IMMUNOELECTROPHORESIS
on agarose gels

IVD CE

Principal and Methodology

Immunoelectrophoresis (IE) combines zone 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 molecular weight. After the electrophoresis monospesific polyclonal antisera are applied to the corresponding troughs (Anti whole proteins, anti IgG, anti IgA, anti IgM, anti Kappa and anti Lambda) and is followed by immunodiffusion (incubation for 18 hours in a humid incubator at room temperature). If the antisera react with the specific antigen an immunoprecipitin arc will be formed.
Generally IE is 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 cannot be accomplished by the Immunofixation Electrophoresis.
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 paraproteinemia, especially when heavy chains in both clones belong to the same class, the interpretation may be difficult.*

Required Reagents and Equipment's included in each kit:

[Warning: All reagents from each kit must be used together]

Product	KIT IE / 10 TESTS
Agarose Gels	**10**
Electrophoresis Buffer [50X concentrated]	**20 ml**
Staining Solution [5X concentrated]	**60 ml**
Destaining solution [500X concentrated]	**8 ml**
Gel Drying sheets	**40**
Gel Blotter sheets	**50**
Antisera kit	**Goat anti-Human antisera**
Serum control	**Ready to use**
Instructions for use in English	

All reagents must be used according to the instructions and until the expiration date indicated on the kit

Preparation, storage and stability of the reagents included in the kit:

a) Agarose Gels: Agarose Gels are in non- barbital buffer and other non-reactive ingredients for long stability and optimum resolution of protein fractions. The Gels must be stored at **15 - 25 ^{0}C on horizontal position** until the expiration date indicated on the kit. Do not freeze the gels. Carefully discover the gel just before use and follow the instructions of the manual.
b) Electrophoresis Buffer: Non-barbital buffer and other non-reactive ingredients. It is in concentrated solution. It must be stored at 15 – 25 ^{0}C until the expiration date indicated on the kit. To prepare working solution dilute the content of the bottle according to the instructions on the bottle. The diluted solution is enough for electrophoresis of all gels of the kit. **The buffer solution is for one use only**. Store the diluted solution at room temperature for 2 months. If crystals appear, place the vial in warm water to dissolve the crystals.
c) Staining Solution: Concentrated Amido Black solution. Store the concentrated solution at 15 - 25 ^{0}C until the expiration date indicated on the kit. To prepare working solution dilute the content of the bottle according to the instructions on the bottle. The diluted solution is enough for the staining of all gels of the kit. It should be stored in a closed flask at room temperature until 3 months.
d) Destaining solution: 2% citric acid solution. To prepare working solution dilute the content of the bottle according to the instructions on the bottle. Store at room temperature until the expiration date indicated on the kit.
e) Washing solution: 0.9% Saline (NaCl).
f) Antisera kit: Each kit contains protein fixation solution, goat anti-Human heavy chain of IgG, IgA and IgM and free+bound anti light chain of kappa (κ) and lambda (λ). Store at -18 ^{0}C (long term condition) until the expiration date indicated on the kit.

Additional Reagents and Equipments required and which can be provided by Hellabio:

Power supply, Electrophoresis tank, Staining-destaining baths, HellabioScan (Gel Analyzer).

Collection and handling of specimens:

The samples should be collected according to the standard hospital methods.

a) Blood: *Analysis is preferably performed on sera in order to avoid the fibrinogen band (in plasma), which migrates in the gamma zone and could lead to a false interpretation. Venous blood is drawn into tube. After centrifugation the supernant fluid (serum) can be stored at 4-8℃ for 48-72 hours. Storage at -20℃ may disturb some of the electrophoretic patterns. Care should be taken to prevent haemolysis in the serum because it will cause false elevation in the α_2 and β-fractions. Samples with much precipitate that will not dissolve on warming to 37℃ should be either centrifuged or allowed to settle before the clear supernant fluid is applied to the gel surface.*

b) Cerebrospinal fluid: *Normally, some 80% of the proteins of cerebrospinal fluid originate from plasma, and the rest have been synthesised locally. The filtration resistance for plasma protein rapidly increases with molecular size and therefore the ratio of large proteins normally is much lower in CSF than in plasma. The protein content of CSF is in the range of 15-40 mg/dl. Therefore it must be concentrated at least 150-fold before electrophoresis*.

c) Urine: *Analysis of the urinary proteins shows that normal urine contains more kind of proteins which are much diluted and therefore it must be concentrated. For the detection of Bence-Jones proteins, free Kappa and Lambda light chains, the sample has to be concentrated to ≥ 100 mg/dl of total protein and to about 80 mg/dl of total proteins for immunoglobulins.*

Procedure of Immunoelectrophoresis

I. Fill the electrophoresis chamber with adequate volume of electrophoresis buffer.
II. Take the agarose gel out of its packaging, uncover it from the plastic plate and put it on the backside of the plate in horizontal position.
III. Apply 2μl of **control serum** [it is ready to use] and from **sample** across each corresponding slit (C= control, S= sample).
IV. Place the gel into the electrophoresis chamber with the samples on the cathodic side, and run electrophoresis for 18'/ 100 volts.
V. After electrophoresis switch off the power supply and place the gel in the plate on a horizontal position.
VI. Add to each anti-serum trough 20μl of the corresponding antiserum *(Anti whole serum, -IgG,- IgA,- IgM, -κ- and -λ-chain).*
VII. Place the plate with the gel in a moist chamber and incubate it for 18 - 20 hours at room temperature, on a horizontal position.
VIII. Take the gel out from the plate.
IX. Put on the gel one gel blotter sheet and one drying blotter sheet; place a development weight (about 2 kg) for 2 minutes.
X. Soak the gel in saline solution for 10 min.
XI. Repeat steps (**IX**) and (**X**) two more times.
XII. Dry the gel at a temperature less than 85 °C and stain it for 3 minutes with staining solution.
XIII. Decolorize the gel for 5 minutes in 3 destaining solution baths, subsequently.
XIV. Dry the gel and evaluate the results.

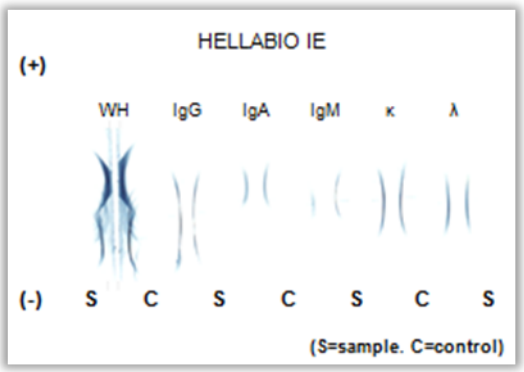
Interpretation of the results:

The qualitative interpretation of the results may be visually interpreted by comparing the sample pattern with the normal control pattern.

Expected Values:

In some malignant disorders a single clone of lymphocytes produces one type of protein - a monoclonal immunoglobulin. This is identifiable as monoclonal by Immunoelectrophoresis. Some people have monoclonal immunoglobulins, but do not have a malignant disorder.

Exemplary Immunoelectrophoresis procedure



References:

- Afonso ,E (1963): Clin Chim Acta, 10, 114-122.
- Joergens, O.S and E. Bock(1975): Scand.J. Immunol. 4 Suppl.2,25-30.
- Normansell D.E (1985): Am I Clin Pathol,84, 469-475.
- Gallango, M.L et al(1980) : Clin Chem 26, 1741-44.

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