Fundamentals of Serology

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SEALS Serology
SEROLOGICAL DIAGNOSIS

DEFINITION

• Measuring antibody in serum or secretions
• Identifying antigens in blood, tissue or secretions using immunochemical techniques
SEROLOGICAL DIAGNOSIS

USES

• Immunity/past infection
• Screening blood /tissue donors
• Recent/Congenital infection
• Epidemiological studies
SEROLOGICAL DIAGNOSIS

APPLICATIONS

• Where organism cannot be cultured
• To confirm an isolation
• To measure vaccine response
• Where disease is sequelae of infection
• To confirm PCR
SEROLOGICAL DIAGNOSIS

PRINCIPLES

• Immune response (rising titre, seroconversion)
• Low avidity IgG
• IgM (recent/congenital infection)
• IgA (mucosal/congenital infection)
• IgE (hypersensitivity/parasites)
• ‘detuned’ EIA
SEROLOGICAL DIAGNOSIS

METHODS USED

- Precipitation
- Nephelometry
- Agglutination
- Neutralisation
- Complement fixation
- Viral haemagglutination inhibition

- Immunofluorescence
- Radio Immunoassay
- Enzyme Immunoassay
- Western Blot
- Recomb. immunoblot
Sensitivity of different serological techniques used for antibody detection (ug/mL)

<table>
<thead>
<tr>
<th>METHOD</th>
<th>SENSITIVITY (ug/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gel-diffusion</td>
<td>30</td>
</tr>
<tr>
<td>Ring precipitation</td>
<td>8</td>
</tr>
<tr>
<td>Bacterial Agglutination</td>
<td>0.05</td>
</tr>
<tr>
<td>Complement Fixation</td>
<td>0.05</td>
</tr>
<tr>
<td>Passive Haemagglutination</td>
<td>0.01</td>
</tr>
<tr>
<td>Haemagglutination Inhibition</td>
<td>0.005</td>
</tr>
<tr>
<td>Immunofluorescence</td>
<td>0.005</td>
</tr>
<tr>
<td>ELISA</td>
<td>0.0005</td>
</tr>
<tr>
<td>Bacterial Neutralisation</td>
<td>0.0005</td>
</tr>
<tr>
<td>ELISA</td>
<td>0.0005</td>
</tr>
<tr>
<td>Bacterial Neutralisation</td>
<td>0.00005</td>
</tr>
</tbody>
</table>
Complement Fixation Test

• **Advantages**
  - High levels do not persist (recent infection)
  - Increased specificity
  - Reproducible (50% cut-off)
  - Antigens stable for many years (>10 years at 4-6 degrees C)
  - Can be automated, or relatively simple & inexpensive equipment

• **Disadvantages**
  - Reduced sensitivity
  - Incorrect storage of sample/bacterial contamination: Anticomplementary activity
  - Non-specific binding of complement: false-positive results (use control antigens)
  - Agglutination of SSRCs (Heterophile antibodies)
  - Serum (C requires calcium & magnesium as co-factors) : acute and convalescent
  - Blood contamination of CSF: false-positive titres
Complement Fixation Test

• 1. Test antigen + Heated test serum/CSF (serial dilutions starting 1:1, 1:4 or 1:8) + Complement incubated

• 2. Sensitized sheep red cells added

• 3. Antigen + Antibody + Complement (Complement Fixation): No lysis of sensitized sheep red cells

• 4. Antigen + Complement: Lysis of sensitized sheep red cells

• 5. Read after fixed incubation with SSRBC, or use biological control (Complement: 2, 1, 0.5 & 0.25 units)
Complement Fixation Test

Complement sequence (C1, C2, C3, C4) in the completion of red cell haemolysis when a suspension of red cells is mixed with corresponding antibody.

1. C1 (heat-labile euglobulin)
   - Absorption in the presence of calcium salts
   - Antigen/Antibody mixture
     - Completion of sequence
     - Taken up in the presence of magnesium salts
     - 2. C4 attachment (heat-stable)
     - 3. C2 (heat-labile)
     - 4. C3 (heat-stable)
Complement Fixation Test

- Haemolysis by a haemolytic antiserum (haemolysin) : due to a specific thermostable antibody acting along with complement (Haemolytic System)

- Red Cells + Specific antibody + Complement = Haemolysis

- Red Cells + Specific antibody = No lysis

- Red cells + Complement = No lysis
Complement Fixation Test –
Establishing diagnostic levels
Complement Fixation Test
AGGLUTINATION

• Microtitre using dyed bacterial suspensions, sensitised erythrocytes, gelatin particles, etc.

• Slide tests using sensitised latex particles, erythrocytes
AGGLUTINATION

• ADVANTAGES
  Simple, inexpensive equipment, quick

• DISADVANTAGES
  Vaccine Induced antibody may persist over long periods at high titres
  Specific agglutinins (H-agglutinins) may persist over prolonged periods
  Anamnestic reactions may result in a “false-positive” rising titre
  Antibiotic treatment early in course of illness may interfere with production of specific Abs
  Cross-reaction among different serotypes of a causative organism
  Costly commercial reagents
  Subjective reading (can be automated)
Immunofluorescence

- IFA developed in 1951
- Solid phase usually a microscope slide
- Ag/Ab complex visible by anti-human globulin-labelled with a fluorochrome (FTIC)
Immunofluorescence

- **Advantages**
  
  - Fixed slide (acetone/methanol) stores many months
  - High sensitivity
  - Enable detection of IgG and IgM class Abs

- **Disadvantages**
  
  - Subjective reading
  - Expensive, well maintained equipment required
  - IgM incubation prolonged
  - IgM FA Abs can persist for months/years
FIG. 1. Indirect method of solid-phase FIA. This assay is a heterogeneous method for the detection of specific antibody in serum.
Immunofluorescence
Enzymeimmunoassay

• Heterogeneous ELISA: antibody is conjugated with an enzyme which upon reacting with its substrate, forms a coloured product

• The coloured product may be directly (non-competitive) or indirectly (competitive) proportional to the amount of antibody in the original test sample
Enzymeimmunoassay

• ADVANTAGES
  Depending on established cut-off, highly specific/sensitive
  Simple and versatile
  Class specific IgG, IgM, IgA assays
  Objective reading
  Can be automated and data reduction programs
  Monoclonal antibodies, recombinant antigens
  Large number of samples with less hands-on time
Enzyme immunoassay – Establishing cut-off OD
ENZYMEOIMMUNOASSAY

• SOLID PHASE
  plastic, nitrocellulose, agarose, glass, cellulose, polyacrylamide, dextran
  (antigens hydrophobically bound)

• CONJUGATES
  Alkaline phosphatase, Horseradish Peroxidase

• SUBSTRATES
  pNPP, ODP, TMB
<table>
<thead>
<tr>
<th>Solid Phase</th>
<th>Enzyme</th>
<th>Substrate</th>
<th>Product</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwells</td>
<td>Alkaline Phosphatase</td>
<td>para-nitro-phenylphosphate (pNPP)</td>
<td>Colour development</td>
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<td>Horseradish peroxidase</td>
<td>3,3′,5,5′-tetramethylbenzidine (TMB)</td>
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<tr>
<td>Microparticles</td>
<td>Alkaline phosphatase</td>
<td>4-methylumbelliferyl phosphate (MUP)</td>
<td>4-methylumbelliferyl (fluorescent product)</td>
</tr>
<tr>
<td>Nitrocellulose</td>
<td>Alkaline phosphatase</td>
<td>5-bromo-4-chloro-3-indolyl phosphate (BCIP) + nitroblue tetrazolium (NBT)</td>
<td>Deposit</td>
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<tr>
<td>PROBLEM</td>
<td>SOLUTION</td>
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<td>----------------------------------------------</td>
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<tr>
<td>Background/Non-specific binding to solid phase</td>
<td>BSA, Casein Hydrolysate etc in diluent</td>
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<tr>
<td>Heterophile antibodies (anti-sheep, anti-goat, anti-rabbit etc)</td>
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<td>Excess IgG (False-negative IgM assays)</td>
<td>IgG absorption (RFRR)</td>
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<td>Hook effect/Excess antigen (False-negative)</td>
<td>IgG absorption (RFRR)</td>
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<tr>
<td>Rheumatoid factor (False positive IgM assays)</td>
<td>IgM capture assays, IgM column separation, RF absorption (RFRR)</td>
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</table>
Enzyme immunoassay
- Indirect method for assay of antibody

**FIGURE 6**
The Indirect Method for assay of antibody

1. Antigen adsorbed to plate
   - Wash

2. Add serum: any specific antibody attaches to antigen
   - Wash

3. Add enzyme labeled antigen; this attaches to antibody
   - Wash

4. Add substrate
   - Amount hydrolyzed = amount antibody present

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Enzyme immunoassay – IgM capture assay
Enzymeimmunoassay
Enzyme immunoassay