Infectious Diseases
Serology Explained

David Dickeson
Centre for Infectious Diseases &
Microbiology Laboratory Services

Why use serology?

- To determine immune status
  - detecting total antibody OR IgG
- To diagnose recent or acute infection
  - detection of IgM
  - fourfold or greater rise in IgG or total titre
  - single high IgG or total Ab titre
- Inability, difficulty or long time to culture organism eg. Hep., HIV, syphilis
- Dangerous to culture (eg. Q fever - P3)
Antibody Structure

Antibody classes are IgG, A, M, E, & D

IgG 6-16 g/L
IgA 0.6-4 g/L
IgM 0.5-3 g/L

IgG has a molecular weight 160,000 Daltons

Antibody binding to epitope.
Typical protein epitope is 3-5 amino acids.
(Molecular Weight 300D).

Problems or Limitations of Serology

- Different methods means different antibodies measured with different sensitivity.
- Different antigens with variable purities means different specificity eg. Pertussis whole cell v PT
- Retrospective diagnosis need acute & convalescent specimens eg. leptospirosis MAT.
- Single high titres or long lasting IgM hard to interpret. Use IgG avidity assays.
- Neonatal and immunosuppressed responses.
- Prior exposure masking infection eg. RRv, BFv
Problems or Limitations of Serology

- **Prozone effect**: Ag-Ab imbalance causing incomplete or blocked reactions eg. Cryptococcus latex tests, RPR, VDRL, brucella SAT.
- **Non specificity**:
  1. Abnormal human Ig eg. RF, ANF.
  2. Heterophile antibodies: Ab with affinity to another nonspecific Ag usually of another species eg. antiavian (egg), antiruminant or antimurine.

Reduced by using F(ab) fragments, removing IgG with anti-human IgG, monoclonal Ab.

Antibodies and Clones

- **Polyclonal Antibodies**
  - Easier to make
  - Less expensive
  - Less epitope specific

- **Monoclonal Antibodies**
  - More difficult to make
  - More expensive
  - More epitope specific

Antibody Response

Primary infection

- Immune response in primary infection. Note early appearance and rapid decline of IgM compared with the appearance and persistence of IgG antibody.
Antibody Response

Re-infection

Immune response in re-infection. Note the short lived negligible IgM response compared with the strong and rapid IgG response.

L. Hueston, ICPMR 2008

Individual Antibody response

Effect of Prevalence

Delta values: large

Distance (d) = mean OD ratio (x) – Cut Off; Delta (δ) = d / sd

Delta values: small

Levey-Jennings Graph
Serology Immunoassays

1. Agglutination / Precipitation reactions
   - Immunodiffusion, Particle, HA, IHA, HAI (sensitivity 0.3 – 100 mg/ml)

2. Lysis reactions
   - Neutralisation, CFT (sensitivity 0.001 mg/ml)

3. Labelled reactions
   - IFAT, ELISA, EIA (sensitivity < 1 ng/ml)

4. Immunoblots
   - Western blots, line blots

Antibody Tests by Serology

1. Agglutination / Precipitation reactions
   - Microscopic agglutination test (MAT) eg. leptospirosis
   - Immunodiffusion – precipitates Ab/Ag eg. fungi
   - Latex or other particles coated with Ag eg. H. pylori

   - Haemagglutination (HA) eg. influenza cultures; total antibody, moderate sensitivity & specificity (not in use)

   - Indirect Haemagglutination (IHA) eg. Echinococcus, Entamoeba

   - Haemagglutination Inhibition (HAI) eg. influenza Ab., arboviruses.

Leptospira
by dark field microscopy
Quantifying Serum Antibodies

- The end-point dilution is the highest dilution of serum still giving a positive reaction.
- Titre = inverse of the end-point dilution.
Serology

2. Lysis reactions

- **Neutralisation of organisms**
  - first ab to appear, persists for years
  - most sensitive, specific & clinically important
  - eg. polio virus neutralised by serum Ab in tissue culture; HBsAg confirmation.

- **Complement Fixation Test (CFT)**
  - involving lysis of RBC; detects total antibody, short lived eg. respiratory viruses, mycoplasma

Complement Fixation Test

Wasserman invented CFT in 1906 for syphilis. Still used for respiratory viruses, mycoplasma, Q fever and chlamydia antibodies.

Reagents:
- Veronal buffer incl. Ca, Mg, NaCl
- Complement: guinea-pig serum
- Viral antigen
- Test and control sera
- Haemolysin: rabbit anti-sheep serum
- Sheep blood in Alsever’s solution

**RESULTS:**

- No haemolysis = Positive
- Haemolysis = Negative
Complement Fixation Test

Serology

3. Labelled reactions

- **Indirect Fluorescent Antibody (IFA)**
  - tests binding fluorochromes (FITC)
  - eg. Legionella, Bartonella, Rickettsiae

- **Enzyme Linked Immunosorbent Assays (ELISA)** – non-competitive or competitive

- **Enzyme Immunoassays (EIA)**
  - using Chemiluminescence, Fluorescence

- **Western Immunoblots** using SDS PAGE

Indirect Fluorescent Antibody Test

Indirect Fluorescent Antibody Test

Indirect Fluorescent Antibody Test

Indirect Fluorescent Antibody Test

ELISA

- Very sensitive method for determining and quantitating antibodies. Currently most widely used test system.
- Indirect assays - similar to IF except microtitre plates are used instead of glass slides.
- Class capture - developed for IgA, IgG and IgM; more commonly used for IgM.
- Competitive assays - very specific (epitope specific) and sensitive assay eg. total Ab HAV, arboviruses.
ELISA

IgM detection:
- Commonly achieved by indirect ELISA
- BUT false positives due to RF or cross-reactivity;
- false negatives due to competition with IgG.
- Overcome by:
  1. Pretreatment with anti-human IgG depending on
     how well the pretreatment removes IgG.
  2. Antibody class capture assays; great for IgM not
     for IgG.
  3. Cross absorption eg. EBV and parvovirus.

Advantages
- Very sensitive
- Quantitative & qualitative
- Very specific
- Easily modified design
- Easily automated

Flaws
- Too sensitive
- Needs standardisation
- Not specific enough - common antigens or cross-reactions
- Too easily modified

Sandwich/Non-competitive ELISA
Sandwich/Non-competitive Assay

- Sandwich/Non-competitive assays measure bound sites.
- Colour product is proportional to antibodies present in sample.

Competitive Enzyme Immuno Assays

1) Coating
2) Pre-incubation
3) Tracer incubation
4) Signal development

Small analytes—drugs, T4

http://www.ch.tum.de/wasser/weller/immunoassay_img_2.htm
Competitive ELISA Dynamics

http://kcampbell.bio.umb.edu/lecture1.htm

Competitive Assay

- Competitive assays measure **unbound sites**.
- Colour product is inversely proportional to antibodies present in sample.

Rubella antibody profile
EIA Detection Methods

- Colour by spectrophotometer (absorbance or optical density)
- Fluorescence (RFV) - ELFA
- Radioisotope (gamma counter) – [not in use]
- Chemiluminescence (RLU) - CMIA
- Immunochromatography – lateral flow bands
- Flow cytometry – multiplex assays (sensitivity to 100 fg/ml)
Different EIAs

<table>
<thead>
<tr>
<th>Methodology</th>
<th>AxSYM®</th>
<th>Architect®</th>
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<tbody>
<tr>
<td>Microplate EIA</td>
<td>ELSA</td>
<td>Chemiluminescent IA</td>
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<tr>
<td>Solid Phase</td>
<td>Microtiter Well</td>
<td>Luminex microparticle</td>
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<td>Separation</td>
<td>Glass fibre matrix</td>
<td>Paramagnetic microparticle</td>
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<td>Signal detection</td>
<td>Absorbance</td>
<td>Fluorescence</td>
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<tr>
<td></td>
<td></td>
<td>Chemiluminescence</td>
</tr>
<tr>
<td>Assay time</td>
<td>Assay dependent, 30-60 minutes</td>
<td>Assay dependent, 30-60 minutes</td>
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<tr>
<td></td>
<td>Fixed, 28 minutes</td>
<td>Fixed, 28 minutes</td>
</tr>
<tr>
<td>Throughput</td>
<td>Varies, depends on sample numbers</td>
<td>35 tests/hr, 1 test/hr</td>
</tr>
</tbody>
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*Abbott Diagnostics*
4. Western Immunoblot

Confirmatory tests detecting specific antibodies according to their molecular weight (MW).

Procedure:
1. Separate proteins by SDS PAGE.
2. Transfer from gel to nitrocellulose.
3. Cut strips.
4. Proceed with ELISA on strips.
5. Read bands according to MW.

Australian HIV Western Blot Interpretation

<table>
<thead>
<tr>
<th>Classification</th>
<th>Description</th>
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<tbody>
<tr>
<td>POSITIVE</td>
<td>gp160/41 PLUS three or more bands</td>
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<tr>
<td>Indeterminate 4</td>
<td>gp160/41 PLUS NO MORE THAN TWO other bands</td>
</tr>
<tr>
<td>IND3</td>
<td>p24 ( \geq ) other bands, NO gp160/41 present</td>
</tr>
<tr>
<td>IND2</td>
<td>p17/18 ( \geq ) other bands, NO gp160/41 or p24 present</td>
</tr>
<tr>
<td>IND1</td>
<td>Bands other than those listed above</td>
</tr>
<tr>
<td>NEGATIVE</td>
<td>NO bands</td>
</tr>
</tbody>
</table>
Positive

Negative

Indeterminate

Line Immunoblot

INNO-LIA™ Syphilis Score

Data reporting sheet

1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20

Serology

- Acute diagnosis eg. measles IgM, VDRL
- Immune status for vaccine use
- Retrospective Dx eg. legionella, flu, chlamydia
- Confirmation eg. HIV, syphilis
- Problems with immunosuppressed, cross-reactions

NAT

- Acute diagnosis during infection – short term
- No immune status
- No retrospective Dx unless long term active infection
- Problems with contamination
- No cross reactions

Conclusions

Why bother with serology at all?

- Serology can provide a wealth of information to clinicians, epidemiologists and government about the incidence and prevalence of disease in a community.
- Allows risk to be determined so health dollars can be spent wisely eg. Vaccine surveys.
- It isn’t the answer to every problem but it does provide valuable information if used appropriately and wisely.
Conclusions

What's the best test to use?
- There is no one best test. The most appropriate test to use depends on the question the clinician wants to answer.
- All test systems have shortcomings – the skill for the laboratory scientist lies in knowing the limitations and learning how to interpret the results in light of them.

The end!