani N, Kew A. Synoptic reporting for protein electrophoresis and immunofixation. *Clin Biochem*. 2018;51:21–28. doi:10.1016/j.clinbiochem.2017.09.020.[104]

were large enough that they recommended not comparing M proteins among different laboratories, even ones using the same method. Although quantitative measurement is considered a gold standard, it will take some time for all laboratories to be harmonized and implementing the best practice procedures.

During the open comment period, issues raised were related primarily to the location of the M protein after electrophoretic separation and the variability in integration methods used for quantitation, as well as remarks on the limitation of the densitometry method used for this purpose. Several comments correctly suggested that not all M spikes are in the γ region: that they can occur in other regions of the SPEP, most frequently in the β region.[77,98,99] Another set of comments related to the best method to perform quantitation of an M spike: is perpendicular drop or tangent skimming the preferred method? The 2 international studies mentioned above suggested that either method provides acceptable accuracy but the same method should be performed within the sample laboratory on follow-up.[96,97] It was suggested that laboratory reports include details on the method used, and if this method changes, a comment should be included in addition to a window period allowing for rebaselining of patients. Ideally, there should be a move toward harmonization across all laboratories on how integration of the M spike is performed. It was stated that M-spike determination can be highly operator dependent,[100] as different technologists even within the same laboratory may use slightly different procedures. In addition, densitometry as a method is inherently problematic for quantitation because it is dependent on the limits of dye binding of proteins on gels, and especially in non–γ-region M proteins.[101] Other methods that perform quantitative immunoglobulin measurement using nephelometry or turbidimetry may provide more accurate quantitation of the M protein.[100]



Testing algorithm for monoclonal gammopathies. The asterisk is used to note that when a neuropathy-associated monoclonal process is suspected, a serum immunofixation should be performed. For patient suspected of having amyloid light chain amyloidosis, both serum and urine immunofixation should be performed. Abbreviations: FLC, free light chain; IFE, immunofixation electrophoresis; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; ISUB, immunosubtraction; MS, mass spectrophotometry; M-spike, monoclonal spike; rFLC, free light-chain ratio; sFLC, serum free light chain; SPEP, serum protein electrophoresis.

Limitations

A key feature of the current guideline is the requirement for sFLC testing for the initial screen. Most of the literature on this subject, including the IMWG recommendations, uses the original Binding Site methodology. At the present time, there are 3 other methods for measuring sFLC available that give concordant, but not identical, results to the original method, and each has unique reference intervals for free κ, free λ, and rFLC.[28] Awareness of this issue and attention to current literature on this topic are strongly encouraged.

Whereas sIFE, ISUB, and MASS-FIX are all included as possible mechanisms to validate the presence and identity of suspicious SPEP patterns, each has unique advantages and disadvantages. Which to use depends on the techniques available and the situation. Serum IFE improves sensitivity over SPEP because the reagent antibodies reacting with the M protein increase the protein content for staining. Immunosubtraction does not add protein for staining, yet it improves sensitivity over SPEP by removing the polyclonal background, which allows detection of subtle M proteins that may be missed with sIFE.[47] The standard of the IMWG is to confirm complete remission by sIFE when the known M-protein band is not observed by SPEP. In that situation, without a polyclonal background, sIFE could be more sensitive than ISUB. With a normal or polyclonal γ-region background, however, a subtle band may be better seen with ISUB. The MASS-FIX technique is relatively new is and not currently broadly available for clinical laboratories, but has greater sensitivity than conventional sIFE.[52]

Measuring non–γ-region M proteins remains a problem. Although the IMWG recommended that IgA M proteins in the β region be followed with a nephelometric or densitometric quantitative measurement of total IgA, it was not clear on the utility of using total IgM and IgG.[80] The literature is weak on this subject. The EP recommends including the total IgA, IgG, and IgM to improve harmonization of these measurements. Though nephelometric quantitative measurements of IgG and IgM are more disparate from M-spike quantitative measurement than those of IgA, following response to treatment with the total IgG or IgM quantitative measurement will reflect the M protein in a similar manner to the total IgA quantitative measurement.[102] Nonetheless, just as with using a perpendicular drop in the γ region, the user needs to be aware that total IgG, IgA, or IgM results include polyclonal immunoglobulins of the same isotype.

Although the use of isotype to predict progression of MGUS to MM or other B-cell lymphoproliferative disorders is recommended, there are relatively few papers in the literature on this subject and there is some confusion about the progression of IgA-related lesions.

The systematic review was further limited by a lack of identified evidence evaluating the collection of urine samples in recent literature. Although the key questions included a query for the feasibility of replacing of 24-hour urine collection with early-morning void collections, no identified studies directly compared the ability to detect monoclonal proteins in these samples. The EP discussed the burden placed on patients required to collect urine during 24 hours, but without sufficient evidence, no guidance could be

provided. Future studies designed to compare the accuracy of diagnosis for both collection methods are needed.

A further limitation was introduced based on a search covering 2008 through 2019. Although all recommendations were informed by the evidence base identified by the systematic review, some landmark studies that were published prior to 2008 were included in the discussion of these recommendations. These studies provide historical context and have been included in order to support the evidence-based statements.

CONCLUSIONS

A comprehensive survey demonstration of heterogeneity of clinical laboratory testing for identifying M proteins indicated likely suboptimal detection of treatable MGs in current practice. Based on a systematic review of the literature, the EP concluded that sFLC is an essential feature to include for the initial detection of an M protein. For conditions with relatively small quantities of M proteins, IFE of serum and occasionally urine is also needed. This guideline provides 9 statements and background details to assist laboratory implementation of techniques to improve and harmonize the initial detection of patients with MGs.

Guideline Revision

This guideline will be reviewed every 4 years from publication, or earlier in the event of publication of substantive and high-quality evidence that could potentially alter the original guideline recommendations. If necessary, the EP will reconvene to discuss potential changes. When appropriate, the panel will recommend revision of the guideline to the CAP and collaborators for review and approval.

Disclaimer

The CAP developed the Pathology and Laboratory Quality Center for Evidence-Based Guidelines as a forum to create and maintain laboratory practice guidelines (LPGs). Guidelines are intended to assist physicians and patients in clinical decision-making and to identify questions and settings for further research. With the rapid flow of scientific information, new evidence may emerge between the time an LPG is developed and when it is published or read. The LPGs are not continually updated and may not reflect the most recent evidence. The LPGs address only the topics specifically identified therein and are not applicable to other interventions, diseases, or stages of diseases. Furthermore, guidelines cannot account for individual variation among patients and cannot be considered inclusive of all proper methods of care or exclusive of other treatments. It is the responsibility of the treating physician or other health care provider, relying on independent experience and knowledge, to determine the best course of treatment for the patient. Accordingly, adherence to any LPG is voluntary, with the ultimate determination regarding its application to be made by the physician in light of each patient's individual circumstances and preferences. The CAP and its collaborators make no warranty, express or implied, regarding LPGs, and specifically exclude any warranties of merchantability and fitness for a particular use or purpose. The CAP and its collaborators assume no responsibility for any injury or damage to persons or property arising out of or related to any use of this statement or for any errors or omissions.

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APPENDIX. Disclosed Interests and Activities From November 2017 to January 2021[a]

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		Celgene
		Janssen
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