Clinical use and interpretation of serum protein electrophoresis and adjunct assays

Serum electrophoresis and related tests have a variety of uses in clinical practice, but their full potential is not often realized. This article summarizes their basis and clinical use, and outlines how to interpret the results.

Introduction: what is the test?

Separation of different analytes in body fluids is useful to distinguish individual components that may be contributing to pathology. Electrophoresis is a useful test to achieve this and helps characterize and identify components of biological fluids that may be a result of, a cause of, or associated with a disease process. Protein electrophoresis is a laboratory test that separates out proteins in an electric field based on their charge. While protein electrophoresis is commonly used for the detection of paraproteins (otherwise known as M-bands) for the diagnosis and monitoring of myeloma, there are several other uses which are often underappreciated (Spickett, 2013).

Electrophoresis can be performed on any body fluids although by far the most common is serum. Separation of proteins in urine and CSF is also possible by another technique called isoelectric focusing that has specific diagnostic indications. This article reviews protein electrophoresis and its associated tests, including indications to order, basic interpretation and steps to take next by the generalist. This is supported by literature and the latest guidelines to facilitate clinical practice.

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The basis of the test

In clinical laboratories, agarose gel is used to separate proteins based on electric charge, which are then visualized using amido black, a protein-binding stain. Serum protein electrophoresis is used to examine the general distribution and quantitation of proteins in serum. In an electrical field, the negatively-charged proteins migrate towards the positive (anode) end and separate according to charge.

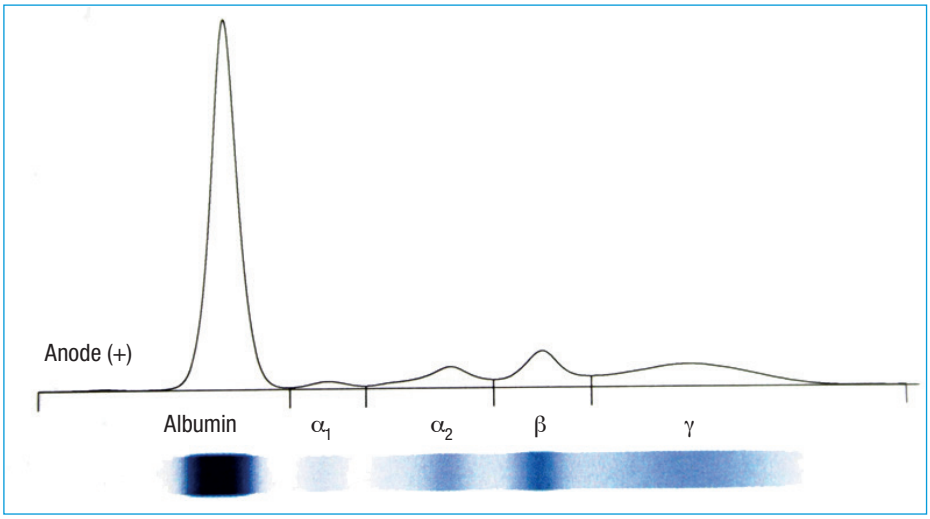
In protein electrophoresis, two major groups of proteins can be distinguished: albumin (50–70% of total serum by weight) and globulins (chiefly immunoglobulin G (IgG) in healthy individuals). Albumin has the greatest negative charge and will travel the furthest of all proteins. Five distinct bands can be appreciated on zone electrophoresis: albumin, alpha-1, alpha-2, beta and gamma (*Figure 1*). Sometimes, distinct areas can be visualized in the beta fraction which can be subdivided into beta-1 and beta-2 components. Most immunoglobulins (IgM, IgG, IgD and IgE) exist in the gamma region.

Immunofixation electrophoresis is an extension of protein electrophoresis, in which the proteins are first separated by electrophoresis and then exposed to antibodies (antisera) specific for certain antigens to identify the components of each band. In most cases, this is used to identify the isotype of heavy (IgG, IgM, IgA, IgD or IgE) and light chains (kappa or lambda) to identify a specific paraprotein detected by protein electrophoresis.

When should the test be requested?

As a diagnostic test, protein electrophoresis has a variety of uses that may facilitate the direction of a diagnostic workup. It is particularly useful in distinguishing monoclonal from polyclonal immunoglobulin expansion when elevated serum immunoglobulin levels are noted. The classical use of serum protein electrophoresis is in the diagnosis of plasma cell dyscrasias in which excess monoclonal immunoglobulins are produced. Conversely, identification of polyclonal increases of

Figure 1. Serum protein electrophoresis on normal serum. The protein fractions are labelled accordingly on this electrophoretic strip and a corresponding densitometry graph is also included.



immunoglobulins suggests further tests to identify an inflammatory disorder such as an infection, autoimmune disease or, more rarely, a malignancy.

As a result, clinical features that suggest these conditions may warrant this test on the patient's serum. These may be isolated and sometimes unexplainable features such as fatigue, recurrent infections, or the presence of CRAB features (hyperCalcaemia, Renal failure, Anaemia and Bone pain/lesions). In addition, serum protein electrophoresis can be useful as a monitoring tool for someone with monoclonal gammopathy of undetermined significance. Supplementary tests, such as measurement of serum free light chains (see below), may then allow stratification into low-risk patients who may be monitored clinically, while intermediate and high-risk patients need a yearly test (Jacobs, 2013). In addition, in those with established M-protein disease, international guidelines also recommend monitoring M-protein by protein electrophoresis on a monthly basis while on treatment, or every 3–4 months when off therapy (Vincent Rajkumar, 2014).

Serum protein electrophoresis may also be used to further characterize persistent protein abnormalities (e.g. elevated IgG). Consequently, it also has a role to play in ambiguous clinical presentations or where standard clinical and laboratory evaluations are unclear. In rheumatology, serum protein electrophoresis is particularly useful in the investigation of general inflammatory conditions resulting in polyclonal gammopathy or as a work-up for joint pain (which may, in fact, be bone pain seen in a plasma cell dyscrasia).

It is important that immunofixation electrophoresis is performed in conjunction with serum protein electrophoresis as it is more sensitive for the detection of a paraprotein and some plasma cell dyscrasia patients may have a normal or equivocal protein electrophoresis (Kyle, 1994). Urine protein electrophoresis and immunofixation electrophoresis can be performed in parallel either on a spot urine sample (morning preferable) or 24-hour collection. The primary use is to detect and characterize free light chains (Bence Jones protein) in the diagnostic workup of plasma cell dyscrasias.

Urinary protein electrophoresis is also useful for investigating proteinuria and distinguishing between glomerular and tubular protein loss. In glomerular kidney disease, large proteins are lost; hence the dominant protein increased on protein electrophoresis will be albumin. In contrast, tubular insult as a result of drug toxicity will result in inefficient reabsorbtion of low molecular weight proteins, producing increases in alpha-1 and beta-2 protein fractions on protein electrophoresis (D'Amico and Bazzi, 2003; Jenkins, 2009).

As an adjunct, serum free light chain analysis is a sensitive assay that detects light chains that may otherwise be missed on protein electrophoresis or immunofixation electrophoresis analysis. This is especially useful in cases where only free light chains are secreted at low concentrations. In addition, serum free light chain analysis has a place in the monitoring, prognostication and diagnosis of monoclonal gammopathies (Dispenzieri et al, 2009), with studies showing high sensitivity and higher detection (screening) rates for this test over urinary protein electrophoresis (Holding et al, 2011; Graziani and Merlini, 2014; Dejoie et al, 2016). Free light chain also showed better concordance to protein electrophoresis and immunofixation electrophoresis over 24-hour urinary protein electrophoresis and is therefore a better monitoring tool (Dejoie et al, 2014).

Despite these findings, however, the latest international guidelines currently do not support the replacement of 24-hour urinary protein electrophoresis with free light chain tests (Dispenzieri et al, 2009). Serum free light chain analysis also needs to be interpreted in the patient's context, as compromised renal function (raised creatinine level) is one common cause of elevated kappa:lambda ratios which can yield false positives (Abadie et al, 2009).

Free light chain and protein electrophoresis analysis is also useful for the diagnosis of primary amyloidosis, since light chains are involved in the pathogenesis (Gertz et al, 2005). Signs and symptoms which suggest this diagnosis, e.g. unexpected heart failure, hepatomegaly, proteinuria, or unexplained peripheral neuropathy, should warrant ordering these tests for possible amyloidosis.

Finally, although less commonly ordered, CSF can be subjected to protein electrophoresis as well in the diagnostic workup for multiple sclerosis. CSF oligoclonal IgG bands (two or more bands) found on protein electrophoresis help support a diagnosis of multiple sclerosis (Polman et al, 2011), but are not specific for this diagnosis as they may be found in other inflammatory and autoimmune neurological disorders (Ebers and Paty, 1980).

Interpretation

Reports of protein electrophoresis are returned with quantitative measurements of each protein group. Protein electrophoresis performed on serum can provide several diagnostic clues depending on which protein fraction is affected (*Table 1*) and the clinician can be guided accordingly in the clinical investigation and management.

In contrast, urine protein electrophoresis is more complicated to interpret (Spickett, 2013). Poor renal function, renal damage or a significant systemic inflammatory disorder may result in a monoclonal band as a result of leakage of an intact monoclonal immunoglobulin (Jenkins, 2009), therefore urine immunofixation electrophoresis is essential to determine if this is Bence Jones protein (monoclonal light chains) from a possible plasma cell dyscrasia, or some other intact protein.

What to do next?

What steps to take when an abnormal protein electrophoresis or immunofixation electrophoresis is received depends on the extent of the derangement and in which fraction(s) it is. If the results hint at a possible plasma cell dyscrasia, then care is vital to ensure all other associated testing has been carried out. This includes serum immunoglobulins (IgG, IgM and IgA at a minimum), β2-microglobulin for prognostication, serum free light chain, full blood examinations, erythrocyte sedimentation rate, serum calcium, urea, creatinine and skeletal surveys (Firkin, 2009). Following this, a referral to a haematologist may be appropriate.

If there is a significant isolated elevation or depression of one of the globulin groups, then investigation according to the corresponding typical proteins found in that fraction may be warranted (*Table 1*). For example, if the alpha-1 fraction is significantly and persistently depressed, then an α_1-antitrypsin level and phenotype test may be requested if clinically appropriate. Elevation or depression of immunoglobulins must be confirmed with serum quantitative tests and a referral to an immunologist may be considered if there is no identifiable secondary cause for the abnormality.

KEY POINTS

- Electrophoresis is a laboratory technique to separate molecules (commonly proteins) according to their charge.
- The main use for serum protein electrophoresis is in the workup of plasma cell dyscrasias by differentiating polyclonal *vs* monoclonal expansion in immunoglobulins.
- Immunofixation assays can identify the precise heavy and light chains of monoclonal (M) proteins.
- Laboratory tests need to be interpreted in conjunction with clinical situation to be of maximal benefit.

Conclusions

Protein electrophoresis and immunofixation electrophoresis are useful tests to consider on serum samples, particularly if there are clinical indications of a possible plasma cell dyscrasia or a perturbation in immunoglobulin levels is found. When there is a strong suspicion of the former, consider requesting a parallel serum free light chain assay (preferred over urine electrophoresis). However, a normal serum protein electrophoresis does not rule out a monoclonal gammopathy.

Serum protein electrophoresis is a complex test that can give a wealth of information and diagnostic clues to the clinician; however, its sensitivity means minor abnormalities can be picked up. If there is a significant abnormality that warrants further testing, and if clinically indicated, the clinician may consider a repeat serum protein electrophoresis a few months later, preferably when the patient is 'well'. Persistent abnormalities may warrant further investigations and/or specialist referral. **BJHM**

Conflict of interest: none.

Table 1. Differential diagnoses for protein fraction deviations on serum protein electrophoresis

Protein fraction	Typical proteins in this fraction	Conditions decreased (absent or faint band)	Conditions increased (increased band)
Albumin	Albumin	Negative acute phase reactant, e.g. inflammation, sepsis, malnutrition, nephrotic syndrome and kidney disease, liver disease, pregnancy haemodilution (artefact)	Dehydration
Alpha-1	α_1-lipoprotein (high-density lipoprotein), α_1-antitrypsin, α-fetoprotein	**α_1-antitrypsin deficiency***, liver disease	Increased α-fetoprotein – liver tumours, germ cell tumours, pregnancy, inflammatory states
Alpha-2	α_2-macroglobulin, haptoglobin, caeruloplasmin	Liver disease, haemolysis, malnutrition, Wilson's disease	Inflammatory states, steroid use, adrenal insufficiency, nephrotic syndrome, severe diabetes mellitus
Beta-1	Transferrin, β_1-lipoprotein (low-density lipoprotein)	Malnutrition	Iron deficiency, pregnancy, inflammatory states, hyperlipidaemia
Beta-2	C3 complement, β_2-microglobulin	C3 consumption	Inflammatory states
Beta-gamma region	Fibrinogen (uncoagulated samples), C-reactive protein, IgM, IgA		Liver disease, inflammatory states
Gamma	IgG, IgM, IgA, IgD, IgE (+/- monoclonal proteins which can also migrate to alpha-2 region)	**Inherited humoral immunodeficiency**, kidney disease, sepsis, malnutrition, viral infections, amyloidosis, leukaemias	**Smear – polyclonal immunoglobulins (infection or inflammation). Monoclonal band – plasma cell dyscrasia, e.g. multiple myeloma, lymphoma, Waldenstrom's**

**It is insufficient to make diagnoses based on protein electrophoresis alone; but diagnoses in bold are particularly important to consider.*

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