6 STORAGE AND STABILITY

Upon receipt immunofixation grade antisera should be stored at 2-8°C, where it will remain stable until the given expiry date. DO NOT FREEZE. Slight precipitation can occur on storage which may be removed by centrifugation and should not affect performance.

7 SPECIMENT COLLECTION AND PREPARATION

Use fresh or deep frozen serum samples. Plasma samples are not recommended as the presence of fibrinogen may confuse interpretation. Blood samples should be collected by venepuncture, allowed to clot naturally and the serum separated as soon as possible to prevent haemolysis. The serum may be stored at 2-8°C for up to 48 hours prior to the assay, or for prolonged storage, aliquotted and kept undiluted at -20°C or below. Repeated thawing and freezing should be avoided. Microbially contaminated, haemolysed, and lipaemic serum and samples containing particulate matter should not be used, as unclear staining patterns may occur.

8 METHODOLOGY

8.1 Materials provided

8.1.1 1 x 1.4mL Anti-Human IgE (Fix)

8.1.2 1 x Instruction leaflet

8.2 Materials required but not provided

8.2.1 The Binding Site IFE Kit (XK002).

8.2.2 Electrophoresis tank and power supply.

8.2.3 Various pipettes, micropipettes, measuring cylinders.

8.2.4 Drying equipment or 45°C Incubator.

8.2.5 Gel washing and drying equipment.

8.2.6 5% v/v acetic acid for destaining solution and protein stain diluent.

8.2.7 Moist box (e.g. sealable plastic box containing damp tissue paper) when not using a gel box and dryer.

8.2.8 Washing solution (0.85% saline).

8.2.9 Distilled water.

8.2.10 Gel press or weight.

8.3 Reagent preparation

The antiserum is supplied ready to use.

8.4 Sample preparation

Serum samples can be assayed in a range from neat up to a 1:10 dilution (e.g., 25µL serum plus 225µL buffer for a 1:10 dilution).

8.5 Instructions

See specific immunofixation kit instructions for electrophoretic conditions/methodology details.

8.6 Antiserum/sample volumes

<table>
<thead>
<tr>
<th>Method</th>
<th>Sample volume</th>
<th>Volume of antiserum per track (1 drop = 50µL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Binding Site IFE</td>
<td>3µL</td>
<td>2 drops/track</td>
</tr>
</tbody>
</table>

8.7 Quality control

Care should be taken at the following stages to maintain good quality control.

8.7.1 Accurate sample application mask alignment.

8.7.2 Accurate sample application.

8.7.3 Correct running time for electrophoresis.

8.7.4 Immediate fixation following electrophoresis, to prevent protein diffusion thus reducing the definition of the bands.

8.7.5 Care should be taken to prevent antiserum spillage and touching the gel surface with the nozzle of the bottle.

8.7.6 Correct incubation in a humid environment at 45°C for the appropriate length of time.

8.7.7 Care during washing and staining.

8.7.8 Where interpretation becomes difficult, the sample must be rerun.

9 RESULTS

The presence of a stained band results from the reaction between the antiserum and the specific protein in the sample. The intensity of the band will depend on the level of the specific protein in the sample.

9.1 Normal samples give light or no staining in the gamma region.

9.2 Elevated polyclonal immunoglobulin may give strong but diffuse staining with no distinct bands.

9.3 Monoclonal protein appears as a sharp distinct band in the reference (TSP) track, and also in the same position in the appropriate IgE class track.
9.4 High protein concentrations can result in a clear region in the centre of a strong band, due to antigen excess. These samples can, if required, be re-assayed at a higher dilution.

10 LIMITATIONS OF PROCEDURE

10.1 Specific test limitations

10.1.1 Plasma samples should not be used due to the presence of fibrinogen, which binds in the beta region of the tracks and can confuse interpretation.

10.1.2 Unusual results can be obtained with samples containing high concentrations of rheumatoid factor or immune complexes.

10.1.3 A dark elongated ring (with pale or clear centre) can indicate antigen excess. The test sample should be diluted and re-run.

10.1.4 Dark background can be due to inadequate washing prior to staining.

10.1.5 This kit is used to aid diagnosis only. A positive result suggests certain diseases which must be confirmed by clinical findings and other serological tests.

10.1.6 The results obtained from this assay are not diagnostic proof of the presence or the absence of disease.

10.2 For FDA (USA) information see front page.

10.3 Trouble Shooting

<table>
<thead>
<tr>
<th>Problem</th>
<th>Possible Cause(s)</th>
<th>Suggested Action(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 No distinct band in track.</td>
<td>Normal sample.</td>
<td>No action required.</td>
</tr>
<tr>
<td>2 Distinct band in all antisera tracks.</td>
<td>a) Fibrinogen present (usually in the beta region).</td>
<td>Repeat IFE using serum.</td>
</tr>
<tr>
<td></td>
<td>b) Monoclonal protein sticking non-specifically to gel.</td>
<td>Identify Monoclonal protein which forms strongest bands.</td>
</tr>
<tr>
<td>3 Dark elongated ring (pale or clear centre)</td>
<td>Monoclonal antigen excess.</td>
<td>Dilute sample and repeat IFE</td>
</tr>
<tr>
<td>4 Poorly resolved band or weak bands.</td>
<td>a) Elevated polyclonal immunoglobulin.</td>
<td>Repeat IFE</td>
</tr>
<tr>
<td></td>
<td>b) Incorrect electrophoresis and incubation times, poor antiserum application technique.</td>
<td>Repeat IFE checking electrophoresis time, incubation time and temperature (45°C) and carefully apply antisera.</td>
</tr>
</tbody>
</table>

If a problem cannot be resolved, please refer to supplier.

11 PERFORMANCE CHARACTERISTICS

11.1 Precision

3 samples were electrophoresed three times on the same batch of gel. These were then fixed each time using the same batch of IgE antiserum. Identical staining patterns were obtained for all identical samples.

11.2 Comparison

5 clinical (elevated IgE) and 2 normal samples were tested by IFE using The Binding Site antiserum and a competitor’s IgE antisera. All samples gave identical results when using both antisera.

12 BIBLIOGRAPHY

