Viral Serology
Viral Serology

- IgM antibody during acute phase
- IgG antibody during convalescence
- IgA mucosal antibody (respiratory infections, GI)
Viral Serology

- Serum sample within 7 days of onset
- Convalescent sample 10-14 days after the first sample
- 4-fold rise in IgG
- Negative to positive IgM
Serology Tests

- Haemagglutination
- Haemagglutination Inhibition
- Complement fixation
- Indirect IFA
- Indirect EIA (ELISA)
Haemagglutination

- Used with specific viral antigens
- Red cells are first sensitized
- Reacted with patient serum
- Agglutination of Ab to antigens produces agglutination
- Agglutination indicates presence of specific antibody in the patient
Haemagglutination

- RBC
- Patient Serum
- Specific Viral Antigen
- Sensitised RBC
- Agglutination
Haemagglutination Inhibition

- Used with viruses that haemagglutinate
- Incubation of virus with RBC causes haemagglutination
Haemagglutination Inhibition

RBC

Haemagglutinating Viral Antigen

Haemagglutination
Haemagglutination Inhibition

- Used with viruses that haemagglutinate
- Incubation of virus with RBC causes haemagglutination
- Preincubation of patient serum with antigen inhibits ag-RBC agglutination
- Inhibition of agglutination indicates presence of antibodies to the viral antigen (virus) used.
Haemagglutination Inhibition

Haemagglutinating Viral Antigen is immobilised

RBC

No agglutination
Complement fixation

- Heat inactivated patient serum mixed with specific virus (antigen) and complement
- Sheep RBC & Haemolysin added
- Incubate 37°C / 45 minutes
- Absence of Ab = lysis
Complement Fixation

Positive Ab

Negative Ab
Complement Fixation

No lysis

Activated RBC

Lysis
Complement Fixation test
Viral Serology

Detection of patient antibodies to a specific virus

- Indirect Immunofluorescence Assay (IFA)
- Indirect Enzyme Immuno Assay (ELISA)
Indirect IFA

- Apply cells infected with a known virus to a microscope slide
- Acetone fix
- Add patient serum, incubate
- Wash 3x
- Add secondary Ab conjugate (anti-human antibody), incubate
- Wash 3x
- Dry & examine
Indirect IFA Ab Detection

1. Positive cells
2. Positive cells fixed
3. Patient serum Applied
4. Conjugate Applied
5. Wash
6. UV
Indirect EIA - IgG

- A specific antigen is coated onto polystyrene microtitration plate
- Block uncoated sites
- Add primary Ab (patient serum) incubate
- Wash 5x
- Add anti-human antibody-enzyme conjugate, incubate
- Wash 6x
- Add substrate (TMB, DNPP)
- Read colour on spectrophotometer
Indirect ELISA

1. Ag coated wells
2. Patient serum
3. Antibody Applied
4. Anti-hu IgG antibody conjugate
5. Substrate Colour
6. Conjugate Applied
7. Colour
Variations of EIA method to detect antibodies

- IgM antibodies - direct detection
  - indirect detection

- Non human antibodies
IgM Ab Detection - Indirect Method

IgM antibody

Anti-hu IgM conjugate

Anti-IgM Conjugate Applied
IgM Ab Detection - Indirect Method

- False Positives due to rheumatoid factor.
- False negatives due to IgG
- IgM capture is superior.
False positives – Rheumatoid Factor

RF is IgM Ab directed to human IgG

IgG

wash

FALSE +

Anti-IgM Conjugate Applied
False negatives - IgG

Early convalescence → high levels of IgG

IgG

IgM

IgG

False -

Anti-IgM Conjugate Applied

wash
IgM Ab Detection – Capture Method

Anti-hu IgM

antigen

Anti-Ag conjugate
Competition ELISA

Test antibody

Anti-Ag conjugate

Reduction in signal