



Diagnostic Serology What Does it Mean?

Michigan Department of Community Health
Bureau of Laboratories

William Crafts, B.S MT
Unit Manager
Bacterial/Parasitic/Viral Serology
Craftsw@michigan.gov
335-8100

Prevent Disease – Promote Wellness – Improve Quality of Life



Objectives

- Immunologic response to infection
- Diagnostic serology
- Titers, A/C testing
- Screening vs confirmatory assays
- Test methods/interpretation

Prevent Disease – Promote Wellness – Improve Quality of Life



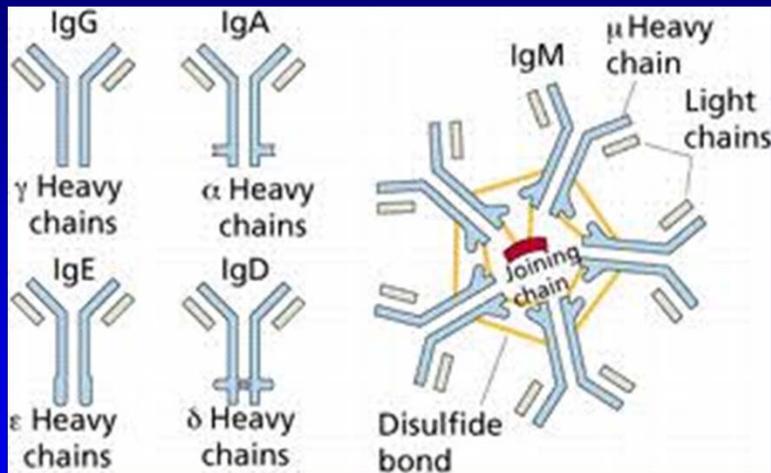
Immunoglobulin Characteristics

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

Prevent Disease – Promote Wellness – Improve Quality of Life



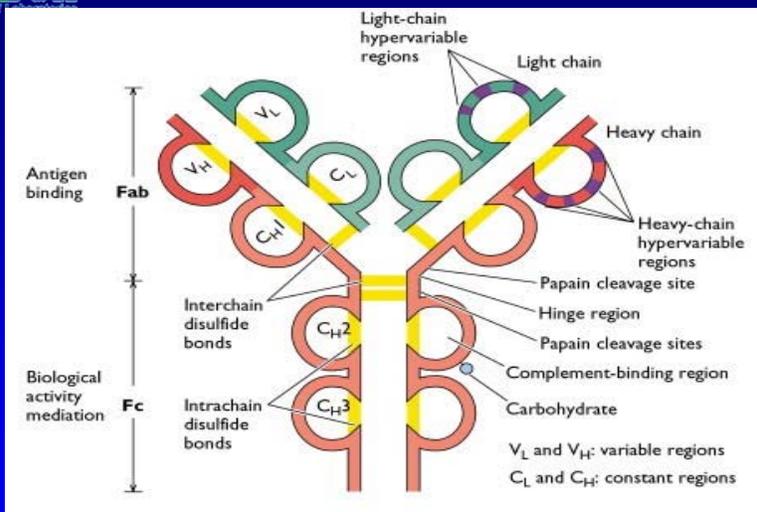
IG Structures



Prevent Disease – Promote Wellness – Improve Quality of Life



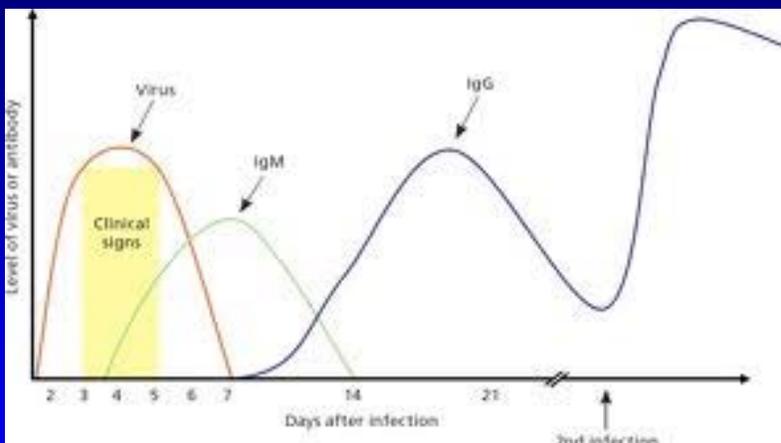
IgG Molecule



Prevent Disease – Promote Wellness – Improve Quality of Life



AB Response



Inpracticebmj.com

Prevent Disease – Promote Wellness – Improve Quality of Life



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

Prevent Disease – Promote Wellness – Improve Quality of Life



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

Prevent Disease – Promote Wellness – Improve Quality of Life



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 |

Prevent Disease – Promote Wellness – Improve Quality of Life



Principle of EIA Assays

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

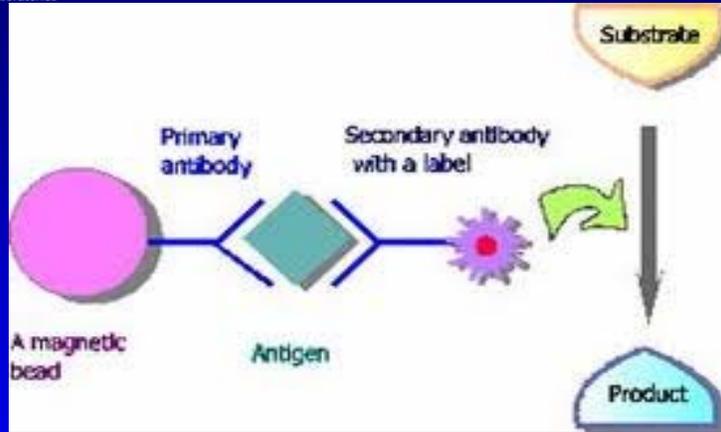
Qualitative assays

Titers not applicable & values are not reported

Prevent Disease – Promote Wellness – Improve Quality of Life



Direct EIA – ag detection



che.un.edu

Prevent Disease – Promote Wellness – Improve Quality of Life



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

Prevent Disease – Promote Wellness – Improve Quality of Life



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

Prevent Disease – Promote Wellness – Improve Quality of Life



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

Prevent Disease – Promote Wellness – Improve Quality of Life



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

Prevent Disease – Promote Wellness – Improve Quality of Life



What is a Titer ??

Antibody titer or endpoint titer

1^o 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

Prevent Disease – Promote Wellness – Improve Quality of Life



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 ↑

Prevent Disease – Promote Wellness – Improve Quality of Life



Screening Assays

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

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

Prevent Disease – Promote Wellness – Improve Quality of Life



Confirmatory Assays

Method

TP-PA, FTA
Western blot
PRNT

Confirm. assay

USR/RPR
Lyme, HIV, HCV
Arbovirus

More specific
Less false positive reactions

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

Prevent Disease – Promote Wellness – Improve Quality of Life



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 (chancres)
 - 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

Prevent Disease – Promote Wellness – Improve Quality of Life



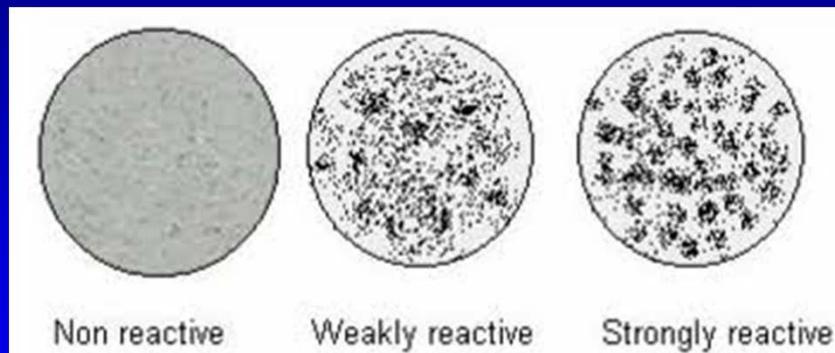
USR Testing

- 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)

Prevent Disease – Promote Wellness – Improve Quality of Life



USR Microscopic Analysis



cajnz1.info

Prevent Disease – Promote Wellness – Improve Quality of Life



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

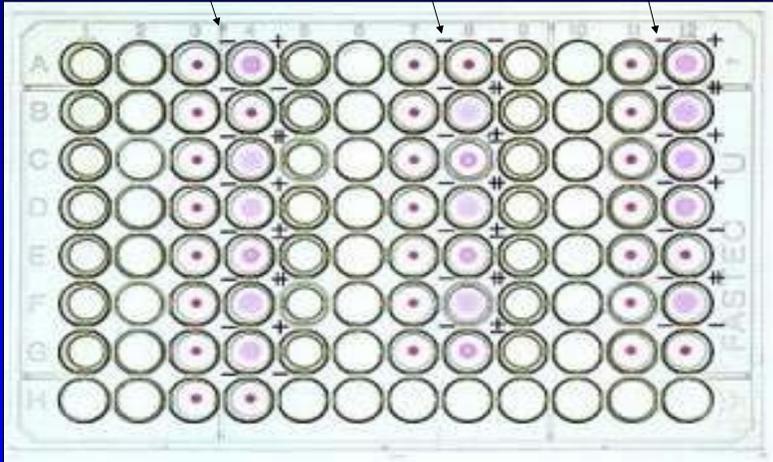
- **False positive - 1 %**
 Most treponemal BFP due to (those in 8th decade of life, drug use, ?)
 False negative if drawn too early/immunocompromised
- **Maternal treponemal IgG crosses the placenta**

Prevent Disease – Promote Wellness – Improve Quality of Life



TP-PA

2+
NR
3+



Prevent Disease – Promote Wellness – Improve Quality of Life



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 %

Prevent Disease – Promote Wellness – Improve Quality of Life



Western Blot - Immunoblot

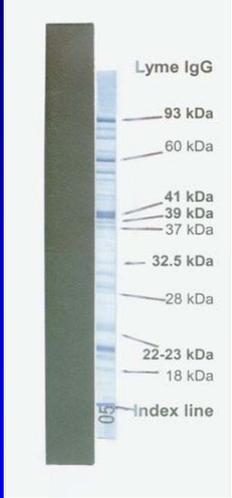
- Treponemal Assay - Immunoblot
 - Species specific proteins (Ag's) transferred to nitrocellulose membrane
 - IgG or IgM AB binds to Ag's on strip
 - Conjugated anti-IgM added to strip
 - Substrate converted to purple band
 - # and/or intensity of reaction determines pos or neg result
 - detects IgM 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 %

Prevent Disease – Promote Wellness – Improve Quality of Life

MOCH
Bureau of Laboratories

Lyme IgG WB



Lyme IgG

93 kDa
60 kDa
41 kDa
39 kDa
37 kDa
32.5 kDa
28 kDa
22-23 kDa
18 kDa
Index line

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

Prevent Disease – Promote Wellness – Improve Quality of Life

27

MOCH
Bureau of Laboratories

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.

Prevent Disease – Promote Wellness – Improve Quality of Life

MOCH
Bureau of Laboratories

Direct Detection DFA-TP



Prevent Disease – Promote Wellness – Improve Quality of Life

MOCH
Bureau of Laboratories

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

DOC	USR	TPPA	FTA	M-WB
8/12	WR	Indet.	Rm 1+	15.5 2+ 47 1+
8/22	1:2	Indet.	Rm 1+	15.5 2+ 47 1+

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

Prevent Disease – Promote Wellness – Improve Quality of Life



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

Prevent Disease – Promote Wellness – Improve Quality of Life



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

Prevent Disease – Promote Wellness – Improve Quality of Life



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

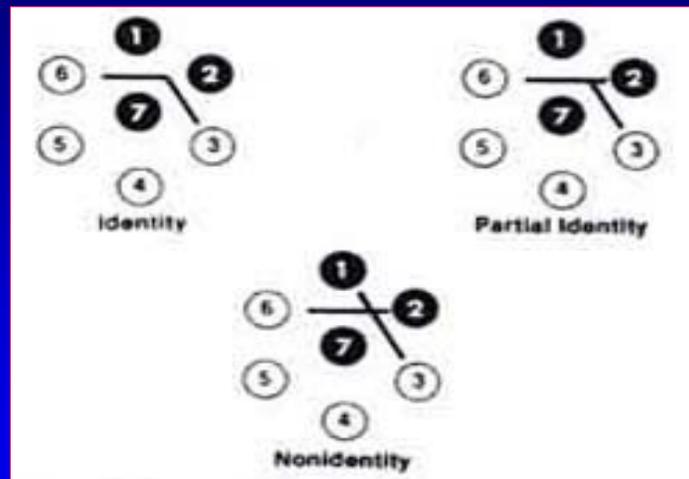
Histoplasma contains H & M antigens.

H band occurs late in disease, extrapulmonary dis
 M band 1st to appear, freq. without H band, can remain pos for months/yrs.

Prevent Disease – Promote Wellness – Improve Quality of Life

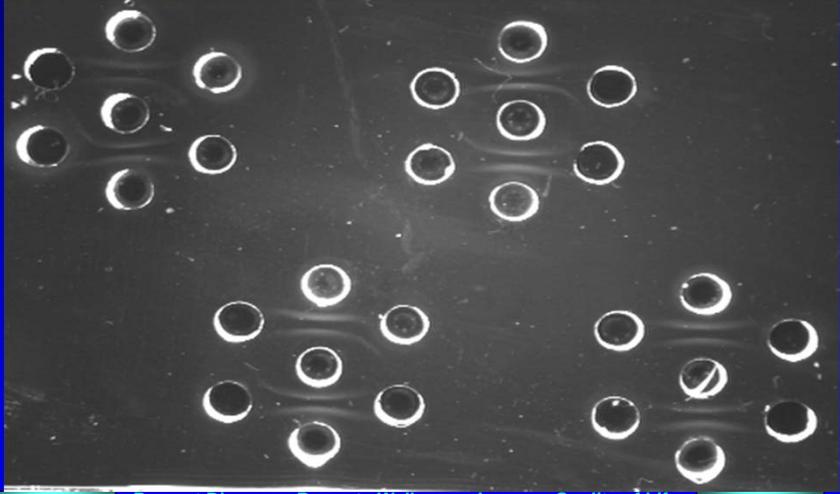


Identity/non-identity bands



Prevent Disease – Promote Wellness – Improve Quality of Life

 **Fungal IDs**



Prevent Disease – Promote Wellness – Improve Quality of Life

 **More information**

- **BOL Lab Services Guide**
www.michigan.gov/mdch

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

Prevent Disease – Promote Wellness – Improve Quality of Life