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

Norman Moore, PhD

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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

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Leading Causes of Deaths in the United States

1. Cardiac events

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Leading Causes of Deaths in the United States

1. Cardiac events

2. Cancer

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Leading Causes of Deaths in the United States

1. Cardiac events
2. Cancer
3. Medical errors

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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

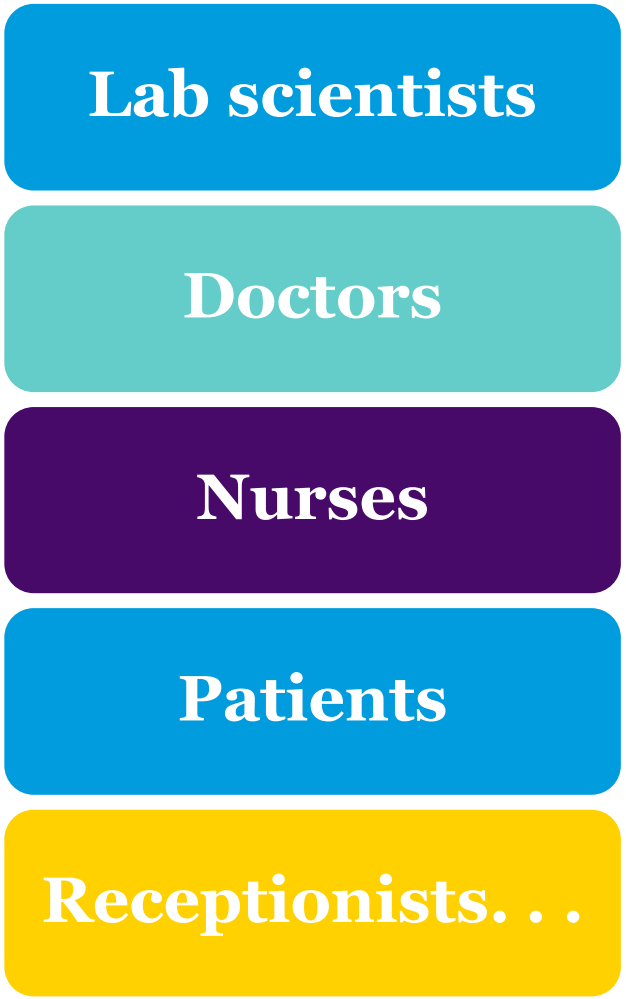
Daniel M and Makary M. Medical error – the third leading cause of death in the US. BMJ 2j016. 353:i2139.|

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



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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 med techs 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 med techs 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 men[1]**
- **Pregnancy – Hgb declines in first trimester and continues to fall in second before rebounding[2]**
- **Collection site – Ear stick can be higher than venous or fingerstick[3]**
- **Type of sample – Capillary blood has more Hgb than venous[1]**
- **Altitude – Hgb increase at high altitudes to make up for lower concentrations of oxygen[2]**
- **Smoking – Proportional change to how much the person smokes[2]**
- **Time of day – Hgb is usually highest in the morning[2]**
- **Body position – Hgb is increased when standing compared to sitting or laying down[1]**
- **Dehydration – Loss of plasma**

[1]Cable RG, Steele WR, Melmed RS, et al. The difference between fingerstick and venous hemoglobin and hematocrit varies by sex and iron stores. NHLBI retrovirus epidemiology donor study II (REDS II). Transfusion. 2012. 52: 1031-1040. doi: 10.1111/j.1537-2995.2011.03389.x
[2]Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Vitamin and Mineral Nutrition Information System. Geneva, Switzerland: World Health Organization, 2011 (WHO/NMH/NHD/MNM/11.1). Available at: www.who.int/vmnis/indicators/haemoglobin.pdf. Accessed October 11, 2017.
[3]Wood EM, Kim Dm, Miller JP. Accuracy of predonation Hct sampling affects donor safety, eligibility, and deferral rates. Transfusion. 2001. **41**: 353-359.

. . .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

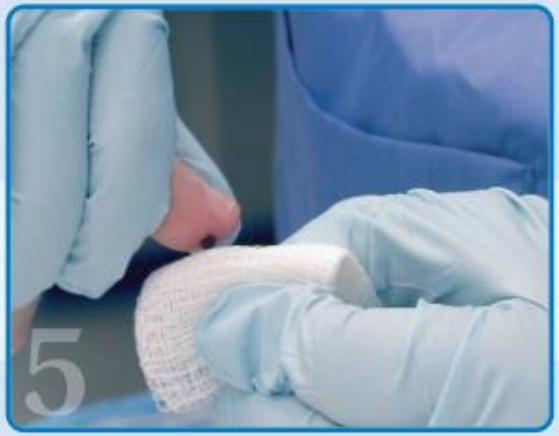
- Clean and disinfect site
- Wipe away alcohol so it doesn’t dilute blood
- Usually wipe away first drop (for Hgb, can be variable for first 3)

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.

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

Scrub the patient's middle finger or ring finger with an alcohol swab. Dry with gauze.

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.



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

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.

DEPARTMENT OF HEALTH & HUMAN SERVICES · USA

For more information visit
www.cdc.gov

CDC
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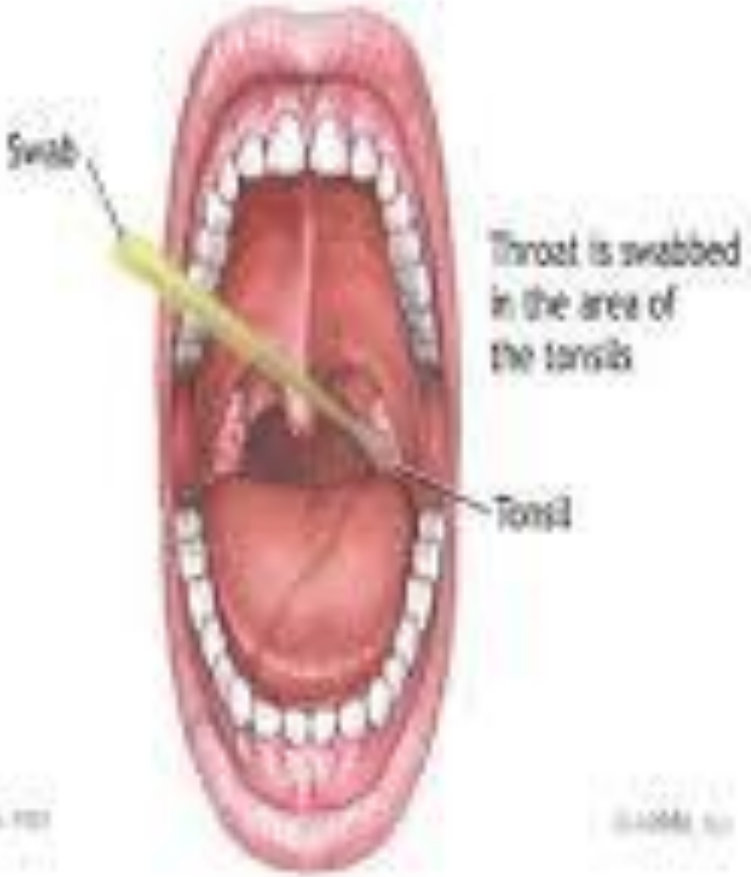
Throat Swabs

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?

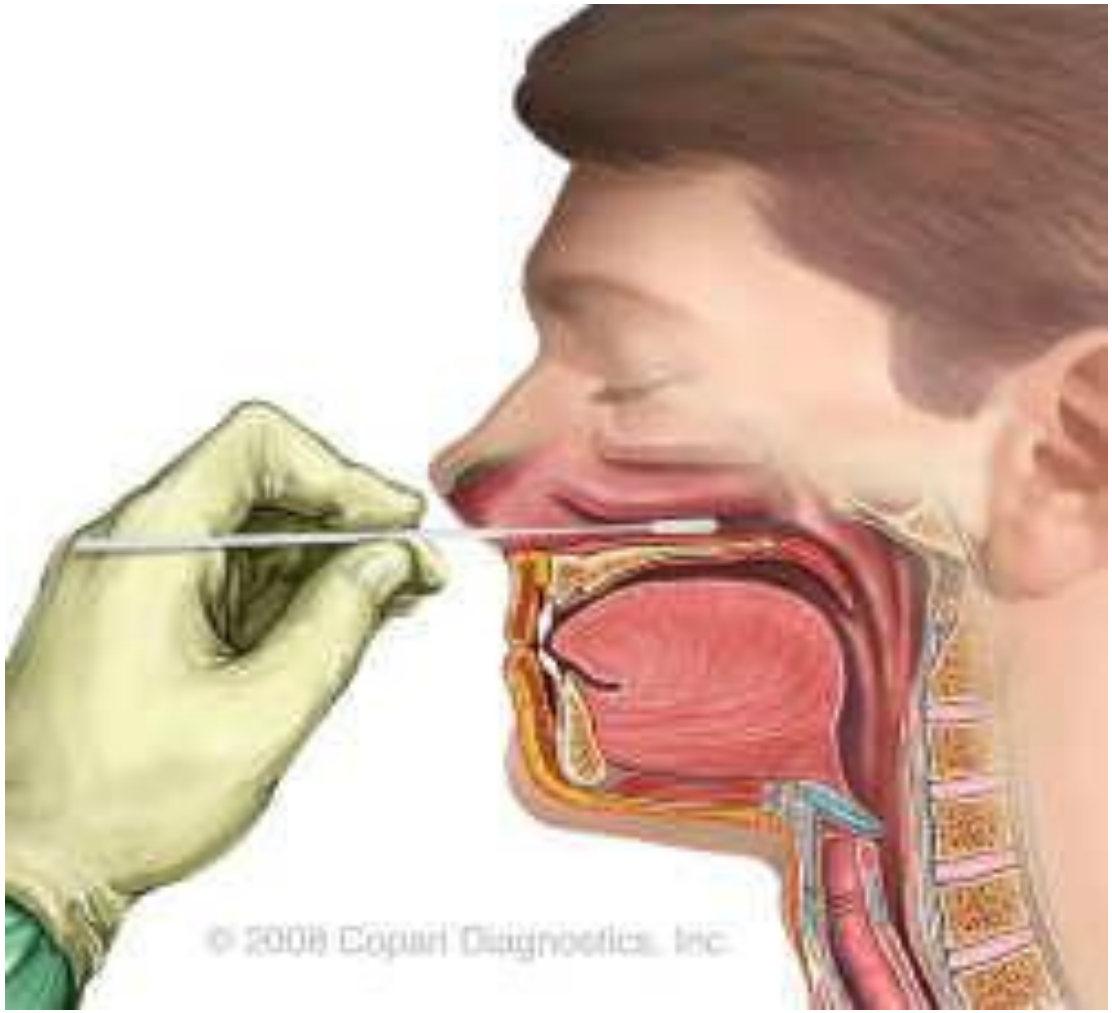
Improper Throat Swabs with Strep A

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

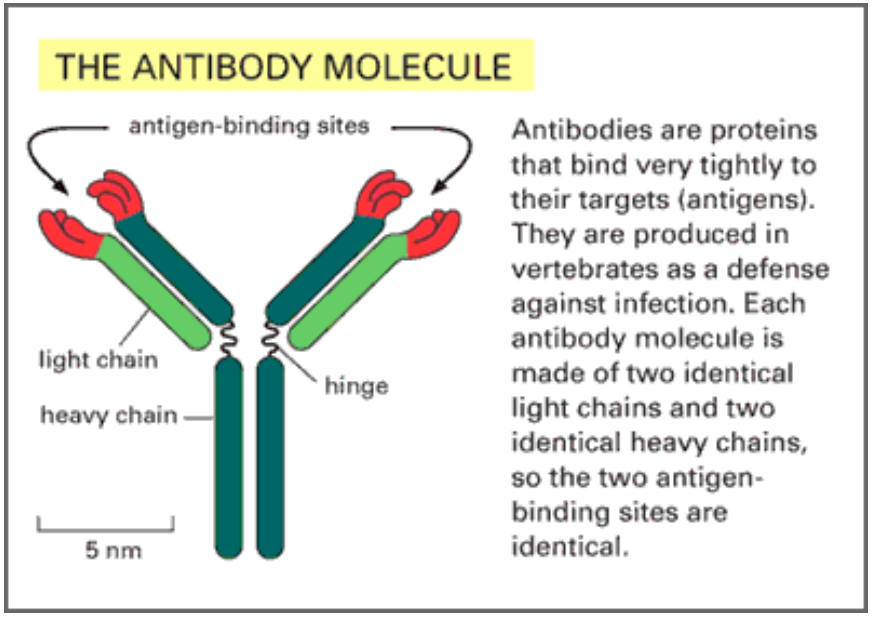

3
Rotate the swab up to 5 times
and hold in place for 5-10 seconds
to collect sample material.

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**



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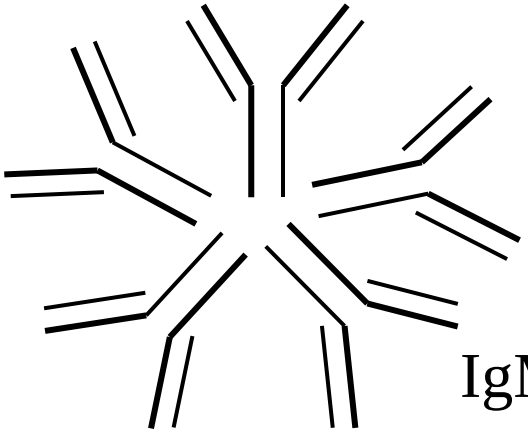
Multiple Types Of Antibodies

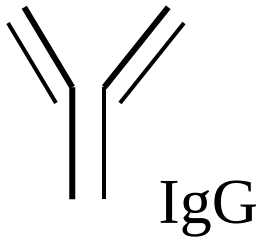
IgM is first antibody to respond

- **characterizes a recent infection**

IgG is second antibody to respond

- **Used for primary and secondary infection**





Polyclonal vs.
Monoclonal

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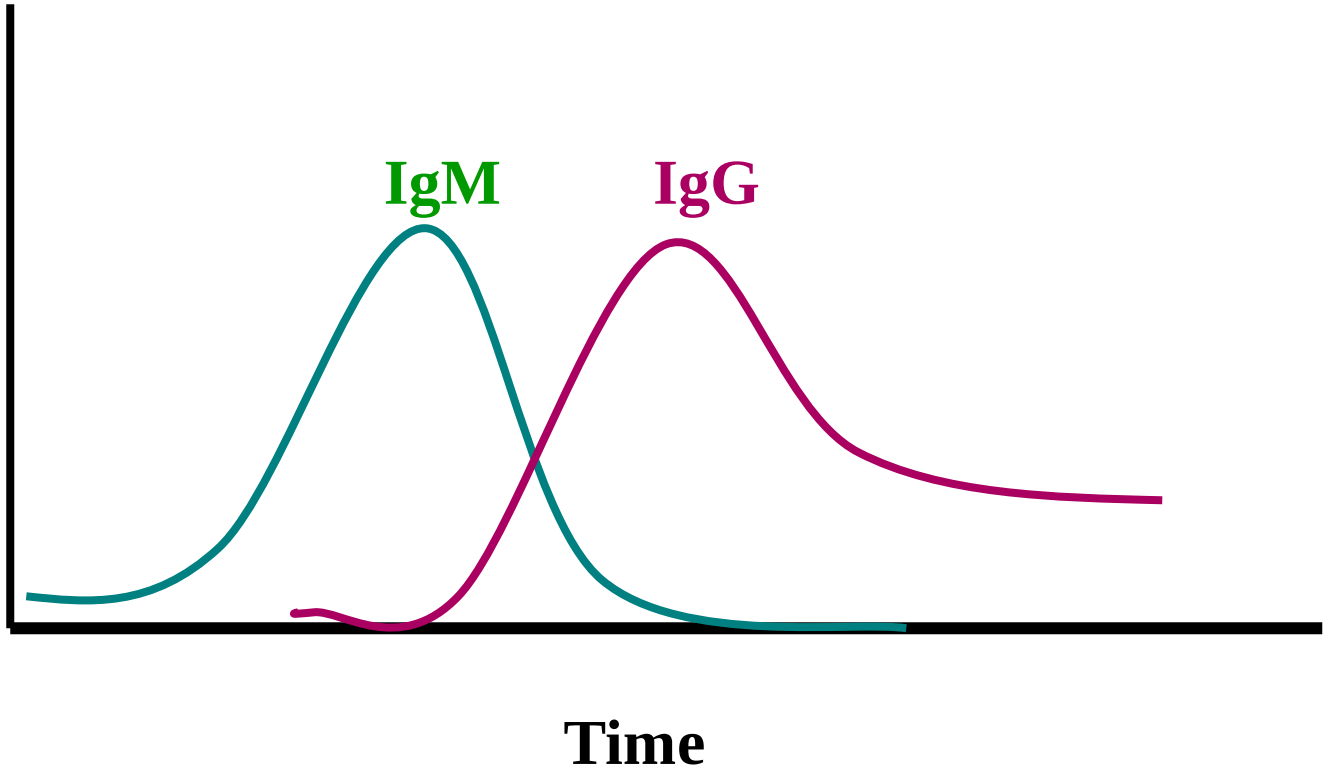
Serological Response To Infection

Antibody concentration



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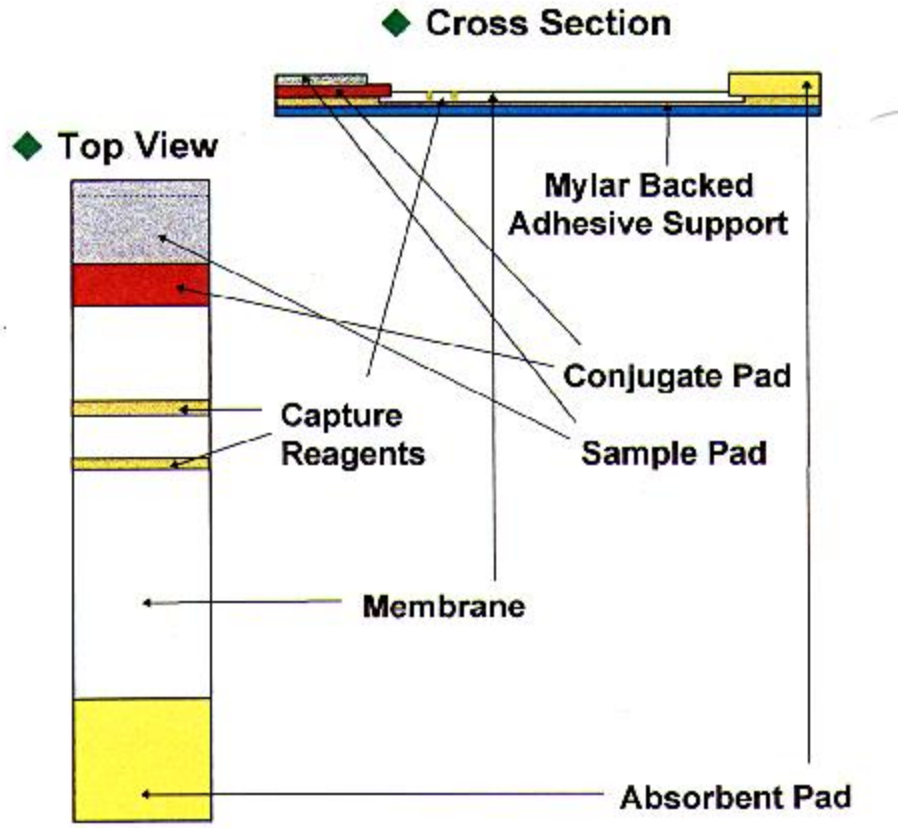
Lateral Flow Schematic



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Lateral Flow Types

Direct antigen

- **Pregnancy, Strep A, and Influenza**

Serological

- **HIV**

Competitive (a negative line means a positive result)

- **DOA**

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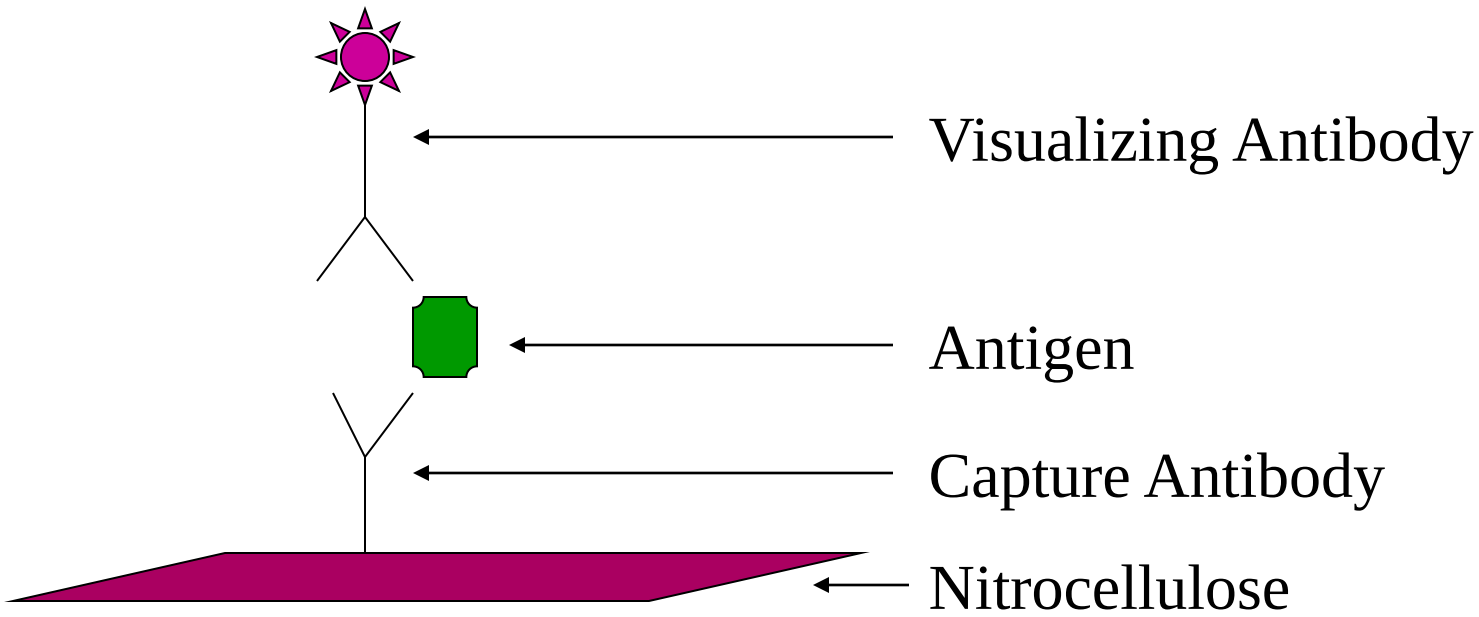
Direct Antigen Detection



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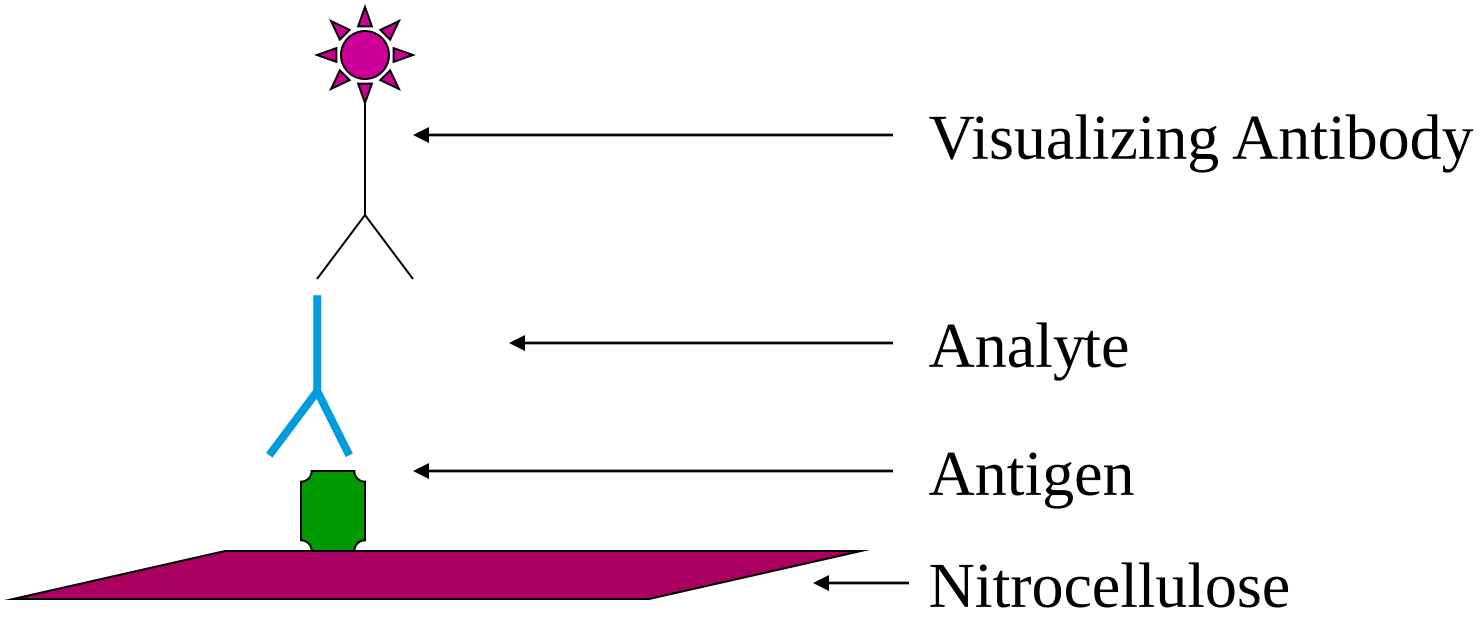
Serology Detection

Looking for a person's antibody response to disease (the blue antibody)



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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**

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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 Tests

Molecular Amplification

Double-stranded DNA

95° Denaturation

Heating separates strands

72°
Extension

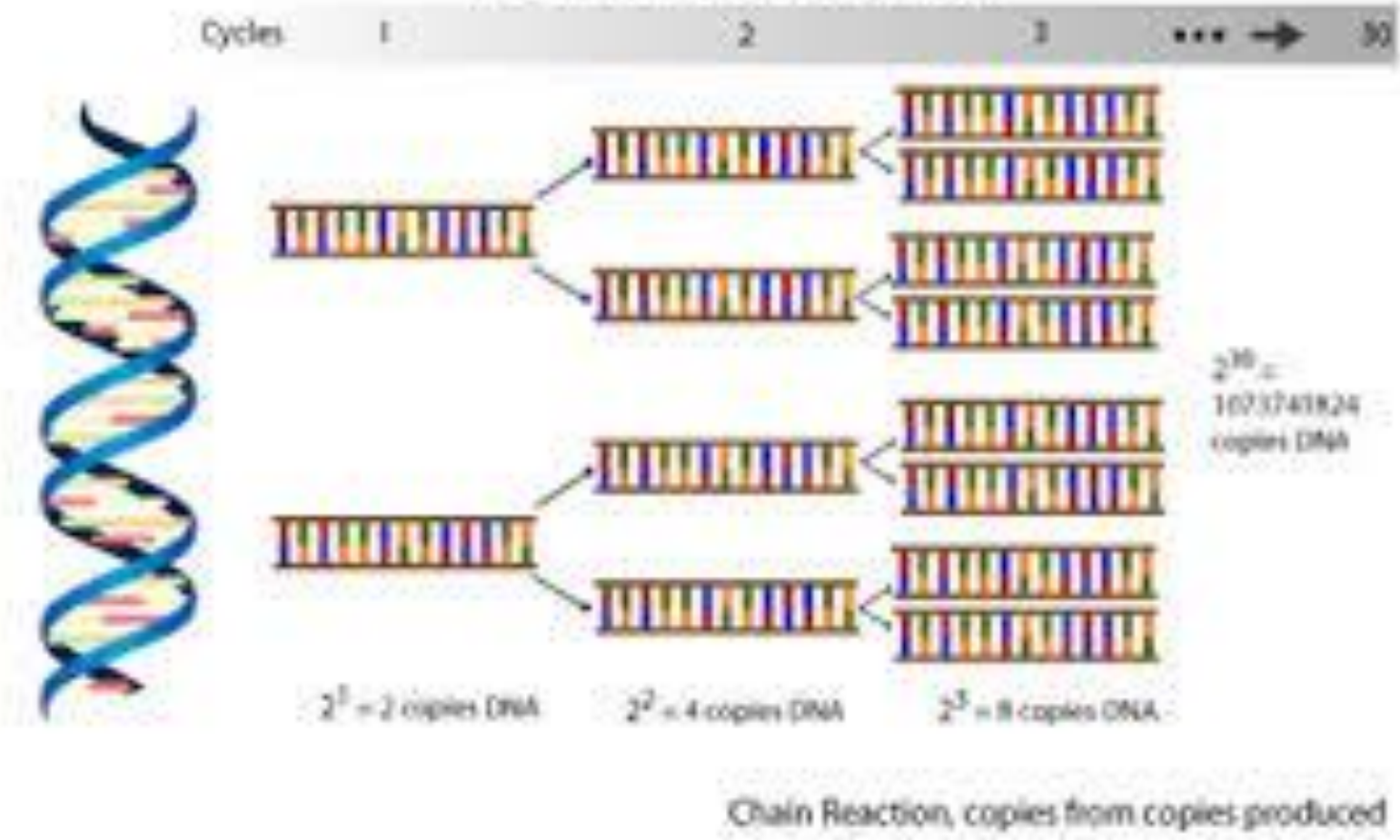
Taq Polymerase reads existing DNA
strand to create a new matching one

57° Annealing

Primers Bind to target sequences

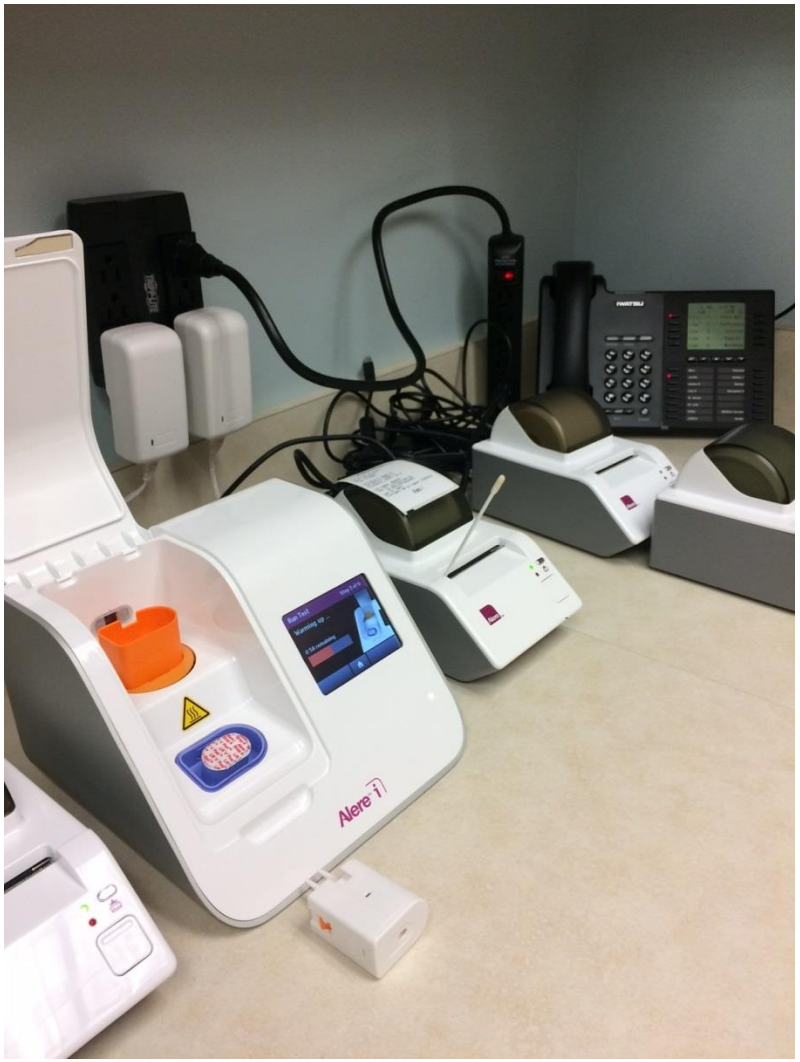
Taq Polymerase Binds at Primer Sites

PCR amplification



Chain Reaction, copies from copies produced

What is Wrong With This Picture?



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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?

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What Can Cause False Positives?

Menopausal women

Post partum women

People taking hCG supplements

Fertility medications that contain synthetic hCG

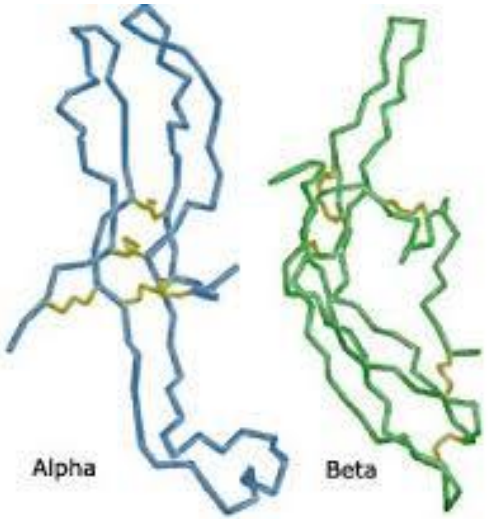
Ovarian cancer

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



hCG subunits

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

What Makes You Ache When You Have Influenza

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

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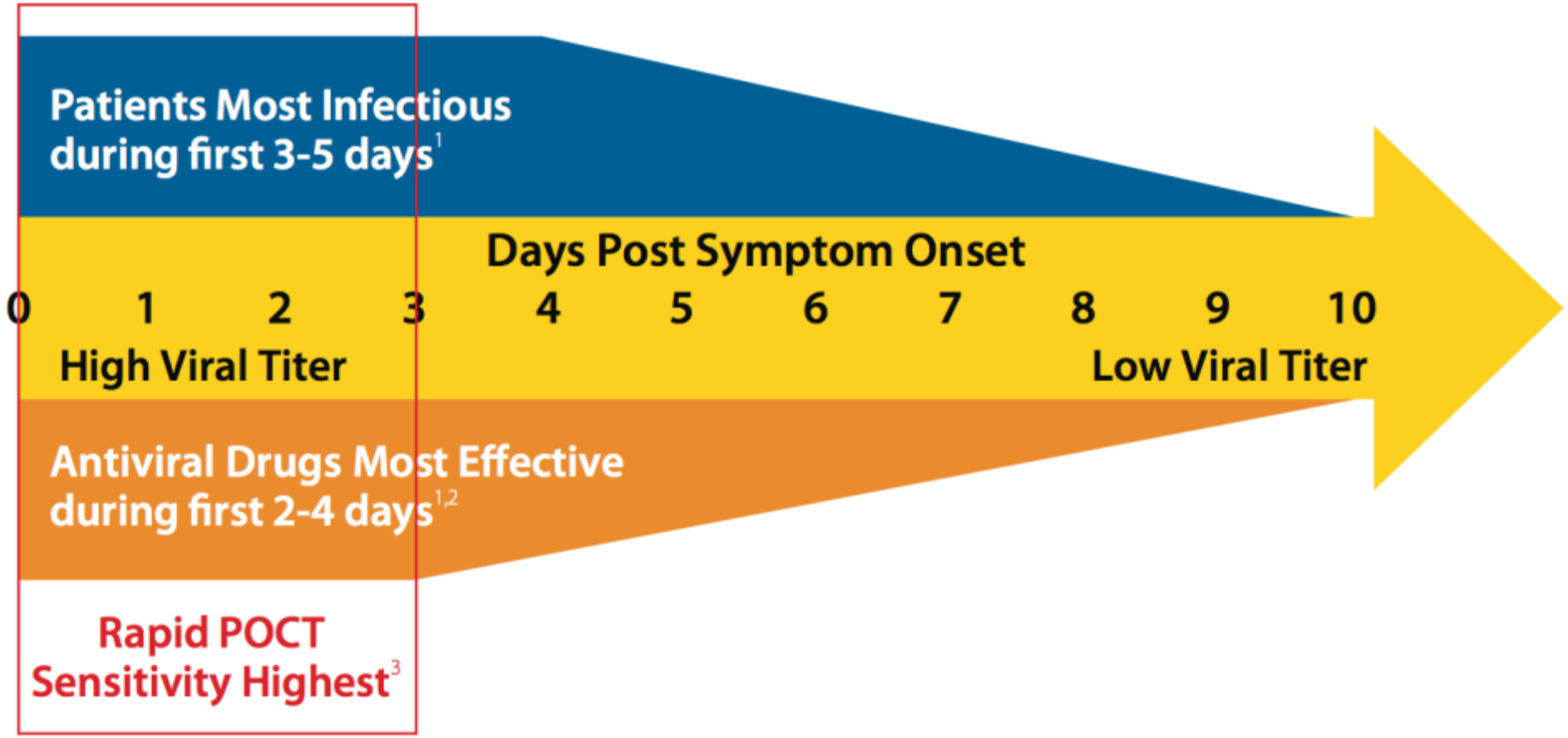

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What Can Increase Cases of Seasonal Influenza?

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

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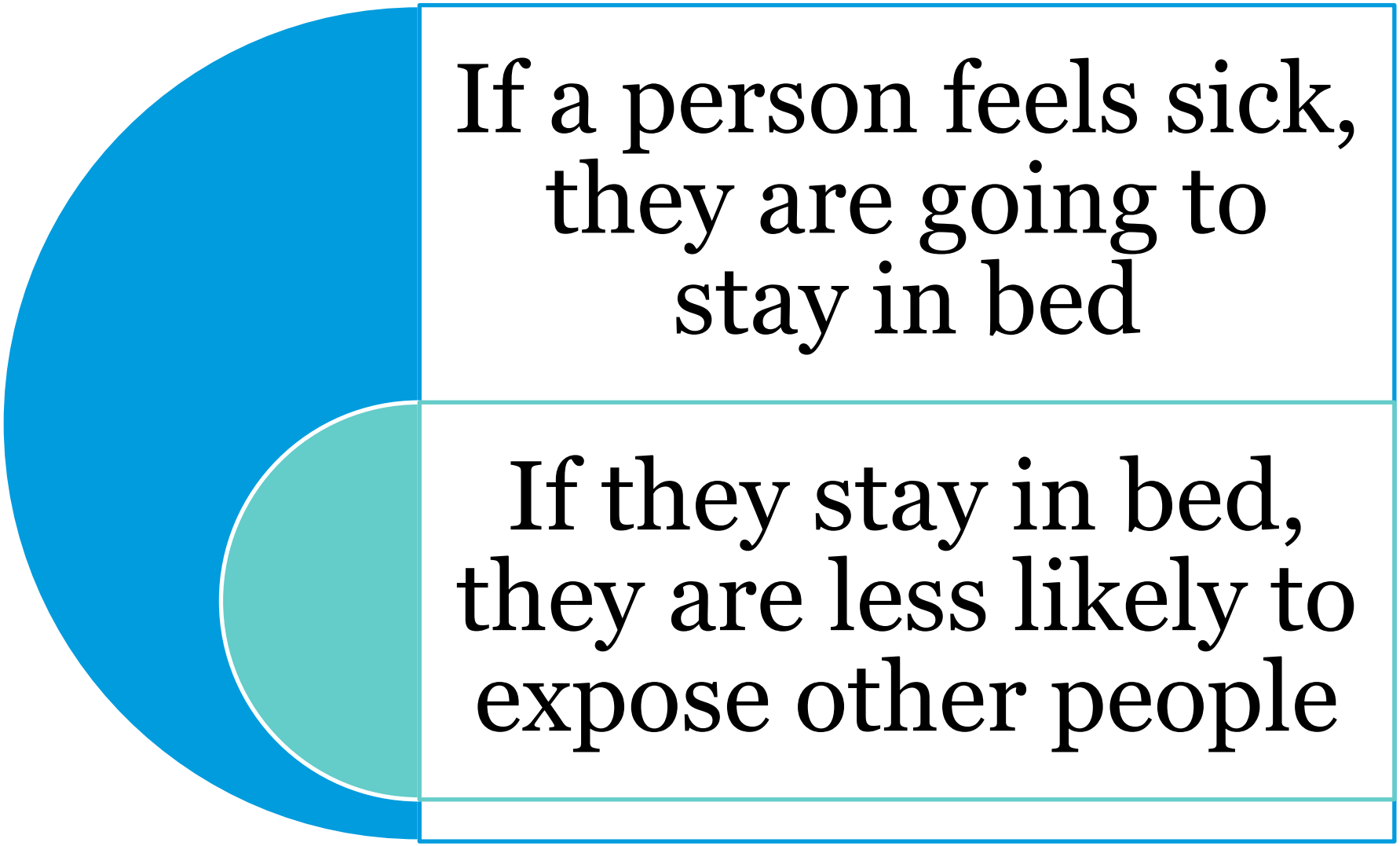
Hypothesis On Evolution of Feeling Bad When You Are Sick



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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. . .

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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

Klein, SL, Hodgson A, Robinson DP. Mechanisms of sex disparities in influenza pathogenesis. Journal of Leukocyte biology. 2012. Jul: 92(1) 67-73

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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

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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

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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%**

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Test 1,000 persons

Test Specificity = 99.6% *(4/1000) P*revalence = 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%

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RSV

How many people have had RSV in their lives?

THE POWER OF now

Almost ALL people in this room

had RSV by the age of 2!

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RSV – Who Do You Test

Under 5?

- **Lateral flow and NAAT tests are available**

Over 65?

- **Don’t do lateral flow**

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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?

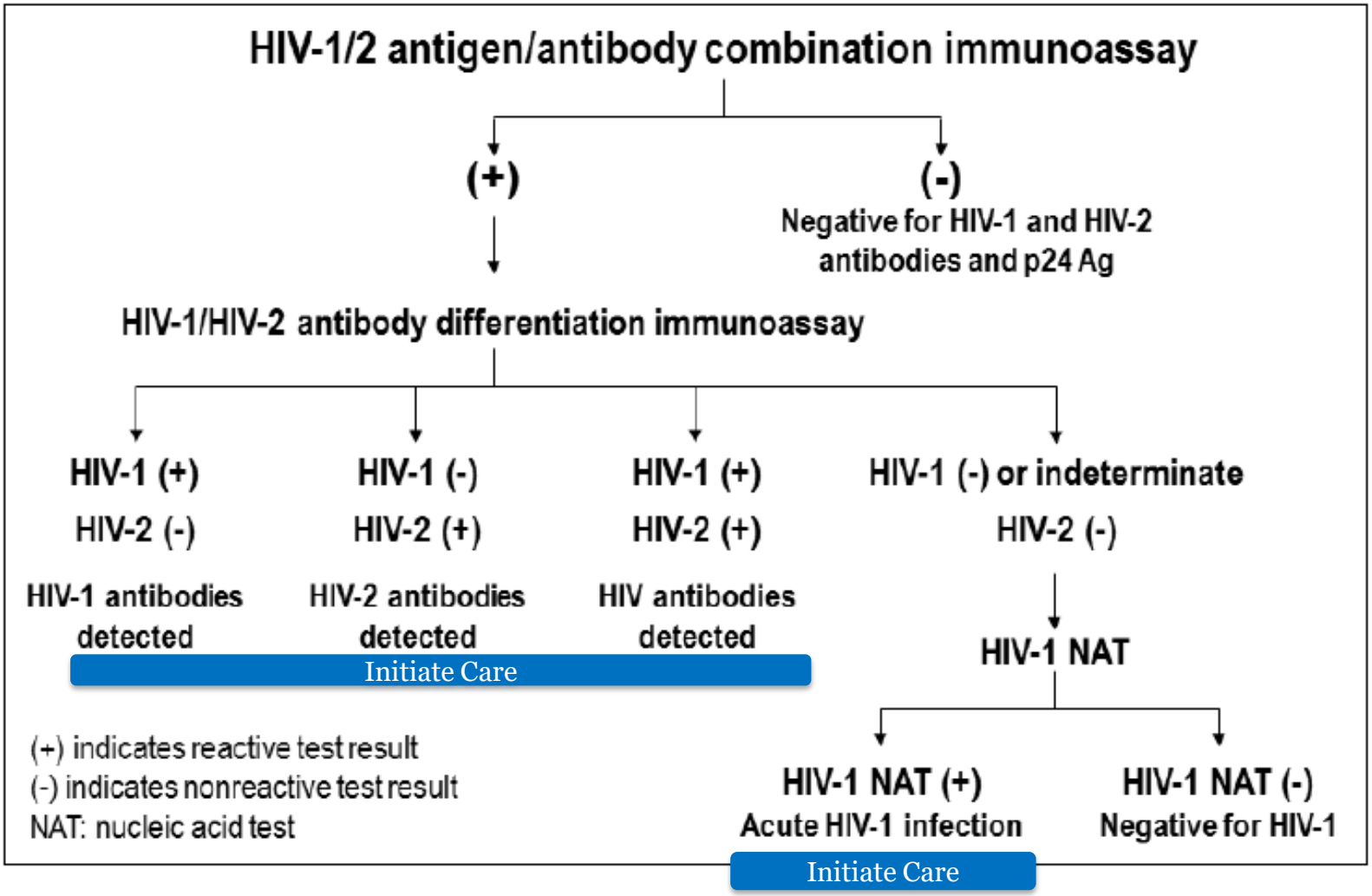
- What happens if caps get exchanged?
- What happens if setting up early?

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[1]



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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

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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

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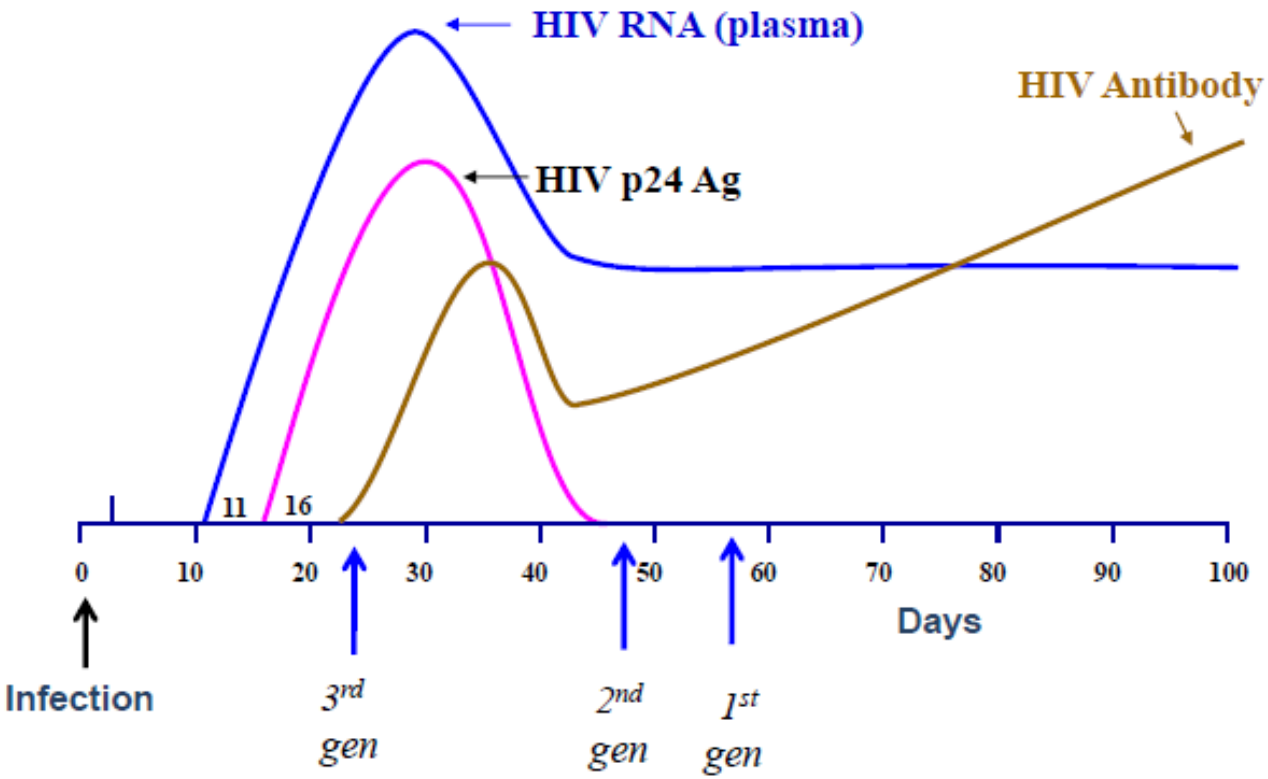
HIV Infection & Laboratory Markers



Modified after Busch et al. Am J Med. 1997

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**

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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. . .

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Advantages of Rapid Testing for Infectious Diseases

Faster directed therapy to reduce:

- **antibiotic resistance**
- **hospital length-of-stay**

Less adverse consequences

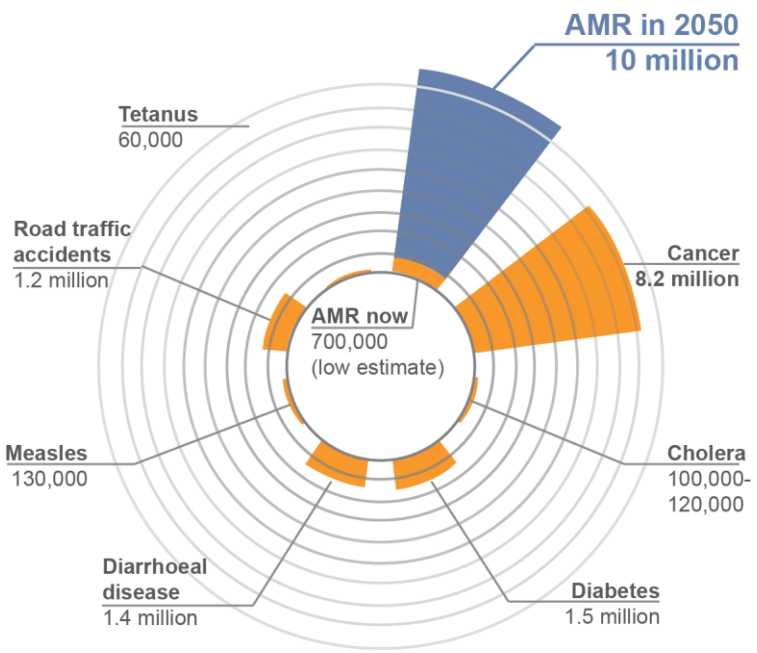
Teachable moment

Reduced length-of-stay in Emergency Department

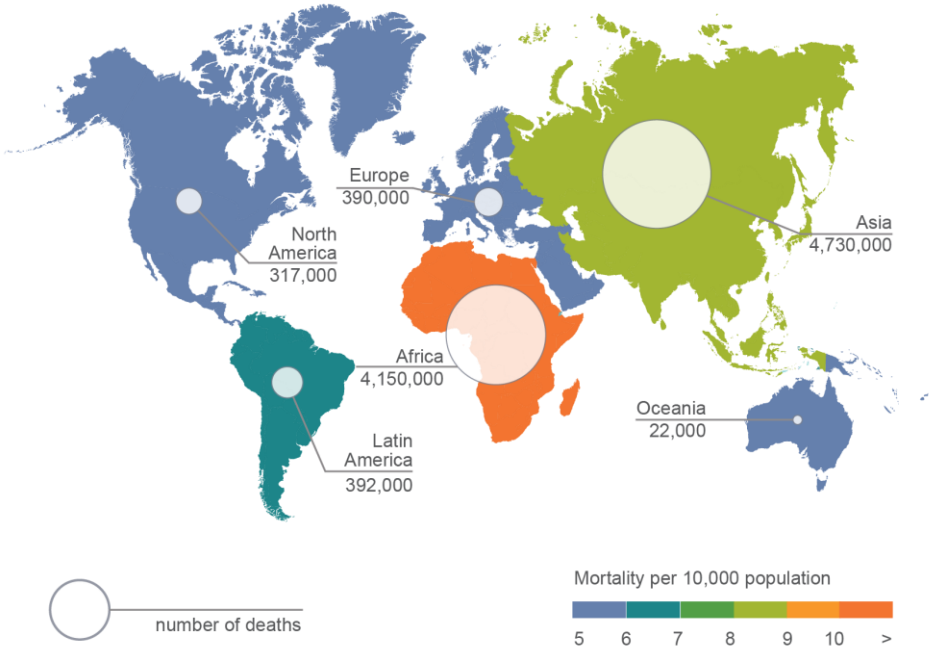
Timely application of **appropriate infection control** procedures

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



Global Antibiotic Resistance Crisis

“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.[12]”

- Jim O’Neill 2016

12. O’Neill, J. Tackling drug-resistant infections globally: Final report and recommendations. The Review on antimicrobial resistance. May 2016.

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

Transition to "patient-centered" value based health service delivery[8]

- 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.[8]

8. Akhmetov, I. and Bubnov, R.V. Assessing the value of innovative molecular diagnostic tests in the concept of predictive, preventive, and personalized medicine. *The EPMNA Journal* (2015) 6:19.

TEST
TARGET
TREAT™

Question:

What is the future of the microbiology laboratory?

Abbott