Report of Survey conducted by IFCC WG Harmonisation of Interpretive Commenting EQA (WG-ICQA) subgroup:

Results of an international survey of the reporting of protein electrophoresis and serum free light chains, and quantification of small monoclonal proteins.

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Introduction:

Clinical laboratory testing plays an important role in the diagnosis, monitoring and prognostication of monoclonal gammopathies. Analytical methods using serum and urine protein electrophoresis (SPEP, UPEP), immunofixation electrophoresis (IFE) and immunosubtraction (IS), serum free light chains (FLC), immunoglobulin and heavy/light chain (HLC) immunoassay, and more recently mass spectrometry, identify and are used to quantify monoclonal proteins (also called M-protein or monoclonal free light chains in keeping with the clinical guidelines, although some countries may use preferred nomenclature such as M-spike, paraprotein, monoclonal component).

While international clinical guidelines for myeloma, AL amyloidosis, and Waldenström macroglobulinemia advise on the required M-protein testing for these monoclonal gammopathies, they do not recommend the exact methodology that clinical laboratories should use for the quantification and reporting of M-proteins.

Results from External Quality Assurance (EQA) programs and other surveys of protein electrophoresis show that there is large variation in the quantification of M-proteins between laboratories and this is reflected in large differences in absolute values for the various protein electrophoresis methods and for serum FLC measured using different manufacturers' assays or different platforms for the same manufacturer's assay (1-3). This variation may be larger than the within-subject biological variation that is typically measured using a single analyser platform. Hence, while we may have good quality control of methods within our laboratory, the variation between laboratories is far wider and may impact the monitoring of disease response if the patient attends different medical centres for their testing and results are linked cumulatively in the electronic health record.

References:

1. Tate JR, Keren DF, Mollee P. A global call to arms for clinical laboratories – Harmonised quantification and reporting of monoclonal proteins. Clin Biochem 2018;51:4-9.

2. Booth R, McCudden CR, Balion C, Blasutig IM, Bouhtiauy I, Rodriguez-Capote K, et al. Candidate recommendations for protein electrophoresis reporting from the Canadian Society of Clinical Chemists Monoclonal Gammopathy Working Group. Clin Biochem 2018;51:10-20.

3. Genzen JR, Murray DL, Abel G, Meng QH, Baltaro RJ, Rhoads DD, et al. Screening and diagnosis of monoclonal gammopathies. An international survey of laboratory practice. Arch Pathol Lab Med 2018;142:507-15.

Aim of survey:

In order to determine how clinical laboratories that perform routine protein testing for monoclonal gammopathy quantitate, interpret, and comment on M-proteins when reporting results, the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) subgroup of the Working Group on the Harmonisation of Interpretive Commenting in EQA (WG-ICQA) developed a survey that was distributed to all IFCC societies through the IFCC secretariat and Survey Monkey in early 2017.

Survey Methodology:

The survey contained a total of 30 questions that addressed specific aspects of the Pre-analytical, Analytical and Post-analytical phases, as well as laboratory demographics (location, affiliation, relevant annual test volumes) of each responding laboratory. Sections A and B (Pre-analytical phase) consisted of six questions related to clinical guidelines and test requesting for the diagnosis and monitoring of disease response in myeloma and AL amyloidosis. Section C (Analytical phase) consisted of 11 questions covering protein electrophoresis methodology, quantitation, limit of detection of M-protein, and interference by therapeutic monoclonal antibody (mAb) immunotherapy. Section D (Post-analytical phase) contained eight questions covering the reporting of M-proteins and interpretative commenting.

The survey took place over approximately 4 months between January and April, 2017. There were 347 responses to the survey; however, 102 responses did not include the country of testing and response rates to questions decreased as the survey progressed. The figure "Response rates to questions" shown in the attachment "IFCC Survey SPEP SFLC 245 labs 31 countries.pdf" indicates the number of questions skipped over during survey progression. Complete responses to all questions were received from 31 countries and included 245 laboratories. As it was uncertain if some laboratories participated more than once, only completed survey responses have been included in this report. Of the 10 countries (217 laboratories) from which more than 5 laboratories participated per country, Italy had the highest number of participants (N=83), followed by the United Kingdom (N=42), then Australia and New Zealand (N=27), followed by The Netherlands (N=22) (refer to second attachment "IFCC Survey SPEP SFLC 217 labs 10 countries.pdf").

Of the 245 participating laboratories that provided their affiliations, 44.7% and 5.3% were located at public and private Metropolitan Hospitals, respectively, 29.5% were at University Hospitals, while 11.1%, 10.7% and 10.2% were either local or national reference laboratories, practised in private pathology or were in General Practice, respectively.

Results and conclusions:

Several questions involved multiple selections and values are expressed as the total number of responses and percent responses (of 245 participants) for each individual selection. Results are presented for some questions only (Table 1). Conclusions are also provided where appropriate. Also refer to "IFCC Survey SPEP SFLC 245 labs 31 countries.pdf" and "IFCC Survey SPEP SFLC 217 labs 10 countries.pdf" for further results.

Table 1. Responses to several of the survey questions.

Question	Options / % labs or responses	Conclusions
Q1: If you are asked to screen for a monoclonal gammopathy, which of the following describe best your laboratory procedure?	a. Perform SPEP and/or UPEP – 14% b. Perform screening IFE (i.e. 1 lane kappa/lambda or pentavalent antiserum) – 3% c. Perform SPEP and reflex to/or request full IFE or IS – 35% d. Perform UPEP and reflex to/ or request full IFE or IS – 0% e. Perform SPEP and reflex to/ or request full IFE or IS, Igs, and serum FLC – 35% f. Perform other tests (please state in free text) – 13%	Clinical guidelines are generally followed. 70% of labs reflexed to or recommended follow-up tests when an M-protein was found on SPEP.
Q's 4&5: What tests are used in your institution to follow-up a treated myeloma case with the M-protein migrating in the gamma fraction and in the beta/alpha-2 fractions? **Select all responses that apply.**	a. SPEP and M-protein quantification – 80-89% b. IFE or IS if M-band visible on SPEP – 28-31% c. IFE or IS if M-band NOT visible on SPEP but previously detected on IFE/IS – 56-60% d. Serum FLC – 60-62% e. Serum heavy-light assay – 3-5% f. Ig quantitation – 52-55% g. Selective Ig quantitation e.g. in gamma if IgA M-protein is <10 g/L (<1.0 g/dL) or IgA M-protein in beta/alpha-2 fraction – 17-32% h. UPEP and IFE (and quantification of BJP if detected) – 41% i. Other tests (please state in free text) – 11-14%	The number of responses recommending selective Ig quantitation varied depending on whether the M-protein was in the gamma or beta/alpha-2 fractions.
Q7: Once a light chain (kappa or lambda) is identified on serum	a. Reflex IFE to serum FLC – 11% b. Run IFE with anti-IgD antiserum – 6%	75-81% of labs would have checked for IgE and IgD, respectively. However,

IFE or IS without a corresponding heavy chain for the first time, with no available history, what is the next step?	c. Run IFE with anti-IgD and anti-IgE antisera – 65% d. Report a monoclonal light chain – 8% e. Send-out to reference lab for confirmation and additional testing – 10%	19% of labs would either have not tested for IgD (and IgE) but would report either a monoclonal light chain or reflexed to serum FLC.
Q8: Do you perform any routine testing to distinguish between an endogenous M-protein and a therapeutic mAb?	a. Yes – 4% b. No – 96%	Only a small minority of labs (4%) perform routine testing to distinguish between an endogenous M-protein and a therapeutic mAb.
Q9: Which method do you use (or think will be able to use in the future) to detect this interference?	a. DIRA, daratumumab-specific immunofixation electrophoresis reflex assay – 18% b. Other IFE-based tests with anti-drug-antibodies immune-complex formation – 12% c. Mass spectrometry method able to identify the mAb molecular mass and compare it to a library – 10% d. We are ready to send out these samples to more specialized labs – 60%	Currently there is not yet a clear consensus of the preferred method to detect mAb interference. 60% of labs will outsource these analyses.
Q11: How do you currently quantitate the M-protein migrating in the gamma fraction?	a. Perpendicular drop of M-spike only, including any polyclonal Ig background – 63% b. Tangent skimming of M-spike, not including the polyclonal Ig background – 23% c. Quantitation not performed - report qualitatively as small, medium or large – 3% d. Ig quantitation by nephelometry or turbidimetry – 11% e. Quantify serum FLC – 0%	Perpendicular (orthogonal) method of gating M-protein is most popular method.
Q15. When do you quantitate the beta-fraction or beta-1 and beta-2 fractions?	a. When there is a shoulder and the M-protein is visible and distinguishable from the normal protein background – 59% b. When the entire beta-fraction is greater than 20 g/L (2 g/dL) – 8% c. Don't quantitate beta-migrating M-proteins using electrophoresis, only total beta-fraction concentration is reported – 36% d. Free text (Other) – 31%	59% of labs quantitate the M-protein if the band can be distinguished from the normal background. However, 68 labs (31%) offered their approaches in the free text indicating the heterogeneity in quantitating bands that migrate in the beta region.
Q16: How do you quantify M-proteins overlapping normal	a. Perpendicular drop of M-spike only, including any normal protein background – 28%	32% of labs prefer to quantify "Total beta/alpha-2 + M-protein"

proteins in the beta and alpha-2 fractions when the M-protein is not clearly separated?	b. Tangent skimming of M-spike, not including the normal protein background – 7% c. Quantitation not performed; rather the total beta or alpha-2 fraction containing the M-protein is reported – 32% d. Recommend or reflex to Ig quantitation by nephelometry or turbidimetry – 11% e. Recommend serum FLC – 4% f. Recommend or reflex to heavy-light chain pairs (e.g. IgAK/IgAL) – 2% g. Other (please write in free text) – 16%	
Q23: How do you report the first presentation of a small abnormal band on SPEP/IFE in a patient with no known M-protein? **Select all responses that apply.**	a. There is a small (type: e.g. IgG kappa) band approximately (amount: e.g. 1 g/L [0.1 g/dL]) – 68% b. Its clinical significance is uncertain – 17% c. Suggest UPEP and IFE, or serum FLC – 41% d. Repeat SPEP in 3–6 months if clinically indicated – 36% e. Other (please write in free text) – 18%	68% of responses recommended putting a comment when a small band was present.
Q24: How do you report a new, small abnormal band with different electrophoretic mobility from the original M-protein in a patient with a known M-protein? **Select all responses that apply.**	a. There is a small (type: e.g. IgG kappa) band approximately (amount: e.g. 1 g/L [0.1 g/dL]) on a background of a polyclonal and / or oligoclonal pattern – 35% b. This band is different from the original M-protein – 49% c. Its clinical significance is uncertain – 9% d. In the case of the band being identified as IgG kappa: "A new small monoclonal IgG kappa) band has been found in the gamma fraction on immunofixation. This could represent a new clone or the presence of a therapeutic monoclonal antibody. Clinical correlation is required" – 31% e. Other (please write in free text) – 22%	35% of responses only recommended putting a comment when a small band was present in a patient with a known M-protein. Fewer labs seemed to understand the clinical significance of these transient small bands.

IFE, immunofixation electrophoresis; Ig, immunoglobulin; IS, immunosubtraction; M-protein (also known as monoclonal component , M-spike, M-band, paraprotein); serum FLC, serum free light chains (by immunoassay); SPEP, serum protein electrophoresis; mAb, therapeutic monoclonal antibody; UPEP, urine protein electrophoresis.

Main Conclusion:

The quantification and reporting of small bands on protein electrophoresis are difficult to harmonise as indicated from the survey. Questions 11, 15, 16, 23 and 24 indicate the heterogeneity of these processes among laboratories. It may be that clinical societies in individual countries will need to work with their haematologists and immunologists to achieve greater harmonisation, at least within a country.

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