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5/85 Bourke Rd, Alexandria NSW, 2015
Telephone: +61 2 9669 6600 Facsimile: +61 2 9669 6607 Email: office@aacb.asn.au

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Original Article

Recommendations for standardized reporting of protein electrophoresis in Australia and New Zealand

Jillian Tate[1], Grahame Caldwell[2], James Daly[3], David Gillis[4], Margaret Jenkins[5], Sue Jovanovich[6], Helen Martin[7], Richard Steele[8], Louise Wienholt[9] and Peter Mollee[10] on behalf of the Working Party on Standardised Reporting of Protein Electrophoresis

[1]Chemical Pathology Department, Pathology Queensland, Royal Brisbane and Women's Hospital, Herston Road, Brisbane, Queensland 4029; [2]Douglass Hanly Moir Pathology, Macquarie Park, New South Wales; [3]Haematology Department, Royal Hobart Hospital, Hobart, Tasmania; [4]Immunology Department, Pathology Queensland, Royal Brisbane and Women's Hospital, Brisbane, Queensland; [5]Biochemistry Department, Austin Hospital, Heidelberg, Victoria; [6]RCPA Immunology QAP, SA Pathology, Flinders Medical Centre, Adelaide; [7]Biochemistry Department, Healthscope Pathology, Wayville, South Australia, Australia; [8]Immunology Department, Wellington Hospital, Wellington, New Zealand; [9]Clinical Immunology, Royal Prince Alfred Hospital, Sydney, New South Wales; [10]Haematology Department, Pathology Queensland, Princess Alexandra Hospital, Brisbane, Queensland, Australia
Corresponding author: Jillian Tate. Email: jill_tate@health.qld.gov.au

Abstract

Background: Although protein electrophoresis of serum (SPEP) and urine (UPEP) specimens is a well-established laboratory technique, the reporting of results using this important method varies considerably between laboratories. The Australasian Association of Clinical Biochemists recognized a need to adopt a standardized approach to reporting SPEP and UPEP by clinical laboratories.

Methods: A Working Party considered available data including published literature and clinical studies, together with expert opinion in order to establish optimal reporting practices. A position paper was produced, which was subsequently revised through a consensus process involving scientists and pathologists with expertise in the field throughout Australia and New Zealand.

Results: Recommendations for standardized reporting of protein electrophoresis have been produced. These cover analytical requirements: detection systems; serum protein and albumin quantification; fractionation into alpha-1, alpha-2, beta and gamma fractions; paraprotein quantification; urine Bence Jones protein quantification; paraprotein characterization; and laboratory performance, expertise and staffing. The recommendations also include general interpretive commenting and commenting for specimens with paraproteins and small bands together with illustrative examples of reports.

Conclusions: Recommendations are provided for standardized reporting of protein electrophoresis in Australia and New Zealand. It is expected that such standardized reporting formats will reduce both variation between laboratories and the risk of misinterpretation of results.

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Introduction

The aim of this paper is to provide laboratories with recommendations to standardize the reporting of serum and urine protein electrophoresis (SPEP, UPEP). Some of this guidance may not be appropriate for all laboratories performing these procedures and individual laboratory circumstances may dictate a different approach.

Methodology

The Working Party on Standardised Reporting of Protein Electrophoresis was an initiative of the Australasian Association of Clinical Biochemists. Working Party members were proposed by the various professional bodies involved in the reporting of protein electrophoresis (Australasian Association of Clinical Biochemists, Royal College of Pathologists of Australasia, Australasian Society of Clinical Immunology and Allergy, and Haematology Society of Australia and New Zealand) and were selected to be representatives of Australian and New Zealand laboratory scientists, pathologists and clinical experts in this field. The Working Party produced the Position Paper which was subsequently revised through a consensus process to allow interested stakeholders an opportunity to contribute to recommendations and guidance for clinical pathology

laboratories. These groups include the Myeloma Foundation of Australia Medical and Scientific Advisory Group, Australasian Association of Clinical Biochemists, Haematology Society of Australia and New Zealand, Australasian Society of Clinical Immunology and Allergy, Royal College of Pathologists of Australasia, and public and private pathology laboratories.

The scope of the Working Party was to consider standardization of the reporting of SPEP and UPEP. This did not include cerebrospinal fluid (CSF) and lipoprotein electrophoresis. Measurement of total immunoglobulins by immunochemical methods as it relates to protein electrophoresis is covered. The serum free light chains (FLC) assay was not considered except where relevant to report commenting. Aspects of appropriate levels of staff expertise and training have been considered as well as ongoing quality assurance and education in the field. The paper is not intended to address clinical indications for SPEP and UPEP and readers are referred to recent guidelines published in this area.[1–8]

Levels and grades of evidence have not been used in this paper as these generally relate to patient treatments or indications for investigations rather than test reporting. There is little guidance in the area of test reporting in the peer-reviewed literature and most of the recommendations that follow are based on evidence from expert committee reports or the laboratory and clinical expertise of respected authorities. As such, most recommendations are grade C, level IV according to National Health Medical Research Council (NHMRC) criteria (NHMRC additional levels of evidence and grades for recommendations for developers of guidelines. December 2008; see http://www.nhmrc.gov.au/_files_nhmrc/file/guidelines/evidence_statement_form.pdf).

Background

The primary reason for performing SPEP and UPEP is to detect monoclonal immunoglobulins associated with plasma cell dyscrasias and lymphoproliferative disorders. As any laboratory report needs to deliver clear information to the clinician to assist them in the management of their patients, an understanding of the clinical requirements of a protein electrophoresis report is essential. Clinicians are primarily interested in whether a paraprotein is present or not, and if present, what its characteristics are and how great its concentration is. Given the role of protein electrophoresis in monitoring plasma cell dyscrasias, the ability to view a cumulative report is also essential. Several recent publications have highlighted the clinical importance of response criteria in the monoclonal gammopathies.[9–17] SPEP and UPEP reports must therefore contain adequate information to enable assessment of partial response, very good partial response, near-complete response and complete response (Table 1). For example, the latter two response categories require performance of immunofixation electrophoresis (IFE) on specimens where a paraprotein was previously detected in order to demonstrate its absence. Other findings of clinical significance on SPEP include increased alpha-1 and alpha-2 globulins indicative of an acute phase response, a decrease in alpha-1 globulins suggestive of alpha-1 antitrypsin deficiency, an increase in the beta-1 region suggestive of increased transferrin and iron deficiency, decreased gammaglobulins, and a polyclonal increase in gammaglobulins reflecting inflammation, infection, autoimmune disease and/or liver disease.[18]

While a number of clinical guidelines exist in relation to the diagnosis, monitoring and treatment of plasma cell dyscrasias,[1–8] these only give passing attention to laboratory

Table 1 Protein electrophoresis and response criteria for multiple myeloma*

Response category	Abbreviation	Response criteria	Comment
Complete remission	CR	Negative IFE of the serum and urine samples	IFE required if paraprotein not visible on electrophoretogram
Near complete remission	nCR	Paraprotein visible by IFE but not on electrophoresis of the serum or urine samples	IFE required if paraprotein not visible on electrophoretogram
Very good partial remission	vgPR	Paraprotein visible by IFE but not on electrophoresis of the serum or urine samples OR $\geq$90% reduction in serum paraprotein plus urine paraprotein $<$100 mg/24 h	IFE required if paraprotein not visible on electrophoretogram
Partial remission	PR	$\geq$50% reduction of serum paraprotein and reduction in 24-h urinary paraprotein by $\geq$90% or to $<$200 mg/24 h	Allow use of quantitative immunoglobulin concentrations in patients in whom the paraprotein measurements are unreliable (e.g. IgA paraproteins co-migrating with the beta region)
Progressive disease	PD	25% increase from nadir of serum paraprotein (the absolute increase must be $\geq$5 g/L) OR 25% increase from nadir of urine paraprotein (the absolute increase must be $\geq$200 mg/24 h)	
Stable disease	SD	Not meeting criteria for CR, vgPR, PR or PD	

SPEP, serum protein electrophoresis; UPEP, urine protein electrophoresis; IFE, immunofixation electrophoresis; FLC, free light chains
*Response criteria adapted from Durie *et al*.[11] and Richardson *et al*.[10] Only those criteria relating to SPEP and UPEP are listed. Other criteria including FLC response, bone marrow plasmacytosis and the presence of soft tissue plasmacytomas, and the definitions of measurable disease are not detailed here and readers are referred to the original articles. Other response criteria for AL amyloidosis[16] and Waldenstrom's macroglobulinaemia[15] have been published

aspects of protein electrophoresis. There is very little in the published literature to guide reporting of SPEP and UPEP[19,20] and, in particular, systematic reporting standards are not available. A recent survey from laboratories across Australia and New Zealand[21] documented extensive variation in the reporting of protein electrophoresis results. This variation was particularly noticeable in the following reporting practices: (a) units for urine Bence Jones protein (BJP); (b) reporting absence of a paraprotein rather than a normal pattern; (c) numerical reporting of all protein fractions or only the paraprotein; (d) warning of possible inaccuracy in the serum immunoglobulin result of the paraprotein type; (e) co-migration of a paraprotein with a normal serum protein; and (f) use of a confirmatory test when a known paraprotein is no longer detectable. The report called for standardization of protein electrophoresis reporting in order to reduce variation between laboratories and the risk of misinterpretation of results.

The Working Party was also aware of other issues which impact on the reporting of protein electrophoresis. Due to the increasing complexity of managing clinical data within an electronic database, there is a need for a standardized structure of reporting formats for laboratory tests, e.g. test name, units and reference intervals. Logical Observation Identifiers Names and Codes (LOINC) is one such system developed for standardizing the terminology describing laboratory observations, and reporting formats will need to be compatible with such endeavours. Clinical laboratories must constantly streamline testing to cope with financial and workload pressures. Reporting practices should not interfere with good workflow practices, but similarly, as a specialized test, protein electrophoresis often requires individualized reporting from supervising scientists and pathologists in order to produce a quality result to clinicians. Reporting practices also need to take into consideration the conditions that apply to payments made by funding agencies.

Nomenclature

Throughout this paper the following terminology and definitions have been used in an attempt to standardize some of the nomenclature used in reporting protein electrophoresis. The Working Party concluded that widespread adoption of common terminology would facilitate standardization of protein electrophoresis reports.

Serum paraprotein or monoclonal immunoglobulin

The monoclonal component in serum is referred to as a 'Paraprotein' (preferable) or 'Monoclonal immunoglobulin', e.g. IgG kappa paraprotein or monoclonal IgG kappa. The use of terminology such as M-protein, M-band and M-spike, while widespread in the literature, is potentially confusing as such terms may be misinterpreted as meaning an IgM paraprotein. In cases where the monoclonal component consists of FLC, then the term 'Monoclonal free light chains' is preferred to 'Bence Jones protein'.

Urinary BJP or monoclonal free light chains

The monoclonal component in urine is referred to generally as 'Paraprotein' or specifically as 'BJP' or 'Monoclonal free light chains'. If the monoclonal component in the urine includes an intact immunoglobulin, it is referred to as 'Monoclonal immunoglobulin' and the term M-band is not used, e.g. monoclonal IgG kappa.

Oligoclonal immunoglobulin G bands

The term 'Oligoclonal IgG bands' refers to two or more bands of gamma mobility on SPEP that appear as two or more groups of lines on isoelectric focusing (IEF).[22,23] IgG paraproteins generally have a pattern of multiple lines in an ordered sequence by IEF whereas oligoclonal IgG typically results in a pattern of multiple randomly distributed sharp lines.[22,23] Whereas oligoclonal patterns that are biclonal can usually be detected on SPEP, as the number of bands increases, they may overlap or one group may be present at much higher concentration and thus be predominant, so much so that the pattern seen on SPEP is that of a single band. It is important to differentiate these low-level groups to ensure that an oligoclonal pattern is not reported as monoclonal.

Recommendations for nomenclature:

- The monoclonal component in serum is referred to as a *Paraprotein* (preferable) or *Monoclonal immunoglobulin* e.g. IgG kappa paraprotein or monoclonal IgG kappa;
- The term *Monoclonal free light chains* is preferred to *Bence Jones protein* when referring to serum monoclonal FLC;
- The monoclonal component in urine is referred to generally as *Paraprotein* or specifically as *Bence Jones protein* or *Monoclonal free light chains*;
- *Oligoclonal IgG bands* refers to two or more bands of gamma mobility on protein electrophoresis. Isoelectric focusing is a useful technique to distinguish monoclonal bands from overlapping oligoclonal bands and polyclonal IgG.

Analytical requirements of a protein electrophoresis report

Detection system for protein electrophoresis

The system used for quantitative electrophoresis should be of high resolution and be able to detect small monoclonal bands that may co-migrate with normal proteins particularly in the beta region. The major commercial manufacturers of electrophoresis systems either separate the beta region into beta-1 and beta-2 globulins or expand the beta and beta–gamma interzone separation, permitting paraproteins to be more readily identified. This covers agarose gel and capillary zone electrophoresis (CZE) methods. Low-resolution systems for SPEP and UPEP should not be used.[5] Low-resolution electrophoresis on cellulose acetate is not a suitable medium for SPEP and UPEP. CZE does not usually resolve fractions differently to agarose gel electrophoresis. It is recommended to use the same method (used by the same laboratory or laboratory network), including ensuring analysts have access to the cumulative reports of

the paraprotein delineation on the densitometric/CZE scan when monitoring the paraprotein concentration for individual patients. This practice improves the reproducibility and comparability of paraprotein estimations, and also avoids unnecessary follow-up (e.g. immunofixation of a 'new' band), easier interpretation of when and if a patient achieves 'remission' as well as simplifying and optimizing cost allocation. The Working Party acknowledges that IEF may occasionally be required in certain situations such as when examining serum samples of patients who are post-stem cell transplantation. For example, IEF may help to ascertain if a low-concentration band detected on IFE is the same as the paraprotein originally found in the patient's serum samples or is a new monoclonal protein, or if the band(s) on SPEP are oligoclonal. If a laboratory does not perform IEF, serum samples of patients should be referred to a reference laboratory in problematic cases.

Recommendations for detection system for protein electrophoresis:

- Gel-based methods and CZE are suitable methods for protein electrophoresis;
- The electrophoretic system should be of high resolution and be able to detect small monoclonal bands that may co-migrate with normal proteins particularly in the beta region. Low-resolution electrophoresis on cellulose acetate is not suitable for protein electrophoresis;
- Clinicians should be encouraged to monitor the paraprotein concentration in individual patients using the same method (used by the same laboratory or laboratory network), hence ensuring analysts have access to the cumulative reports of the paraprotein delineation on the densitometric/CZE scan;
- Specimens requiring IEF should be referred to a reference laboratory.

Serum protein and albumin quantification

The presence of significant paraproteinaemia initially may be detected in patients undergoing routine testing that includes the measurement of serum albumin and total protein. The early recognition of a paraprotein disorder by performing electrophoretic studies on patients with a raised globulin concentration is preferable to a later diagnosis in patients who present with the clinical manifestations of more advanced disease. To this end, it is recommended that both albumin and total protein measurements together with calculation of globulin are at least performed for any older adult patients in response to a request for routine liver function tests.[24,25]

Serum total protein quantification is by biuret-based methods determined by an automated analyser. Non-linearity can occur at very high concentrations in the presence of a paraprotein and appropriate sample dilution is required. Serum albumin is also quantified on an automated analyser; both bromocresol green (BCG) and bromocresol purple (BCP) methods are in common use. BCG[26,27] may significantly overestimate albumin concentrations in sick individuals[26–29] and both methods are prone to interference from free fatty acids and may underestimate albumin. Albumin measurement by BCP generally correlates better with SPEP albumin than by BCG.[29,30] Measurement of albumin by densitometry from the SPEP may be overestimated at high paraprotein concentration or in the presence of cryoglobulin. In the event of major discrepancies between the chemical and electrophoretic albumin quantifications, laboratories should investigate the discrepancy by cross checking the chemical albumin against the densitometer albumin calculated from the total protein. Immunochemical measurement of albumin by immunonephelometric (INA) and immunoturbidimetric (ITA) assays or CZE, where the absorbance of the peptide bond is used and thus the problem of differential stain uptake is eliminated, give more accurate values and offer the most reliable methods. In addition, CZE albumin coefficients of variance (CVs) are usually 1.1–1.2% compared with the best BCG/P methods (Abbott Diagnostics, Abbott Park, IL, USA) at 1.9%. Whatever albumin value is used on an SPEP report, it requires a consistent approach by the laboratory. Except when using CZE albumin on electrophoresis reports, it is preferable to report the same result on the SPEP report as on the General Chemistry report to avoid differing values that may cause confusion to clinicians. This may require adjustment of the densitometric albumin concentration using the total globulin concentration. Total protein and albumin should be quantified in g/L to the nearest whole number.

Recommendations for serum protein and albumin quantification:

- To facilitate the early recognition of a paraprotein disorder, it is recommended that both albumin and total protein measurements together with calculation of globulin are performed for older adult patients in response to a request for routine liver function tests;
- Total protein and albumin quantification as determined by an automated analyser be available for assessment of the protein electrophoresis;
- Serum albumin quantification by BCP or CZE is preferable to quantification by BCG although all are acceptable;
- Providing the same albumin result on the SPEP report as on the General Chemistry report is preferable but may not be possible depending upon the available Laboratory Information System;
- Whatever albumin value is used, the reporting of albumin on an SPEP report requires a consistent approach;
- Laboratories should investigate major discrepancies between the chemical and electrophoretic albumin quantifications;
- Total protein and albumin should be quantified in g/L to the nearest whole number.

Quantitative reporting of SPEP fractions

The core elements of SPEP that need quantitative reporting are the total protein, albumin and paraprotein. While the presence and amount of a paraprotein (see the following section) is of primary interest to clinicians, other important information can also be derived from the other protein fractions (alpha-1, alpha-2, beta and gamma regions). This information can be conveyed by reporting the quantitative values of each protein fraction together with interpretive commenting or by reporting only the total protein and albumin (± paraprotein) together with appropriate interpretive commenting. However, the reporting of other protein fractions is optional and will depend on local clinical

utility. Protein fractions should be quantified in g/L to the nearest whole number. Laboratories should determine their own reference intervals or validate published reference intervals. In view of the less common occurrence of plasma cell dyscrasias with multiple paraproteins, up to three quantitative fields should be available for reporting abnormal bands. There should be consistent reporting of the paraprotein(s) in the same reporting field to facilitate long-term cumulative review of the progress of a patient's disease; substitution of new bands into the same field(s), e.g. post-transplantation, may result in misinterpretation of results. The documentation of the position of known paraproteins either with the concentration of the relevant fraction, commenting on the report, or a scanned database image is useful for retrospective identification of original paraproteins by laboratories (see the section Interpretive commenting).

Recommendations for quantitative reporting of SPEP fractions:

- The minimal quantitative fields to be reported are total protein, albumin and, if present, the paraprotein(s);
- The quantitative reporting of all SPEP fractions is optional;
- Protein fractions should be quantified in g/L to the nearest whole number;
- Laboratories should determine their own reference intervals or validate published reference intervals;
- In view of the less common occurrence of plasma cell dyscrasias with multiple paraproteins, 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 long-term cumulative review of the progress of a patient's disease and avoid misinterpretation of results.

Serum paraprotein quantification

Quantification of the paraprotein provides a good surrogate for monitoring the size of the population of malignant cells in an individual patient. It is recommended to use the same laboratory and the same method to improve the reproducibility and comparability of serial paraprotein estimations. Delineation of the paraprotein peak on the densitometric/CZE scan should be made with reference to how it was delineated before. The paraprotein can be quantified from the SPEP (densitometric measurement from the agarose gel electropherogram or quantification from CZE and calculated as a percentage of the total protein) or by immunochemical methods (INA and ITA). In the sections below, unless specifically stated, where reference is made to quantification by densitometry, it is acknowledged that quantification by CZE is an equally valid method. Occasionally, however, agarose gel and CZE may give differing results at high IgG concentrations, possibly due to saturation of staining of the agarose gel.[31,32] The paraprotein should be quantified in g/L to the nearest whole number and use of qualitative terms such as low, medium or high paraprotein concentration should be avoided. The minimum concentration for quantification is 1 g/L, although it is accepted that quantification of paraproteins at this level is imprecise (see Quantification of small bands).

Analytical issues

Analytical limitations of electrophoresis and immunochemical methods in the quantification of paraproteins

Quantification of paraproteins by SPEP and immunochemical methods does not always yield the same result. Immunoglobulin quantification by INA/ITA can give results that are quite disparate to SPEP, which may be due to the dilutional properties of monoclonal proteins or the presence of other polyclonal immunoglobulins of the same class as the paraprotein. INA/ITA methods measure both the monoclonal and polyclonal immunoglobulins whereas densitometry is more specific for the paraprotein unless hidden by other co-migrating proteins, e.g. in alpha and beta regions or by fibrinogen. Nephelometry has been reported to overestimate IgM at higher concentrations, presumably due to the pentameric structure of IgM forming increased antigen–antibody complexes with increased light scatter patterns.[33–36] IgG and IgA may also be overestimated by both INA and ITA methods.[33] The most marked effects of non-linearity occur at very high paraprotein concentrations where serial sample dilutions can result in final levels greater than the globulin concentration. Some studies have found a close correlation between densitometry and INA measurements of IgA paraproteins,[35] whereas others have found method discrepancies of 25% or more for paraprotein concentrations of >40 g/L.[37] It is desirable that laboratories perform a comparison of their quantitative electrophoresis and immunochemical method for monoclonal IgG, IgA and IgM immunoglobulins that migrate in the gamma region to determine the upper limit of agreement for monoclonal immunoglobulins measured by INA/ITA methods.[34]

Densitometry has been reported to underestimate higher concentrations of IgG in comparison with immunonephelometry, possibly due to a dye saturation effect of the electrophoretic gel[35] or due to non-linearity of scanning densitometry of the gel.[38] This results in overestimation of the albumin, alpha and beta and residual gammaglobulin fractions and requires sample dilution prior to quantitative electrophoresis analysis. Sometimes, specific monoclonal protein amino acid sequences may not react with protein stains or immunochemical reagents, causing spurious results. As IgD and IgE are not readily measured by INA/ITA methods, these paraproteins are generally quantified by densitometry.

Paraproteins, in particular IgM paraproteins, may occasionally be cryoglobulins. Cryoglobulins are immunoglobulins that form a gel or precipitate on cooling and dissolve again when warmed to 37°C.[39] In the presence of these precipitating cryoglobulins (type I: monoclonal immunoglobulin; type II: a mixture of immunoglobulins of different isotypes, including at least one monoclonal directed at the Fc portion of normal IgG), quantification of paraproteins may be inaccurate by both electrophoresis and immunochemical methods. If a cryoglobulin is suspected, the appropriate sample collection and handling is critical to reduce this artefact.[40] Laboratories should ensure that specimen temperature is maintained at 37°C from the time of

collection until the serum or plasma is separated. Samples that have been stored refrigerated require warming to 37°C and re-solubilization of the cryoglobulin prior to analysis by electrophoresis and INA/ITA to avoid loss of the paraprotein and its underestimation.

Taking into account the technical limitations of both electrophoresis and immunochemical methods, at the time of diagnosis of a plasma cell dyscrasia, it is recommended that the paraprotein be quantified from the electrophoretogram and that immunoglobulins (G, A, M) be measured by INA/ITA methods. It is preferred that a paraprotein be monitored by using densitometric quantification, unless a low-level paraprotein is obscured by other proteins (see the following section).[4,5,11,41] The rationale for this recommendation is that paraprotein resolution by densitometry is more specific for the monoclonal protein than immunoglobulin quantification which includes polyclonal as well as monoclonal immunoglobulins. This specificity is particularly apparent when a low-level paraprotein exists in the presence of a polyclonal expansion of immunoglobulins.

Paraproteins co-migrating with other normal proteins in alpha and beta regions

When a paraprotein is located in the non-gamma regions (most commonly in the beta region), the quantification should be reported to include total 'beta+paraprotein' concentration. Attempts to provide an estimate of the 'true' paraprotein concentration by subtracting other beta-globulins are inherently unreliable due to the non-constant levels of the co-migrating proteins and this practice is not encouraged. In these cases, quantification of total IgG, IgA and IgM by INA or ITA may be more useful to monitor the paraprotein.[5,7,11,17] It is important to ensure that the results of densitometric and INA/ITA quantification are not used interchangeably. In some cases, paraproteins in the alpha and beta regions may be best monitored by reporting both the densitometric (beta/alpha+paraprotein) and INA/ITA quantification (according to a laboratory's upper limit of agreement for monoclonal immunoglobulin) with an educational strategy to indicate the uncertainty of measurement of both approaches. Spurious overestimated values should not be reported.

The clinical importance of having both densitometric and immunochemical quantification of paraproteins in the alpha and beta regions is highlighted in health systems where drug funding and availability is linked to paraprotein response. For example, in Australia (see bortezomib restrictions on the Pharmaceutic Benefits Scheme website, http://www.pbs.gov.au/pbs/home) and the UK (see final appraisal determination for bortezomib on National Institute for Health and Clinical Excellence website, http://www.nice.org.uk/guidance/index.jsp?action=folder&r=true&o=37093), ongoing supply of bortezomib is only funded for patients with myeloma who achieve a partial remission after four cycles of therapy. While the assessment of partial remission is straightforward for most paraproteins in the gamma region, patients with low-level paraproteins in the beta region may have their response underestimated by densitometric quantification. To illustrate this point, worked example 2 shown in the Appendix indicates the value of inclusion of both densitometric and INA/ITA quantification for a myeloma patient receiving bortezomib. At presentation, SPEP 'beta+IgA lambda paraprotein' concentration was 28 g/L and total IgA by INA was 21 g/L. Following treatment, 'beta+IgA lambda paraprotein' concentration was 16 g/L (<50% decrease) and total IgA by INA was 9 g/L (>50% decrease). The inclusion of the normal beta region proteins in the densitometric paraprotein quantification has, in this case, prevented accurate response assessment. Use of the immunochemical measurement where IgA results are less spurious at low concentrations would, to some extent, overcome this problem. Immunochemical quantification of the paraprotein would enable the patient to continue to receive bortezomib whereas the SPEP results would not. The complementary use of immunochemical quantification of paraproteins in such situations may facilitate response assessment and allow the patient to access treatment.[17]

In the case of monoclonal FLC diseases such as light chain myeloma, measurement of serum FLC may be helpful when the paraprotein co-migrates with other normal proteins or is present in trace amounts on IFE.

Quantification of small bands

Laboratories should know the limit of detection of their SPEP, UPEP and IFE methods (Table 2) and the between-run imprecision (also refer to the section Laboratory performance of SPEP, UPEP and IFE). The total imprecision quoted by manufacturers in package inserts for gel electrophoresis indicates a range of imprecision of around 3–11% CV for gamma and beta fractions containing polyclonal and monoclonal proteins. Similarly for CZE, depending on the position of the band, the between-run imprecision is between 3% and 11% CV at protein concentrations ≤10 g/L and 1–2% at higher concentrations (personal communication, Ken Sikaris). A low imprecision of 2.5% CV corresponds to ±1 g/L at a band level of 20 g/L whereas a 10% CV at 2 g/L is ±0.4 g/L. If, as recommended, the reporting of bands is in whole numbers and not to decimal places, then concentrations between 1 and 2 g/L cannot be effectively differentiated (e.g. when a band of 1.5 g/L is rounded up to 2 g/L the rounding error is 33%). At these low levels, the results will be semi-quantitative. Bands of <1 g/L which are visible but cannot be reliably quantified, or small bands that are difficult to resolve, especially if there is a polyclonal background of globulins, should be quantified as <1 g/L or 'trace' with comments such as 'small band cannot be quantified reliably'.

Bands that are visible only by immunofixation should not be quantified in the separation report but should be referred to with comments such as 'IgG kappa band only visible by immunofixation electrophoresis' (see the section Interpretive commenting – specimens with a paraprotein). Such reporting enables the distinction between paraprotein responses such as 'very good partial remission' (>90% reduction in paraprotein), 'near complete remission' (paraprotein only visible by immunofixation) and 'complete remission' (paraprotein not visible by immunofixation; see Table 1). When paraproteins are quantified in a cumulative

Table 2 Detection limits for paraproteins*

Method	Immunofixation (IFE)/immunosubtraction	Limit of detection†
Agarose gel electrophoresis		
Serum protein	Without IFE	≃0.5 g/L
	With IFE	0.1 g/L
Urine protein	Without IFE	20 mg/L‡
	With IFE	3–5 mg/L
Capillary zone electrophoresis		
Serum protein	With immunosubtraction	0.25 g/L
Urine protein	Without immunosubtraction	20 mg/L

*Information from manufacturers' data on file

†The detection limit may vary depending on the position of the paraprotein and the polyclonal background (in gamma region on agarose gel electrophoresis) or depending on the proximity and magnitude of interfering proteins on capillary zone electrophoresis. For an IgG paraprotein diluted in a polyclonal IgG-containing diluent, the level of detection is not as low and is influenced by the total IgG concentration. For an IgM paraprotein diluted in a polyclonal IgG-containing diluent, similar results are obtained. The level of detection by IFE of an IgM paraprotein is not affected by the level of polyclonal IgG but the sensitivity of the light chain reaction will be affected

‡Depending on the gel electrophoresis method and stain used, Bence Jones protein can be quantitated down to a concentration of 20 mg/L without concentration of the urine[42]

report, it is essential that this data field be restricted to reporting of the original paraprotein and not to any other small bands or paraproteins that develop. When paraprotein quantities become small, IFE (Table 1) may be required to confirm the presence or absence of the originally identified paraprotein.

Recommendations for serum paraprotein quantification:

- Paraproteins in the gamma region should be quantified by densitometric or CZE measurement in g/L rounded to the nearest whole number;
- Paraproteins of <1 g/L visible on SPEP or CZE cannot be quantified reliably especially if there is a polyclonal gammaglobulin background and should be referred to as '<1 g/L' or 'trace' with comments such as 'small band cannot be quantified reliably';
- Paraproteins visible only by immunofixation should be described in the comment section (e.g. IgG kappa paraprotein only visible by immunofixation) rather than being given a quantified value;
- If a paraprotein is in the non-gamma regions, the beta region being the most common region for IgA paraproteins, report the total protein in the beta region (beta + paraprotein) quantification at presentation and during monitoring;
- Quantification of total IgG, IgA and IgM by INA/ITA provides an approximate concentration of the paraprotein, which may be overestimated due to non-linearity at higher sample dilution. However, they are particularly useful in situations where densitometry cannot reliably quantify a paraprotein (e.g. low-level paraproteins in the beta region). It is recommended that laboratories perform a comparison of their quantitative electrophoresis and immunochemical method for monoclonal IgG, IgA and IgM paraproteins to determine the upper limit of agreement for monoclonal immunoglobulins measured by INA/ITA methods;
- For patients with known plasma cell dyscrasias and paraproteins in the non-gamma regions, laboratories should support reporting of both the densitometric paraprotein and INA/ITA immunoglobulin measurement to facilitate disease monitoring according to a laboratory's upper limit of agreement for monoclonal immunoglobulin. Spurious overestimated values should not be reported.

Urine paraprotein separation and quantification

UPEP, unlike SPEP, produces a heterogeneous range of urine protein patterns depending on the presence and relative concentration of urine albumin, glomerular and tubular proteins, BJP, serum paraprotein, polyclonal FLC, and possibly myoglobin and haemoglobin. Urine may require preconcentration (recommended 10- up to 100-fold) to achieve a detection limit for BJP of 10 mg/L (by IFE).[7,42–44] If the laboratory method of detecting BJP is to use UPEP followed by IFE only if a band is observed in addition to albumin (as opposed to those who immunofix all urines as the initial investigation), then the UPEP needs to be able to detect 10 mg/L BJP in all samples.[7]

Urines from patients who have multiple myeloma with Bence Jones proteinuria will show a recognizable pattern of one or more discrete alpha-2, beta or gamma bands on UPEP and trace albumin. In multiple myeloma with Bence Jones proteinuria and non-selective glomerular proteinuria, the pattern may show albumin, alpha-1 antitrypsin, transferrin and BJP bands on UPEP while in AL amyloidosis, the same glomerular proteinuria pattern can occur but BJP may be visible only on IFE. Hence, if on UPEP there are protein bands visible in addition to albumin, which may obscure BJP or in fact be BJP, IFE is needed to detect the BJP. Note that often patients with tubular proteinuria, especially those having an inflammatory type pattern, show a 'light-chain ladder' on IFE of concentrated urine which may be mistaken for BJP.[22,45–49]

The College of American Pathologists recommends a 24-h urine specimen for the detection and quantification of BJP.[5] The collection of a 24-h urine specimen, however, is not necessarily popular with clinicians and patients for pragmatic reasons. BJP is particularly prone to bacterial degradation and there may be a lack of compliance in collecting the requisite specimen. For these reasons, the Working Party believes that the first voided urine is satisfactory for screening purposes. For the staging and monitoring of the plasma cell dyscrasias, a 24-h urine specimen is recommended by all guidelines[1,11,16,17,50] rather than a random or early morning specimen. It should be noted that a 24-h urine collection is also required to access certain myeloma treatments through the Australian Pharmaceutical Benefits Scheme and may be required for clinical trial purposes.

Quantification of BJP provides a good surrogate for monitoring the size of the population of malignant cells in an

individual patient. The BJP concentration in an individual patient can be quantified by densitometric measurement from the UPEP unless other urine proteins co-migrate with the BJP when quantification is not possible. Immunochemical methods for urinary FLC (using the serum free light chains assay) can result in gross overestimation of BJP and are not recommended.[43] Urine creatinine should be performed on every random or first void specimen sent for BJP and the concentration of BJP expressed relative to urine creatinine (BJP/creatinine in mg/mmol). For monitoring purposes, there is no literature from clinical trials that provides evidence about the BJP/creatinine ratio corresponding to disease response, e.g. for partial response that requires a 90% reduction in the 24-h urinary BJP excretion. The 24-h urine specimens should report BJP in mg/24 h in line with the proposed recommendations for reporting of proteinuria and albuminuria in Australia and New Zealand (personal communication, Australasian Proteinuria Consensus Working Group).

BJP concentration is calculated in mg/L as a percent of the urine total protein measurement. Differences in reactivity for BJP exist between the various urine total protein precipitating reagents (e.g. benzethonium chloride) and dye-binding reagents (e.g. pyrogallol red-molybdate, pyrocatechol violet-molybdate, Coomassie blue, etc.). This is due to differences in reaction of tubular versus glomerular proteins (e.g. BJP versus albumin) and differences in calibrator composition, resulting in the underestimation of BJP concentration relative to a total protein or albumin calibrator.[51] For example, the 2010 RCPA Immunology QAP Urine Paraprotein Specimen 14–01 gave a range of urine total protein from 500 to 2000 mg/L and a range of BJP concentration from 310 to 1540 mg/L depending on the UPEP and urine total protein methods.[52] Because of this variability, it is recommended that clinicians use the same method (including the same laboratory or laboratory network) to monitor BJP concentration in individual patients.

As well as reporting the urine total protein, there also should be an indication as to whether the urine specimen has glomerular or tubular proteinuria or if it is a mixed glomerular/tubular proteinuria, and a comment as to whether BJP is detected or not. A cut-off of 200 mg/L for total protein is suggested for when such commenting is appropriate.[53] Any intact monoclonal immunoglobulin should also be quantified and reported (see worked example 3 in Appendix).

Recommendations for urine paraprotein separation and quantification:

- First voided urine is suitable for screening UPEP;
- A 24-h urine specimen is preferred for staging and monitoring of the plasma cell dyscrasias, although first voided specimens are acceptable if a 24-h specimen is not available or practical;
- Laboratories should be able to detect BJP at a level of 10 mg/L with levels <10 mg/L reported as 'trace';
- As well as reporting the urine total protein, it is recommended that there be an indication as to whether the urine specimen has glomerular and/or tubular proteinuria, and a comment as to whether BJP is detected or not. Any intact monoclonal immunoglobulin should also be quantified and reported;
- Creatinine should be performed on first voided urine specimen and the concentration of BJP expressed relative to urine creatinine (BJP/creatinine in mg/mmol);
- For timed excretion, urine total protein and BJP should be reported in mg/24 h.

Paraprotein characterization

IFE or immunosubtraction is required to identify bands to confirm their monoclonality and to characterize the paraprotein. IFE should be performed with antisera against G, A and M heavy chains, and kappa and lambda light chains. All patterns that demonstrate monoclonal light chains without an associated heavy chain should be immunofixed with antisera to IgD[54] and, if clinically indicated, IgE. In subsequent specimens, IFE or immunosubtraction do not need to be repeated unless there is a change in the electrophoretic mobility, there is an additional visible band or if the paraprotein is no longer visible.[5] Small paraproteins in the non-gamma region or in a polyclonal background also may require IFE on each presentation in order to confirm their presence. This is particularly important for the oligosecretory diseases, e.g. AL amyloidosis, IgD myeloma, plasmacytoma, and post-allogeneic bone marrow transplantation. IFE is therefore required to be repeated to confirm the absence of a previously reported paraprotein or to detect disease relapse (Table 1).[11]

The sensitivity of IFE depends on the medium chosen. High-resolution agarose gel electrophoresis with IFE is able to detect monoclonal protein concentrations as low as 0.1 g/L (depending on the broadness of the band, the stain used, the level of background polyclonal immunoglobulin and other co-migrating proteins; see Table 2). Immunosubtraction has been shown by commercial suppliers to detect 0.25 g/L of monoclonal protein (Table 2). Because of this difference in sensitivity, IFE is recommended over immunosubtraction in certain clinical situations.[11,41] This includes when there is a strong clinical suspicion of a plasma cell dyscrasia and a normal electrophoretogram, and for confirmation of complete remission following therapy.

IEF is a useful technique to identify small bands detected on SPEP or UPEP (especially post-stem cell transplantation) as being monoclonal, oligoclonal and/or polyclonal patterns. Broad-range IEF (pH gradient 3.5–10) is used routinely for detection of oligoclonal immunoglobulin in CSF and can be applied to the detection of low concentrations of monoclonal and oligoclonal immunoglobulin in serum and urine. IEF is able to detect 0.01 g/L of IgG monoclonal protein.[55] Low levels of monoclonal IgA protein and monoclonal IgM protein are better detected by IFE using high-resolution gel electrophoresis than IEF. Paraprotein investigation by IEF is a useful tool and is available in specialist laboratories.

Because IFE or immunosubtraction often is performed subsequent to the electrophoretogram (and in most cases IFE is not required at all), the electrophoretogram is reported first with a subsequent report issued after IFE. It is recommended that a final integrated report combining

both the electrophoretogram and IFE (and IEF if required) results is issued with commenting that this supersedes the previous report(s). SPEP and UPEP reports should be issued separately.

Recommendations for paraprotein characterization:

- IFE or immunosubtraction are required to characterize all new bands and to confirm their monoclonality;
- In subsequent specimens, IFE or immunosubtraction does not need to be repeated unless there is a change in the electrophoretic mobility, there is an additional visible band or if the paraprotein is no longer visible;
- Small paraproteins in the non-gamma region or in a polyclonal background also require IFE on each presentation in order to confirm their presence;
- IFE is required to confirm the absence of a previously reported paraprotein (to enable calculation of the response criteria 'complete remission'). In general, once complete remission has been confirmed, IFE is not required on each subsequent occasion unless a new band is visible or IFE is specifically requested;
- If the paraprotein is detected in the serum by immunofixation only, refer to this in the comment rather than in the quantification, e.g. 'IgG kappa band visible only by immunofixation' (refer also to Commenting section);
- If the paraprotein is detected in the urine by immunofixation only, report this as 'trace' and refer to in the comment as only visible by immunofixation, e.g. 'kappa BJP is only visible by immunofixation' (refer also to worked examples in Appendix);
- Problematic samples requiring identification of small protein bands can be referred to a reference laboratory for IEF;
- A final integrated report combining both the electrophoretogram and IFE should be issued.

Laboratory performance of SPEP, UPEP and IFE

Laboratories should determine the measurement uncertainty of their SPEP, UPEP and IFE methods. This includes the assessment of (1) analytical imprecision at different paraprotein concentrations and when superimposed on different background levels of polyclonal immunogobulin, to determine the method repeatability within one run by one operator and the reproducibility between runs, days and different operators; (2) limit of detection of serum and urine protein quantitative electrophoresis and IFE (Table 2); and (3) linear range of scanning densitometry.

The intraindividual biological variation (CVi) determined in healthy subjects with no known paraprotein is on average approximately 5% for IgG, IgA and IgM immunoglobulins.[56] For a CVi of 5%, the desirable analytical imprecision (CVa) should be no greater than 2.5%. Such a CVa is difficult to achieve, especially for low-level paraproteins where the CVa can range between 3% and 11%. An alternative approach to setting quality specifications is to calculate a reference change value (RCV) at a 95% confidence level to determine the likelihood of a significant change in clinical status. For a CVi of 5% and a CVa varying between 3% and 11%, the calculated RCV ranges between 16% and 33%.[57]

The RCV can be applied to interpreting changes in consecutive paraprotein levels during treatment to determine a significant change. Clinical disease progression requires that a paraprotein concentration increases by $\geq$5 g/L and is $\geq$25% above the nadir paraprotein concentration following treatment. In the case of a nadir concentration of 6 g/L and a subsequent concentration of 11 g/L, the change indicates a RCV of more than 33% and hence 11 g/L indicates disease progression. In another case where nadir and follow-up concentrations are 20 and 25 g/L, respectively, a laboratory with the higher imprecision will not be able to distinguish these two concentrations.

Recommendations for laboratory performance of SPEP, UPEP and IFE:

- An assessment of laboratory performance of SPEP and UPEP requires determination of
  - analytical imprecision at different paraprotein concentrations to determine method repeatability and between-day and operator reproducibility;
  - limit of detection of protein electrophoresis and immunofixation;
  - the linear range of scanning densitometry.

Laboratory expertise and staffing

The minimum analytical techniques for laboratories performing protein electrophoresis are agarose gel SPEP, UPEP and IFE, or CZE and immunosubtraction, and immunoglobulin quantification by INA/ITA. A minimum competency-based standard is required for those who review and interpret protein electrophoresis patterns. Protein laboratories are encouraged to have an educational module suitable for continuing professional development.

In relation to the expertise of staff reporting protein electrophoresis, the following recommendations are made:

- It is not necessary for a pathologist to report all specimens requested for SPEP and UPEP;
- The initial reporting should be performed by a scientist who has been appropriately trained by a senior scientist, and who has worked in a protein laboratory performing all areas of bench work for preferably 12 months;
- The protein electrophoresis report should be validated by a second scientist who has worked in the protein area for at least three years. Any difficult or unusual specimens, or any specimen where the numerical results for tests such as FLC and protein electrophoresis do not agree or have changed unexpectedly since the previous specimen should be referred to either a senior scientist or a pathologist. Repeat testing of the specimen received or on another specimen collected from the patient should resolve any discrepancy;
- Communication with clinicians is to be encouraged.

Recommendations for laboratory expertise and staffing:

- Minimum analytical techniques for laboratories performing protein electrophoresis are
  - agarose gel SPEP, UPEP and IFE, or;
  - CZE and immunosubtraction;
  - immunoglobulin quantification by INA/ITA.

- A minimum competency based standard is required for those who review and interpret protein electrophoresis patterns;
- The initial reporting should be performed by a scientist who has been appropriately trained by a senior scientist, and who has worked in a protein laboratory performing all areas of bench work for preferably 12 months;
- Protein laboratories are encouraged to have an educational module suitable for continuing professional development.

Interpretive commenting in protein electrophoresis reports

The Working Party noted that interpretive commenting in protein electrophoresis reports varied considerably. For specimens without a paraprotein, minimal descriptive comments are usually adequate. Specimens with a paraprotein, oligoclonal or other low-concentration bands can be more complex and may sometimes require individualized commenting. The Working Party also acknowledges that depending upon the level of knowledge of the requesting clinicians and their patient demographics, the comments may require further expansion. In the Interpretive commenting recommendations below, we have given the minimal information to be provided which is most appropriate to laboratories servicing specialist physicians in tertiary-level hospitals. Laboratories performing protein electrophoresis predominantly for general practitioners, however, are likely to require additional commenting which is educational in nature and may specify the significance of a new paraprotein and appropriate further investigations and follow-up. It is recommended that any laboratory reporting protein electrophoresis should have an education strategy in place suitable for the requesting clinicians. An example of an algorithm for the investigation of a newly detected paraprotein that can be made available to requesting physicians can be found in UK Myeloma Forum and Nordic Myeloma Study Group guidelines.[7] In addition, physicians requesting protein electrophoresis should be encouraged to provide a suitable patient history, especially if it is the first specimen for a particular patient to be examined by the laboratory. Table 3 is not meant to be exhaustive or prescriptive, but lists minimal comments for various SPEP patterns and situations. Bird *et al*.[7] suggest other ways of providing information to non-haematological physicians.

Interpretive commenting recommendations: samples with a paraprotein

The Working Party recommends that SPEP and UPEP be reported in a format suitable for cumulative viewing. This allows clinicians to view the paraprotein trends more easily, thus facilitating patient management. Such a report would contain a field to document whether a paraprotein is detected or not and another field to document quantification of that specific paraprotein. That quantification field label could specify the paraprotein type (e.g. IgA kappa paraprotein) or could be a more general heading (e.g. paraprotein 1) in which case additional commenting is required to describe the paraprotein when the IFE result is available (see additional comments in square brackets in Table 4). Laboratories also require a system to easily demonstrate the location of known paraproteins in prior specimens from individual patients. Ideally this would be a scanned image linked to the patient's data file. Some laboratories may not have this facility, in which case commenting on the location of the paraprotein(s) in the comments section may be the most efficient way to record and access this information. In this circumstance, such commenting has more utility for the laboratory than the clinician.

Special circumstances

Paraproteins in the non-gamma regions

As discussed in section 'Serum paraprotein quantification', paraproteins co-migrating with other normal proteins in alpha and beta regions (most commonly IgA in the beta region) pose particular problems for both electrophoretic and immunochemical quantification. Attempts to subtract the beta region from the total quantity to provide an estimate of the 'true' paraprotein concentration are inherently unreliable, while not reporting INA/ITA values at higher concentrations may be unhelpful to clinicians. The Working Party decided that this situation is best addressed by supporting the reporting of both the electrophoretic and INA/ITA immunoglobulin measurement to facilitate disease monitoring. Spurious overestimated INA/ITA values should not be reported and an explanatory comment added to the report such as 'At concentrations >X g/L (specify according to a laboratory's upper limit of agreement for monoclonal immunoglobulins measured by INA/ITA methods), IgA (or IgG or IgM) cannot be accurately quantified by immunoassay. Refer to the quantification from the protein electrophoresis report'. An education strategy may be required to explain the uncertainty of measurement that applies to both electrophoretic (beta+paraprotein) and immunochemical (total polyclonal and monoclonal immunoglobulin) quantification of the paraprotein. It is important that the results of electrophoretic and INA/ITA quantification are not used interchangeably, that is, when monitoring disease progress electrophoretic quantification is only compared with electrophoretic quantification and INA/ITA values are only compared with INA/ITA values.

New, small abnormal bands on SPEP in patients with a known paraprotein, especially post-stem cell transplantation

Small abnormal protein bands are frequently seen on SPEP following autologous and allogeneic haematopoietic stem cell transplantation.[23,58–60] Often immunofixation reveals not only oligoclonal bands but small discrete bands with the appearance of a paraprotein which are typically ≤1 g/L but may occasionally be larger. These small bands are likely due to transient dysregulation of the regenerating B-cell compartment during haematopoietic recovery post-transplant[58,60] and typically persist from between one to 18 months. The appearance of these new bands in patients

Table 3 General interpretive commenting recommendations: all specimens

Pattern	Minimal information to be provided in the interpretive comment
Normal pattern	Normal pattern. Paraprotein not detected
Normal pattern (and clinical context suggests suspicion of plasma cell dyscrasia)	Normal pattern. Paraprotein not detected. Suggest urine protein electrophoresis and immunofixation, and/or serum free light chains if clinically indicated (if not already done/ordered)
Decreased alpha-1 globulins	Decreased alpha-1 globulins. Suggest alpha-1 antitrypsin quatitation and genotyping/phenotyping if clinically indicated
Decreased albumin and increased alpha-2 and beta globulins	Pattern is consistent with nephrotic syndrome (if corroborated by serum lipid results)
Increased alpha-1 and alpha-2 and/or gammaglobulins	Pattern is consistent with an acute inflammatory process
Increased beta-1 globulin (if IFE performed and paraprotein excluded)	Paraprotein not detected. If indicated, suggest iron studies
Polyclonal hypergammaglobulinaemia	A polyclonal increase in immunoglobulins is present
Polyclonal hypergammaglobulinaemia and acute phase pattern	Pattern is consistent with a chronic inflammatory process
Beta–gamma bridging	Beta–gamma bridging is present due to raised IgA or sometimes IgM. Causes may include cirrhosis, mucosal or cutaneous inflammation
Hypogammaglobulinaemia (first presentation)	Hypogammaglobulinaemia is present. Suggest serum immunofixation and urine protein electrophoresis including immunofixation (or serum free light chains) together with quantitation of total serum immunoglobulins (if not already done/ordered)
Hypogammaglobulinaemia (subsequent presentation)	Hypogammaglobulinaemia is present
Fibrinogen present	Fibrinogen present. Please send repeat serum specimen. (No clinical comment is required if laboratory can run a repeat serum specimen, otherwise needs IFE to ensure small band is fibrinogen and there is no underlying paraprotein; optimally needs repeat serum specimen as a small paraprotein cannot be quantitated by agarose gel SPEP when masked by the presence of fibrinogen)
Oligoclonal banding pattern with 2 or more bands on a polyclonal immunoglobulins background	Oligoclonal bands are present. This can occur in a number of infectious or autoimmune conditions. Suggest review in 3–6 months if clinically indicated
'Inflammatory-type' pattern with increased tubular proteins, i.e. alpha-1, alpha-2, and/or beta-2 microglobulins, and polyclonal FLC on IFE ('ladder-type' gamma pattern on UPEP)	Excess polyclonal free light chains present on immunofixation. Suggest repeat testing if clinically required when acute illness has resolved

UPEP, urine protein electrophoresis; IFE, immunofixation electrophoresis; FLC, free light chains

Table 4 General interpretive commenting recommendations: specimens with a paraprotein and/or small abnormal band

Pattern	Minimal information to be provided in the interpretive comment*
First detection of a paraprotein	Suggest total serum immunoglobulins and urine protein electrophoresis and immunofixation (if not already done/ordered) [Typing and numerical quantitation, e.g. 'An IgG kappa paraprotein was detected in the gamma region']
Follow-up of a known paraprotein which is still present	Nil required [A comment should be made on the original band and its current status, e.g. 'The previously reported IgG kappa paraprotein was detected']
Paraprotein detected only by immunofixation electrophoresis	The previously reported IgG kappa paraprotein is now only visible by immunofixation
If paraprotein has disappeared	A comment is required to confirm the absence of the previously detected paraprotein, e.g. 'The previously reported IgG kappa paraprotein was not detected by immunofixation'
New, small abnormal band with different electrophoretic mobility from the original paraprotein in a patient with a known paraprotein	There is a small (type: e.g. IgG kappa) band approximately (amount: e.g. 1 g/L) on a background of a polyclonal and/or oligoclonal pattern. This band is different from the original paraprotein. Its clinical significance is uncertain
First presentation of small abnormal band (and no known paraprotein)	There is a small (type: e.g. IgG kappa) band approximately (amount: e.g. 1 g/L). Its clinical significance is uncertain. Suggest urine protein electrophoresis and immunofixation (or serum free light chains) and repeat serum protein electrophoresis in 3–6 months if clinically indicated
First presentation of small abnormal bands in polyclonal/oligoclonal background (and no known paraprotein)	There is a small (type: e.g. IgG kappa) band approximately (amount: e.g. 1 g/L) on a background of a polyclonal and/or oligoclonal pattern. Its clinical significance is uncertain but may reflect an inflammatory/reactive process. Suggest urine protein electrophoresis and immunofixation (or serum free light chains) and repeat serum protein electrophoresis in 3–6 months if clinically indicated

*Comments in square brackets refer to reporting formats where the quantitation field label does not specify the paraprotein type (e.g. see worked example 1A compared with 1B shown in the Appendix)

with myeloma can pose significant difficulty to the laboratory as they may be mistakenly reported to suggest relapse when in fact they are associated with improved remission depth and outcome.[23,58,59] More recently, they have been described following novel agent combination regimens where appearance of small bands also correlated with better haematological response.[61] As such, these small bands need to be recognized but should not be reported as new paraproteins (i.e. terms such as 'paraprotein' or 'monoclonal protein' should be avoided as they can be a potential source of confusion to clinicians).

First presentation of a small abnormal band on SPEP in patients without a known paraprotein

High-resolution SPEP detects 0.5–1 g/L size bands, the clinical significance of which is often uncertain. Very low-level paraprotein has potential implications for diagnosis of lymphoma (if IgM), AL amyloidosis or oligosecretory myeloma.[62] Alternatively, oligoclonal bands and small discrete bands are a common occurrence on SPEP due to infectious and autoimmune diseases in which case they are often transient and do not require the lifetime follow-up that a diagnosis of monoclonal gammopathy of undetermined significance may dictate. Overcalling these small bands can precipitate a cascade of investigations because of clinician concern that such small bands indicate clonal plasma cell or lymphoproliferative disease. Not reporting these small bands may lead to delayed diagnosis of important oligosecretory plasma cell disease such as AL amyloidosis. One approach recommended by Keren[41] is that when commenting on such a finding, note the following: (a) a restriction (small band rather than a paraprotein or monoclonal band) is present; (b) its significance is not known; (c) suggest urine testing to rule out BJP; and (d) note if the gamma region is increased, decreased or normal. The report also could include a comment that consideration could be given to repeat/follow-up testing in a clinically appropriate time frame such as 3–6 months.

Conclusion

Although protein electrophoresis of serum and urine specimens is a well-established laboratory technique, the reporting of results varies considerably between laboratories. Recommendations are provided for standardized reporting of protein electrophoresis in Australia and New Zealand. While not all of this guidance may be appropriate for all laboratories, it is expected that standardized reporting formats and interpretive commenting especially for complex cases will reduce both variation between laboratories and the risk of misinterpretation of results.

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Appendix: Worked examples showing two reporting format styles for SPEP and UPEP

Below are listed some worked examples of how SPEP and UPEP reports may appear based on the recommendations in this document. This is done in order to better visualize some of the suggested reporting formats. The worked examples are not, however, intended to be prescriptive. Two reporting formats have been provided (A and B), but individual laboratory circumstances and information systems may dictate different reporting fields.

Example 1: SPEP: initial presentation, e.g. IgG kappa paraprotein and monoclonal kappa free light chains both in gamma region and residual hypogammaglobulinaemia

1A. SPEP report format

Serum protein electrophoresis		Unit	Ref
Specimen date	#1		
Total protein	85	g/L	
Albumin	22	g/L	
Paraprotein	DET		
Paraprotein 1	41	g/L	
Paraprotein 2	Trace	g/L	
Immunoglobulins			
IgG	45	g/L	
IgA	0.5	g/L	
IgM	0.2	g/L	

DET, detected; ND, not detected; SEEC, see comment; FLC, free light chains; Trace, <1 g/L

Comments:

Paraprotein 1: An IgG kappa paraprotein was detected in the gamma region.

Paraprotein 2: A monoclonal kappa FLC was detected in the gamma region.

Decreased residual gammaglobulins. Suggest urine protein electrophoresis and immunofixation.

1B. SPEP report format

Serum protein electrophoresis		Unit	Ref
Specimen date	#1		
Total protein	85	g/L	
Albumin	22	g/L	
Total globulin	63	g/L	
Paraprotein	DET		
Alpha-1	3	g/L	
Alpha-2	8	g/L	
Beta	8	g/L	
Gamma	44	g/L	
IgG kappa paraprotein	41	g/L	
Monoclonal kappa FLC	Trace	g/L	
Residual gamma	3	g/L	
Immunoglobulins			
IgG	45	g/L	
IgA	0.5	g/L	
IgM	0.2	g/L	

DET, detected; ND, not detected; SEEC, see comment; FLC, free light chains; Trace, <1 g/L

Comments:

Decreased residual gammaglobulins. Suggest urine protein electrophoresis and immunofixation.

Example 2: SPEP: monitoring multiple myeloma, e.g. IgA lambda paraprotein in beta region

2A. SPEP report format

Serum protein electrophoresis			Unit	Ref
Specimen date	#1	#2		
Total protein	76	64	g/L	
Albumin	30	34	g/L	
Paraprotein	DET	DET		
Paraprotein 1	28	16	g/L	
Immunoglobulins				
IgG	4.0		g/L	
IgA	21	9	g/L	
IgM	0.5		g/L	

DET, detected; ND, not detected; SEEC, see comment; FLC, free light chains; Trace, <1 g/L

Comments:

Date #2:

Paraprotein 1: The previously reported IgA lambda paraprotein was detected. Quantification includes both the beta region and the paraprotein.

Hypogammaglobulinaemia is present.

Date #1:

Paraprotein 1: An IgA lambda paraprotein was detected in the beta region. Quantification includes both the beta region and the paraprotein.

Hypogammaglobulinaemia is present. Suggest urine protein electrophoresis and immunofixation.

2B. SPEP report format

Serum protein electrophoresis			Unit	Ref
Specimen date	#1	#2		
Total protein	76	64	g/L	
Albumin	30	34	g/L	
Total globulin	46	30	g/L	
Paraprotein	DET	DET		
Alpha-1	3	2	g/L	
Alpha-2	10	9	g/L	
Beta+IgA lambda paraprotein	28	16	g/L	
Gamma	5	3	g/L	
Immunoglobulins				
IgG	4.0		g/L	
IgA	21	9	g/L	
IgM	0.5		g/L	

DET, detected; ND, not detected; SEEC, see comment; FLC, free light chains; Trace, <1 g/L

Comments:

Date #2:

Hypogammaglobulinaemia is present.

Date #1:

Hypogammaglobulinaemia is present. Suggest urine protein electrophoresis and immunofixation.

Example 3: UPEP: monitoring IgG kappa multiple myeloma with kappa Bence Jones proteinuria

3A. UPEP report format

Urine protein electrophoresis				Unit	Ref
Specimen date	#1	#2	#3		
Bence Jones protein	DET	DET	DET		
Monoclonal immunoglobulin	DET	DET	ND		
Concentrations					
Urine total protein	1000	500	200	mg/L	
Urine creatinine	10.0	10.0	10.0	mmol/L	
Paraprotein 1	700	100	Trace	mg/L	
Paraprotein 2	50	SEEC		mg/L	
Creatinine ratios					
Paraprotein 1	70	10		mg/mmoL creat	
Paraprotein 2	5			mg/mmoL creat	
Timed excretion					
Time period	24.0	24.0	24.0	h	
Volume	2.00	2.00	2.00	l	
Urine total protein	2000	1000	400	mg/24 h	
Paraprotein 1	1400	200		mg/24 h	
Paraprotein 2	100			mg/24 h	

DET, detected; ND, not detected; SEEC, see comment; Trace, <10 mg/L

Comments:

Date #3:

Paraprotein 1: The previously reported kappa BJP was detected.

Paraprotein 2: The previously reported IgG kappa paraprotein was not detected by immunofixation.

Date #2:

Paraprotein 1: The previously reported kappa BJP was detected.

Paraprotein 2: The previously reported IgG kappa paraprotein is only visible by immunofixation.

Date #1:

Paraprotein 1: Kappa BJP was detected.

Paraprotein 2: An IgG kappa paraprotein was detected.

3B. UPEP report format

Urine protein electrophoresis				Unit	Ref
Specimen date	#1	#2	#3		
Bence Jones protein	DET	DET	DET		
Monoclonal immunoglobulin	DET	DET	ND		
Concentrations					
Urine total protein	1000	500	200	mg/L	
Urine creatinine	10.0	10.0	10.0	mmol/L	
Kappa Bence Jones protein	700	100	Trace	mg/L	
Monoclonal IgG kappa	50	SEEC		mg/L	
Creatinine ratios					
Kappa Bence Jones protein	70	10		mg/mmoL creat	
Monoclonal IgG kappa	5			mg/mmoL creat	
Timed excretion					
Time period	24.0	24.0	24.0	h	
Volume	2.00	2.00	2.00	l	
Urine total protein	2000	1000	400	mg/24 h	
Kappa Bence Jones protein	1400	200		mg/24 h	
Monoclonal IgG kappa	100			mg/24 h	

DET, detected; ND, not detected; SEEC, see comment; Trace, <10 mg/L

Comments:

Date #3:

The previously reported kappa BJP is only visible by immunofixation.

Date #2:

The previously reported IgG kappa paraprotein is only visible by immunofixation.