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ORIGINAL ARTICLE

Survey on serum protein electrophoresis and recommendations for standardised reporting

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Abstract

Introduction: Serum protein electrophoresis (SPE) is a well-established laboratory technique. However, reporting of results varies considerably between laboratories. The variation in reporting can cause confusion to the clinician with a potential of adversely impacting patient care. The purpose of the survey was to find out the variation in reporting and to prepare recommendations to the Malaysian laboratories based on the survey to reduce both the variation in reporting between laboratories and the risk of misinterpretation of reports. *Materials and Methods:* To determine the extent of variation in reporting of protein electrophoresis results questionnaires were distributed to the pathologists of various laboratories in Malaysia regarding the method, quantification of paraprotein concentrations and immunoglobulin assays, and information regarding current laboratory electrophoresis practices. *Results:* Variation was found in the following reporting practices: (a) screening protocol; (b) reporting of serum albumin; (c) numerical reporting of protein fractions and paraprotein; (d) co-migration of a paraprotein with a normal serum protein; (e) reporting of multiple paraprotein bands (f) appearance of small abnormal band and oligoclonal bands and (g) communication about of interferences. *Conclusion:* The pathologists of the country made recommendations on the reporting of protein electrophoresis. Harmonised reporting will reduce inconsistency, variation in reporting, improve the quality of the report and most importantly improve patient care.

Keywords: Multiple myeloma, protein electrophoresis, standardised reporting

INTRODUCTION

The monoclonal gammopathies (MG) are a group of disorders characterised by the proliferation of one clone of plasma cells. These disorders range from the benign (pre-malignant) to the malignant plasma cell dyscrasias (PCD) to the lymphoproliferative disorders, e.g. benign monoclonal gammopathy of undetermined significance (MGUS), smouldering myeloma to symptomatic multiple myeloma (MM), AL (light-chain) amyloidosis, Waldenström macroglobulinaemia (WM) and plasmacytoma.[1] Monoclonal proteins or paraproteins are the key biomarkers of MG. Protein electrophoresis (PE) is commonly used as an aid in the diagnosis of MG. The term paraprotein is used to describe a discrete band containing immunoglobulins of a single light and/or heavy chain class, which are visible on electrophoretic separations of serum or urine. The International Myeloma Working Group (IMWG) has published recommendations for diagnosis, prognosis and treatment of myeloma.[2] The diagnostic tests included in the guidelines are assessments of monoclonal protein in serum and urine and serum-free light chain (sFLC) levels. Nevertheless, the guideline does not discuss

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the methodologies that should be used by the clinical laboratories for the quantification and reporting of monoclonal proteins. Interpretative comments are always included in the serum/urine PE results. Wide variations in quantification and reporting of serum/urine PE were revealed by the survey conducted in Canada, Australia and New Zealand and by the International Federation of Clinical Chemistry (IFCC).[3,4,5] The variation in reporting can cause confusion for the clinician with a potential to adversely impact patient care.

The first guideline to assist the clinical laboratory in supporting the diagnosis and monitoring of multiple myeloma and other plasma cell dyscrasias was published by the College of American Pathologists in 1998[6] and this was revised and updated in the year 2012.[7] The IFCC has formed a working group on Harmonization of Interpretive Commenting EQA (WG-ICQA). Although there is no formal recommendation by this group, in a special issue of Clinical Chemistry and Laboratory Medicine (CCLM), they have published an article on laboratory testing in the diagnosis and monitoring of plasma cell disorders.[8] In 2012, the Australasian Association of Clinical Biochemists (AACB) developed comprehensive recommendations for standardised reporting of Protein Electrophoresis[9] and have proposed an addendum to their recommendations in 2019.[10] The Canadian Society of Clinical Chemists Monoclonal Gammopathy Working Group also published candidate recommendations for protein electrophoresis reporting in 2017.[11]

In Malaysia, protein electrophoresis is performed only in tertiary care centres and in specialised laboratories. Interpretative comments are provided by the pathologists. There are no formal guidelines or recommendations available on the reporting of protein electrophoresis in our country. The aim of this study was to assess whether variation in reporting was significant within Malaysia. Hence, an online survey was conducted to gather information regarding the method, quantification of paraprotein concentrations by serum protein electrophoresis (SPE) and immunoglobulin assays, and information regarding current laboratory electrophoresis practices. To have uniform reporting of PE by the pathologists throughout the country, a group of chemical pathologists decided to prepare recommendations. This paper summarises the results of the survey and the recommendations for standardised reporting of protein electrophoresis.

MATERIALS AND METHODS

The pathologists reporting protein electrophoresis were invited by email to participate in the survey in April 2019. The questionnaire consisted of 24 simple questions (Table 1). Framing of the questionnaire was done by referring to AACB and the IFCC survey forms.[4,5] Questions were addressed to the methods, nomenclature, quantification and reporting of co-migrating paraproteins and the presence of small bands. Most of the questions were simple in the form of multiple-choice questions and a few questions were of free text option.

RESULTS

Only seven laboratories report protein electrophoresis in the country. All the laboratories participated in the survey.

1. **Nomenclature**
   Different terms are used to represent the monoclonal component identified by protein electrophoresis. However, the term "paraprotein" was used by all laboratories.

2. **Serum protein electrophoresis and immunotyping methods**
   The popular method used for SPE and the typing of paraprotein in the country is the agarose gel-based system. Capillary electrophoresis is not used.

3. **Screening for paraprotein**
   The protocol for screening for MG differs among the seven laboratories. Four of the laboratories perform serum and urine protein electrophoresis with reflex immunofixation (IFE). Serum and urine electrophoresis combined with IFE and sFLC measurement are performed by one laboratory, while another laboratory performs only serum and urine protein electrophoresis. One laboratory performs SPE combined with IFE and sFLC but urine protein electrophoresis is not part of the screening protocol and is performed only when necessary.

4. **Reporting individual fractions**
   The method used for protein fraction identification and quantification should clearly identify individual fractions. The respondents report on all of the common serum protein electrophoresis fractions.

5. **The analytical methodology used to quantify serum albumin**
   It should be clearly indicated if serum albumin levels are also reported from the same laboratory using other techniques. Serum albumin measured either by bromocresol Purple (BCP) or bromocresol green (BCG) method is reported by three laboratories and the remaining laboratories report serum albumin obtained by SPE.

6. **Number of decimal places reported in quantitative fractions**
   The number of decimal places reported for quantitative fractions is not uniform. One laboratory does not use decimal fractions at all, while other laboratories use either one or two decimal places.

7. **Serum immunoglobulin results in electrophoresis reporting**
   Serum immunoglobulins are only performed upon specific request and not routinely included as part of serum electrophoresis by four laboratories whereas it is reported with SPE by three laboratories.

8. **Paraprotein quantification in the gamma region**
   Even though there are different methods used to quantify the paraprotein migrating in the gamma region, the perpendicular method (PD) is the method of choice for quantifying the paraprotein concentration.

9. **Number of decimal places used in reporting paraprotein if the quantification is > 10 g/L**
   The majority report a single decimal place when the paraprotein concentration is > 10 g/L whereas one laboratory reports as a whole number.

10. **Number of decimal places used in reporting monoclonal protein if the quantification is < 10g/L**
    The reporting is similar to that reported when paraprotein concentration is > 10 g/L.

11. **Quantification and reporting of co-migrating paraproteins in the beta or alpha-2 region**
    There is variation in reporting these co-migrating paraproteins. Four of the laboratories report the beta-migrating paraproteins as "total beta + paraprotein", but the others report it as paraprotein concentration.

12. **Reporting a small paraprotein in the beta region that cannot be distinguished from the normal beta proteins**
    IFE was performed by five of the laboratories and these laboratories reported the quantity of the beta globulin. IFE was not performed by two laboratories and they do not report the presence of paraprotein co-migrating in the beta region.

13. **Reporting of multiple paraproteins**
    When multiple paraproteins are present, two laboratories do not report the individual concentrations of the paraproteins but instead report the sum of the paraprotein concentrations.

14. **Reporting of small abnormal bands**
    The way of reporting and the comments are different amongst the laboratories.

15. **Reporting significant changes in monoclonal proteins and when it is significant**
    When reporting serial protein electrophoresis, a 50% change in the monoclonal protein concentration is considered a significant change. Some interpreters do not report significant change.

16. **Reporting normal SPE and other pathological patterns**
    There is consensus in the reporting of normal SPE and other pathological patterns. The normal pattern is reported as a "normal pattern and no paraprotein detected". Other specific pathological patterns are reported by the interpreters.

17. **Reporting oligoclonal bands**
    There is no consensus in reporting of oligoclonal bands. Oligoclonal bands are reported by three laboratories as "Oligoclonal bands can appear in a number of infections or autoimmune conditions. Suggest review after 3 to 6 months".

18. **Reporting of the interferences to the clinician**
    Comments mentioning the interferences are generally not included in the report by any of the laboratories.

DISCUSSION

Serum protein electrophoresis is reported by a limited number of laboratories in this country, but there are variations in reporting. The following aspects (1) screening for monoclonal gammopathies (2) decimal places used in reporting the fractions as well as paraproteins (3) reporting serum albumin (4) reporting of serum immunoglobulins with SPE report (5) reporting of paraproteins co-migrating in beta or alpha 2 regions (6) reporting of multiple paraproteins and (7) oligoclonal bands need to be harmonised in reporting of protein electrophoresis.

Only the agarose gel method is being currently used by laboratories for performing PE and typing and quantification of paraprotein, unlike what has been reported in other audit findings.[4,5] While screening for paraproteins, the initial investigations include quantitative immunoglobulin levels (IgG, IgA, and IgM), SPE, IFE, as well as sFLC ratio to obtain information about the type and quantity of the paraprotein.[1] Urinalysis includes a 24-hour urine total protein and UPE, which remains a part of the IMWG criteria for the diagnosis of light-chain only disease.[1] We realised that the screening protocol used in our country differs from the IMWG criteria. Following IMWG criteria would help in a better screening for multiple myeloma.

The purpose of doing SPE is to know whether a paraprotein is present. However, reporting the total protein, albumin and other fractions are also important. Reporting these fractions to provide additional information other than the presence or absence of paraprotein alone. When reporting fractions to clinical users, laboratories should include appropriate reference intervals and significant digits.[11] We noted in this audit that all laboratories provided quantitative values for protein fractions (albumin, alpha, beta and gamma) when SPE was requested. However, there is a difference in the number of decimal places used in the reporting. Salamatmanesh *et al*. suggested that the number of decimals that should be reported for the various fractions should be based on the analytical precision.[12]

Serum albumin reported with protein electrophoresis is either by densitometry from SPE or quantified by an automated analyser using BCG or BCP method. Albumin quantification by densitometry from the SPE may be overestimated in the presence of high paraprotein concentration.[13] When the laboratory reports albumin by both methods, there may be inconsistency and it may confuse the clinician. To avoid this, the analytical methodology should be indicated when reporting protein fractions if the laboratory is using both methods to report serum albumin.[11] Irrespective of the albumin method used, there should be consistent reporting of serum albumin in the SPE report.

Investigations for screening and monitoring of MG include quantitative immunoglobulin analysis. Immunoglobulin quantification may be useful in monitoring monoclonal immunoglobulins, but the measurement may be positively biased as the measurement includes both the monoclonal and the polyclonal immunoglobulins. IgG paraprotein quantification by densitometry has been shown to underestimate at higher concentrations of IgG compared to immunonephelometry, probably due to a dye saturation effect.[14] Laboratories should be aware of the limitation of both methods. The recommendations for standardised reporting of protein electrophoresis in Australia and New Zealand states that at the time of diagnosis of a plasma cell dyscrasia, paraprotein should be quantified by the electrophoretogram and immunoglobulins (G, A, M) be measured by immunonephelometric or immunoturbidimetric method.[9] In this audit, we observed that serum immunoglobulins are only performed upon specific request and not routinely included as part of SPE. In the case of IgA multiple myeloma, when the paraprotein band overlaps the beta region, reporting IgA levels with the SPE will help the clinician in monitoring the response to treatment.

Quantification of paraprotein is by the integration of the paraprotein peak in the electropherogram. Integration is usually performed by perpendicular drop (PD) method or by tangent skimming (TS). Since no reference method is available for paraprotein quantification, it is not possible to assess which gating method is accurate. This survey indicated that only PD method is used in the quantification of paraprotein.

Tate *et al*.[9] have recommended that paraproteins in the gamma region quantified by densitometry should be reported in g/L and rounded to the nearest whole number. At lower concentration, when rounding to the nearest whole number is done, the rounding error will also contribute to the total error. The Canadian Society of Clinical Chemists Monoclonal Gammopathy Working Group has recommended[11] when the paraprotein concentration is > 10 g/L it has to be reported as a whole number, whereas when

the concentration is < 10 g/L one decimal place should be reported. This survey revealed that most of the laboratories use a single decimal place in the report whether the paraprotein is > or < 10g/L.

Quantification of a small paraprotein band can be problematic due to the significant contribution of background polyclonal immunoglobulins and can result in overestimation. There is no guideline available regarding the lower limit of reporting the paraprotein level. Currently, an IFCC-sponsored project is assessing the functional sensitivity of SPE and immunotyping methods, hence information on method sensitivity may be available later. Based on the lower limit of detection and imprecision by SPE and IFE, Tate *et al*.[9] recommend the lower limit of quantification of paraprotein to be 1 g/L. This is supported by the Canadian group also.[11] However, we noticed that most of the laboratories report the actual value that is obtained by peak integration. The peak integration below the lower limit of detection may not be precise and can lead to inconsistency when reported by different pathologists.

Paraprotein can migrate with α2 and β globulins and when it co-migrates with these proteins, the quantification of paraprotein is difficult. In these situations, there will be an overestimation of the paraprotein. An estimate of the 'true' paraprotein concentration by subtracting other beta globulins may not be a reliable way of reporting paraprotein concentration. Hence when the paraprotein migrates in the non-gamma region in alpha-2 or more commonly in the beta region, it is recommended to report the paraprotein as "total beta + paraprotein".[9] No consistency in reporting these paraproteins was noted by the survey. This may lead to over or underestimation of the paraprotein concentration. When the paraprotein level is low, it is impossible to differentiate paraprotein from the normal alpha-2 or beta globulins. IFE and immunoglobulin levels may help in these situations. However, there is inconsistency in the reporting when small paraprotein migrates in these regions. This may classify the response to treatment wrongly.

The IMWG[2] defines a "very good partial response" when there is ≥ 90% reduction in serum paraprotein, "a partial response" as 50% decrease and "progressive disease" when 25% increase from the lowest response value of serum paraprotein (the absolute increase must be >5 g/L). These recommendations are based largely on expert opinion. Only limited studies are available regarding the biological variation[12,15] and the biological variation that has been quoted in these studies are very different. Salamatmanesh *et al*.[12] noted the reference change value ranged from 36.7% to 39.6% depending on the monoclonal protein concentration. As we noted in our survey, reporting 50% change as a significant change may not be appropriate. The response to the treatment may be misclassified and will affect the management of the patient.

When multiple paraprotein bands are present, each should be quantified if possible and the value of each paraprotein should be correlated with the type of paraprotein.[11] Failure to identify may lead to mismanagement of the patient. We noticed in this audit that a couple of laboratories sum up the individual paraprotein and report as a single concentration.

Oligoclonal patterns appear as multiple distinct immunoglobulin bands often with different heavy chains and different light chains in SPE and IFE. Such oligoclonal patterns have been noticed in infection with human immunodeficiency virus, cytomegalovirus infection, after haematopoietic stem cell transplantation and in solid organ transplantation with immunosuppression.[16] In the post-autologous stem cell transplant setting, it may represent a benign regenerative process.[17] Hence it is important to recognise and report oligoclonal patterns. The survey revealed that there are inconsistencies in the reporting of oligoclonal bands.

Like many other laboratory tests, serum and urine protein electrophoresis are also not exempted from interferences.[18] Erroneous results can be reported due to interferences and it may lead to unnecessary clinical investigations. Many of the interferences can be identified by performing IFE. However, the interpreters of SPE and IFE should be aware that some interferences can affect IFE also. The new class of myeloma drugs, such as Daratumumab and Elotuzumab can appear as paraproteins in SPE and as IgG Kappa in IFE. [18] Hence clinicians and pathologists should communicate with each other when such therapies are given to the patient. We noticed in our audit that there is inconsistency in the reporting of interferences to clinicians.

This survey highlights that reporting of protein electrophoresis varies substantially between laboratories. Reducing the variability in reporting practices between laboratories will ease the task of clinicians and reduce the chance

of misinterpretation of reports, thus providing an improved service to patients. The pathologists of the country hence made recommendations on the reporting of protein electrophoresis. Implementation of such recommendations should reduce both report variation between laboratories and the risk of misinterpretation of reports.

These recommendations are based on the following publications:

1. **Recommendations for standardized reporting of protein electrophoresis in Australia and New Zealand**[9, 10]
2. **Candidate recommendations for protein electrophoresis reporting from the Canadian Society of Clinical Chemists Monoclonal Gammopathy Working Group**[11]

Recommendations for the Laboratory Reporting of Serum Protein Electrophoresis

1. Screening for monoclonal gammopathies should include serum protein electrophoresis (SPE), immunofixation electrophoresis (IFE) and serum-free light chains (sFLC) and screening for amyloidosis (AL) should also include 24 hours urine electrophoresis and urine IFE.
2. Nomenclature: The monoclonal component in serum shall be referred as a paraprotein (preferable) or monoclonal immunoglobulin e.g. IgG kappa paraprotein or monoclonal IgG kappa.
3. The system used for quantitative electrophoresis should be of sufficiently sensitive and be able to detect small paraprotein bands (<1g/L) that may co-migrate with polyclonal immunoglobulins.
4. Protein fractions should be quantitated during laboratory analysis and interpreted against an appropriate reference interval. When reporting fractions to clinical users, laboratories should include: (i) appropriate reference intervals and (ii) protein fractions should be quantified in g/L to the nearest whole number. Laboratories shall determine their own reference intervals or validate published reference intervals.
5. When reporting albumin, the analytical methodology should clearly be indicated if it is reported from the same laboratory using bromocresol green (BCG) or bromocresol purple (BCP) method or albumin by SPE.
6. The presence of an abnormal or monoclonal-appearing band upon electrophoresis should prompt further investigation by IFE automatically.
7. Paraproteins in the gamma-region should be quantified by densitometric measurement in g/L.
8. The perpendicular drop method for quantification is preferable for gating of gamma-region paraproteins, and most importantly, the same method must be used during the follow-up of the patient. Where multiple paraproteins are present, each paraprotein should be independently quantified when possible; up to three quantitative fields should be available for reporting abnormal bands. Paraprotein(s) should be consistently reported in the same quantitative field to facilitate a long-term cumulative review of the progress of a patient's disease.
9. When the concentration of the paraprotein is > 10 g/L, it should be reported to the nearest whole number. When the concentration of paraprotein is < 10 g/L, one decimal place should be used.
10. When a paraprotein is located in the non-gamma regions, the quantification should be reported for e.g. as total 'beta + paraprotein' concentration. For patients with paraproteins in the non-gamma-regions, laboratories shall recommend the measurement of immunoglobulin to facilitate disease monitoring.
11. Small paraprotein visible on SPE, which cannot be quantified reliably, especially if there is a polyclonal gamma globulin background, should be referred to as "< 1 g/L" or commented as "Small band that cannot be quantified reliably".
12. Paraprotein visible only by immunofixation should be described as "Paraprotein only visible by immunofixation".
13. When a new, small abnormal band with different electrophoretic mobility from the original paraprotein band or oligoclonal band appears, the laboratory report should mention "Appearance of small band/ oligoclonal band noted" after IFE testing
14. Pathologists and clinicians should communicate about reporting of results in patients receiving monoclonal therapies and about the history of the patient
15. Laboratory shall report other protein electrophoretic patterns that are not related to monoclonal gammopathies.
    - Decreased albumin and increased alpha-2 and beta globulins - Pattern is consistent with nephrotic syndrome

- Increased alpha-1 and alpha-2 and/or gamma globulins -Pattern is consistent with an acute inflammatory process
- Polyclonal hypergammaglobulinaemia and acute phase pattern - Pattern is consistent with a chronic inflammatory process
- Beta–gamma bridging - Beta–gamma bridging may be due to raised IgA level (Causes may include cirrhosis, mucosal or cutaneous inflammation)
- Hypogammaglobulinaemia - Suggest to IFE, sFLC and urine protein electrophoresis including immunofixation
- When an oligoclonal banding pattern with 2 or more bands on a polyclonal immunoglobulin background is present, the laboratory shall report "Oligoclonal bands are present. This can occur in a number of infectious or autoimmune conditions. Suggest review in 3–6 months if clinically indicated".

TABLE 1: (Responses to survey questions regarding protein electrophoresis)

Questions	Responses
1. Which method do you use for serum protein electrophoresis?	
A. Gel electrophoresis	7
B. Capillary electrophoresis	
2. Which method do you use for serum immunotyping?	
A. Immunofixation	7
B. Immunosubtraction	
3. What is the most common approach in your laboratory in order to screen an individual for the presence of a monoclonal gammopathy in the initial evaluation?	
A. Serum protein electrophoresis only	None
B. Serum protein electrophoresis with reflex to immunofixation or immunosubtraction	4
C. Serum protein electrophoresis and immunofixation or immunosubtraction	None
D. Serum protein electrophoresis combined with serum protein immunofixation and serum-free light chain	2
E. Serum protein electrophoresis and urine examination for Bence-Jones protein	1
4. Do you perform screening using urine protein electrophoresis?	
A. Yes	6
B. No	1
5. Which method do you use to quantitate a paraprotein in the gamma region on serum protein electrophoresis?	
A. Perpendicular (orthogonal)	7
B. Corrected perpendicular	
C. Tangent skimming (valley to valley)	
D. Other (please specify)	
6. What would be the next test step you do, when you detect a monoclonal band in the beta region?	
A. Immunofixation	7
B. Immunosubtraction	
C. Other (please specify)	
7. The nomenclature used in reporting monoclonal components detected in protein electrophoresis	
A. Paraprotein	7
B. Monoclonal protein	
C. M-protein	
D. M-band	
E. M-spike	
F. Monoclonal immunoglobulin	

8. Do you report the other fractions of serum protein electrophoresis? A. Yes B. No	 7
9. Please specify the decimal places reported in quantitative fractions A. None B. One C. Two	 1 4 2
10. Do you report total immunoglobulin concentration with protein electrophoresis (e.g. IgG, IgA or IgM)? A. Yes B. No	 3 4
11. Albumin reporting based on A. BCG or BCP method B. Based on Serum protein electrophoresis	 3 4
12. Number of decimal places used in reporting monoclonal protein if the quantification is > 10g/L A. None B. One	 1 6
13. Number of decimal places used in reporting monoclonal protein if the quantification is < 10g/L A. None B. One	 1 6
14. How do you report the concentration of a medium to large monoclonal protein in the beta region on serum protein electrophoresis? A. Monoclonal protein concentration B. Monoclonal protein concentration after subtracting a predetermined value for beta (beta-1 or beta-2) C. 'Monoclonal protein + total beta'	 3 None 4
15. How do you report a small paraprotein in the beta region that cannot be distinguished from the normal beta proteins? A. Do immunofixation and report the presence of paraprotein B. Do not proceed with immunofixation	 4 3
16. Do you report multiple monoclonal proteins? A. Yes B. No	 5 2
17. How do you report monoclonal protein less than 1g/L? A. Numerical value B. As <1g/L	 4 3
18. Do you report a significant change in the value of paraprotein level? A. Yes B. No	 5 2
19. When do you call it a significant change? Specify A. None B. 15% change C. 50% change	 2 2 3
20. How do you report a normal serum protein electrophoresis pattern? A. Normal pattern B. Normal pattern. M-protein not detected	 7

21. Do you report other pathological patterns? A. Yes B. No	 7
22. Do you report an oligoclonal banding pattern? A. Yes B. No	 5 2
23. Do you report about the interferences to the clinician? A. Yes B. No	 4 3
24. Do you report small abnormal band (<1g/L) seen for the first time in a patient with no known monoclonal gammopathy? A. Yes B. No	 5 2
25. 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? A. Yes B. No	 5 2

Authors' contribution: Pavai STHANESHWAR is the principal author and developed the questionnaire and analysed the questionnaire used in the survey. All other authors contributed to the writing of this paper and the finalisation of the questionnaire used in the survey.

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