CLIA-Waived Tests Mean Nothing Can Go Wrong. . .Right?

Norman Moore, PhD
Learning Objectives

- Explain sample collection and the bearing it can have on test results
- List potential issues with cross-reactivity
- Define issues with particular disease states that could give inaccurate results
Leading Causes of Deaths in the United States

1. Cardiac events
Leading Causes of Deaths in the United States

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2. Cancer
Leading Causes of Deaths in the United States

1. Cardiac events
2. Cancer
3. Medical errors
Medical Errors

200,000 American deaths each year are associated with preventable harm in hospitals

Cost associated with errors exceeds $17 billion annually

Up to 70% of clinical decisions influenced by laboratory results

Changes in Medical Testing
Where Are Medical Tests Being Done?

- Laboratory
- Emergency Department
- Doctors’ Offices
- Ambulances
- Urgent Care Centers
- Pharmacies
- Home
- Public Health Vans
- Nightclubs, concerts, . . .
Who Is Doing Testing

- Lab scientists
- Doctors
- Nurses
- Patients
- Receptionists...
Crisis In The Laboratory

Lack of medical scientists is a national issue

- The US Department of Labor’s Bureau of Labor Statistics is estimating demand to increase for medtechs by 22% from 2012 to 2022
- Current programs expected to give half of what is needed
- Less than 5,000 people are graduating each year from accredited programs
- The number of accredited programs is declining
- About 50% of medtechs are within 10 years of retirement

Current medical scientists

- Senior people
- On the job training
Why Aren’t People Staying In the Field?

**Education**
- Medical technologist requires baccalaureate and year of training in accredited or approved laboratory training
- Medical technician requires associate degree and a year of training

**Money**
- Median salary is below registered nurses, physical therapists, and pharmacists.
Let’s Talk Clinical Sampling
# Capillary Blood Sampling

## Why?
- Less pain for the patient
- Less invasive
- Easy and fast to collect

## Who
- Pediatric patients so less blood volume restriction
- Obese
- Geriatric
- Anxious patients
- Severe burns
- Veins are hard to get – fragile or inaccessible
What Can Affect Measuring Hemoglobin?

Gender – Women are lower than men¹

Pregnancy – Hgb declines in first trimester and continues to fall in second before rebounding²

Collection site – Ear stick can be higher than venous or fingerstick³

Type of sample – Capillary blood has more Hgb than venous¹

Altitude – Hgb increase at high altitudes to make up for lower concentrations of oxygen²

Smoking – Proportional change to how much the person smokes²

Time of day – Hgb is usually highest in the morning²

Body position – Hgb is increased when standing compared to sitting or laying down¹

Dehydration – Loss of plasma

And Then There is Sample Collection

Use the appropriate finger

• Usually middle or ring finger. Ring finger has less pain

Don’t milk the finger as you get more interstitial fluid

• Appropriate size lancet
• Can massage up to first knuckle

Get right sample

• Clean and disinfect site
• Wipe away alcohol so it doesn’t dilute blood
• Usually wipe away first drop (for Hgb, can be variable for first 3)
1. Place all collection materials on top of a disposable pad. Open the lancet, alcohol swabs, gauze, bandage, and other items. Have all items ready for blood collection.

2. Put on powder-free gloves. Turn patient’s hand upward. Massage patient’s hand and lower part of the finger to increase blood flow.

3. Scrub the patient’s middle finger or ring finger with an alcohol swab. Dry with gauze.
4. Hold the finger in an upward position and lance the palm-side surface of the finger with proper-size lancet (adult/child). Press firmly on the finger when making the puncture. Doing so will help you to obtain the amount of blood you need.

5. Apply slight pressure to start blood flow. Blot the first drop of blood on a gauze pad and discard pad in appropriate biohazard container.

6. Keep the finger in a downward position and gently massage it to maintain blood flow. Hold the Microtainer® at an angle of 30 degrees below the collection site and use the scoop on the Microtainer® to fill it to the 250-500 μL level.
Cap the Microtainer® and gently invert it 10 times to prevent clots from forming. Properly discard all used materials and refrigerate the specimen until shipment or analysis.

Apply a sterile adhesive bandage over the puncture site.
Throat Swabs

Collecting the Swab

An adequate view of throat should be ensured by good lighting conditions and the use of a disposable wooden spatula or a tongue depressor to pull outwards and so depress the tongue.
Improper Throat Swabs with Strep A

What happens with cheek swab?
What happens with saliva on the swab?
Improper Throat Swabs with Strep A

What happens with back of throat?
Nasopharyngeal Swabs
What Happens With Improper NP Swab

Must use NP rather than nasal swab!

Small tip swab collects less sample in a site with less virus.
Nasal Swab

3

Rotate the swab up to 5 times and hold in place for 5-10 seconds to collect sample material.
Immunological Tests
Defining Immunological Testing

**Antigen**: the part of a molecule that an antibody binds to

**Antibody**: a molecule the body makes to bind to an antigen
Multiple Types Of Antibodies

*IgM* is first antibody to respond
- characterizes a recent infection

*IgG* is second antibody to respond
- Used for primary and secondary infection

Polyclonal vs. Monoclonal
Serological Response To Infection

Antibody concentration

Time
Lateral Flow Schematic
Lateral Flow Types

Direct antigen

- Pregnancy, Strep A, and Influenza

Serological

- HIV

Competitive (a negative line means a positive result)

- DOA
Direct Antigen Detection

- Visualizing Antibody
- Antigen
- Capture Antibody
- Nitrocellulose
Serology Detection

Looking for a person’s antibody response to disease (the blue antibody)
Issues With Antibody Based Reactions

Heterophile antibodies, such as HAMA (human anti-mouse antibodies)
• Can cause false results
• Some tests can incorporate a HAMA blocker

Rheumatoid factors
• Autoantibodies in clinical sample, usually IgM that can bind to IgG antibodies

Hook effect
• Analyte is in high concentrations capture and detector antibodies are saturated
• Creates False negatives

Antigen break-down
• If antigen denatures, antibodies may not be able to sandwich the target
What are Common Mistakes For Running Lateral Flow

**Storage**
- Is it stored refrigerated? If so, is it supposed to warm up first?
- Was it not stored refrigerated when it should

**Read time**
- Are you using a timer?
- What is the problem when you read too early?
- What is the problem when you read too late?

**Contaminating reagents**
- Where did you put the sample?
- Is it in contact with your hands or reagent bottles?
Issues with People Reading the Test

- Light lines can be missed by some people
- Multitasking can lead to not reading at the right time
- Not having clinical samples or tests properly identified
- Transcription errors
What is Wrong With This Picture?
Or This One?
Molecular Amplification

Double-stranded DNA

95° Denaturation

Heating separates strands

72° Extension

Taq Polymerase reads existing DNA strand to create a new matching one

57° Annealing

Primers Bind to target sequences

Taq Polymerase Binds at Primer Sites
PCR amplification

Cycles 1 2 3 50

2^1 = 2 copies DNA  2^2 = 4 copies DNA  2^3 = 8 copies DNA

Chain Reaction, copies from copies produced

2^{30} = 1073741824 copies DNA
What is Wrong With This Picture?
Molecular Testing Near Patient

What can go wrong running positive and negative control swabs?

- Technician touches swab head and then touches other samples
- Sample can be laid down on bench so can contaminate other areas

What Can Go Wrong During?

- Anything that interacts with enzymes
- Point mutations

What can go wrong AFTER running a molecular test?

- Amplicon!
Pregnancy
What Does the Pregnancy Test Measure

Pregnancy tests have different hCG cutoffs

- High sensitivity tests
- Low sensitivity tests

Why?
What Can Cause False Positives?

- Menopausal women
- Post partum women
- People taking hCG supplements
- Fertility medications that contain synthetic hCG
- Ovarian cancer
What Can Cause False Negative?

Testing too early

Potentially testing late pregnancies!

- Breakdown of hCG into α and β subunits
- Publications have shown this can happen in late pregnancies
Influenza
Influenza A versus Influenza B

**Influenza A**
- More severe disease than B
- Can cause disease in a wide variety of animals

**Influenza B**
- Causes a milder flu, usually in the spring months
## Influenza

- Is attacking epithelial cells in the nose, throat, and respiratory system

## Body’s reaction

- Releases histamine which widens the blood vessels near infection
- Allows immune responses like antibodies to get to the infection better
- Histamines also end up in other body parts like muscles
- Cytokines are also released that help coordinate the body’s attack on virus

## The problem

- Histamines and cytokines can affect pain receptors
Patients Most Infectious during first 3-5 days\textsuperscript{1}

Days Post Symptom Onset

0 1 2 3 4 5 6 7 8 9 10

High Viral Titer

Low Viral Titer

Antiviral Drugs Most Effective during first 2-4 days\textsuperscript{1,2}

Rapid POCT Sensitivity Highest\textsuperscript{3}
What Can Increase Cases of Seasonal Influenza?

**Vaccine Mismatch**
- Vaccine is made by predicting strains for next season so may not be accurate

**Multiple strains hitting at the same time**
- Can have multiple strains as well as overlap of influenza A and B

**Virulence of Strains**
- Some strains can cause an extreme immune response
Hypothesis On Evolution of Feeling Bad When You Are Sick

If a person feels sick, they are going to stay in bed

If they stay in bed, they are less likely to expose other people
Should You Get the Fever Down?

**Why Do You Get a Fever?**
- Your immune system releases chemicals called pyrogens
- The hypothalamus portion of the brain get the pyrogens and raises the temperature

**Increased temperature**
- Can kill some bacteria
- Can inhibit the replication of some viruses

**When you reduce the fever**
- Don’t reduce the amount of virus
- Research is suggesting that tens of thousands of more people can be then infected!

**Do you reduce the fever?**
- If too high, yes!
- If not too high...
Differences Between the Sexes

Women tend to generate stronger immune responses than men

- Helps clear virus faster from the system

The good

- Lower virus can shorten intensity and duration of illness
- Especially important if pregnant

The bad

- More likely to have hyperimmune response so could have higher morbidity/mortality in outbreak or pandemic
- Chronic infections (like HIV) have been linked to accelerating the aging process

Influenza Sample Collection

Appropriate specimens

• Nasal wash/aspirate, nasopharyngeal swab, or nasal swab
• Throat swabs have dramatically reduced sensitivity

Samples should be collected within first 24 to 48 hours of symptoms since that is when viral titers are highest and antiviral therapy is effective

• Reduction in sensitivity over days – NOT related to how people feel
Sensitivity vs Specificity vs PPV vs NPV

**Sensitivity:**
Probability test=positive if patient=positive

**Specificity:**
Probability test=negative if patient=negative

**PPV:** Probability patient=positive if test=positive

**NPV:** Probability patient=negative if test=negative
Flu is seasonal. Prevalence of the disease is different in June than in January. This will impact the perceived performance of the test

Test 1,000 persons
Test Specificity = 99.6% \((4/1000)\)
Prevalence = 10%

True positive: \(100\)  False positive: 4

Positive predictive value: \(100/104 = 96\%\)
Test 1,000 persons

Test Specificity = 99.6% (4/1000) Prevalence = 10%
True positive: 100 False positive: 4
Positive predictive value: 100/104 = 96%

Prevalence = 0.4%

True positive: 4 False positive: 4
Positive predictive value: 4/8 = 50%

www.cdc.gov/hiv/rapid_testing
How many people have had RSV in their lives?
Almost ALL people in this room had RSV by the age of 2!
RSV – Who Do You Test

**Under 5?**
- Lateral flow and NAAT tests are available

**Over 65?**
- Don’t do lateral flow
Strep A
Strep A Reagents
Strep A Issues

Reagent 1 and 2

- When mixed, make unstable nitrous acid
- The acid is meant to expose the antigen

How can 2 bottles be an issue?

- What happens if caps get exchanged?
- What happens if setting up early?
Questions on Strep A

• What is sensitivity of rapid tests?
  • Cohen 2016 meta-analysis said 85.6%
• What pharyngitis are we supposed to treat for?
  • Strep A
  • Exceptions like gonorrhea
• Treating anyway?
HIV
CDC/APHL HIV Diagnostic Algorithm

- **HIV-1/2 antigen/antibody combination immunoassay**
  - (+) → Initiate Care
  - (-) → Negative for HIV-1 and HIV-2 antibodies and p24 Ag

- **HIV-1/HIV-2 antibody differentiation immunoassay**
  - HIV-1 (+) and HIV-2 (-) → HIV antibodies detected
  - HIV-1 (-) and HIV-2 (+) → HIV antibodies detected
  - HIV-1 (+) and HIV-2 (+) → HIV antibodies detected
  - HIV-1 (-) or indeterminate and HIV-2 (-) → Initiate Care
  - HIV-1 (-) or indeterminate and HIV-2 (+) → HIV antibodies detected

- **HIV-1 NAT**
  - HIV-1 NAT (+) → Acute HIV-1 infection
  - HIV-1 NAT (-) → Negative for HIV-1

*Initiate Care*
How do rapid tests fit into HIV algorithm?

**Use the algorithm when practical**

- Most situations where HIV testing is done does not have large instrumentation

**Rapid tests**

- If negative, no further testing
- If positive, start at beginning of algorithm
Role for Rapid HIV Tests

- Increase receipt of test results
- Increase identification of HIV-infected pregnant women so they can receive effective prophylaxis
- Increase feasibility of testing in acute-care settings with same-day results
- Increase number of venues where testing can be offered to high-risk persons
HIV Infection & Laboratory Markers

Clostridium difficile
What to Test

1. Tests for *C. difficile* or its toxins should be performed ONLY diarrheal (unformed) stool, unless ileus due to *C. difficile* is suspected

2. Do not test stool from asymptomatic patients

3. Do not perform “test of cure” testing

4. Repeat testing during same episode of diarrhea is of limited value and should be discouraged.....one week following initial testing

5. Do not test babies
How Can Improper Samples Be Tested?

- Bully the lab
- Lie
  - Don’t report that the patient had a laxative
  - Say that this is their third stool
- Alter the sample
So Why Do CLIA-Waived POC Tests?
Let’s Talk Pandemics...
Advantages of Rapid Testing for Infectious Diseases

- Faster directed therapy to reduce:
  - antibiotic resistance
  - hospital length-of-stay

- Less adverse consequences

- Reduced length-of-stay in Emergency Department

- Teachable moment

- Timely application of appropriate infection control procedures
AMR: If We Don’t Take Action Now

Deaths attributable to AMR every year compared to other major causes of death

Deaths attributable to AMR every year by 2050

- AMR in 2050: 10 million
- AMR now: 700,000 (low estimate)
- Tetanus: 60,000
- Road traffic accidents: 1.2 million
- Measles: 130,000
- Diarrhoeal disease: 1.4 million
- Cancer: 8.2 million
- Cholera: 100,000-120,000
- Diabetes: 1.5 million

Number of deaths

Mortality per 10,000 population

- North America: 317,000
- Europe: 390,000
- Africa: 4,150,000
- Latin America: 392,000
- Asia: 4,730,000
- Oceania: 22,000
There aren’t enough good rapid tests to confirm the professional judgment of the doctor,.. this is not acceptable: we need to encourage more innovation and ensure that useful products are used. I call on the governments of the richest countries to mandate now that by 2020, all antibiotic prescriptions will need to be informed by a rapid diagnostic test wherever one exists.\textsuperscript{12}

\textbf{- Jim O’Neill 2016}

What’s driving the need for rapid accurate diagnostic tests?

Transition to “patient-centered” value based health service delivery

- Get the diagnosis right the first time
- Diagnose in an actionable timeframe
- Early optimal treatment selection
- Avoid the waste of unnecessary investigations
- Avoid the waste of over treating
- Avoid the consequences of incorrect patient management
- Better health outcomes and reduced healthcare costs

The results of diagnostic tests are immensely influential, affecting around 60–70% of all clinical decisions, although they still amount for only 4–5% of healthcare costs.

Question:

What is the future of the microbiology laboratory?