



Paraproteins

General consideration

The term paraprotein was introduced by Apitz in 1940 and is given to describe the abnormal proteins that are produced by **myeloma cells** and occur in the blood, urine and tissues.

Paraproteins are the earliest described **tumour markers** and remain an essential part of the investigation, diagnosis and monitoring of patients with B cell dyscrasias.

Electrophoresis is the only reliable way of detecting a paraprotein in biological fluids. This test is the initial screening procedure and therefore should have sufficient resolution to do this adequately. Serum electrophoresis should always be accompanied by measurement of serum IgG, IgA and IgM concentrations. Samples with raised IgA and IgM concentrations that cannot be confirmed as polyclonal by the electrophoresis pattern should be analysed by immunofixation to exclude small paraprotein bands obscured by one of the normal zones.

The majority of serum paraproteins will be found in the region from the start of the beta to the end of the gamma zones. Occasionally paraprotein bands appear in the alpha-zones and in the post gamma region.

Presence of monoclonal free light chains or Bence Jones protein in urine is suggestive of B cell malignancy and may be the only tumour marker. Whatever analytical system is being used, a trace of albumin should be visible in every urine sample to indicate adequate sensitivity.

The detection of a paraprotein in serum or urine must be followed-up with typing. Immunofixation remains the method of choice for paraprotein typing because it is fast, specific, flexible and easy to interpret. It is also more sensitive than electrophoresis and may detect paraprotein bands that are not visible on routine electrophoresis.

The method of choice for quantification of paraprotein is densitometric scanning of the electrophorogram (HellabioScan). Immunochemical quantitation is unreliable.

Detection of paraproteins

Agarose electrophoresis is the most common method currently in use in clinical diagnostic for the detection of paraproteins in serum and urine.

Paraprotein bands may be 'missed' if they are at low serum concentration (<5.0 g/l) and or where their mobility coincides with other bands such as beta globulins. It is also possible to miss paraproteins where there is no suppression of normal immunoglobulin concentrations

Immunofixation should be done on samples when no obvious paraprotein band is detected but where there is raised IgA or IgM without the increased staining of the beta-gamma region that is associated with a polyclonal increase in IgA or IgM.

IgD paraproteins and free heavy chains are susceptible to post-synthetic degradation, which results in diffuse paraprotein bands on electrophoresis. These may be missed if present at low concentrations or if there is an expectation of seeing a clearly defined band.

There are a number of situations where a band is seen in a serum electrophoretic separation that is not monoclonal immunoglobulin; these include:

- additional bands in the alpha-1 region due to allotypic variation in α -1 antitrypsin
- split alpha-2 zone due to the different mobility of the haptoglobin-haemoglobin complex after intravascular haemolysis
- an additional band in the beta-gamma region due to high concentrations of C-reactive protein
- additional bands in the fast gamma region due to the presence of fibrinogen.

It is also worth noting that some paraproteins precipitate at temperatures below 37°C - so called cryoproteins [Cryoglobulins]. Samples where cryoprotein is being considered must be collected, transported and separated at 37°C. Failure to do this may result in the precipitation of the cryoprotein which will be subsequently discarded with the call pellet.

Identification (typing) of paraproteins

The detection of a paraprotein band must always be followed up with the specific typing of the band. It is important that the heavy and light chain components are identified because this confirms monoclonality and **the paraprotein type may give the clinician additional information about the underlying tumour and prognosis**. Electrophoretic pattern of a patient's sample may change during the course of their disease or treatment so the initial investigations can serve as a point of reference. Complete **disappearance of the paraprotein** is rare but is occurring increasingly with treatment regimens using high dose chemotherapy and bone marrow or stem cell transplantation. An oligoclonal-banding pattern is sometimes seen in patients after bone marrow transplantation and it is important to distinguish this from the original paraproteinaemia.

Quantification of serum paraproteins

Immunochemical quantification of paraproteins is unreliable. The densitometric scan (HellabioScan) of gel electrophoretic separation is recommended for measurement of paraprotein concentration. It is important to be aware that there is a differential dye binding between albumin and the globulins, therefore the most precise estimation of paraprotein is derived from the percentage of relative dye-binding of the

paraprotein band compared with the total globulin fraction rather than the total protein. It is also important to note that there is a non-linear relationship between dye-binding and protein concentration at high paraprotein concentrations.

Measurement of serum total protein and albumin are generally reliable and it can be useful to use these two concentrations as a 'rough check' of the paraprotein quantitation. The albumin concentration added to the paraprotein concentration cannot exceed the total protein concentration and (accepting that there may be differential albumin to globulin binding) bands of similar areas should ultimately be of similar concentrations.

Quantification of Bence-Jones protein

Quantitation of BJP is being recommended as a criterion for response, progression or relapse of multiple myeloma treated by high-dose therapy and stem cell transplantation. This must be done like serum paraprotein quantitation, by densitometry of the urine electrophoresis and calculation of the paraprotein band with respect to the urine total protein (either random or 24 hour).