COLLEGE of AMERICAN PATHOLOGISTS

ARCHIVES
of Pathology & Laboratory Medicine

EARLY ONLINE RELEASE

Note: This article was posted on the *Archives* Web site as an Early Online Release. Early Online Release articles have been peer reviewed, copyedited, and reviewed by the authors. Additional changes or corrections may appear in these articles when they appear in a future print issue of the *Archives*. Early Online Release articles are citable by using the Digital Object Identifier (DOI), a unique number given to every article. The DOI will typically appear at the end of the abstract.

The DOI for this manuscript is doi: 10.5858/arpa.2017-0128-CP

The final published version of this manuscript will replace the Early Online Release version at the above DOI once it is available.

Screening and Diagnosis of Monoclonal Gammopathies

An International Survey of Laboratory Practice

Jonathan R. Genzen, MD, PhD; David L. Murray, MD, PhD; Gyorgy Abel, MD, PhD; Qing H. Meng, MD, PhD; Richard J. Baltaro, MD; Daniel D. Rhoads, MD; Julio C. Delgado, MD; Rhona J. Souers, MS; Christine Bashleben, MT(ASCP); David F. Keren, MD; Mohammad Q. Ansari, MD

• ***Context.***—**Serum tests used for the screening and diagnosis of monoclonal gammopathies include serum protein electrophoresis (SPE; agarose gel or capillary zone), immunofixation (IFE) and immunosubtraction capillary electrophoresis, serum free light chains, quantitative immunoglobulins, and heavy/light–chain combinations. Urine protein electrophoresis and urine IFE may also be used to identify Bence-Jones proteinuria.**

Objective.—**To assess current laboratory practice for monoclonal gammopathy testing.**

Design.—**In April 2016, a voluntary questionnaire was distributed to 923 laboratories participating in a protein electrophoresis proficiency testing survey.**

Results.—**Seven hundred seventy-four laboratories from 38 countries and regions completed the questionnaire (83.9% response rate; 774 of 923). The majority of participants (68.6%; 520 of 758) used agarose gel electrophoresis as their SPE method, whereas 31.4% (238 of 758) used capillary zone electrophoresis. The most common test approaches used in screening were SPE with reflex to IFE/immunosubtraction capillary electrophoresis (39.3%; 299 of 760); SPE only (19.1%; 145 of 760); SPE and IFE or immunosubtraction capillary electrophoresis (13.9%; 106 of 760); and SPE with IFE, serum free light chain, and quantitative immunoglobulins (11.8%; 90 of 760). Only 39.8% (305 of 767) of laboratories offered panel testing for ordering convenience. Although SPE was used by most laboratories in diagnosing new cases of myeloma, when laboratories reported the primary test used to follow patients with monoclonal gammopathy, only 55.7% (403 of 724) chose SPE, with the next most common selections being IFE (18.9%; 137 of 724), serum free light chain (11.7%; 85 of 724), and immunosubtraction capillary electrophoresis (2.1%; 15 of 724).**

Conclusions.—**Ordering and testing practices for the screening and diagnosis of monoclonal gammopathy vary widely across laboratories. Improving utilization management and report content, as well as recognition and development of laboratory-directed testing guidelines, may serve to enhance the clinical value of testing.**

(*Arch Pathol Lab Med.* doi: 10.5858/arpa.2017-0128-CP)

Monoclonal gammopathies are hematologic disorders characterized by abnormal production of one or more immunoglobulin clones.[1] They range from asymptomatic, benign disorders (such as monoclonal gammopathy of undetermined significance) to malignant plasma cell and lymphoid disorders, including multiple myeloma and Waldenström macroglobulinemia.[2] Laboratories use a variety of methods to detect, quantify, and characterize immunoglobulins as part of the laboratory's role in the screening, diagnosis, and monitoring of these disorders.[1,3–6]

Serum protein electrophoresis (SPE) and urine protein electrophoresis—which may be conducted using either agarose gel electrophoresis (AGE) or capillary zone electrophoresis (CZE) methods—are commonly used to screen for monoclonal proteins (M proteins; originally referred to as myeloma proteins). These methods are also used to quantify the amount of M protein present when performed in conjunction with scanning densitometry and total protein measurement.[4,7] Clonal characterization of identified M proteins is typically performed using immunofixation electrophoresis (IFE) or immunosubtraction capillary electrophoresis (IS-CE). Turbidimetric and/or nephelometric assays are also frequently ordered, including quantitative immunoglobulin assays (total immunoglobulin [Ig] G, IgA, and IgM), serum free light-chain (sFLC) assays,[8] and heavy/light–chain assays.[9]

Accepted for publication July 11, 2017.

From the Department of Pathology, University of Utah, Salt Lake City, and ARUP Laboratories, Salt Lake City, Utah (Drs Genzen and Delgado); the Department of Laboratory Medicine, Mayo Clinic, Rochester, Minnesota (Dr Murray); the Department of Laboratory Medicine, Lahey Hospital & Medical Center, Burlington, Massachusetts (Dr Abel); the Department of Laboratory Medicine, University of Texas MD Anderson Cancer Center, Houston (Dr Meng); the Department of Pathology and Laboratory Medicine, East Carolina University, Greenville, North Carolina (Dr Baltaro); the Department of Pathology, Case Western Reserve University, Cleveland, Ohio (Dr Rhoads); the Department of Surveys, College of American Pathologists, Northfield, Illinois (Mss Souers and Bashleben); the Department of Pathology, University of Michigan, Ann Arbor (Dr Keren); and the Department of Pathology and Laboratory Medicine, Louis Stokes Veterans Affairs Medical Center, Cleveland, Ohio (Dr Ansari).

The authors have no relevant financial interest in the products or companies described in this article.

Reprints: Jonathan R. Genzen, MD, PhD, Department of Pathology, University of Utah, ARUP Laboratories, 500 Chipeta Way, Mail Code 115, Salt Lake City, UT 84108 (email: jonathan.genzen@path.utah.edu).

In 2009, the International Myeloma Working Group evaluated studies that compared the diagnostic sensitivity and specificity of different combinations of testing components, and the group concluded that the evidence supported the use of a combination of SPE, serum IFE, and sFLC testing for screening for monoclonal disorders.[10] If light chain amyloidosis is suspected, then urine studies should also be added.[10] Subsequently, others have proposed algorithms for monitoring monoclonal disorders after they have been detected.[11–13]

Current practices in ordering, testing, and interpretation of assays used for the detection and characterization of monoclonal disorders are not well chronicled. In an attempt to understand current practices, the College of American Pathologists Diagnostic Immunology Resource Committee distributed a voluntary, supplemental questionnaire to laboratories enrolled in an electrophoresis proficiency testing survey. It should be noted that this survey was designed to focus primarily on the use of these techniques in the assessment of monoclonal disorders (eg, in the arena of hematology and oncology) and did not include questions specifically directed toward specialties such as rheumatology, gastroenterology, and/or nephrology, which may also use interpretative information from electrophoretic and related techniques in clinical care.

MATERIALS AND METHODS

In April 2016, a voluntary supplemental questionnaire was distributed to 923 laboratories as part of the 2016 A mailing of a College of American Pathologists proficiency testing survey for electrophoresis (survey ELP). Survey questions were designed as multiple-choice responses, permitting one or more selections by the participant depending on the question. Open-ended choices (eg, "Other, specify") were included in some questions. These qualitative questions were not recategorized into quantitative tabulations of multiple-choice question responses. They are included in the Results section, as applicable, with subtabulation of thematic content across responses. Questions that allowed participants to select multiple responses were tabulated according to total number of responses, as well as the percentage of laboratories that selected individual response items. Percentage totals were rounded to 1 decimal place. Differences between US and non-US responses were assessed using the χ^2 test with a significance level of $P < .05$. All summaries and analyses were performed with SAS 9.3 (SAS Institute, Cary, North Carolina).

RESULTS

Demographics

A total of 774 laboratories from 38 countries and regions completed the questionnaire (84% response rate). Most respondent laboratories (85.7%) were located in either the United States (n = 614) or Canada (n = 49). Survey respondents who provided a facility type description (n = 760) were from voluntary nonprofit hospitals (33.9%; n = 258), university hospitals (16.4%; n = 125), regional/local independent laboratories (except clinic or group practice and not owned by national corporation[s]; 13.2%; n = 100), city/county/state hospitals (12.9%; n = 98), national/corporate laboratories (owned by national corporation[s]; 8.9%; n = 68), veterans' hospitals (6.4%; n = 49), proprietary hospitals (5.9%; n = 45), Army/Air Force/Navy hospitals (1.2%; n = 9), and public health nonhospital facilities (1.1%; n = 8). The distribution of institution types (academic versus nonacademic) did not differ between US and non-US participant laboratories ($P = .16$).

Laboratory Method Questions

Questions directed toward understanding methods used by participant laboratories are included in Table 1. A total of 68.6% of respondents (520 of 758) reported using AGE methods for SPE, whereas 31.4% (238 of 758) reported using CZE methods (Table 1, question A). Use of SPE methods differed between 600 US laboratories (AGE 72.5% [435]; CZE 27.5% [165]) and 158 non-US laboratories (AGE 53.8% [85]; CZE 46.2% [73]) ($P < .001$). A total of 71.7% of respondents (536 of 748) used IFE for clonal characterization, 7.5% (56 of 748) used IS-CE, and 17.8% (133 of 748) did not reflexively use either method when a monoclonal band was observed by AGE or CZE (Table 1, question B).

When an M protein was identified by either AGE or CZE methods, the vast majority of respondents (77.3%; 521 of 674) used a perpendicular-drop method of M-spike quantification from the electropherogram (Table 1, question C; see Figure, A and B, for illustration). A much smaller number of participants used a tangent-skimming approach for quantification (9.1%; 61 of 674). Method of quantification differed slightly between US (perpendicular drop 80.2% [425 of 530]; tangent skimming 7.4% [39 of 530]) and non-US (perpendicular drop 66.7% [96 of 144]; tangent skimming 15.3% [22 of 144]) laboratories ($P = .007$). Distribution of 28 responses in the "other" category for this question included primarily different combinations of the multiple-choice items listed. A total of 65 participants reported that quantitation was not performed.

More than half (55.0%) of respondents (410 of 745) provided or suggested additional testing for possible IgD or IgE when reactivity to only κ or λ light chains was observed on routine clonal characterization, and 45.0% of respondents (335 of 745) did not test for IgD or IgE in that situation (Table 1, question D). This practice of providing (or suggesting) testing for IgD or IgE reactivity differed slightly between US (53.0%; 312 of 589) and non-US (62.8%; 98 of 156) laboratories ($P = .03$). Few respondent laboratories (4.0%; 30 of 745) offered any tests that could detect the presence of monoclonal therapeutics (Table 1, question E).

Test Selection Questions

Questions directed toward understanding testing approaches are included in Tables 2, 3, 4, and 5. The most common strategy used at participant institutions in the initial screening for the presence of a monoclonal immunoglobulin was SPE (AGE or CZE) with reflex to IFE or IS-CE (39.3% [299 of 760]; Table 2, question F). Serum protein electrophoresis was included in answer choices selected by nearly all participants (95.8%; 728 of 760), with variation directed toward the content of additional reflexive or panel-based testing (Table 2, question F). Practice differences were observed between US and non-US laboratories. The order of the 2 most frequent responses was reversed between US (SPE with reflex to IFE/ISE 42.8% [257 of 600]; SPE only 16.3% [98 of 600]) and non-US (SPE with reflex to IFE/ISE 26.3% [42 of 160]; SPE only 29.4% [47 of 160]) laboratories ($P < .001$). Practice patterns were also analyzed for participants who responded to question F and also provided a facility type designation in our survey. Serum protein electrophoresis with reflex to IFE/ISE was the most frequent response chosen by respondents at nearly all facility types (Table 3). Although SPE only was the most frequent response chosen by participants at national/corporate laboratories (owned by national corporation[s]), SPE with

Table 1. Laboratory Method Questions		
Question	**No.**	**%**
A. What method do you use for serum protein electrophoresis?		
AGE	520	68.6
CZE	238	31.4
Total	**758**	
B. Does your laboratory perform immunofixation or immunosubtraction when an apparent monoclonal band is identified by serum protein electrophoresis (AGE or CZE)?		
Yes, for immunofixation	536	71.7
Yes, for immunosubtraction	56	7.5
Yes, other	23	3.1
No	133	17.8
Total	**748**	
C. How does your laboratory routinely quantitate monoclonal protein?		
Perpendicular drop of M-spike only (including area below the spike)	521	77.3
Tangent skimming of M-spike only (not including underlying polyclonal immunoglobulin)	61	9.1
Quantitative Ig class (nephelometry or turbidimetry)	42	6.2
Serum free light-chain ratio	13	1.9
Heavy/light chains (nephelometry or turbidimetry)	9	1.3
Other	28	4.2
Total	**674**	
Quantitation not performed[a]	65	NA
D. If an apparent monoclonal band only reacts with antisera to either κ or λ light chain (and not to γ, α, or μ heavy chain), does your laboratory perform or suggest additional testing to verify that it is a monoclonal free light chain (and not a monoclonal IgD or IgE immunoglobulin)?		
Yes	410	55.0
No	335	45.0
Total	**745**	
E. Does your laboratory offer a test to detect monoclonal therapeutics used to treat myeloma?		
Yes	30	4.0
No	715	96.0
Total	**745**	

Abbreviations: AGE, agarose gel electrophoresis; CZE, capillary zone electrophoresis; Ig, immunoglobulin; IgD, immunoglobulin D; IgE, immunoglobulin E; M-spike, monoclonal protein; NA, not applicable.

[a] Results not included in total number of responses for question-specific percentages.

reflex to IFE/ISE was still the second most frequent response in that setting (20.6%; 13 of 63; data not shown).

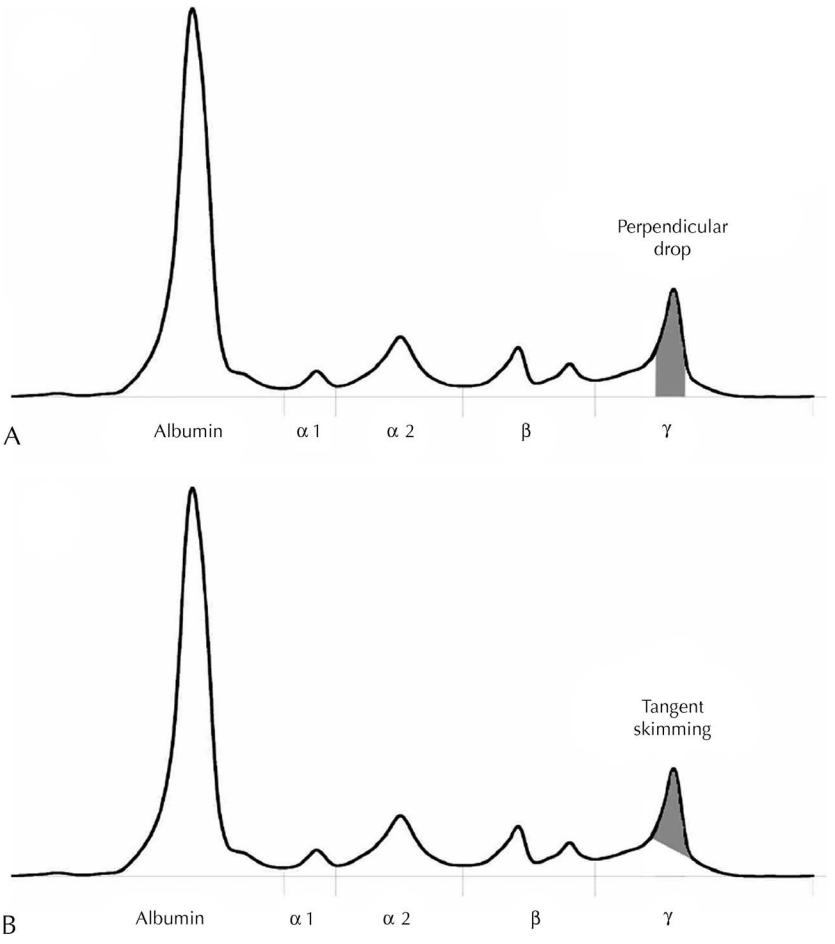
The majority of participant laboratories (60.2%; 462 of 767) did not provide panels of these tests for clinician ordering convenience (Table 2, question G). As the combination of test choices listed in question F may not fully represent laboratory practice across all facilities, a "fill all that apply" version of this question ("Which testing does your laboratory perform for *new* cases of multiple myeloma to *detect* serum monoclonal proteins") was also included in the survey (Table 2, question H). This allowed additional data analyses (Table 2, question H) based on the number of responses and the percentage of participant laboratories. Additionally, a matrix that displays the most common combination of tests selected (Table 4) was created using response data from Table 2, question H. Serum protein electrophoresis (88.1%; 656), IFE (79.1%; 589), and sFLC (34.4%; 256) were used in the highest percentage of 745 laboratories (Table 2, question H). The combination of SPE and IFE was chosen by participants most frequently (36.9% [275 of 745]; Table 4), followed by SPE, IFE, and sFLC (26.6%; 198 of 745), and SPE alone (11.9%; 89 of 745). Of the 47 "other" responses to question H, 19 mentioned that quantitative immunoglobulins (eg, IgG, IgA, IgM) were also commonly ordered for new patients.

A forced-choice question (Table 2, question I) was included to determine which test was primarily used by laboratories to follow patients with monoclonal gammopathy. Most of the 724 participants chose SPE (55.7%; 403), followed by IFE (18.9%; 137) and sFLC (11.7%; 85). However, 84 respondents selected "other." Of this subset, 50 listed a combination of multiple tests, most of which (44) also included SPE.

Participants were also asked which methods were most frequently ordered for monoclonal free light-chain testing and/or monitoring (Table 2, question J). Serum free light-chain (48.4%; 355), urine IFE on 24-hour urine (47.1%; 345), and urine IFE on an early-morning void (43.5%; 319) were used in the highest percentage of 733 laboratories. Of the 70 "other" responses, 19 noted that testing was performed on a "random" urine specimen, whereas 25 noted that free light-chain testing was not performed in house. The "fill in all that apply" format of this question also permitted creation of a matrix displaying the most frequently selected combination of methods used for free light-chain testing (Table 5).

Interpretation Questions

Interpretation of test results (eg, SPE, IFE, and IS-CE) at most participant laboratories is conducted by pathologists who have MD degrees (72.3% [540 of 747]; Table 6, question K). A smaller number of the 747 participant laboratories noted that interpretations were conducted by medical laboratory scientists (11.9%; 89) or scientists with PhD degrees (8.2%; 61). Open-ended text in the 44 "other" responses made thematic or specific reference to interpretation by those with MDs (n = 30), those with PhDs (n = 19), and medical laboratory scientists and/or technologists (n =



Monoclonal protein (M-protein) gating. Electropherograms demonstrating 2 approaches to M-protein quantification. A, Perpendicular-drop method. B, Tangent-skimming method.

19), often in collaboration (eg, "medical laboratory scientist does interpretation, pathologist reviews"). Practice differences in who performed interpretations existed between US and non-US laboratories. For example, MD pathologists were far more likely to perform interpretations in US laboratories (80.7%; 486 of 602) than in non-US laboratories (37.2%; 54 of 145) ($P < .001$).

A majority of participant laboratories (66.6%; 499 of 749) kept a file on all known monoclonal gammopathy patients to assist in review (Table 6, question L). Most laboratories (76.9%; 576 of 749) did not cancel IFE orders on previously characterized specimens with M proteins (Table 6, question M). This was true even if the M protein had the same migration as the originally described M protein (78.2% [577 of 738] did not cancel; Table 6, question N). Trends in the 73 "other" responses to question M included 13 responses noting that intervals of time were used in deciding upon cancellation (eg, "if IFE has been done in last 30 days"), 10 responses noting that a change in results might be used in deciding whether an IFE is required (eg, "if change in electrophoresis pattern"), 20 responses noting that IFE was either nonorderable by clinicians or done reflexively, 7 responses noting that IFE testing might be performed based on ordering clinician specialty (eg, "hematology"), patient location, or specific clinician request, and 4 responses noting that IFE testing was performed at the discretion of the pathologist. Immunofixation electrophoresis order cancellation on specimens with previously characterized M proteins was less common in US (18.6%; 109 of 585) than in non-US (34.0%; 52 of 153) laboratories ($P < .001$). More than half of participant laboratories (59.6%; 442 of 742) did not comment on whether an M protein in a follow-up had "increased, decreased, or not changed" from a previous quantitation (Table 6, question O).

DISCUSSION

Agarose gel electrophoresis remains the most common method of SPE used by participant laboratories, although CZE methods are gaining widespread adoption, particularly in non-US laboratories. It should be noted that a limitation of the survey design is that laboratory size and focus (eg, inpatient versus outpatient) was not assessed and therefore could not be used to assess practice variations in facilities of different sizes or practice types. The distribution of methods reported by respondents in our questionnaire (Table 1, question A) is concordant with analytical methods reported in the 2016 ELP-A proficiency testing challenge, the survey that contained our questionnaire. For example, the proficiency testing event included ~66% AGE and ~34% CZE methods for the total γ globulin challenge.[14] This provides supportive evidence that the voluntary questionnaire responses accurately reflect current laboratory practices by participants. CZE methods are gaining a more widespread adoption by clinical laboratories, as studies have shown that they provide an analytically acceptable alternative to AGE for most routine analysis.[15] Additionally, CZE methods and instrumentation are more automated (eg, specimen/bar code traceable) than traditional gel-based techniques.

Table 2. Test Selection Questions		
Question	**No.**	**%**
F. What is the *most common* approach used by physicians who send specimens to your laboratory in order to screen an individual for the presence of a monoclonal immunoglobulin in the *initial* evaluation?		
Serum protein electrophoresis (AGE or CZE) with reflex to immunofixation or immunosubtraction	299	39.3
Serum protein electrophoresis (AGE or CZE) only	145	19.1
Serum protein electrophoresis (AGE or CZE) and immunofixation or immunosubtraction	106	13.9
Serum protein electrophoresis (AGE or CZE) combined with serum protein immunofixation, serum free light-chain, and immunoglobulin (IgA, IgG, IgM) quantitation	90	11.8
Serum protein electrophoresis (AGE or CZE) combined with serum protein immunofixation and serum free light chain	46	6.1
Serum protein electrophoresis (AGE or CZE) and urine examination for Bence-Jones protein	29	3.8
Serum protein electrophoresis (AGE or CZE) and serum free light chain	13	1.7
Serum free light chain only	1	0.1
Urine examination for Bence-Jones protein only	0	0
Other	31	4.1
Total	**760**	
G. Does your laboratory offer any of the above combinations as a panel for ordering convenience?		
Yes	305	39.8
No	462	60.2
Total	**767**	
H. What testing does your laboratory perform for *new* cases of multiple myeloma to *detect* serum monoclonal proteins? (Multiple responses allowed; n = 745 laboratories)		
Serum protein electrophoresis	656	88.1
Immunofixation	589	79.1
Serum free light-chain quantitation and ratio	256	34.4
Immunosubtraction	72	9.7
Other	47	6.3
Testing not performed[a]	10	NA
I. What testing does your laboratory perform to follow patients with known monoclonal gammopathy?		
Serum protein electrophoresis	403	55.7
Immunofixation	137	18.9
Serum free light-chain quantitation and ratio	85	11.7
Immunosubtraction	15	2.1
Other	84	11.6
Total	**724**	
J. What methods are most frequently ordered in your laboratory for monoclonal free light-chain testing and/or monitoring (Bence-Jones protein)? (Multiple responses allowed; n = 733 laboratories)		
Serum free light-chain quantitation (nephelometry or turbidimetry)	355	48.4
Immunofixation on concentrated urine from 24-hour collection	345	47.1
Immunofixation on concentrated urine from an early-morning void	319	43.5
Urine free light-chain quantitation (nephelometry or turbidimetry)	71	9.7
Other	70	9.5

Abbreviations: AGE, agarose gel electrophoresis; CZE, capillary zone electrophoresis; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; NA, not applicable.

[a] Results not included in total number of responses for question-specific percentages.

The survey also showed that laboratories are conducting clonal characterization most commonly by IFE as opposed to IS-CE (Table 1, question B). This distribution is also roughly concordant with analytical methods reported in the 2016 ELP-A proficiency testing challenge (~81% IFE, ~3% immunoelectrophoresis, and ~16% IS-CE).[14] Slightly higher percentages in the challenge likely reflect the fact that respondents who answered no to survey question B would

Table 3. Most Common Approach by Laboratory Type[a]		
Facility Type	**Most Frequently Selected Response for Question F**	**% of Responses (No./Total)**
Voluntary nonprofit hospitals	SPE (AGE or CZE) with reflex to IFE or IS-CE	42.4 (109/257)
University hospitals	SPE (AGE or CZE) with reflex to IFE or IS-CE	37.4 (46/123)
Regional/local independent laboratories	SPE (AGE or CZE) with reflex to IFE or IS-CE	28.9 (28/97)
City/county/state hospitals	SPE (AGE or CZE) with reflex to IFE or IS-CE	51.0 (49/96)
National/corporate laboratories	SPE (AGE or CZE) only	31.7 (20/63)
Veterans hospitals	SPE (AGE or CZE) with reflex to IFE or IS-CE	65.3 (32/49)
Proprietary hospitals	SPE (AGE or CZE) with reflex to IFE or IS-CE	27.3 (12/44)

Abbreviations: AGE, agarose gel electrophoresis; CZE, capillary zone electrophoresis; IFE, immunofixation electrophoresis; IS-CE, immunosubtraction capillary electrophoresis; SPE, serum protein electrophoresis.

[a] Laboratory types with fewer than 10 participants are not shown (Army/Air Force/Navy hospitals, n = 9; public health nonhospital facilities, n = 8).

Table 4. Combination Testing Performed for New Cases of Multiple Myeloma[a]						
SPE	IFE	IS-CE	sFLC	Other	No.	%
+	+	−	−	−	275	36.9
+	+	−	+	−	198	26.6
+	−	−	−	−	89	11.9
−	+	−	−	−	59	7.9
+	−	+	−	−	16	2.1
+	−	+	+	−	16	2.1
+	+	−	+	+	15	2.0
−	−	−	−	+	12	1.6
+	+	+	−	−	12	1.6
+	+	+	+	−	11	1.5
−	−	+	−	−	10	1.3
+	+	−	−	+	10	1.3
Additional combinations					22	3.0
Total					**745**	

Abbreviations: IFE, immunofixation electrophoresis; IS-CE, immunosubtraction capillary electrophoresis; sFLC, serum free light chain; SPE, serum protein electrophoresis.

[a] + indicates that the test corresponding to the column header was included in the test combination selected by respondents (test combinations organized by row); − indicates that the test was not included in the test combination selected by respondents.

not be participating in that particular component of the proficiency testing event, as they were presumably not offering IFE, immunoelectrophoresis, and/or IS-CE methods in house. IS-CE methods provide a more automated alternative for clonal characterization than IFE, although some performance differences between methods exist, particularly in the detection and characterization of small or atypical M proteins.[16,17]

The vast majority of participants reported using a perpendicular-drop approach to M-protein quantitation, and a smaller percentage use tangent skimming (Table 1, question C). Perpendicular drop includes the entire area under the M-protein gate on the electropherogram, whereas tangent skimming provides one way to exclude potential underlying polyclonal immunoglobulin from the overall M-protein quantitation (Figure 1).[7,18] Much smaller percentages of participants reported using quantitative immunoglobulins, sFLC ratios, or heavy/light chains as methods of M-protein quantitation. Not offering quantitation was noted by a minority of laboratories (8.8% of respondents; 65 of 739), although the survey did not assess whether quantitation was available in these settings as a send-out test. M-protein identification without quantitation does not provide adequate information to clinicians in establishing diagnoses or evaluating the outcome of therapeutic intervention. Because of the differences in actual measurement with these techniques, one should use the same technique when following patient M proteins.

There are several scenarios in which IFE and/or IS-CE results may be misleading or inaccurate. One such scenario is IgD and IgE myeloma,[19] as routine IFE/IS-CE testing does not include antisera to detect δ or ε heavy chains. Only 55.0% of participant laboratories provide or suggest additional testing to exclude these rare disorders when only free light chains are observed by routine testing (Table 1, question D). This suggests that IgD and/or IgE M proteins may go mischaracterized as free light chain disease in a subset of patients.

Growing use of monoclonal antibody therapeutics (MATs) for clinical care has led to increased detection of these drugs as interferents on AGE, CZE, IFE, and IS-CE methods.[20–22] Monoclonal antibody therapeutics such as daratumumab (human anti-CD38 IgG_1-κ)[23] and elotuzumab (humanized anti-SLAMF7 IgG_1-κ)[24] are now being used for treatment of multiple myeloma and may be detected as false-positive bands on electrophoresis.[25]

Table 5. Most Frequent Methods for Free Light-Chain Assessment[a]						
sFLC	IFE on Concentrated Urine Early-Morning Void	IFE on Concentrated Urine 24-h Collection	Urine FLC Quantitation	Other	No.	%
+	−	−	−	−	142	19.4
−	+	−	−	−	112	15.3
−	−	+	−	−	104	14.2
−	+	+	−	−	77	10.5
+	+	+	−	−	64	8.7
+	−	+	−	−	61	8.3
−	−	−	−	+	60	8.2
+	+	−	−	−	33	4.5
+	−	−	+	−	19	2.6
+	+	+	+	−	14	1.9
+	−	+	+	−	9	1.2
+	+	−	+	−	9	1.2
−	−	−	+	−	8	1.1
Additional combinations					21	2.9
Total					**733**	

Abbreviations: FLC, free light chain; IFE, immunofixation electrophoresis; sFLC, serum free light chain.

[a] + indicates that the test corresponding to the column header was included in the test combination selected by respondents (test combinations organized by row); − indicates that the test was not included in the test combination selected by respondents.

Table 6. Interpretation Questions		
	No.	**%**
K. Who performs interpretation of monoclonal gammopathy screening and monitoring in your laboratory?		
MD pathologist	540	72.3
Medical laboratory scientist	89	11.9
PhD scientist	61	8.2
Nonpathologist physician	13	1.7
Other	44	5.9
Total	**747**	
L. Does your laboratory keep a file on all known monoclonal gammopathy patients?		
Yes	499	66.6
No	250	33.4
Total	**749**	
M. When a clinician orders an immunofixation on a previously characterized M protein, does your laboratory cancel the immunofixation?		
Yes	100	13.4
No	576	76.9
Other	73	9.7
Total	**749**	
N. Does your laboratory cancel an order for an immunofixation requested on a follow-up serum that contains an M protein that has the same migration as the original M protein?		
Yes	161	21.8
No	577	78.2
Total	**738**	
O. Do you comment on whether the M protein on a follow-up sample has increased, decreased, or not changed since the previous sample?		
Yes	300	40.4
No	442	59.6
Total	**742**	

Abbreviation: M protein, monoclonal protein.

Although a method to detect one MAT (daratumumab-specific immunofixation electrophoresis reflex assay) has recently been developed,[26,27] few respondents reported that they offer any methods for the detection of MATs. Laboratories should consider the effect that MATs may have on test interpretation and downstream patient care. Medication history and clinical notes may also serve to identify possible MATs in facilities where that information is available (eg, via the electronic health record) to those responsible for interpreting test patterns and results.

Serum protein electrophoresis is used by virtually all participant institutions, and most often as a reflex to IFE or IS-CE. It is interesting to note that most facilities do not provide panels for ordering convenience, although this may be intentional to prevent overuse that can occur with panel-based testing. Use of sFLC testing for screening and monitoring is also quite common. Urine studies are still widely used in the detection and assessment of free light chain disease, although recent studies support the value of sFLC assays over urine studies in many circumstances.[28] Light-chain testing is another area of laboratory medicine that is evolving, and laboratories should work together with ordering providers to perform the most appropriate testing for their patients.

The present survey demonstrated that a variety of test combinations are used for screening and follow-up of patients with monoclonal disorders (Tables 2, 3, 4, and 5). When asked about the most common approach used in screening for the presence of an M protein in an initial evaluation (Table 2, question F), 6.1% mentioned the combination of SPE, serum IFE, and sFLC for screening. A larger percentage (11.8%) included quantitative immunoglobulins (IgA, IgG, and IgM) in addition to the testing above. In a separate "fill in all that apply" question to select which testing was used to detect serum M proteins (see Table 2, question H), the matrix analysis (Table 4) revealed a larger percentage of respondents (26.6%) who chose the test combination of SPE, serum IFE, and sFLC, although it should be noted that quantitative immunoglobulins were not included as a choice in question H, a limitation of the present questionnaire. An additional 2.1% chose the 3-test combination but replaced IFE with IS-CE, and 2.0% chose the 3-test combination with the addition of an "other" test. A limitation of Table 2, question H, and Table 4 that cannot be excluded in our analysis is the possibility that some respondents may have interpreted the question as asking which tests were available on their respective test menus, and not which tests were commonly performed, which was the intended goal of the question.

Another limitation of the current survey is that not all combinations of panel and/or reflexive testing were listed in individual question options, and not all laboratories performed all components of test panels or options. Different-sized facilities (particularly smaller laboratories) may not have the capacity or staffing to support an extensive menu of specialized testing. Respondents also may not have expertise or knowledge of send-out tests (eg, Table 2, question J; "other" included 25 respondents who noted that they did not perform free light-chain testing in house), and respondents may have not incorporated send-out tests into the responses for questions in Tables 2, 3, 4, and 5. These factors may influence responses to questions related to test practices in these areas.

Our survey demonstrated that most tests were interpreted by MD pathologists (Table 6, question K), although medical laboratory scientists, PhD scientists, and nonpathologist physicians were often also involved. Analysis of open-ended responses suggested that interpretation may also have been conducted as a collaborative effort, with initial interpretation by a medical laboratory scientist and final review and interpretation conducted by an MD. The predominance of MD interpretation in US facilities may be influenced by requirements associated with Medicare Part B professional component billing.[29] Differences in MD versus PhD interpretations may also reflect differences in the number and/or distribution of physicians and pathologists versus clinical chemists in practice (or assigned to these tasks), although this is difficult to assess with available data.[30–32]

Most laboratories kept a file on all known monoclonal gammopathy patients to assist with interpretation. The survey did not specifically address other methods of accessing prior test information (eg, databases of results or images associated with electrophoresis instrumentation, electronic health record lookup); thus, the percentage of laboratories with access to historical patient information may actually be higher than reported. Regardless, fewer reported actually using that information to comment on whether an M protein had increased, decreased, or not changed since a previous sample. This would require clinicians to trend results in the patient chart or electronic health record to make that determination for the purpose of clinical care. Harmonization of reporting structure and

interpretative comments, as well as interfacing of results to the laboratory information system, could provide better clarity and more consistent information to clinicians.[33,34] Approximately one-third of laboratories reported not keeping such files of known monoclonal gammopathy patients. Without the ability to refer to prior information about the migration and shape of the M protein, it would be difficult to detect changes in migration that could suggest a second M protein, monoclonal antibody therapy, or even a mislabeled sample.

Regarding potential cancellation of testing, most laboratories did not cancel follow-up IFEs even if the M protein migrated to the same position on SPE as a previous sample. The ability to cancel testing may be limited by compliance and billing considerations.[35] In many settings, the laboratory may be considered a "service" that must follow all order requests based on a fee-for-service approach. In a more practical sense, lack of access to clinical information may also hinder the ability to integrate meaningful utilization strategies. Additionally, patient enrollment in clinical trials (with corresponding test requirements) may also conflict with optimization strategies that are otherwise intended to limit unnecessary testing. Test cancellation practices in the clinical laboratory were the focus of a recent College of American Pathologists Q-probes study, which emphasized the importance of having defined policies and procedures.[36]

Extraordinary advances have been made in the detection and treatment of monoclonal disorders such as multiple myeloma, and these advances are linked to appropriate diagnostic testing. All cases of multiple myeloma are preceded for many years by a monoclonal gammopathy of undetermined significance demonstrated by SPE, IFE, and/or sFLC testing.[37] Treating high-risk cases of smoldering multiple myeloma in patients who are clinically asymptomatic improves both progression-free and overall survival of these patients.[38] Availability of current novel therapies in additional to stem cell transplantation has added years of survival for patients with these disorders.[39] Because early detection and monitoring of monoclonal gammopathy of undetermined significance, smoldering multiple myeloma, and multiple myeloma are heavily dependent on optimal use of the clinical laboratory,[6,40,41] we hope that describing baseline differences in current practices, as evident in our survey, may serve as an impetus for clinical laboratories and providers to consider and/or adopt evidence-based guidelines for monoclonal gammopathy testing as appropriate for their setting and practice characteristics.

A limitation of the present survey, however, is that questions were designed to primarily focus on the use of electrophoretic techniques for the diagnosis and management of monoclonal disorders. Serum protein electrophoresis and urine protein electrophoresis have also been used for decades to aid clinicians in identification of a wide variety of other conditions that alter electrophoretic patterns. For example, SPE has been used to report acute-phase reactions, cirrhosis, nephrotic syndrome, α_1-antitrypsin deficiency, and other clinically relevant findings.[42] In many of these areas, more sensitive and specific assays are now available, but may not be considered in the absence of recognizing an unusual electrophoretic pattern. Further studies may be required to assess the ongoing use of electrophoretic testing for such purposes.

In conclusion, the present survey results provide an overview of current laboratory and clinical practice in the context of monoclonal gammopathy ordering and testing. These results suggest significant variation across facilities. Further efforts at harmonization may help to streamline and simplify ordering and testing practices and create greater clarity for clinicians who use this information in patient care.

The authors would like to thank Mu Shan, MS, for statistical assistance in the initial survey analysis. Drs Genzen, Murray, Abel, Meng, Baltaro, Rhoads, Delgado, Bashleben, and Ansari are members of the College of American Pathologists Diagnostic Immunology Resource Committee.

References

1. Harris NS, Winter WE. *Multiple Myeloma and Related Serum Protein Disorders: An Electrophoretic Guide*. New York, NY: Demos Medical Publishing; 2012.

2. Swerdlow SH, Campo E, Harris NL, et al. *WHO Classification of Tumours of Haematopoietic and Lymphoid Tissue*s. 4th ed. Lyon, France: International Agency for Research on Cancer; 2008.

3. Linden M, McKenna R, eds. *Plasma Cell Neoplasms: A Morphologic, Cytogenetic and Immunophenotypic Approach*. Cham, Switzerland: Springer International Publishing; 2016.

4. Keren D. *Protein Electrophoresis in Clinical Diagnosis*. Chicago, IL: ASCP Press; 2012.

5. Rajkumar SV. Updated diagnostic criteria and staging system for multiple myeloma. In: Dixon D, ed. *2016 ASCO Educational Book*. http://asco.org/edbook. Accessed March 21, 2017.

6. Rajkumar SV, Dimopoulos MA, Palumbo A, et al. International Myeloma Working Group updated criteria for the diagnosis of multiple myeloma. *Lancet Oncol*. 2014;15(12):e538–e548.

7. Keren DF, Schroeder L. Challenges of measuring monoclonal proteins in serum. *Clin Chem Lab Med*. 2016;54(6):947–961.

8. Carr-Smith HD, Jenner EL, Evans JA, Harding SJ. Analytical issues of serum free light chain assays and the relative performance of polyclonal and monoclonal based reagents. *Clin Chem Lab Med*. 2016;54(6):997–1003.

9. Bradwell AR, Harding SJ, Fourrier NJ, et al. Assessment of monoclonal gammopathies by nephelometric measurement of individual immunoglobulin kappa/lambda ratios. *Clin Chem*. 2009;55(9):1646–1655.

10. Dispenzieri A, Kyle R, Merlini G, et al. International Myeloma Working Group guidelines for serum-free light chain analysis in multiple myeloma and related disorders. *Leukemia*. 2009;23(2):215–224.

11. Willrich MA, Katzmann JA. Laboratory testing requirements for diagnosis and follow-up of multiple myeloma and related plasma cell dyscrasias. *Clin Chem Lab Med*. 2016;54(6):907–919.

12. Katzmann JA, Dispenzieri A, Kyle RA, et al. Elimination of the need for urine studies in the screening algorithm for monoclonal gammopathies by using serum immunofixation and free light chain assays. *Mayo Clin Proc*. 2006;81(12):1575–1578.

13. Katzmann JA, Kyle RA, Benson J, et al. Screening panels for detection of monoclonal gammopathies. *Clin Chem*. 2009;55(8):1517–1522.

14. *ELP-A 2016 Participant Summary: Electrophoresis*. Northfield, IL: College of American Pathologists; 2016.

15. McCudden CR, Mathews SP, Hainsworth SA, et al. Performance comparison of capillary and agarose gel electrophoresis for the identification and characterization of monoclonal immunoglobulins. *Am J Clin Pathol*. 2008;129(3):451–458.

16. Yang Z, Harrison K, Park YA, et al. Performance of the Sebia CAPILLARYS 2 for detection and immunotyping of serum monoclonal paraproteins. *Am J Clin Pathol*. 2007;128(2):293–299.

17. Poisson J, Fedoriw Y, Henderson MP, et al. Performance evaluation of the Helena V8 capillary electrophoresis system. *Clin Biochem*. 2012;45(9):697–699.

18. Schild C, Wermuth B, Trapp-Chiappini D, Egger F, Nuoffer JM. Reliability of M protein quantification: comparison of two peak integration methods on Capillarys 2. *Clin Chem Lab Med*. 2008;46(6):876–877.

19. Pandey S, Kyle RA. Unusual myelomas: a review of IgD and IgE variants. *Oncology*. 2013;27(8):798–803.

20. McCudden CR, Voorhees PM, Hainsworth SA, et al. Interference of monoclonal antibody therapies with serum protein electrophoresis tests. *Clin Chem*. 2010;56(12):1897–1899.

21. Genzen JR, Kawaguchi KR, Furman RR. Detection of a monoclonal antibody therapy (ofatumumab) by serum protein and immunofixation electrophoresis. *Br J Haematol*. 2011;155(1):123–125.

22. Willrich MA, Ladwig PM, Andreguetto BD, et al. Monoclonal antibody therapeutics as potential interferences on protein electrophoresis and immunofixation. *Clin Chem Lab Med*. 2016;54(6):1085–1093.

23. Lokhorst HM, Plesner T, Laubach JP, et al. Targeting CD38 with daratumumab monotherapy in multiple myeloma. *N Engl J Med*. 2015;373(13):1207–1219.

24. Lonial S, Dimopoulos M, Palumbo A, et al. Elotuzumab therapy for relapsed or refractory multiple myeloma. *N Engl J Med*. 2015;373(7):621–631.

25. Murata K, McCash SI, Carroll B, et al. Treatment of multiple myeloma with monoclonal antibodies and the dilemma of false positive M-spikes in peripheral blood [published online ahead of print September 21, 2016]. *Clin Biochem*. doi: 10.1016/j.clinbiochem.2016.09.015.

26. McCudden C, Axel AE, Slaets D, et al. Monitoring multiple myeloma patients treated with daratumumab: teasing out monoclonal antibody interference. *Clin Chem Lab Med*. 2016;54(6):1095–1104.

27. van de Donk NW, Otten HG, El Haddad O, et al. Interference of daratumumab in monitoring multiple myeloma patients using serum immunofixation electrophoresis can be abrogated using the daratumumab IFE reflex assay (DIRA). *Clin Chem Lab Med*. 2016;54(6):1105–1109.

28. Dejoie T, Corre J, Caillon H, et al. Serum free light chains, not urine specimens, should be used to evaluate response in light-chain multiple myeloma. *Blood*. 2016;128(25):2941–2948.

29. Chapter 12—physicians/nonphysician practitioners. In: *Medicare Claims Processing Manual*. https://www.cms.gov/Regulations-and-Guidance/Guidance/Manuals/downloads/. Updated August 12, 2016. Accessed May 2, 2017.

30. Smit E, Beastall G, Bjorses UM, et al. The number of consultant clinical chemists in the 15-nation European Union. *Clin Chem Lab Med*. 2005;43(3):335–341.

31. Robboy SJ, Weintraub S, Horvath AE, et al. Pathologist workforce in the United States, I: development of a predictive model to examine factors influencing supply. *Arch Pathol Lab Med*. 2013;137(12):1723–1732.

32. American Association of Medical Colleges. *2015 State Physician Workforce Data Book*. http://members.aamc.org/eweb/upload/2015StateDataBook%20(revised).pdf. Published November 2015. Accessed January 17, 2017.

33. Moss MA. Moving towards harmonized reporting of serum and urine protein electrophoresis. *Clin Chem Lab Med*. 2016;54(6):973–979.

34. Mathur G, Haugen TH, Davis SL, Krasowski MD. Streamlined sign-out of capillary protein electrophoresis using middleware and an open-source macro application. *J Pathol Inform*. 2014;5(1):36.

35. CMS Manual System. Pub 100-02 Medicare benefit policy: transmittal 80. https://www.cms.gov/Regulations-and-Guidance/Guidance/Transmittals/downloads/R80BP.pdf. Published January 11, 2008. Accessed January 18, 2017.

36. Darcy TP, Barasch SP, Souers RJ, Perrotta PL. Test cancellation: a College of American Pathologists Q-Probes study. *Arch Pathol Lab Med*. 2016;140(2):125–129.

37. Landgren O, Kyle RA, Pfeiffer RM, et al. Monoclonal gammopathy of undetermined significance (MGUS) consistently precedes multiple myeloma: a prospective study. *Blood*. 2009;113(22):5412–5417.

38. Mateos MV, Hernandez MT, Giraldo P, et al. Lenalidomide plus dexamethasone for high-risk smoldering multiple myeloma. *N Engl J Med*. 2013;369(5):438–447.

39. Ozaki S, Handa H, Saitoh T, et al. Trends of survival in patients with multiple myeloma in Japan: a multicenter retrospective collaborative study of the Japanese Society of Myeloma. *Blood Cancer J*. 2015;5:e349.

40. Dispenzieri A, Stewart AK, Chanan-Khan A, et al. Smoldering multiple myeloma requiring treatment: time for a new definition? *Blood*. 2013;122(26):4172–4181.

41. Chesi M, Bergsagel PL. Advances in the pathogenesis and diagnosis of multiple myeloma. *Int J Lab Hematol*. 2015;37(suppl 1):108–114.

42. Vavricka SR, Burri E, Beglinger C, Degen L, Manz M. Serum protein electrophoresis: an underused but very useful test. *Digestion*. 2009;79(4):203–210.