

A brief look at electrophoresis

Electrophoretic analysis of proteins in serum, urine and cerebrospinal fluid, and the characterisation of haemoglobinopathies is a standard laboratory technique. This article provides a brief overview of methodology and its use in routine practice.

Electrophoresis separates proteins according to their different electrical charges, usually by adding serum samples to cellulose acetate or agarose gel and then applying a current for a set time. The pattern of serum proteins found in humans is mirrored in many other species, which demonstrates that these proteins are highly conserved throughout evolution (Fig 1).

Visual evaluation of focused bands characteristically present on agarose gel electrophoretograms provide valuable diagnostic information about physiological and pathological processes. Any detected variation in concentration of one or more bands and/or the appearance of additional bands, often paraproteins, have important clinical meaning (eg monoclonal bands, nephrotic syndrome and decreased α 1-antitrypsin).

Serum protein electrophoresis (SPE) performed at pH 8.9 normally yields five bands. The first, and most intense, band is albumin, followed by four globulins (α 1, α 2, β and a broad γ band), each of which can contain a number of different proteins.

SERUM PROTEINS IN HEALTH AND DISEASE

Normally, over 50% of serum proteins comprise the single carrier protein albumin. The α 1-globulin band is made up almost entirely of α 1-antitrypsin. Inspection of this band can reveal abnormalities such as α 1-antitrypsin deficiency. The α 2-globulin band consists mainly of α 2-microglobulin and haptoglobin.

The β band can be further separated into two bands. The β 1 band consists mainly of transferrin, with some low-density lipoprotein

(LDL). Transferrin plays an important role in the metabolism of iron, facilitating ferric iron transport from intracellular stores to the bone marrow, where the precursor cells of erythrocytes and lymphocytes bear receptors for transferrin on their cell membranes.

Various types of transferrin have been described according to their different structural features, all showing β 1 mobility in the electrophoretic pattern. Splitting of the β 1 band can be observed as a result of the presence of paraproteins. While pathological increase in a transferrin band is seen in anaemia, diminished concentration of transferrin is of poor diagnostic utility and generally only reflects reduced hepatic synthesis.

The β 2 band is the C3 component of complement. Decrease in C3 is observed in autoimmune diseases (eg systemic lupus erythematosus [SLE] and rheumatoid arthritis). γ globulins comprise immunoglobulins and some may be present in the α 2 and β regions. Malignancies of B-lymphocyte origin produce monoclonal

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Proteins found in plasma are synthesised largely in the liver; however, immunoglobulins are the exception as they are derived from B cells of the immune system. Most plasma proteins are catabolised naturally after pinocytosis by endothelial cells and monocytes. Small proteins are lost through the intestinal wall and renal glomerulus into urine.

MULTIPLE MYELOMA

Serum and urine protein electrophoresis is performed most commonly when multiple myeloma (MM) is suspected. The latest statistics from Cancer Research UK (CRUK) show there were just over 3700 cases of myeloma diagnosed in the UK in 2003. Overall, it is the 17th most common cancer. The CRUK website also states that MM, a B-cell malignancy, is about twice as common in black populations as it is in Caucasian

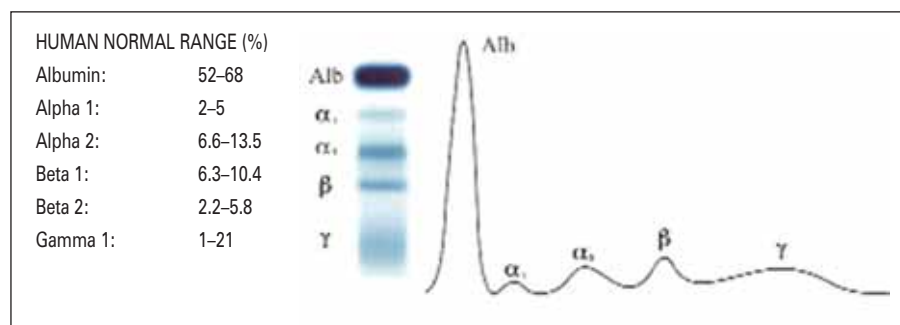


Fig 1. Normal serum protein characterisation and quantification using gel electrophoresis visualised by amido black staining.

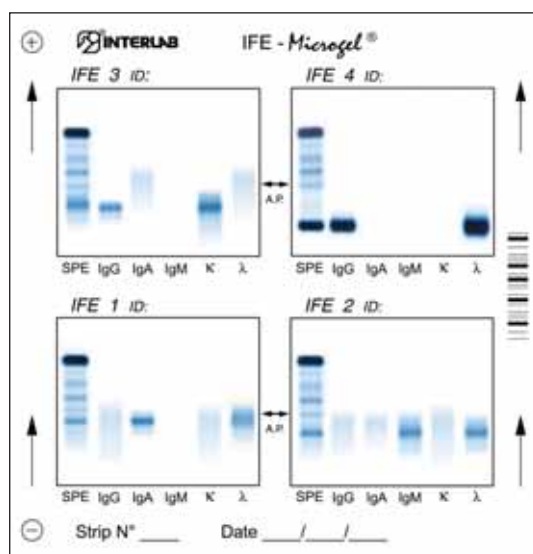


Fig 2. (Left) Immunofixation electrophoresis of four samples on a single gel carried out using a modern automated electrophoresis system. The left-hand lane shows the SPE reference pattern, followed by, from left to right, the heavy-chain and light-chain patterns.

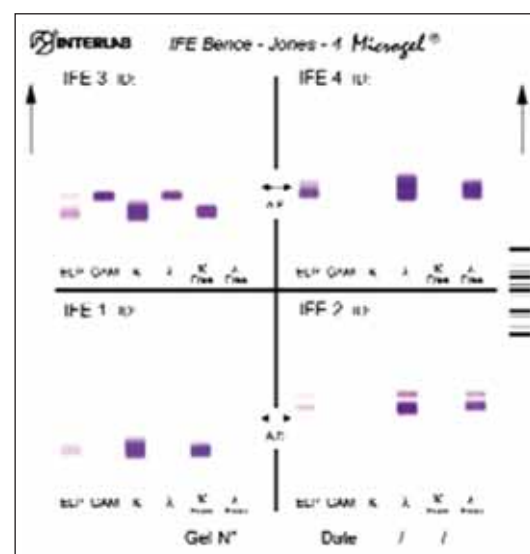


Fig 3. (Right) Immunofixation electrophoresis of Bence Jones protein visualised by an acid violet stain.

populations, with the risk increasing with age. In MM, the malignant clone of B cells in bone marrow produces monoclonal immunoglobulin, resulting in a characteristic band in the γ zone on the gel. A rare condition termed monoclonal gammopathy of unknown significance (MGUS) is associated with an increased risk of developing MM.

IMMUNOFIXATION ELECTROPHORESIS

Paraproteins produce one or more sharp bands in the electrophoretic patterns obtained from serum and/or urine samples. Although monoclonal components are typical of myeloma, they also appear on the electrophoretic pattern of patients suffering from other conditions (eg amyloidosis, plasmacytoma, MGUS, infection and autoimmune diseases) in which their presence may be transient. Confirmation of the presence of a monoclonal immunoglobulin, through characterisation of the immunoglobulin type, is therefore of fundamental importance for a definitive diagnosis of MM.

Immunofixation electrophoresis (IFE) is the laboratory method used to define the biochemical identity and homogeneity of immunoglobulins when suspected monoclonal components are detected in protein electrophoretic patterns of biological fluids. The principle of IFE is based on the visualisation of specific proteins labelled with antisera to immunoglobulin class and light

chains to confirm clonality and light chain restriction following protein separation by electrophoresis (Fig 2).

One lane is treated with a fixative solution to create an SPE reference pattern. Antisera specific for the heavy chains (anti γ [IgG], anti α [IgA], anti μ [IgM]) and for the light chains (anti κ [kappa] and anti λ [lambda]; free and bound) are applied on the other five lanes of each patient test. Quantitative evaluation of the bands is obtained by scanning the SPE gel plate using a densitometer. The densitometric data are then used to calculate the percentage value of each fraction, which then can be expressed as a proportion (g/L) of the total protein concentration of the sample.

BENCE JONES PROTEIN

The appearance of plasma proteins in urine (proteinuria) is of great value in the evaluation of renal function. Physiological proteinuria is defined as approximately 150 mg protein in a 24-hour urine collection.

Henry Bence Jones first diagnosed MM by identifying a classical monoclonal protein in the urine of a patient in 1845. Some 15–20% of MM patients exhibit only a small monoclonal protein fragment in urine, with no associated serum protein. Such a monoclonal protein is termed Bence Jones protein. Electrophoresis of urine remains the only reliable way to detect abnormal proteins in such patients, with essential complementary identification of Bence Jones protein by immunofixation (Fig 3).

Binding between a specific antibody and the monoclonal immunoglobulin results in the formation of a band of precipitate in the corresponding lane that identifies the type of immunoglobulin, following use of acid violet, which is a sensitive stain for proteins.

Immunoglobulin free light chains, or Bence Jones protein, pass from serum into urine and are therefore considered to be a tumour marker for MM. Thus, its detection in urine provides valuable information for initial diagnosis, monitoring and follow-up during

treatment. Bence Jones protein may comprise immunoglobulin fragments of heavy chains and light chains or light chains alone in free or polymerised form.

HAEMOGLOBIN ELECTROPHORESIS

Three main types of haemoglobin are present in the red blood cells of the normal adult. HbA is the main type, with small amounts of HbA₂ and HbF also present. The two abnormal haemoglobins seen most commonly are HbS and HbC. Acid and alkali haemoglobin electrophoresis enable haemoglobinopathy characterisation.

FURTHER READING

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