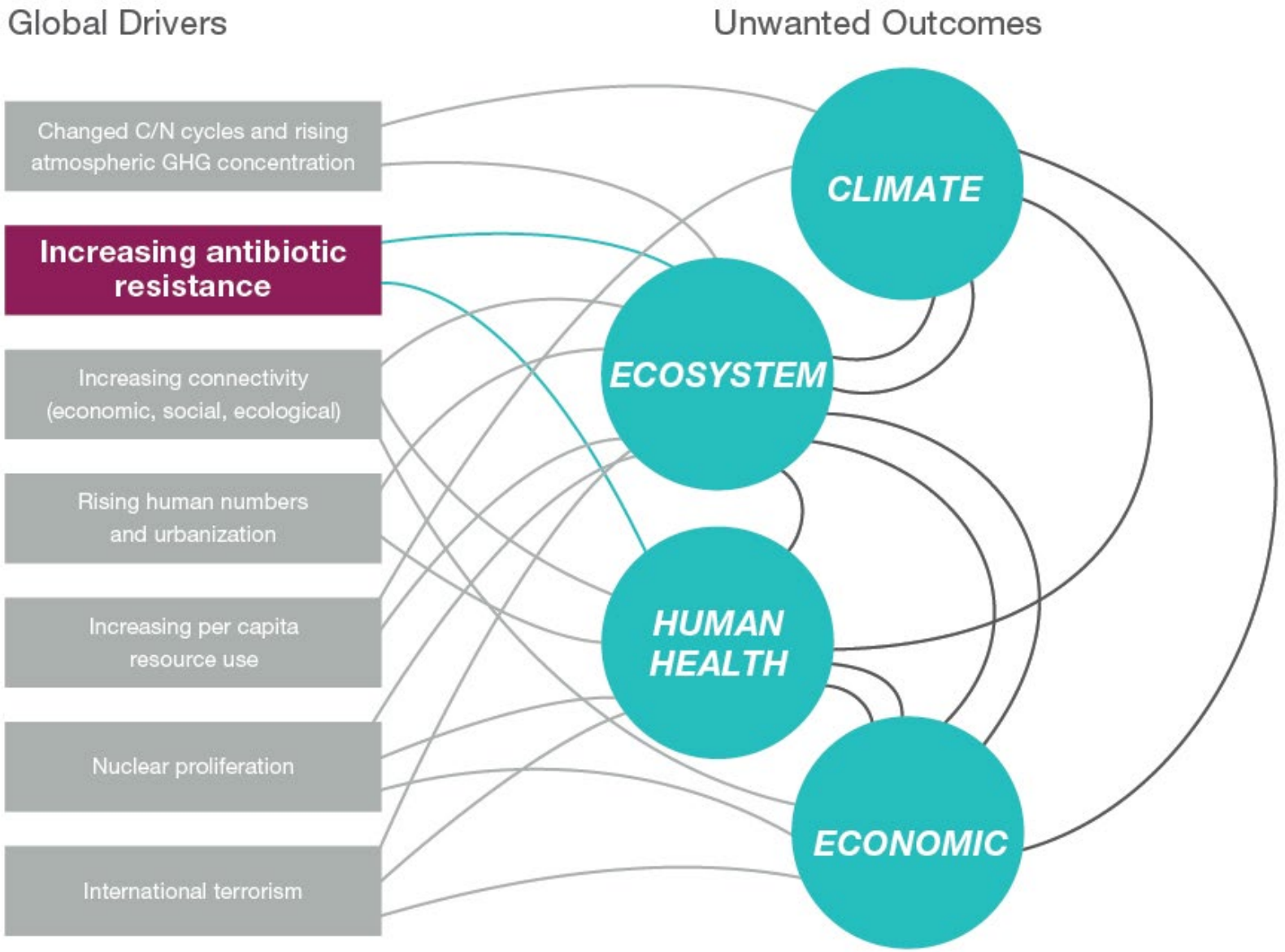
Molecular Testing at Point-of-Care

Norman Moore, PhD
Director of Scientific Affairs, Infectious Diseases

Proprietary and confidential — do not distribute

What do you think are the top 7 threats to the human race?

One of the top 7 issues that threatens the human race



Source adapted from: Science, Vol 325, September 2009
Available at: http://www.sciencemag.org/content/325/5948.cover-expansion

Infectious Disease in the US

1970: William Stewart, the Surgeon General of the United States declared the U.S. was “ready to close the book on infectious disease as a major health threat”; modern antibiotics, vaccination, and sanitation methods had done the job.

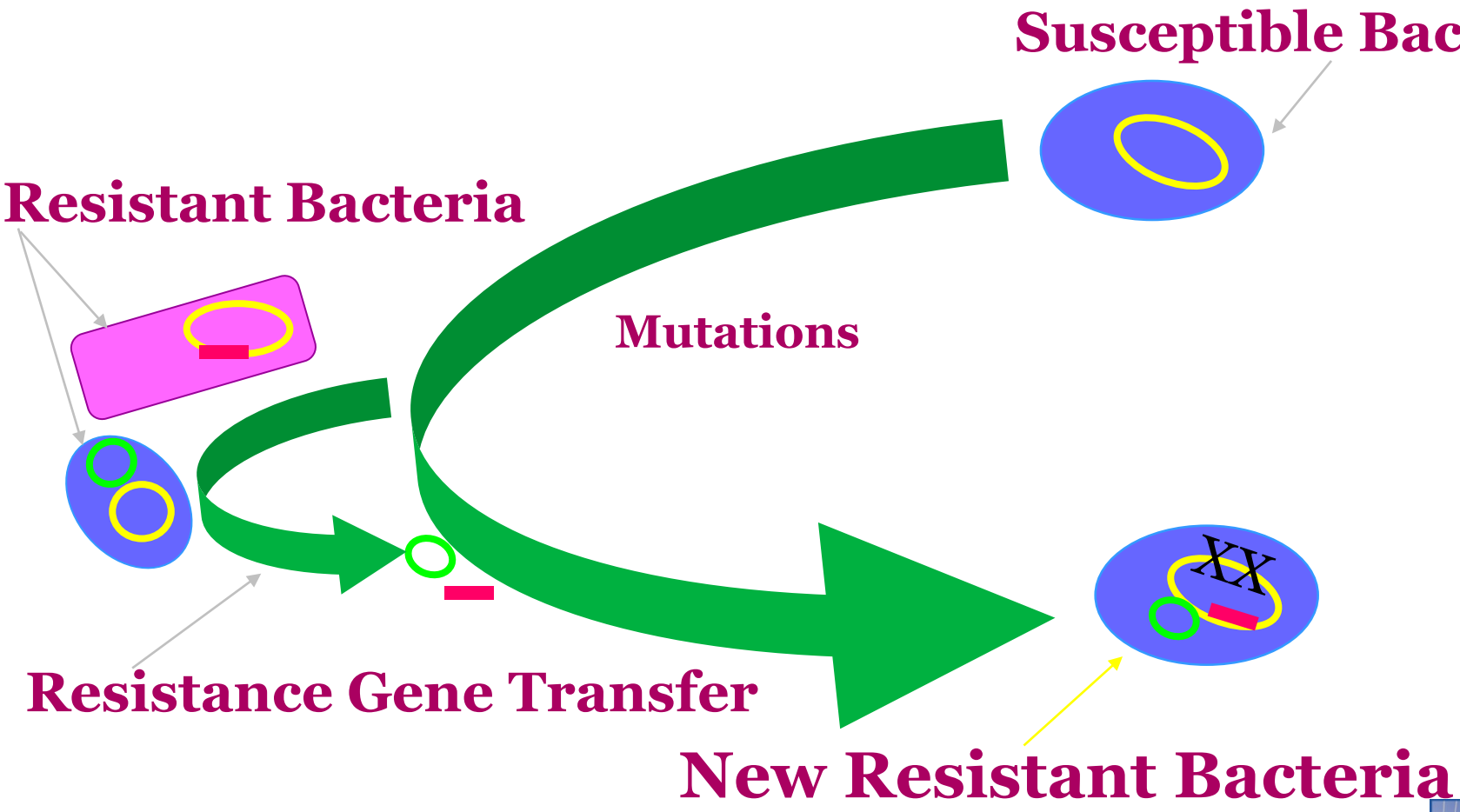
1995: Infectious disease had again become the third leading cause of death, and its incidence is still growing!

“A post-antibiotic era means, in effect, and end to modern medicine as we know it. Things as common as strep throat or a child’s scratched knee could once again kill.”

Margaret Chan, WHO Director General

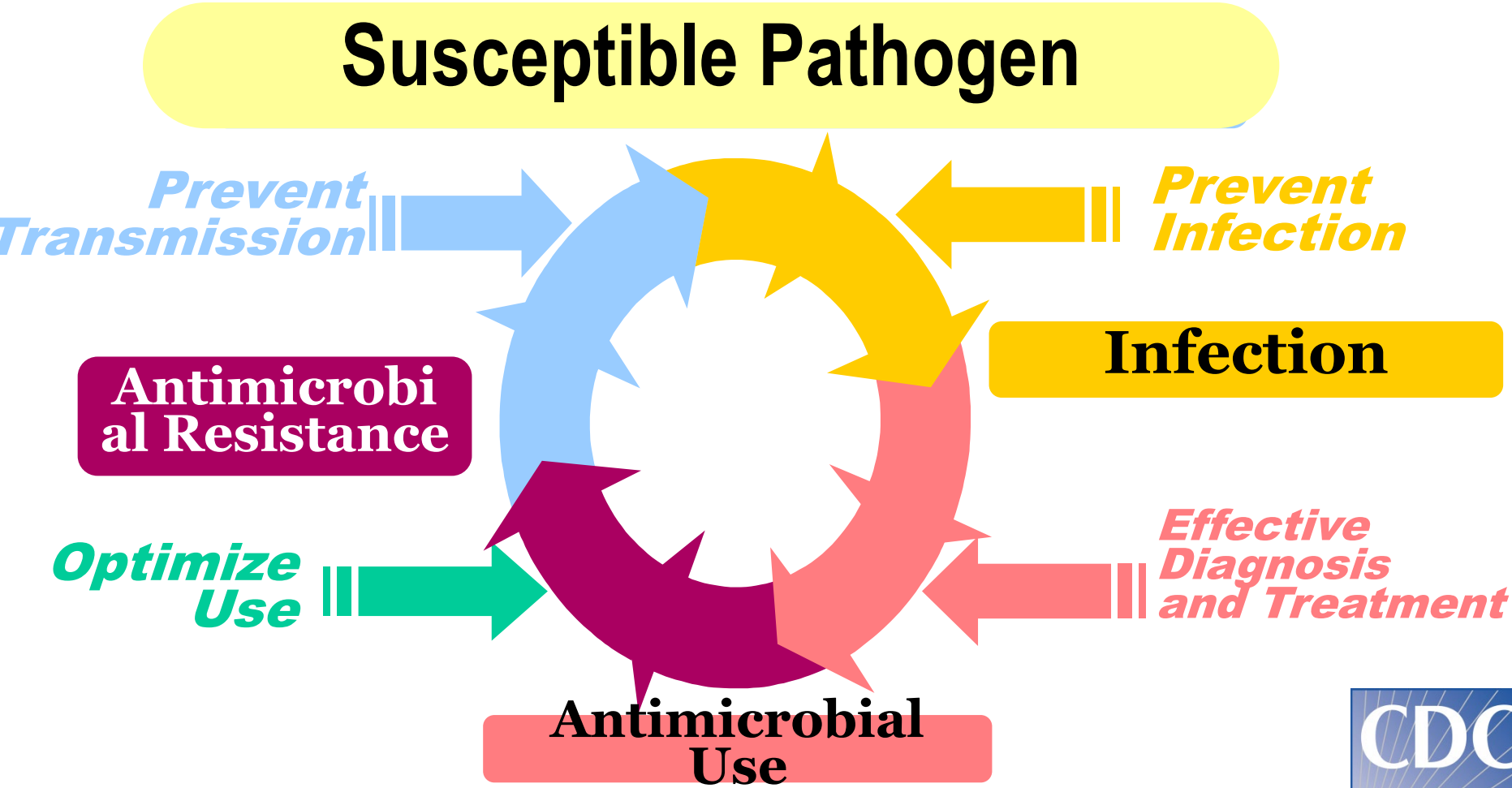
Test Target Treat model

EMERGENCE OF ANTIMICROBIAL RESISTANCE



CDC
Centers for Disease Control and Prevention

ANTIMICROBIAL RESISTANCE: KEY PREVENTION STRATEGIES



CDC
Centers for Disease Control and Prevention

Study on CAP Patients and Therapy

Retrospective study on 175 CAP patients in New York

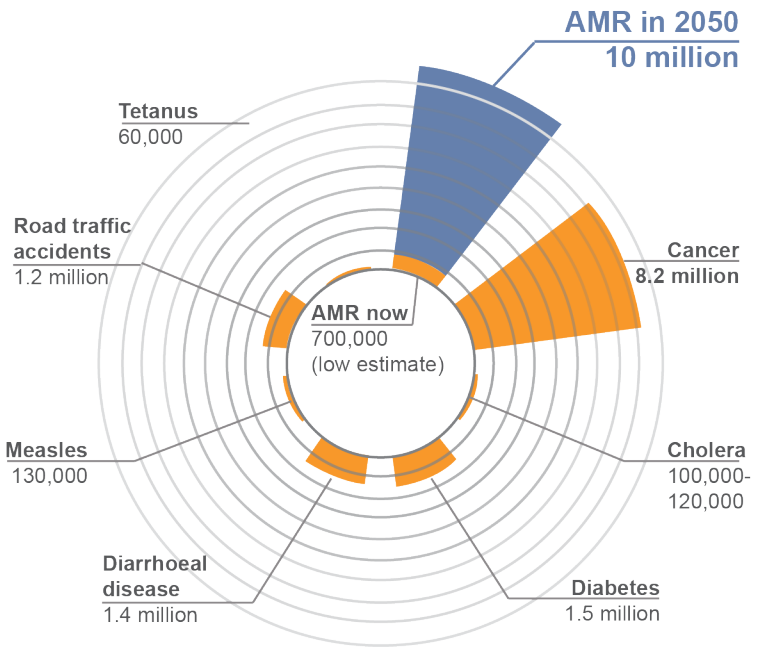
- Exclusion criteria
  - Hospitalization ≥ 2 days within 90 days
  - Residence in nursing home
  - Prior isolation of MDR organism

Rate of multidrug resistant organism detected within 90 days

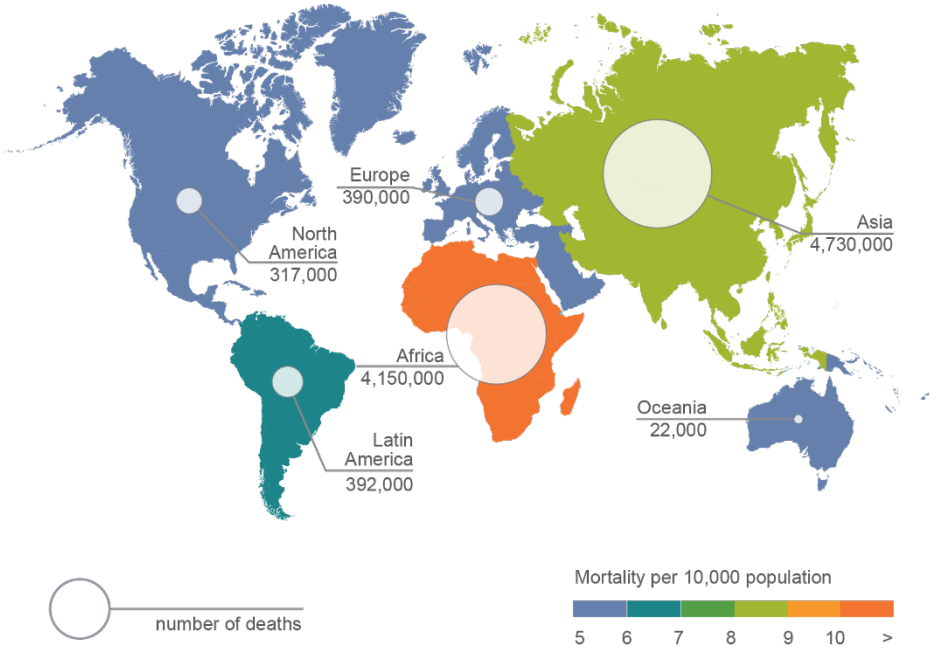
- 15% patients on fluoroquinolone
- 4% of patients on cephalosporin/macrolide

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



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

Molecular Mechanisms

Proprietary and confidential — do not distribute

Pros and Cons of Molecular

Pros

Good for pathogens that you only have when you are sick

- Influenza

Good for living things which would have RNA/DNA

Good to see if active infection & can test where the infection is

- Not things like sepsis

Cons

May only be a screen for bacteria/viruses that people may normally carry

- *Clostridium difficile, S. pneumoniae*

Bad for non living things

- Protein, DOA

Bad for past infection

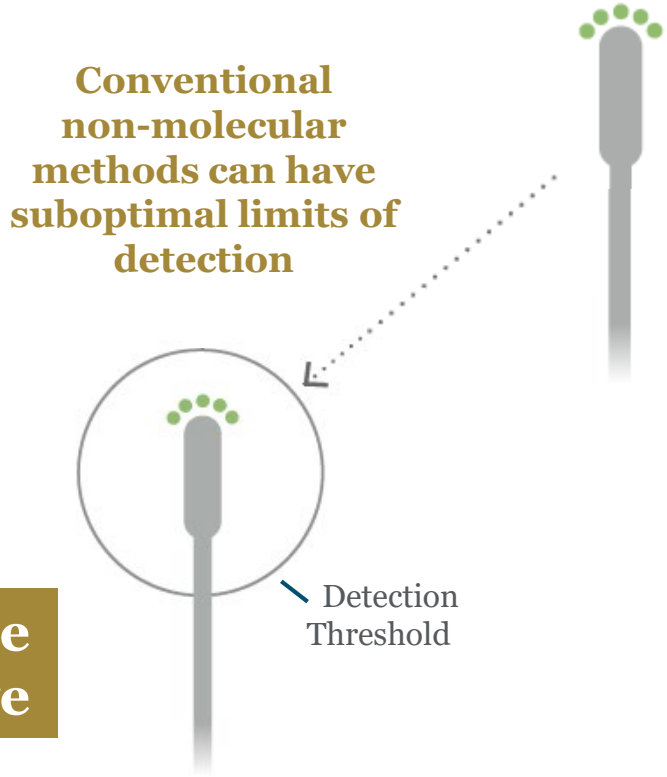
- Want test that detects antibody

Why molecular? The power of sample amplification

Positive Patient Sample

Why molecular? The power of sample amplification

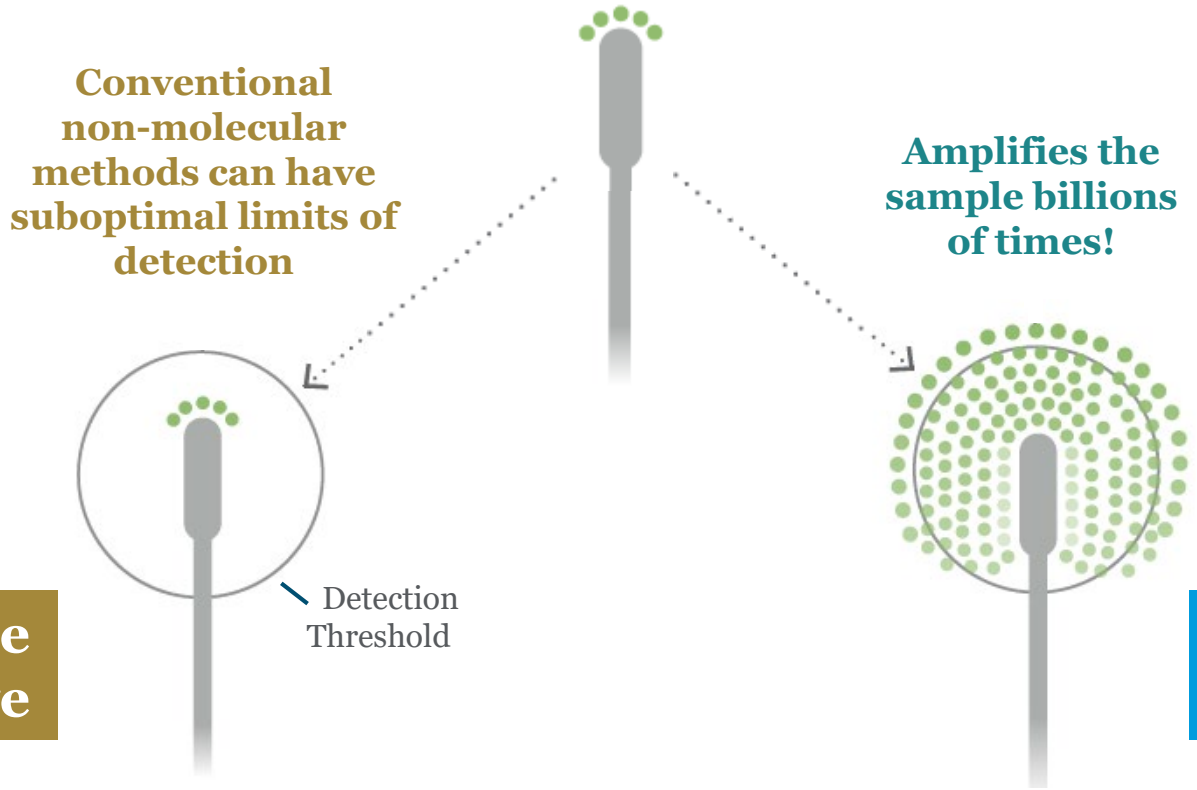
Positive Patient Sample



False Negative

Why molecular? The power of sample amplification

Positive Patient Sample



False Negative

True Positive

Why molecular? The power of sample amplification

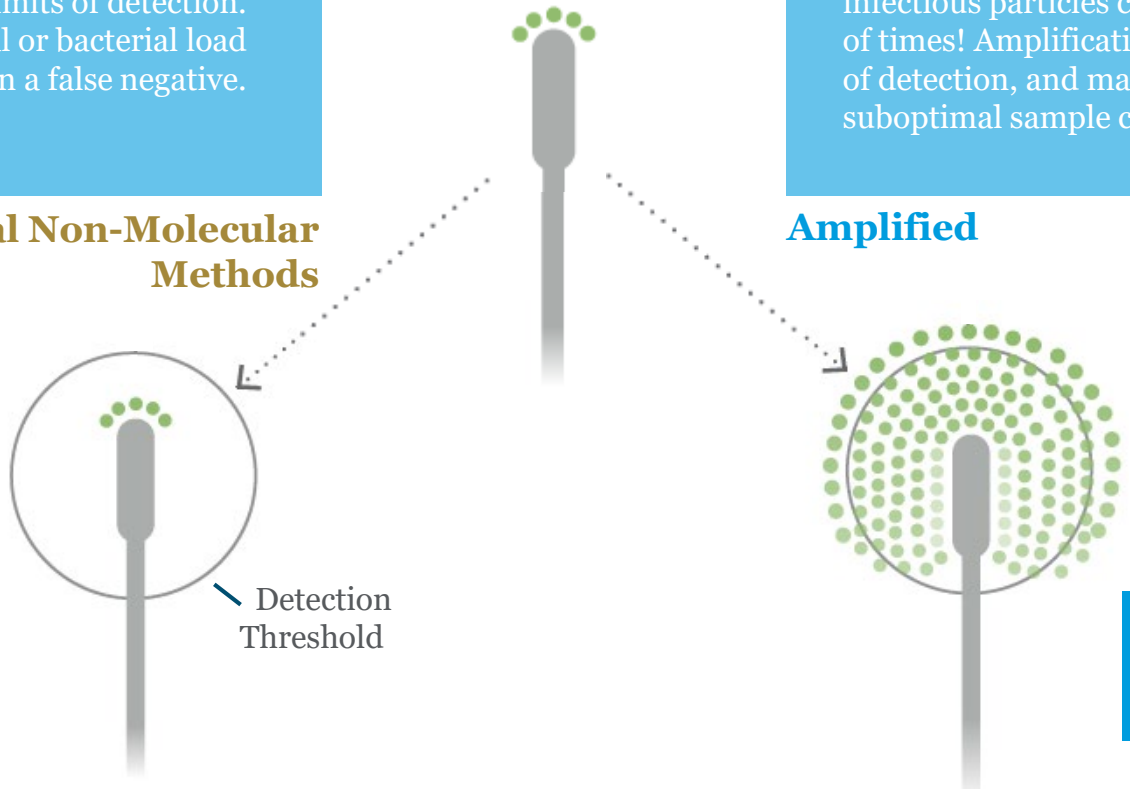
Positive Patient Sample

Conventional *non-molecular* methods can have suboptimal limits of detection. Samples with low viral or bacterial load could result in a false negative.

With *molecular*, even a few hundred infectious particles can be amplified billions of times! Amplification increases likelihood of detection, and may compensate for suboptimal sample collection.

Conventional Non-Molecular Methods

Amplified



False Negative

True Positive

Molecular Tests on the Market

PCR – Polymerase Chain Reaction

- Rely on the ability to amplify due to temperature cycling
- Many traditional molecular companies
- Cepheid – GeneExpert
- Roche LIAT – Lab in a tube

Isothermal

- Rely on the ability to do the reaction at a single temperature
- Meridian's LAMP (loop mediated isothermal amplification)
- Quidel Solana – HDA (Helicase dependent amplification)
- Abbott ID NOW – NEAR / RPA (Nicking enzyme amplification rxn/Recombinase polymerase amplification)

PCR Cycle

Double-stranded DNA

95° Denaturation

Heating separates strands

57° Annealing

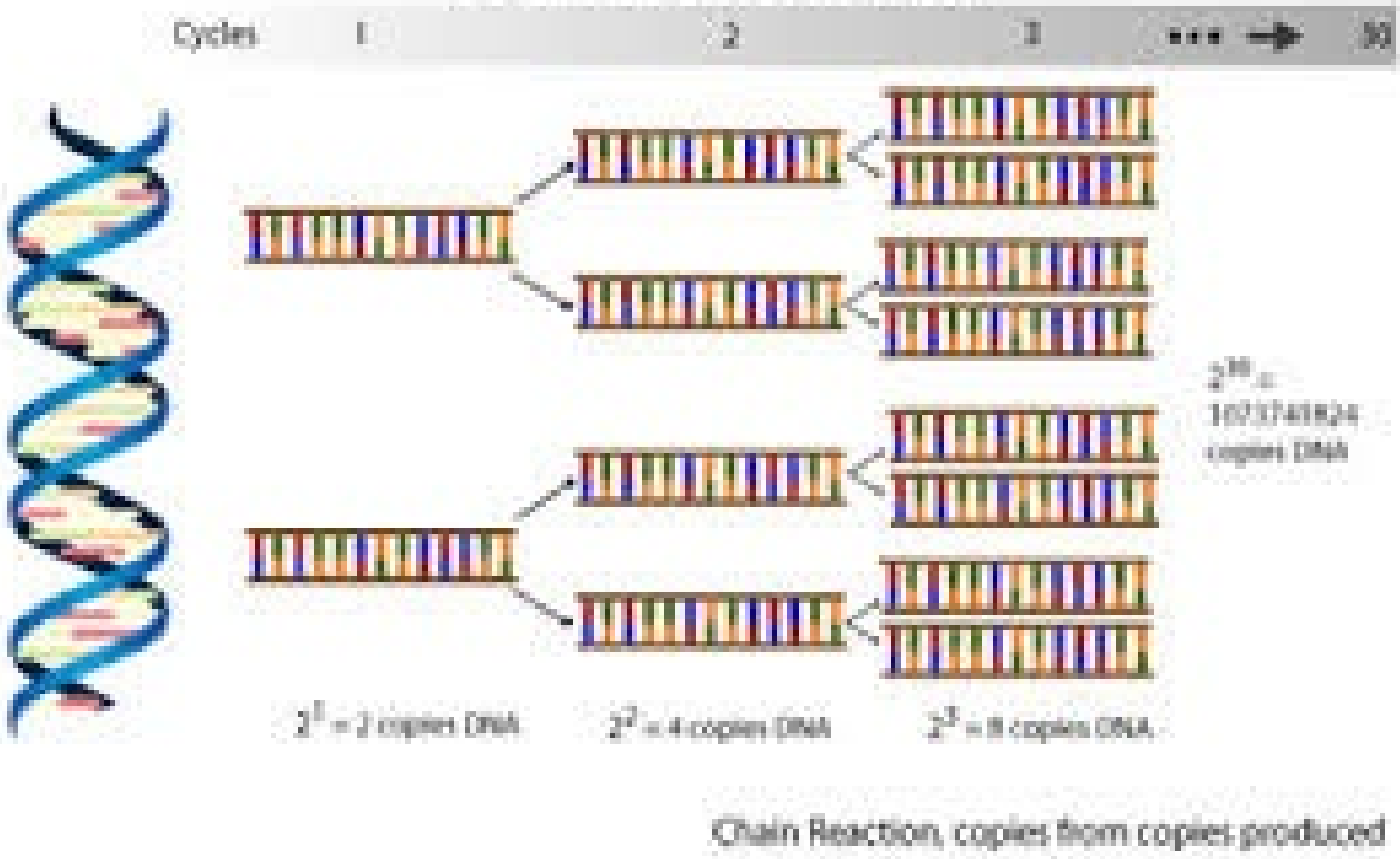
Primers Bind to target sequences

Taq Polymerase Binds at Primer Sites

72° Extension

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

PCR amplification



Chain Reaction, copies from copies produced

GeneXpert - Cepheid

- Minimal hands-on time
- Broad molecular menu
- Multiple Versions

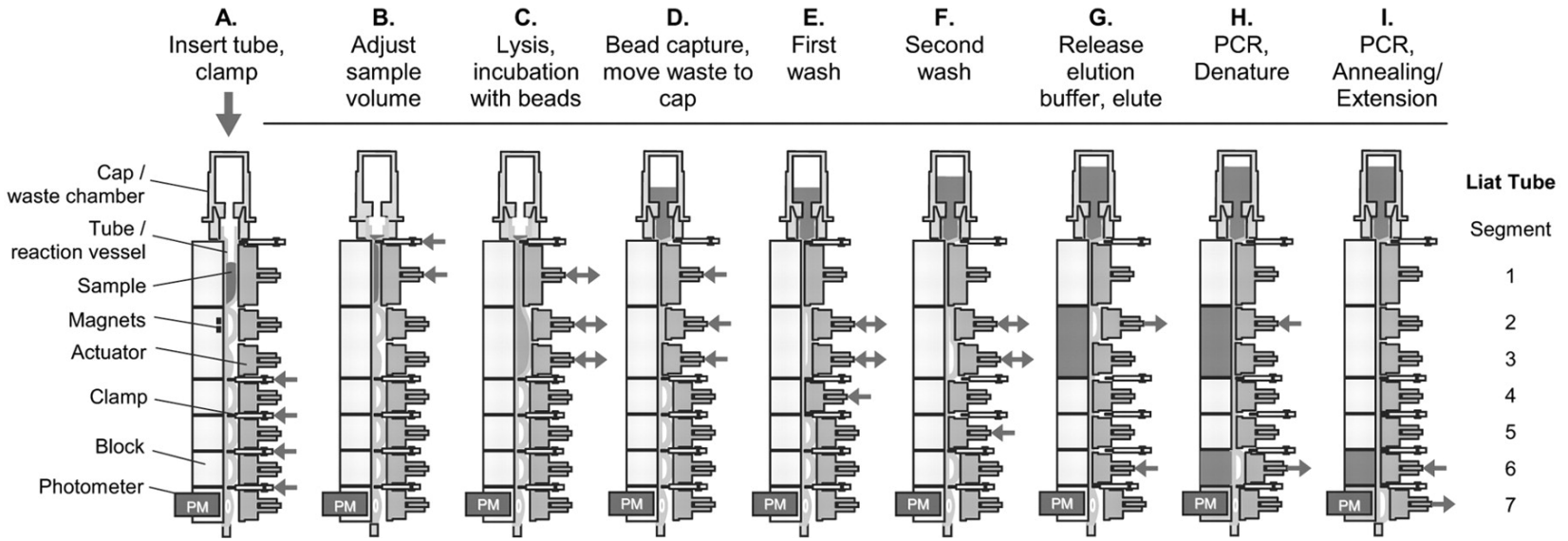
LIAT - Lab In a Tube

Modified PCR

Faster than traditional PCR

Small footprint

Sample processing in the Liat Tube.



Sultan Tanriverdi et al. J Infect Dis. 2010;201:S52-S58

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Sekisui Silaris

- **PCR amplification with lateral flow amplification**
- **CLIA-waived**

Isothermal Molecular Technologies

cHDA : Circular Helicase-dependent amplification
HDA : Helicase-dependent amplification
IMDA : Isothermal multiple displacement amplification
LAMP : Loop-mediated isothermal amplification
MPRCA : Multiply-primed rolling circle amplification
NASBA : Nucleic acid sequence based amplification
NEAR: Nicking enzyme amplification reaction
RAM : Ramification amplification method
RCA : Rolling circle amplification
SDA (RPA): Strand displacement amplification
SMART : Signal mediated amplification of RNA technology
SPIA : Single primer isothermal amplification
TMA : Transcription mediated amplification

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Isothermal Molecular Technologies

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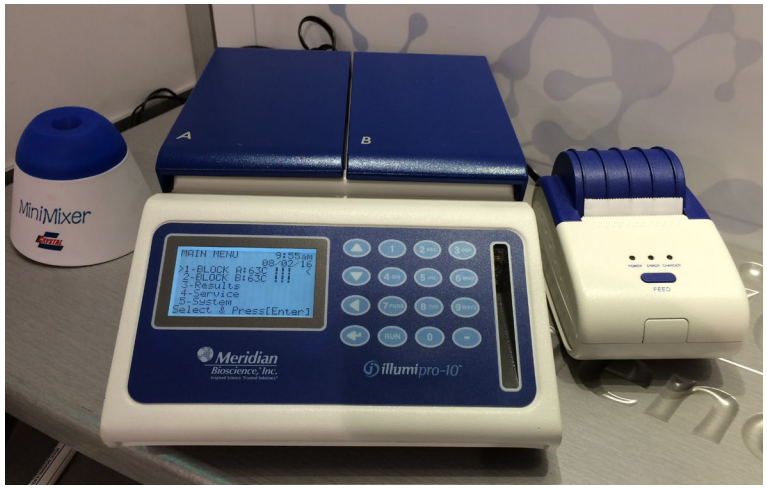
Illumigene – Meridian Bioscience



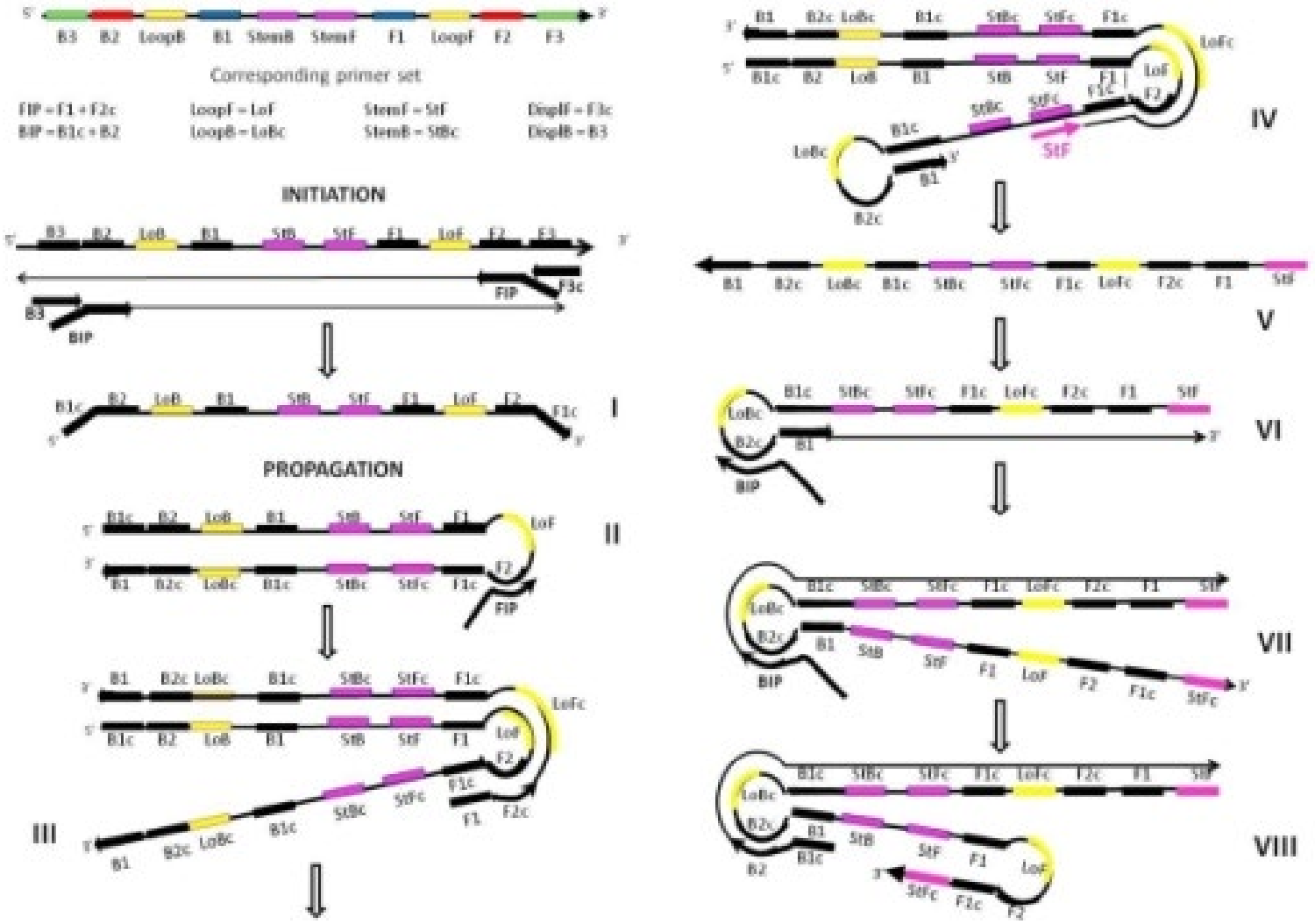
< 60 minutes to results

- Including heat pretreatment step

< 2 minutes hands on time

Small footprint (8.5” x 11”)

Loop Mediated Isothermal Amplification (LAMP)



Solana® - Quidel

35 minutes to results

- Including heat pretreatment step

Small footprint (9.4” x 9.4” x 5.9”)

8.8 lbs

Battery pack available

Data sourced from Quidel Product Labeling

Proprietary and confidential — do not distribute

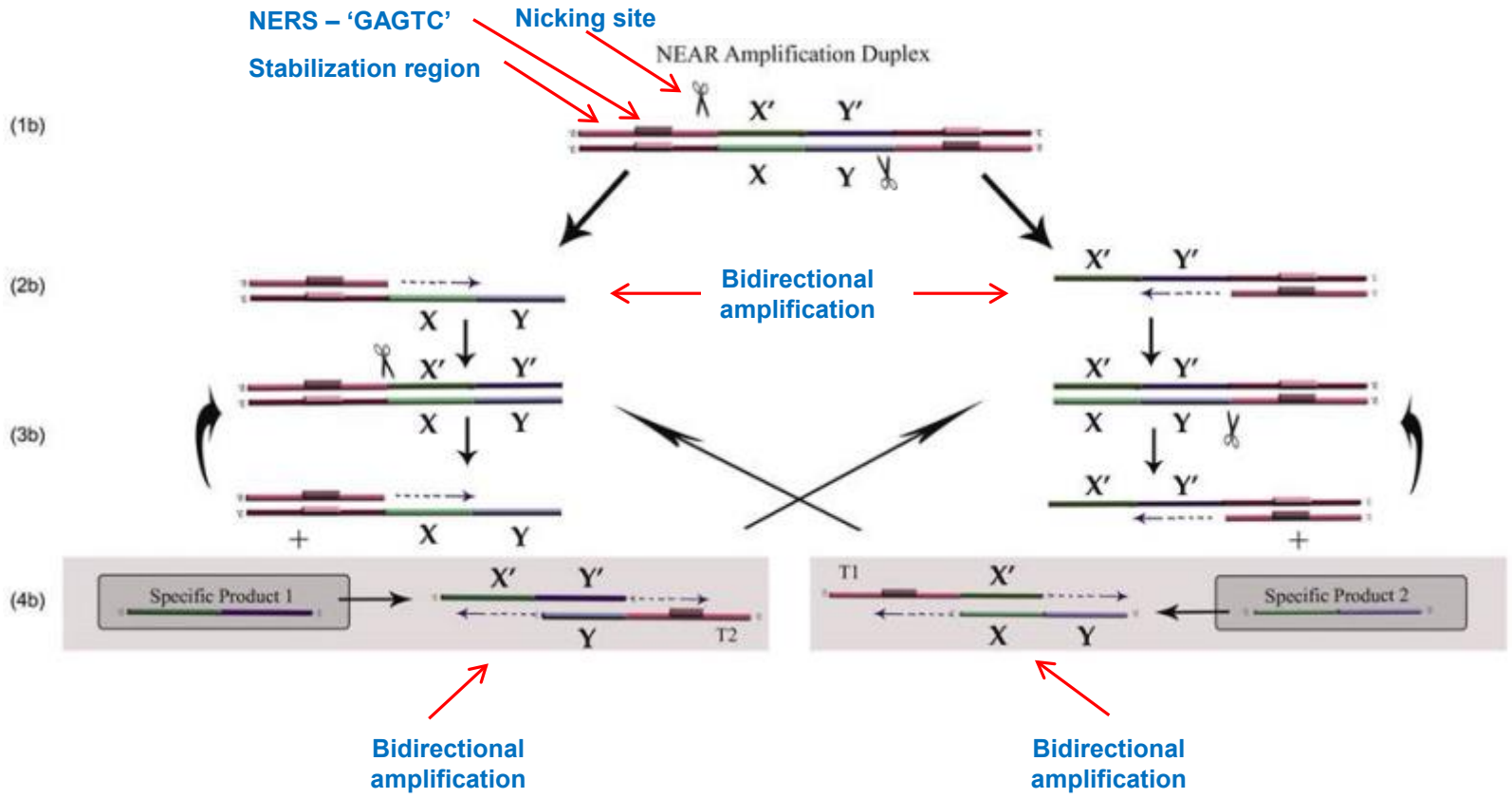
ID NOW System

Currently fastest assay

Minimal hands-on time

Small footprint

NEAR Amplification Duplex – Bidirectional Amplification



Multiplexing Assays

Pros

Able to do multiple pathogens at the same time

- Many pathogens give similar symptoms
- Don't have to do one assay at a time

Cons

Longer time than other rapid molecular

Doesn't do well with commensal bacteria

- *S. pneumoniae* and H. *influenzae*
- *C. difficile*

Not all pathogens are created equally

- Things like influenza, RSV, and hMPV are rare in asymptomatic children and adults
- Rhinovirus and coronavirus can be present in asymptomatic patients and as part of co-infection

Strep A

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How Sensitive is Lateral Flow?

- **Meta-analysis entitled "Rapid antigen detection test for group A streptococcus in children with pharyngitis."**
  - Searched CENTRAL, MEDLINE, EMBASE, Web of Science, CDSR, DARE, MEDION, and TRIP from January 1980 to July 2015.
  - 98 studies with 116 test evaluations and 101,121 participants
- **Results**
  - Lateral flow had sensitivity of 85.6% (CI of 83.3 to 87.6)
  - Lateral flow had a specificity of 95.4% (CI of 94.5 to 96.2)
- **Conclusion**
  - If 1000 children come in and prevalence of group A Strep (GAS) is 30%, 43 will be missed

Cohen JF, Bertille N, Cohen R, Chalumeau M.
Rapid antigen detection test for group A streptococcus in children with pharyngitis.
Cochrane Database of Systematic Reviews 2016, Issue 7. Art. No.: CD010502.
DOI: 10.1002/14651858.CD010502.pub2.

Sore Throats

Strep throat

- 15-30% of childhood cases
- 10% of adult cases
- Transmission in close contact is around 35%
- Incubation is 2 – 4 days

Symptoms of Strep throat

- Sore throat with difficulty swallowing
- Red, swollen tonsils, maybe with white patches
- Fever (usually 101°F or above)
- Swollen lymph notes on neck
- Small red spots on roof of mouth or area in back
- Headache with potential nausea or vomiting
- Body ache or rash

http://www.cdc.gov/Features/strepthroat/

Credit: National Library of Medicine

Scarlet Fever

General information

- Tends to be a mild infection
- Breaks out 2 to 5 days after exposed
- Starts with a fever and sore throat

Symptoms

- May have chills, vomiting, or abdominal pain
- Tongue may be “strawberry-like”
- Rash before illness or up to 7 days later
  - Starts usually neck, underarm, and groin and then spreads over body
  - Small blotches that turn into bumps that feel like sandpaper
  - Lasts about 7 days

Long-term health problems are uncommon, but possible

- Abscesses around tonsils, pneumonia, arthritis, kidney disease, rheumatic fever

https://www.cdc.gov/groupastrep/diseases-public/scarlet-fever.html

Pictures of Scarlet Fever

https://en.wikipedia.org/wiki/Scarlet_fever

Rheumatic fever

General information

- Disease that affects heart, joints, brain, and skin if strep throat not treated properly
- 1 to 5 weeks later
- Thought to be due to body's response to strep throat or scarlet fever

Symptoms

- Fever
- Painful joints (arthritis)
- Symptoms of congestive heart failure
- Fatigue
- Jerky, uncontrollable body movements
- May have heart murmur, enlarged heart, or fluid around heart

Major complication

- If rheumatic fever not properly treated, may weaken the heart valves so need heart surgery

https://www.cdc.gov/groupastrep/diseases-public/rheumatic-fever.html

PANDAS

Pediatric autoimmune neuropsychiatric disorders associated with Streptococcal infections

- Obsessive compulsive disorder (OCD) and/or tic disorders following strep throat
- Symptoms get worse aftr strep throat

Cause

- Strep A hides from immune system by having molecules look like "self," in heart, joints, skin, and brain tissue
- "Anti-brain" antibodies can cause PANDAS

https://www.nimh.nih.gov/health/publications/pandas/index.shtml

Sore Throat Etiology

Bacteria

- *Strep* A
- *Strep* G & C
- *Arcanobacterium haemolyticum*
- *Corynebacterium diptheriae*
- *Neisseria gonorrhea*
- Yersinia enterocolitica

Viruses

- Adenovirus
- Rhinovirus
- Coronavirus
- Epstein-Barr Virus
- Herpes
- Enterovirus
- HIV
- Coxsackie virus
- Cytomegalovirus
- Parainfluenza virus

2012 IDSA Guidelines Pharyngitis. Table 3.

Culture. . .An Imperfect Gold Standard

Culturing of Strep A requires living bacteria

Potential issues

- Patient starts taking antibiotic from previous illness
- Delay in time before throat swab is put on blood agar plate
- Poor conditions transporting swab to laboratory

IDSA: What An Accurate Diagnosis Means

Prevention of rheumatic fever

Prevention of pus-forming complications

- Peritonsillar abscess, cervical lymphadenitits, mastoiditis, etc.

Improve clinical symptoms and signs

Decrease in contagiousness

- Less transmission to family members, schools, daycares

Rapid resumption of usual activities

Minimize potential of inappropriate antimicrobial therapy

Clinical practice guideline for the diagnosis and management of Group A Streptococcal pharyngitis: 2012 update by the Infectious Diseases Society of America. CID 2012: 55. Page e89.

Influenza A & B

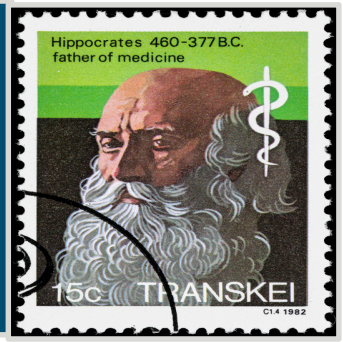
The old thinking. . . “it’s just the flu”

A bit of history

Flu History[1]:

- Flu epidemics: every 1 to 3 years for at least the last 400 years
- Pandemics (worldwide) occur around every 10 to 50 years

Hippocrates described flu back in the 5th century BC.



Columbus brought a devastating flu on his second voyage to the new world.

1. Saunders-Hastings PR, Krewski D. Reviewing the History of Pandemic Influenza: Understanding Patterns of Emergence and Transmission. Young LS, ed. *Pathogens*. 2016;5(4):66. doi:10.3390/pathogens5040066

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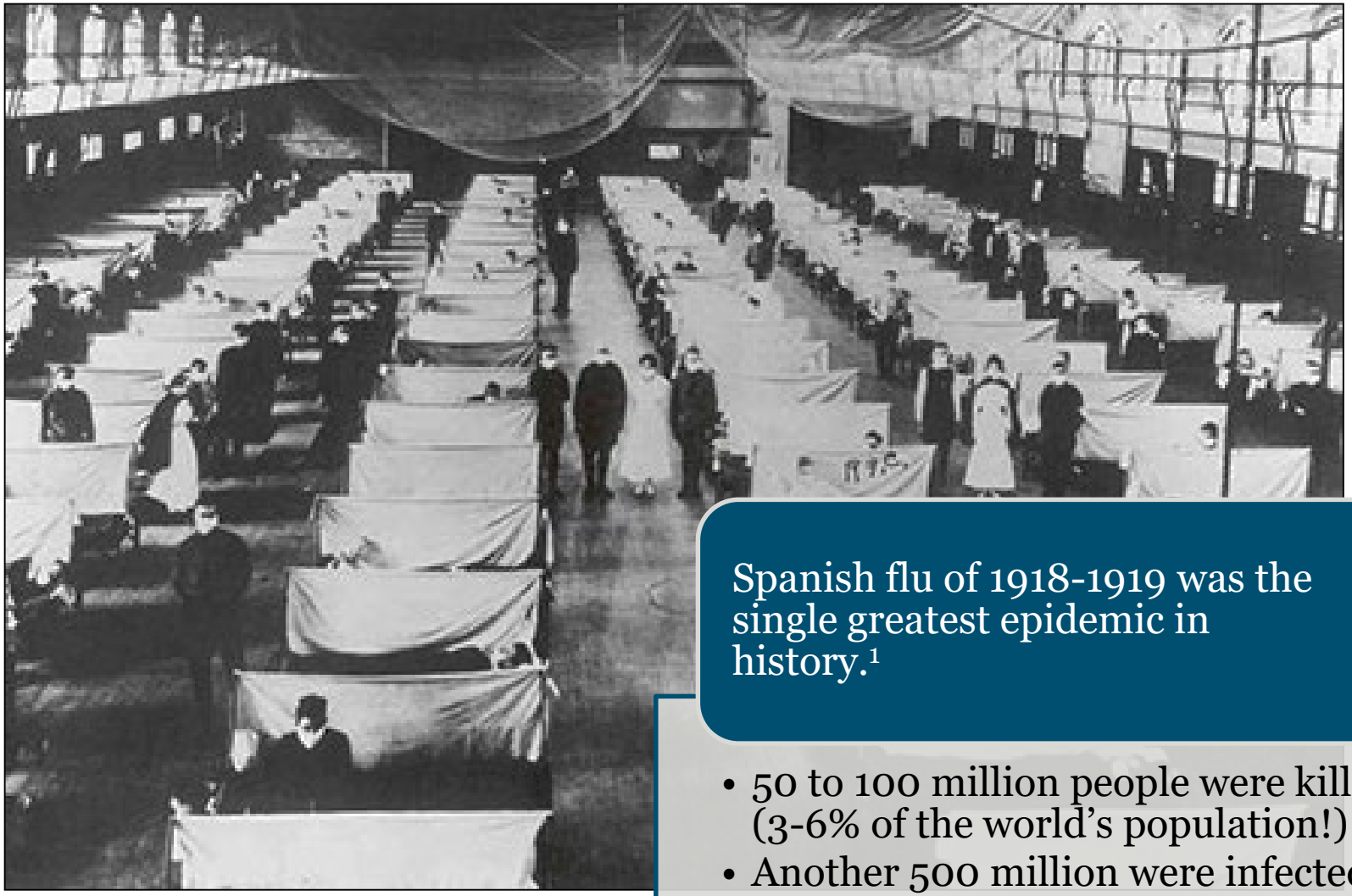
1918 Flu Pandemic – Spanish Flu



Spanish flu of 1918-1919 was the single greatest epidemic in history.[1]

- 50 to 100 million people were killed[1] (3-6% of the world's population!)
- Another 500 million were infected[1] (1/3 of the world's population)

1. CDC. Influenza (Flu). 1918 Pandemic. https://www.cdc.gov/flu/pandemic-resources/1918-pandemic-h1n1.html

Proprietary and confidential — do not distribute

1918 Flu Pandemic – Spanish Flu

1918 Spanish Flu Pandemic broke out during WWI. Image of WWI soldiers.

Policemen in Seattle wearing masks made by the Red Cross, during the influenza epidemic. December 1918.

National Archives at College Park, MD. Record number 165-WW-269B-25.

Proprietary and confidential — do not distribute

Average Disease Burden of Influenza A&B in the US[1]

Annual Impact of influenza in the US

- Cases 9,200,000 – 35,600,000
- Hospitalizations up from 140,000 – 710,000
- Deaths between 12,000 – 56,000
- Influenza target population: 188MM in US

5-20% of US population affected by influenza each year

Most deaths affect elderly and young children

- Also affects otherwise healthy individuals

Proprietary and confidential — do not distribute

1. Centers for Disease Control and Prevention. www.cdc.gov/flu/keyfacts.htm

What are the issues of respiratory disease?

The symptoms of respiratory diseases are vague

- Pneumonia symptoms
  - Cough
  - Fever
  - Chills
  - Difficulty breathing
- Influenza
  - Cough
  - Fever
  - Chills
  - Malaise

Treatment is different

- Bacteria
  - Broad spectrum antibiotic
  - Narrow spectrum antibiotic
- Influenza
  - Antiviral
  - Treat symptoms only

Complications of mistreatment

- Mistreatment of bacterial etiology
  - May increase morbidity/ mortality
  - May have longer hospital stay
  - May get *C. difficile*
- Mistreatment of influenza
  - May have increased resistance and *C. difficile*

Treatment

- Ideally, antivirals should be taken within first 48 hours. Greatest benefit is when started as close to onset as possible.
- Some people do not go in on first day of symptoms.
- Treatment window can be small.
- Some influenza strains are resistant to Tamiflu

Pregnancy

In pregnancy, the immune system adapts to tolerate a foreign body

- Allows for increased risk in infections such as influenza

Pregnant women

- Increased morbidity & mortality
- Increased risk of hospitalization due to influenza complications
- More likely to have acute cardiopulmonary illness when there is a seasonal influenza epidemic

2009 Novel H1N1 Outbreak

- Pregnant women are about 1% of population yet 5% of deaths were pregnant women
- Antiviral treatment in first 2 days reduced mortality

Postpartem Risk

- Increased risk of complications especially in first week

Rasmussen, SA, Jamieson DJ, and Uyeki TM. Effects of influenza on pregnant women and infants. American Journal of Obstetrics & Gynecology. 2012.

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Influenza Effect on Newborns

Data from a study suggests women with respiratory illness during flu season more likely to have lower birthrate

During novel H1N1 outbreak, the UK reported increased perinatal mortality rate

Potential preterm birth

Potential lower birth weight

Infants less than 6 months

- Higher rate of severe complications
- Higher rate of excess hospitalizations for cardiopulmonary conditions when influenza circulating

Rasmussen, SA, Jamieson DJ, and Uyeki TM. Effects of influenza on pregnant women and infants. American Journal of Obstetrics & Gynecology. 2012.

Elderly Patients

Influenza/pneumonia is eighth leading cause death in US[1]

- Risk of hospitalization and death is increased for elderly[2]

Symptoms

- Elderly patients may not present with fever[2]

Vaccine effectiveness

- Can be drop in the effectiveness of the vaccine for elderly people[2]

[1]https://www.cdc.gov/nchs/fastats/leading-causes-of-death.htm
[2]Elliot AJ, and Fleming DM. Influenza and respiratory syncytial virus in the elderly. Expert Reviews. 2008.

RSV

How many people have had influenza in their lives?

How many people have had RSV in their lives?

Abbott

Almost ALL people in this room

had RSV by the age of 2!

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Prevalence / Incidence

RSV accounts for
1 in every 13 visits to pediatrician[3]

177,000 hospitalizations & 14,000 deaths
per year in over 65[6]

126,000 infants hospitalized every year with RSV[7]

20% are premature infants

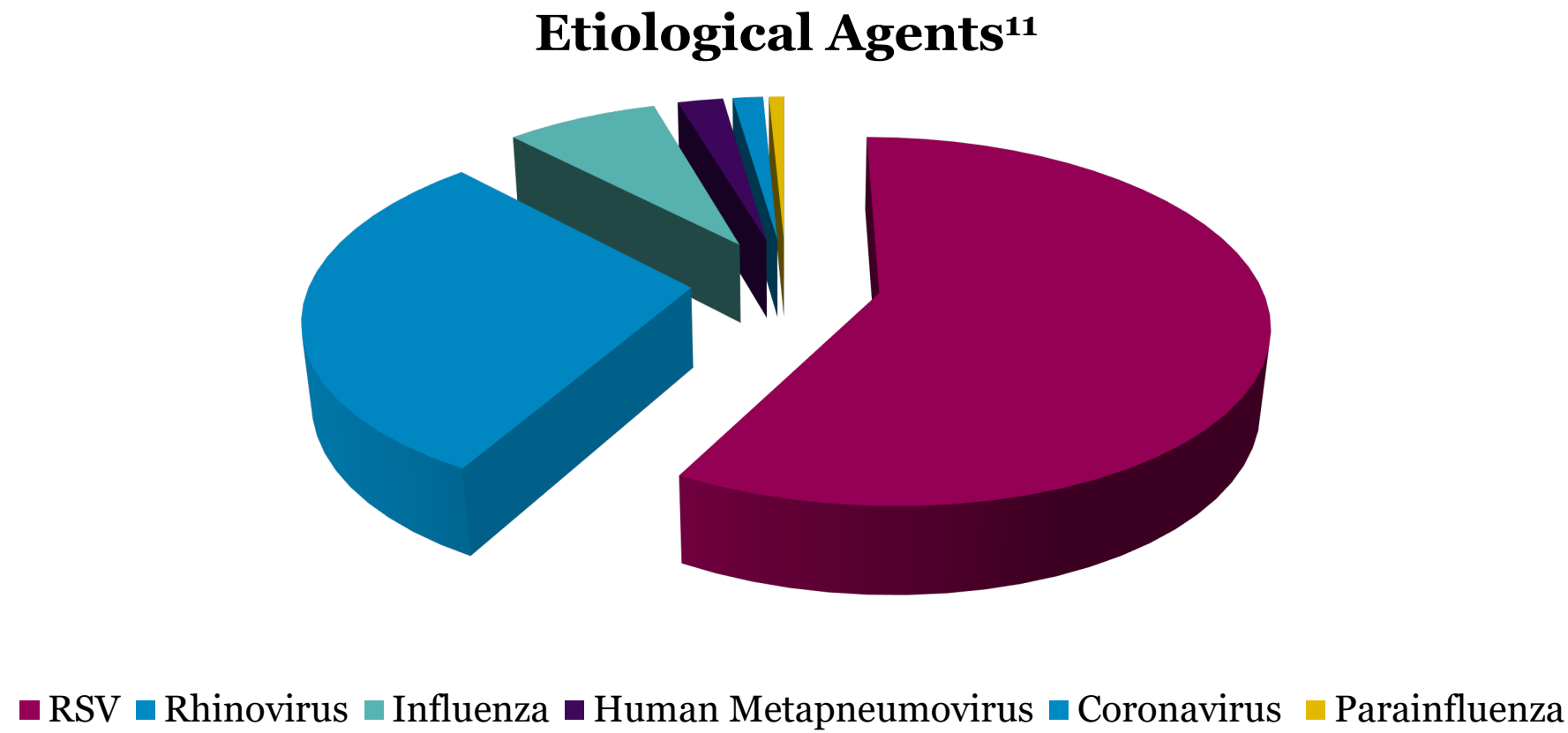
Most common cause of pneumonia in < 1 year old

By age 3, virtually every child has had RSV!

Infects 50% infants in first year of life

400 children each year[7]
under the age of 1 die due to RSV

Causes of Bronchiolitis



In children under 2 years of age presenting with acute bronchiolitis – RSV is the etiologic agent in 60-80% of cases.[12]

Data sourced from: Ralston SL, Lieberthal AS, Meissner HC, et al. Clinical Practice Guideline: The Diagnosis, Management, and Prevention of Bronchiolitis. Pediatrics. 2014;134(5):e1474–e1502. DOI:10.1542/peds.2014-2742

Why Care about RSV in the Elderly?

Estimated that RSV
kills 10,000 people
over the age of 65 every year in the United States![5]

170,000 hospitalizations[5]

Responsible for
17 of every 100 deaths
that happen in a nursing home[5]

$150 to $698 million
in hospitalization costs annually in 1998[14]

$11,000 per RSV hospitalization in 1998[14]

RSV in Elderly[15]

In the elderly population, immune systems wane and they can be prone to getting diseases again

- **Clinical impact of RSV is similar to non-pandemic influenza**
- **Elderly risk groups**
  - Have underlying cardiopulmonary disease
  - Frailty
  - Living in long-term care facilities
  - Immune compromised
- **Symptoms**
  - Mild cold to severe respiratory distress
  - Oncology wards
- **Hospital outbreaks**
  - Oncology wards, bone marrow transplant, surgical wards…

Why Test for RSV?

Reduce transmission to others

- Shouldn't bring child to daycare
- Grandparent to baby or baby to grandparent

Appropriate care

- Antibacterial and antiviral medications don't help
- Potential over-the-counter medications. . .consult your doctor

Monitor child

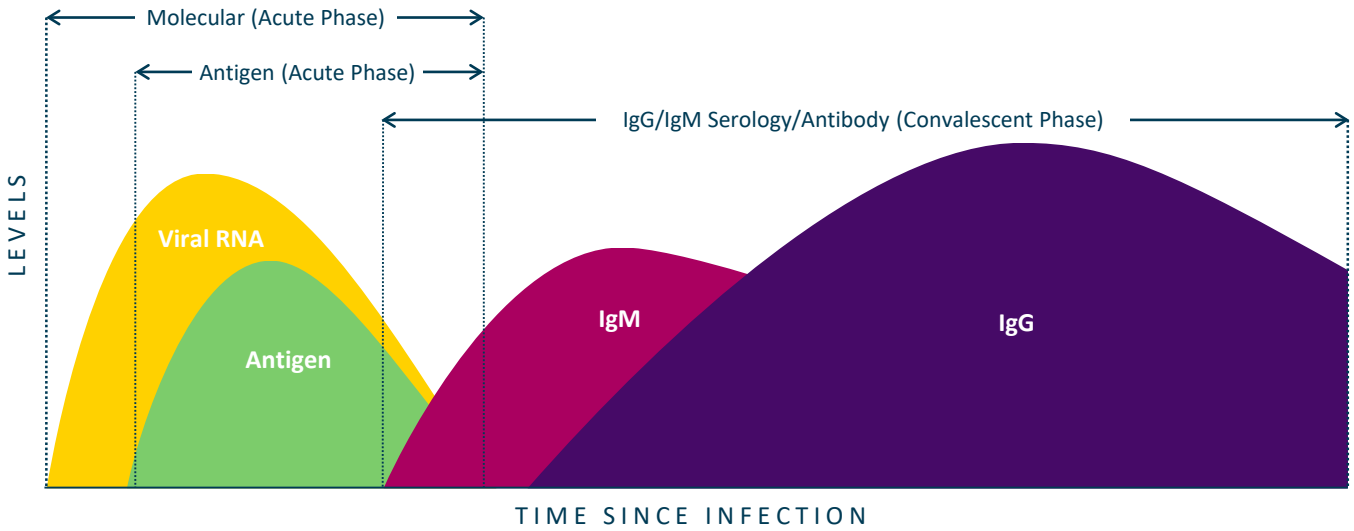
- May need to be hospitalized if have trouble breathing or dehydrated

Peace of mind what is going on!

https://www.cdc.gov/rsv/about/symptoms.html

COVID-19

Immunological response to viral infections



NOTE: This is a typical viral load patient response and is not specific to COVID-19

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SARS-CoV-19 Viral Titers

- **Incubation period**
  - Not fully known
  - The range is usually 2-14 days
- **Time you are infectious**
  - Not fully known
- **How long is virus shed**
  - A study from Wuhan reported that virus was detected for a median of 20 days (up to 37 days among survivors) after symptom onset, but infectiousness may decline significantly 8 days after symptom onset[1]
- **CDC recommendation on returning to work options[2]**
  - 3 days since recovery (fever) or
  - 10 days since symptoms first appeared or
  - Negative on molecular test up on two collections taken over 24 hours apart

[1]https://www.nature.com/articles/s41591-020-0869-5
[2]https://www.cdc.gov/coronavirus/2019-ncov/hcp/return-to-work.html

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Questions?