



IMMUNOELECTROPHORESIS

Serum immunoelectrophoresis detects the presence or absence of immunoglobulins in the blood and assess the type (polyclonal or monoclonal) of immunoglobulins.

Immunoelectrophoresis (IE) combines electrophoresis with immunodiffusion and can be used to identify and characterize serum proteins. IE runs in two main steps. Proteins of sample are first separated by electrophoresis on agarose support according to their charge and MW. After that nonspecific antiserum is applied to corresponding troughs (Anti Globulin's, Anti IgG, Anti IgA, Anti IgM Anti Kappa and Anti Lambda) and is followed the immunodiffusion. If the antigen is present a precipitin (immunoprecipitin arc) will be formed after 18 hours incubation in a humid incubator. By comparison with a known control serum, separated on the same film, tentative identification of individual proteins can be made.

IE is generally a useful technique for qualitative and semi quantitative studies of immunoglobulin 's and is the method of choice for routine clinical services, as it clearly distinguishes a normal arc from an abnormal one, and a pure protein from a contaminated protein, which the Immunofixation Electrophoresis is not able to accomplish. However, there are several faults existent with the IE (therefore the diagnosis should be supplemented by Immunofixation).

Immunoelectrophoresis may be successfully used :

- ◆ *when results of immunofixation are equivocal*
- ◆ *when one suspects a monoclonal protein*
- ◆ *to detect a small monoclonal protein in the presence of normal background or polyclonal increase in immunoglobulin 's*
- ◆ *to detect a small monoclonal immunoglobulin or monoclonal light chain in suspected amyloidosis.*
- ◆ *In cases of early IgA myelomas or IgM macroglobulinemia, for instance, the changes in light chain may be masked by the predominant light chain of IgG, the so called " umbrella effect ".When the monoclonal band is located in the α - or β -zone, especially when it is superimposed with a normal band, the identification may be difficult.*
- ◆ *In cases of biclonal gammopathies, especially when heavy chains in both clones belong to the same class, the interpretation may be difficult.*

HELLABIO IMMUNOELECTROPHORESIS KITS

INCLUDED IN KIT IE 01	
Agarose Gels (10)	Electrophoresis Buffer (for 1or 3 L)
Drying Blotter Sheets	Staining Solution (for 250 ml)
Gel Blotter Strips	Sample Templates
Protein Diluent Solution	Normal control serum (0.5 ml)
Manual	
ANTISERA TO HUMAN PROTEINS	
Whole serum	IgG (γ -chain)
IgA (α -chain)	IgM (μ -chain)
κ -chain (free and bound)	λ -chain (free and bound)

Preparation of Reagents:

• **Electrophoresis Buffer:**

Dilute the content of the bottle in deionized water according to the instruction on the bottle. Store it at room temperature.

• **Staining Solution:**

Dilute the content of the bottle according to the instruction on the bottle. Store it in a good closed flask at room temperature.

• **Distaining Solution:**

Three subsequential baths of 250 ml 10% acetic acid solution. Store it at room temperature.

Collection and handling of specimens

Immunelectrophoresis

1. Fill the electrophoresis chamber with adequate volume of electrophoresis buffer.
2. Take the agarose gel film out of its packaging, uncover it from the plastic plate and put it on a horizontal position.
3. Blot the film with a gel blotter strip in the zone of sample - application.
4. Place the sample template on the application zone carefully. Rub the template with forefinger gently to eliminate trapped air bubbles.
5. Dilute the sample 1:2 with proteins diluent solution and using a 5 μ l pipette, apply 5 μ l of normal control serum and the serum dilution's across each corresponding slit *[The application of the samples should be done as quick as possible. The application slits should not be allowed to dry].*
6. Let the sample absorb into the gel for 5 mins and blot the superfluous sample with a gel blotter strip .
7. Remove both the sample template gently and gel blotter strip and discard them.
8. Place the gel film into the electrophoresis chamber with the samples on the cathodic side, and run electrophoresis for 18 mins/ 100 volts.
9. After electrophoresis switch off the power supply and place the gel film on a horizontal position.
10. Add to each anti-serum trough 20 μ l of the corresponding antiserum (Anti -whole, -IgG,-IgA,-IgM, - κ - and - λ -chain) and incubate it for 18- 20 hours at room temperature in a moist chamber, on a horizontal position.
11. Place the agarose gel film on a horizontal position, put on the gel one gel blotter sheet (Cod No IFE 01.3) and one drying blotter sheet (Cod No IFE 1.6) press them (with about 2 Kg) for 3 mins..
12. Soak the film in saline solution for 10 mins.
13. Repeat steps 11 and 12 two times again.
14. Dry the film at a temperature less than 60°C and stain it for 3 mins with proteins staining solution.
15. Decolorize the film for 2 mins in 3 distaining solution baths, subsequently.
16. Dry the film and evaluate the results

Normal Values : No monoclonal antibodies are detected.