Diagnostic Serology
What Does it Mean?

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Objectives

• Immunologic response to infection
• Diagnostic serology
• Titers, A/C testing
• Screening vs confirmatory assays
• Test methods/interpretation
## Immunoglobulin Characteristics

<table>
<thead>
<tr>
<th>IG Class</th>
<th>Mole. Weight</th>
<th>Serum Concent. (mg/dl) adults</th>
<th>% of Total IG</th>
<th>Cross Placenta</th>
<th>Primary location</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>900,000</td>
<td>50-200</td>
<td>10</td>
<td>N</td>
<td>* Blood</td>
</tr>
<tr>
<td>IgG</td>
<td>160,000</td>
<td>800-1600</td>
<td>70-75</td>
<td>Y</td>
<td>* Blood &amp; extravascular spaces (tissue)</td>
</tr>
<tr>
<td>IgA</td>
<td>360,000</td>
<td>150-240</td>
<td>15-20</td>
<td>N</td>
<td>Tears, saliva, breast milk, GI tract</td>
</tr>
<tr>
<td>IgE</td>
<td>200,000</td>
<td>.002-.05</td>
<td>&lt; 1</td>
<td>N</td>
<td>Binds to mast cells (hist.) mediates allergic rx.</td>
</tr>
<tr>
<td>IgD</td>
<td>160,000</td>
<td>1.5-40</td>
<td>5</td>
<td>N</td>
<td>Surface of B lymphocytes</td>
</tr>
</tbody>
</table>

## IG Structures
**IgG Molecule**

- **Antigen binding**
- **Fab**
- **Antibody activity mediation**
- **Fc**
- **Light chain hypervariable regions**
- **Heavy chain hypervariable regions**
- **Carbohydrate**
- **V, and V, variable regions**
- **C, and C, constant regions**

*Prevent Disease – Promote Wellness – Improve Quality of Life*

**AB Response**

- **Clinical signs**
- **Virus**
- **IgM**
- **IgG**

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Current vs Past Exposure

1. Current exposure
   - Presence of pathogenic antigen(s)
   - Presence of IgM (detectable 1-3 wks)
   - Significant rise in IgG antibody (4-fold)

2. Past exposure
   - Unknown duration
   - Presence of IgG antibody (detectable 2-4 wks)
   - May imply immunity

Basic Serology & Methods

Detection of AB (G/M) against pathogenic proteins (Ag)

Detection of pathogenic proteins

AB/Ag Detection Methods:

- Flocculation
- Agglutination
- Immunofluorescence (IFA/DFA)
- Western blot

- ELISA/EIA
- Complement fixation
- Immunodiffusion
- IgM Capture assays
AB/Ag Detection Principles

AB or Ag attached to solid-phase:

- Microtiter plate: 96 well, EIA most common
- Latex particles/beads: agglutination & EIA
- Blood cells: hemagglutination
- Membranes: western Blot

Examples: Indirect, direct, sandwich, competitive

Antigen detection:
AB attached to microtiter well, add pt. sera (Ag), add AB conj. to enzyme, add substrate, converts to colored compound.
Optically measured to obtain optical density (OD) value
HBsAg, HIV

Antibody detection:
Ag attached to microtiter well, add pt. sera (AB – IgG and/or IgM), add anti-AB conj. to enzyme, add substrate, converts to colored compound.
Optically measured to obtain optical density (OD) value
HCV, HIV, Lyme, Immune status assays

OD converted to:
Index value (IU/ml), serum to cutoff ratio (S/CO), high values may indicate recent exp.
Clinical studies performed to correlate index value/cutoff to disease

Qualitative assays: Titers not applicable & values are not reported

Principle of EIA Assays
Direct EIA – ag detection

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Qualitative vs Quantitative EIA

**Qualitative**
- Report as:
  - Present/Absent
  - React./non-reactive
  - Positive/Negative
- Specimen OD compared to est. cutoff value (no std. curve)
- Cutoff value is assay specific

**Quantitative**
- Report as:
  - Present 2.3 IU/ml
  - Absent < 0.1 IU/ml
- Known standards est. curve
- Specimen OD compared to std. curve to obtain quant. value
Hepatitis C Antibody - EIA

HCV OD (nc) + 0.60 = cutoff value

\[ .006 + 0.60 = 0.606 \]

Patient OD/cutoff = S/CO ratio
- If pt. OD > 0.606 Reactive
- If pt. OD < 0.606 Non-reactive
- If S/CO > 3.8 RIBA not required
- If S/CO < 3.8 conf. RIBA required

Combined quant/qual. assay
RIBA back order situation

Hepatitis A & B Testing

HAV-M detects IgM (capture)
Indicates recent exposure
Not routine testing, outbreak invest. Only, EPI approved

HBsAg detects HB Ag
Indicates current disease/chronic carrier states
HB confirmation test required
Not for immune status testing

Anti- HBsAg detects HB IgG antibody
Indicates past/recent exposure & immune status
Immune Status Testing

- Anti-HBs/Measles/Mumps/Rubella/VZV
- IgG antibody detection by EIA
- Report:
  - Present implies immunity
  - Absent no immunity, require boost
  - Equivocal some protection, require boost, redraw
- There are no titers (S/CO or IU/ml)
- Qualitative – IU/mL values not useful

What is a Titer??

Antibody titer or endpoint titer

1º applies to agglutination and IFA assays
Concept of serial dilutions (undil, 1:1, 1:2)
Last dilution with positive reaction = titer
Measures immunologic response, AB concentration
Higher titers indicate recent/current infection

Used to:
- Establish baseline titer/immunity
- Clinical significance of titers (e.g., CF)
- Monitor treatment success (4-fold drop)

2 tube vs 4-fold rise/drop in titer
1:2 to 1:8 or 1:32 to 1:8
Acute/Convalescent Parallel Testing

Acute serum  DOC near onset date
Conval. Serum  DOC 2-4 weeks after acute

Accurate parallel testing:
- Same time, same lab, same method.
- Cannot accurately analyze results (titers) from different labs, methods, or days (e.g., RPR, USR).

Recent infection:  4-fold IgG ↑

Screening Assays

- Agglutination  USR, VDRL, RPR
- EIA’s  HCV, HIV, Lyme

Less specific but highly sensitive
More false positive reactions
Require confirmatory testing
Confirmatory Assays

<table>
<thead>
<tr>
<th>Method</th>
<th>Confirm. assay</th>
</tr>
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<tbody>
<tr>
<td>TP-PA, FTA</td>
<td>USR/RPR</td>
</tr>
<tr>
<td>Western blot</td>
<td>Lyme, HIV, HCV</td>
</tr>
<tr>
<td>PRNT</td>
<td>Arbovirus</td>
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</tbody>
</table>

More specific
Less false positive reactions

TAT
Arbovirus EIA (approx. 1 wk)
PRNT (1-2 weeks), CSF

USR Unheated Serum Reagin

- Nontreponemal Assay – microscopic flocculation test
  - Others include: RPR, TRUST, VDRL
- Detects total AB produced against cardiolipin (reagin)
  Wasserman Test
  - Cardiolipin released as a result of tissue destruction (chancre)
  - Nonspecific antibody (not vs *T. pallidum*) hence nontreponemal

- Reported as titers, used to monitor tx.
- Biologic false positive (BFP) – 6%
  - Most nontreponemal BFP due to (EBV, collagen disorders, TB, drug use, viral infection)
  - Most false pos = USR titer < 1:8

- Maternal nontreponemal IgG crosses the placenta
USR Testing

- 1 drop serum/1 drop VDRL antigen
- Pt. AB binds to Ag forming microscopic clumps
  - Nonreactive: no clumping
  - Reactive: sl. clumping/rough
  - Weakly rx.: sl. clumping undilute
  - Perform serial dilution & det. titer

- Report endpoint titer:
  - Weakly Reactive (sl. clumping)
  - 1:1 undilute, 1:2, 1:4, etc. (> 1:8 recent)

USR Microscopic Analysis

Non reactive  Weakly reactive  Strongly reactive
TP-PA

Treponemal pallidum particle agglutination

Agglutination test
Detects specific *T. pallidum* AB hence treponemal

- Confirmatory performed on all + USR sera
  - Specific and sensitive
- Reported as:
  - Reactive recent or past exposure to syphilis
  - Nonreactive no serologic evidence of exposure
  - Indeterminate cross-rx AB, redraw in 2-4 weeks

FTA is performed on all indet. results

- False positive - 1%
  - Most treponemal BFP due to (those in 8th decade of life, drug use, ?)
  - False negative if drawn too early/immunocompromised

- Maternal treponemal IgG crosses the placenta
FTA-ABS DS

• Treponemal Assay – indirect fluorescent antibody test (IFA)
• Detects IgG AB produced against T. pallidum
• Confirmatory
  – Specific and sensitive (especially for early primary syphilis)
• Reported as:
  Reactive: recent/past exposure to syphilis
  Nonreactive: no serologic evidence of exposure
  Reactive minimal: cross-rx AB, repeat in 2-4 weeks
• False positive - 1 %

Western Blot - Immunoblot

• Treponemal Assay - Immunoblot
  Species specific proteins (Ag’s) transferred to nitrocellulose membrane
  IgG or IgM AB binds to Ag’s on strip
  Conjugated anti-IgM added to strip
  Substrate converted to purple band
  # and/or intensity of reaction determines pos or neg result
  detects IgM AB produced against T. pallidum
• Confirmatory
  – Highly specific and sensitive, not FDA approved
• Reported as:
  Reactive: 2/3 bands present = recent exposure
  Nonreactive: no serologic evidence of exposure
  Equivocal: 1/3 bands present = DOC too early, repeat 2-4 wks
• False positive - 1 %
Lyme IgG WB

IgM or IgG AB bind to species specific Ag’s

- IgM – 2/3 bands Positive
- IgG – 5/10 bands Positive

IgM – false + if > 30 days onset

Direct detection assays

DFA assays
Clinical specimen (tissue, fluid) fixed onto slide
Add conjugated AB, observe micro.

Examples:
Syphilis, Legionella/pertussis DFA

Syphilis normal flora treponemes- oral/rectal genital lesion – diagnostic if pos.
Case

26 y/o male
- Inguinal lymphadenopathy, high risk, multiple partners
- Painful lesion present for 1 wk. (DFA – TP not performed)
- HSV/GC/Chlamydia/HBV/HCV all negative

Is this primary case? What to do?
- Pt. tx. on 8/12
- Four-fold rise but not > 1:8
- IgM + for both specimens
- HIV pos patient

<table>
<thead>
<tr>
<th>DOC</th>
<th>USR</th>
<th>TPPA</th>
<th>FTA</th>
<th>M-WB</th>
</tr>
</thead>
<tbody>
<tr>
<td>8/12</td>
<td>WR</td>
<td>Indec</td>
<td>Rsc 1+</td>
<td>15 G. 2+ 4F 1+</td>
</tr>
<tr>
<td>8/22</td>
<td>1.2</td>
<td>Indec</td>
<td>Rsc 1+</td>
<td>15 G. 2+ 4F 1+</td>
</tr>
</tbody>
</table>
Fungal antibody testing

Blastomyces/Coccidioides/Histoplasma
- CF reported as titers
  - Reactive > 1:8
  - Non-reactive < 1:8 (except cocci < 1:2)
- Fungal precipitan (immunodiffusion) reported as pos/neg
- Detects IgM and IgG AB

Complement fixation
- Add serum + specific fungal Ag + complement
- Add sheep RBC
  - If AB present AB-Ag-C complex forms (no lysis)
  - If AB absent complement available to lyse cells
- The percentage of lysed cells is inversely proportional to the amount of antibody (detects total AB) present in patient serum.
- Two day test performed once per week
Immunodiffusion (ID)

**Principle of double diffusion:**

1. Serum is placed in one well
2. Ag placed in adjacent well
3. AB & Ag diffuse thru agar, form ppt. line

**Histoplasma contains H & M antigens.**

- **H band** occurs late in disease, extrapulmonary dis
- **M band** 1st to appear, freq. without H band, can remains pos for months/hrs.

Identity/non-identity bands

- **Identity**
- **Nonidentity**
- **Partial Identity**
Fungal IDs

More information

- BOL Lab Services Guide
  [www.michigan.gov/mdch](http://www.michigan.gov/mdch)

  Immunology & Serology in Lab Medicine
  Turgeon, M., L. 3rd ed.