Molecular Diagnostics

USAID PEER/Liberia ID Clinical Curriculum

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Goals

• Review basic techniques for diagnosis of (mainly) infectious diseases
• Consider differences between methods
• Discuss when to use one method over another
• Recognize limitations
We used to have *only* microscopy

...but we still use it!
| Cell Components          | Techniques                                      |
|-------------------------|-------------------------------------------------|
| • DNA                   | • PCR                                           |
| • RNA                   | • ELISA                                         |
| • Antibodies            | • (Southern blot—DNA)                          |
| • Antigens              | • (Northern blot—RNA)                          |
| • Other proteins (eg CA 19-9) | • Western blot—protein                        |
|                         | • (FISH; In Situ Hybridization)                |
|                         | • (Microarray)                                 |

Note: you can run most of these tests even after formalin or paraffin use
PCR

• Amplifies nucleic acid “signals” (DNA or RNA)
• Just requires four basic ingredients:
  • Sample of interest
  • “Primers”—a bit of synthetic DNA/RNA matching the sample’s code
  • “dNTPs”—nucleic acid “letters” that can be added to a new strand
  • Taq Polymerase—the enzyme that synthesizes new strands
Nucleotide

5' 3' 5' 3'

Denaturation

3' 5' 3' 5'

DNA primer

Original DNA
PCR con’t

- Can be used for detection of one agent (eg HSV-1 in spinal fluid)
- Can be used to detect two agents (eg influenza A & B)
- Can be used for multiple agents (“multiplex” PCR)
- Can get viral loads (eg HIV, Hep C, Hep B, Ebola)
- Can get genotype (eg Hep C)
- Can get sequences to search for resistance (eg HIV)
PCR tests & other

- Respiratory: influenza, COVID, “respiratory viral panel”; TB (rifampin resistance)
- CSF: HSV-1, HSV-2, enteroviruses
- STD: HIV, chlamydia
- GI: C diff
- Highly sensitive, highly specific
- Several may be sendouts
- PCR is fast (unlike culture)
- Tests are changing constantly
Serology

• ELISA & Western blot
• Looks for antibodies or antigens
• Has to capture antibody (with antigen) or antigen (with antibody)
• Then links to a (separate) antibody aimed against what got “captured”
• That antibody can be chemically activated to emit light & detected
• Thus, if the person isn’t infected, they won’t have made the antibody (or antigen), and it won’t be captured, and then no separate light-activating antibody, and thus no light detected
• But if an infection led to antibodies, then light detection occurs
Serology con’t

• Thus, if the person isn’t infected...
• …they won’t have made the antibody (or antigen)...
• …and it won’t be captured...
• …and then no separate light-activating antibody...
• …and thus no light detected
• But if an infection led to antibodies, then light detection occurs
• IgM antibodies measure acute response (often less specific)
• IgG antibodies measure adaptive response
ELISA schematic

(a) 

- **Anti-human antibody** 
- Light activation 
- antibody capture 

(b) 

- **Anti-human antibody** 
- Light-activating molecule 
- Light activation
HIV Western Blot

- First step is HIV ELISA (p24)
- ELISA not very specific
- IE lots of false positives
- Western blot can evaluate multiple separate proteins
- Highly specific
- “Two-tier” testing
- (Can also use HIV PCR quant)
Serologic testing

• Antibody serology is like a footprint!
• You can’t “see” the organism directly (that’s PCR or culture)
• You *can* see evidence that it was present (Abs produced *by* infection)
• *Antigen* testing measures active infection however
footprints can be fuzzy: the false-positive problem

- Some things look *like* footprints but aren’t
- Lupus
- Other inflammatory disorders
- High Ab-production states
- Random Ab generation
footprints can be fuzzy con’t

• Some things are footprints, but are from different organisms
• i.e. cross-reactive treponemal Abs can produce false positive results
• *Treponema pallidum* test: RPR
• Oral treponemes, e.g. *Treponema denticola*
36 yo F with fever, cough, malaise x 48 hr

Which of the following tests would be indicated?

• A. influenza PCR
• B. influenza ELISA
• C. COVID PCR
• D. COVID Western Blot
• E. COVID ELISA
• F. *Hatchus badmannus* PCR
• G. Toxoplasmosis ELISA
28 yo M with unexplained rash and generalized malaise

What tests might be appropriate?

- A. HIV PCR
- B. Toxoplasma IgG (ELISA)
- C. Cryptococcus antigen
- D. HIV ELISA
- E. HIV Western Blot
- F. Hepatitis B Surface antigen
- G. Hepatitis B Surface antibody
- H. RPR
- I. Syphilis PCR