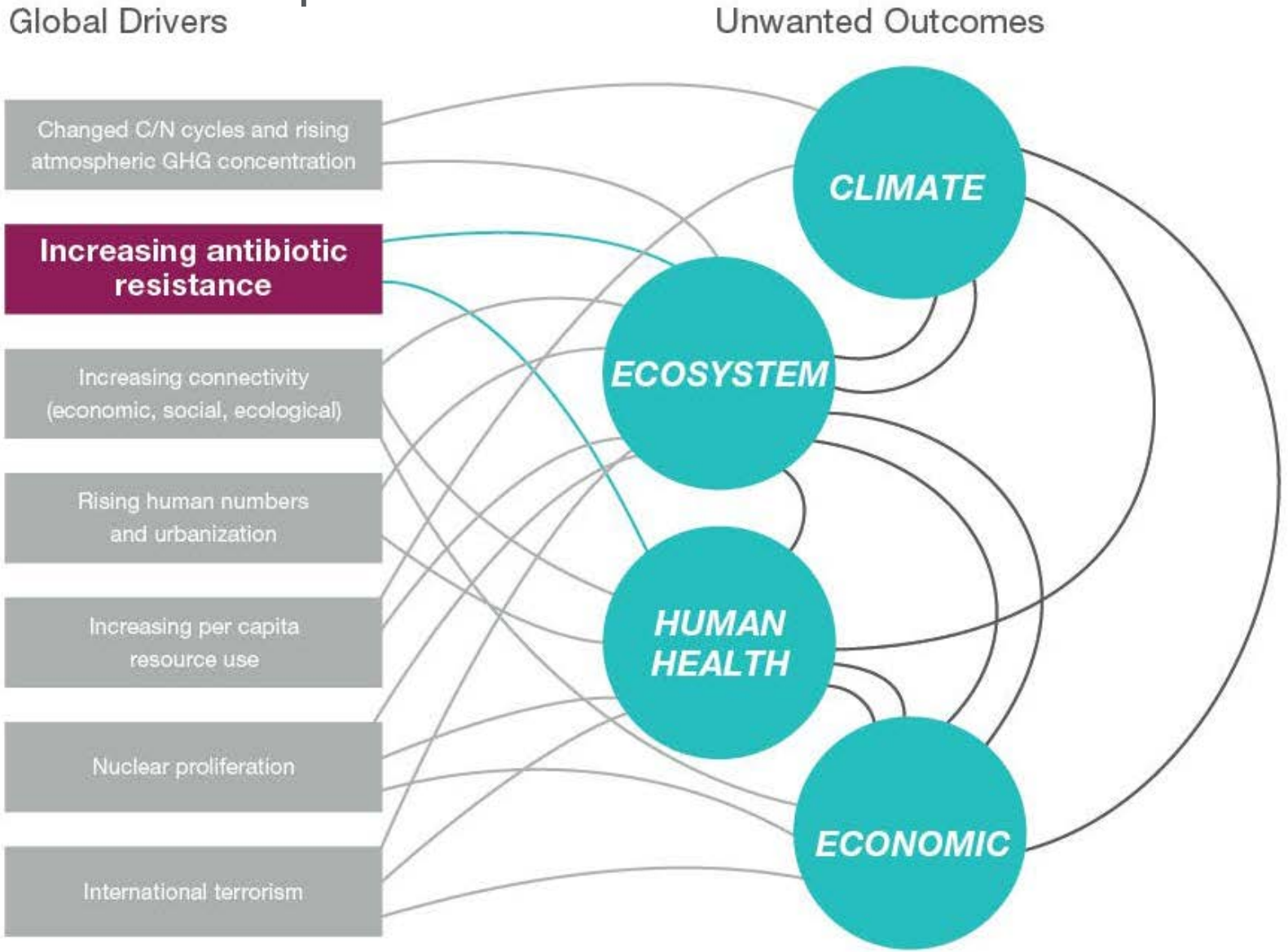
TEST
TARGET
TREAT™

Molecular Testing at Point-of-Care

The Case for Diagnostics to Better Direct Therapy

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

One of the top 7 issues that threatens the human race



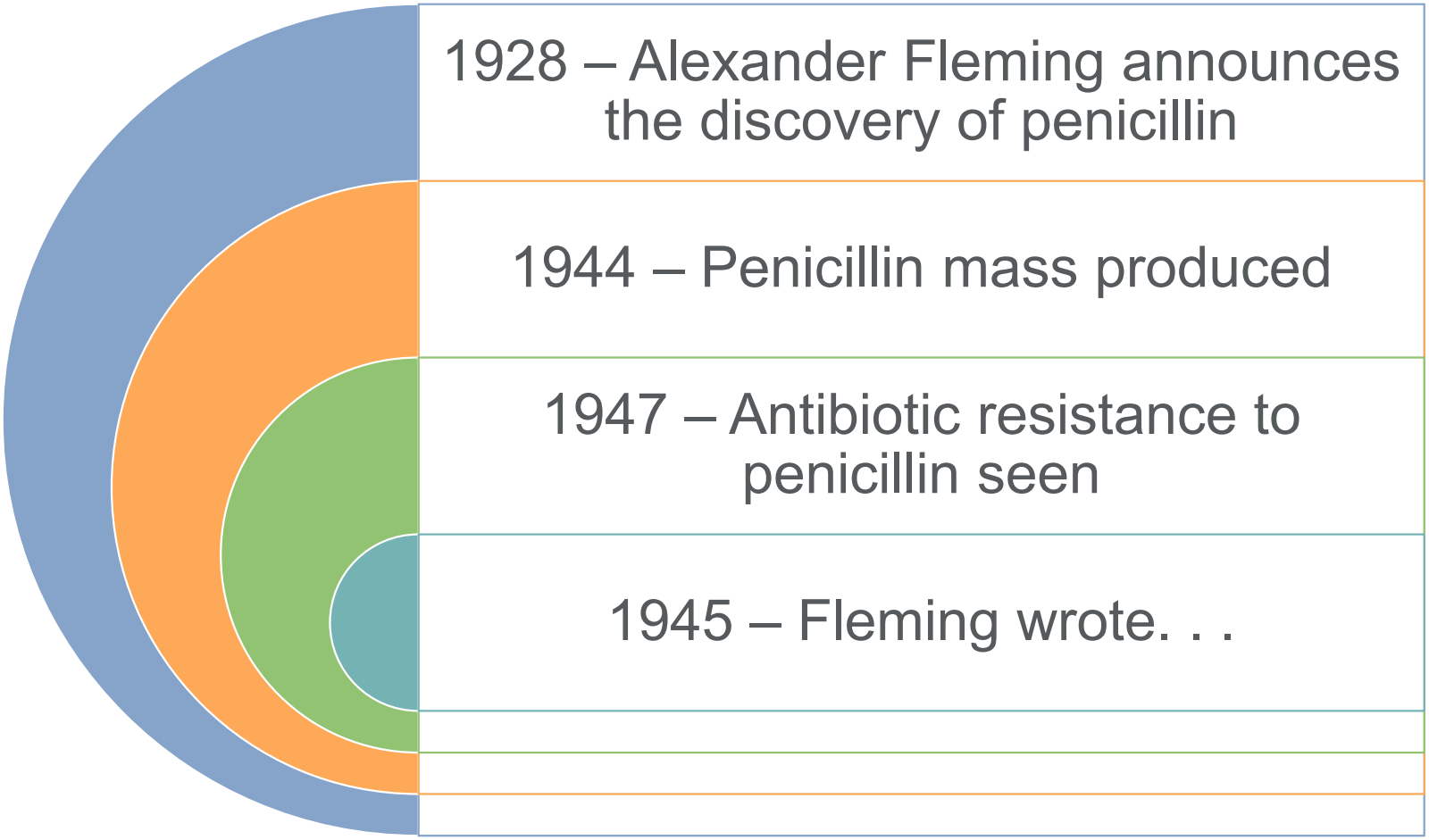
Source adapted from: Science, Vol 325, September 2009
Available at: http://www.sciencemag.org/content/325/5948.cover-expansion

Infectious Disease in the US

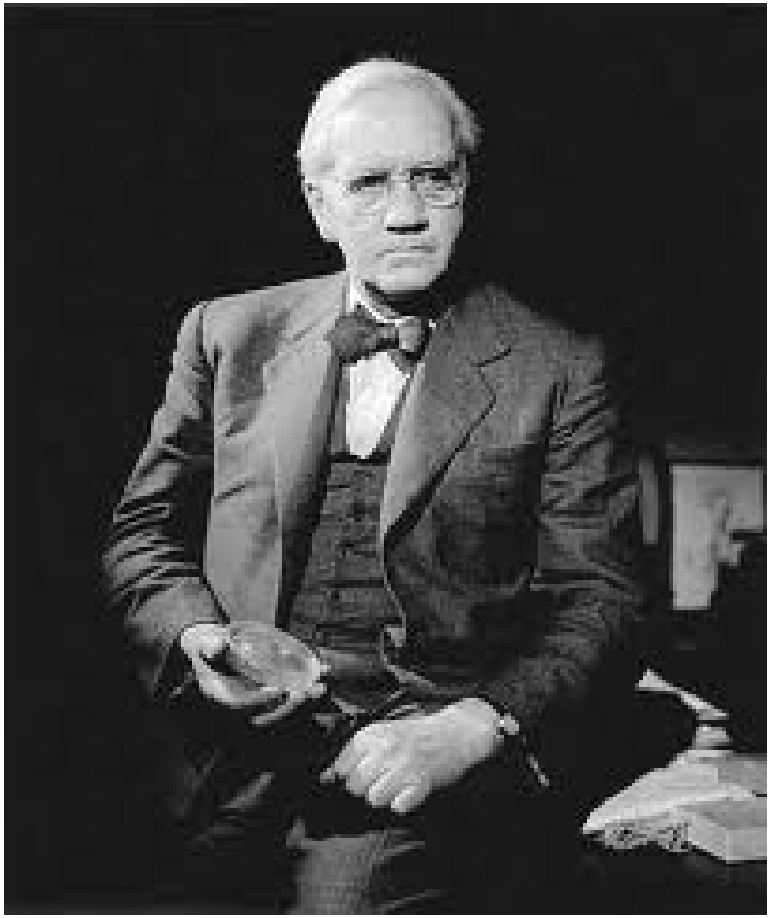
1970: William Stewart, the Surgeon General of the United States declared the U.S. was “ready to close the book on infectious disease as a major health threat”; modern antibiotics, vaccination, and sanitation methods had done the job.

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

Drug Resistance Rates Can Occur Quickly!



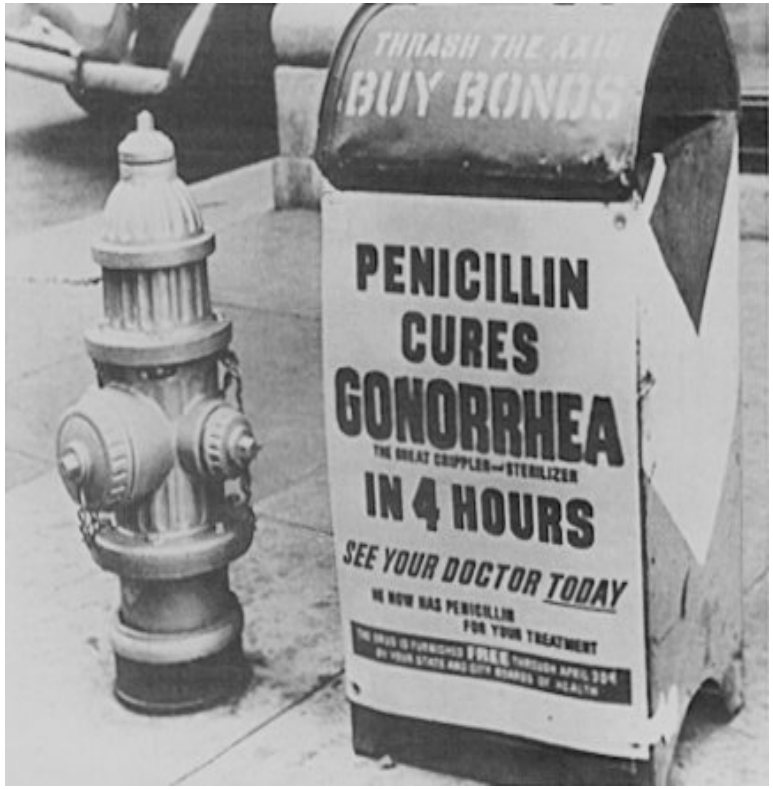
Sir Alexander Fleming

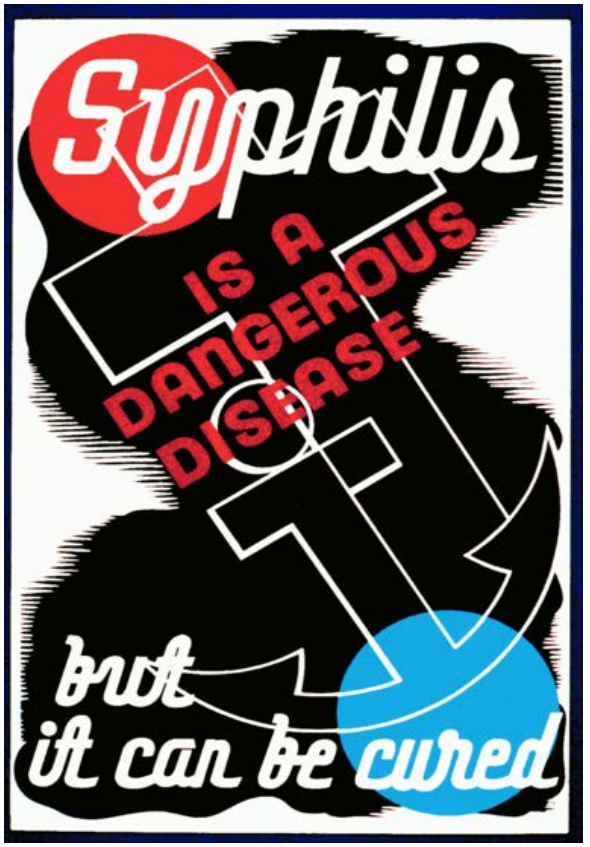


The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily under dose himself and, by exposing his microbes to non-lethal quantities of the drug, educate them to resist penicillin.

Nobel lecture, 1945

How it was





Drug store in Mexico

The Costs of Antibiotic Resistance

Antibiotic resistance increases the economic burden on the entire US healthcare system

- Resistant infections cost more to treat and can prolong healthcare use

More than $1.1 billion is spent annually on unnecessary antibiotic prescriptions for respiratory infections in adults

In total, antibiotic resistance is responsible for:

- $20 billion in excess healthcare costs
- $35 billion in societal costs
- 8 million additional hospital days

CDC – Get Smart Campaign

Inpatient Settings

One in every three patients will receive two or more antibiotics in the course of their hospital stay

Of the patients receiving antibiotics, three out of every four will receive unnecessary or redundant therapy, resulting in excessive use of antibiotics

CDC – Get Smart Campaign

Outpatient Settings

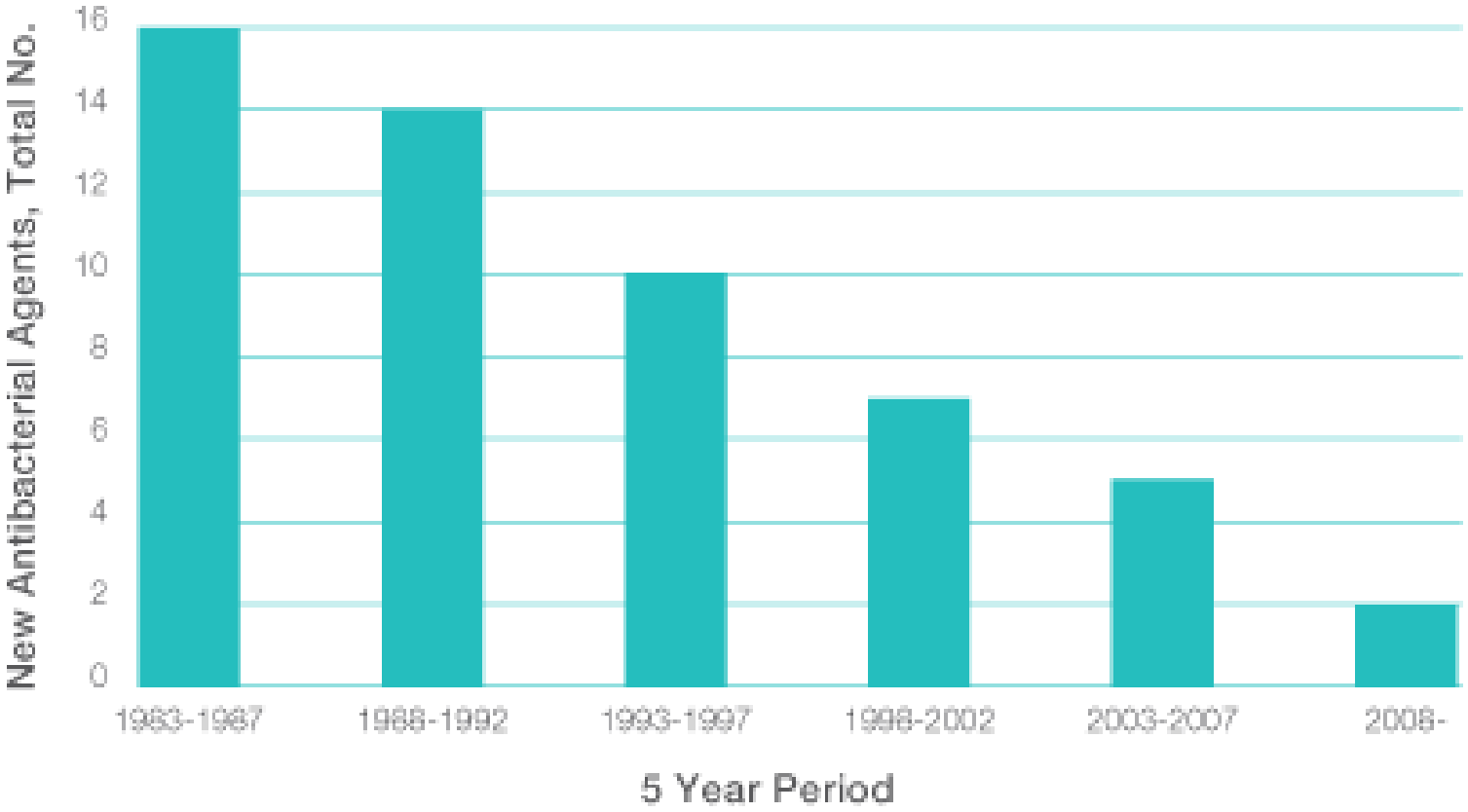
Each year, tens of millions of antibiotics are prescribed unnecessarily for upper viral respiratory infections

Antibiotic use in primary care is associated with antibiotic resistance at the individual patient level

The presence of antibiotic-resistant bacteria is greatest during the month following a patient's antibiotics use and may persist for up to 1 year

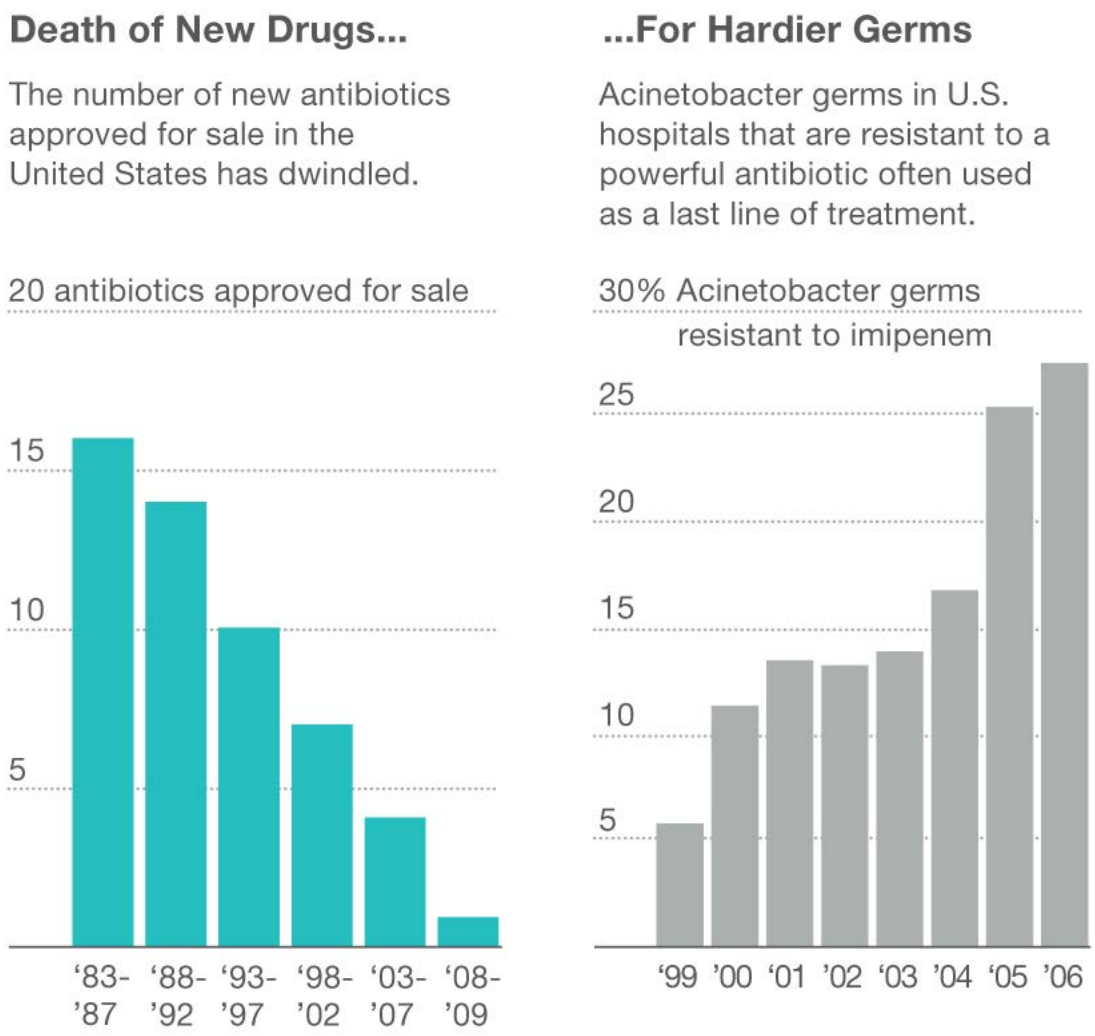
CDC – Get Smart Campaign

New drugs



New antibacterial agents approved in the United States, 1983–2013, per 5-year period].
Source: adapted from Spellberg et al (2008) Clin Inf Dis 46:155-64

New drugs vs. Resistant organisms



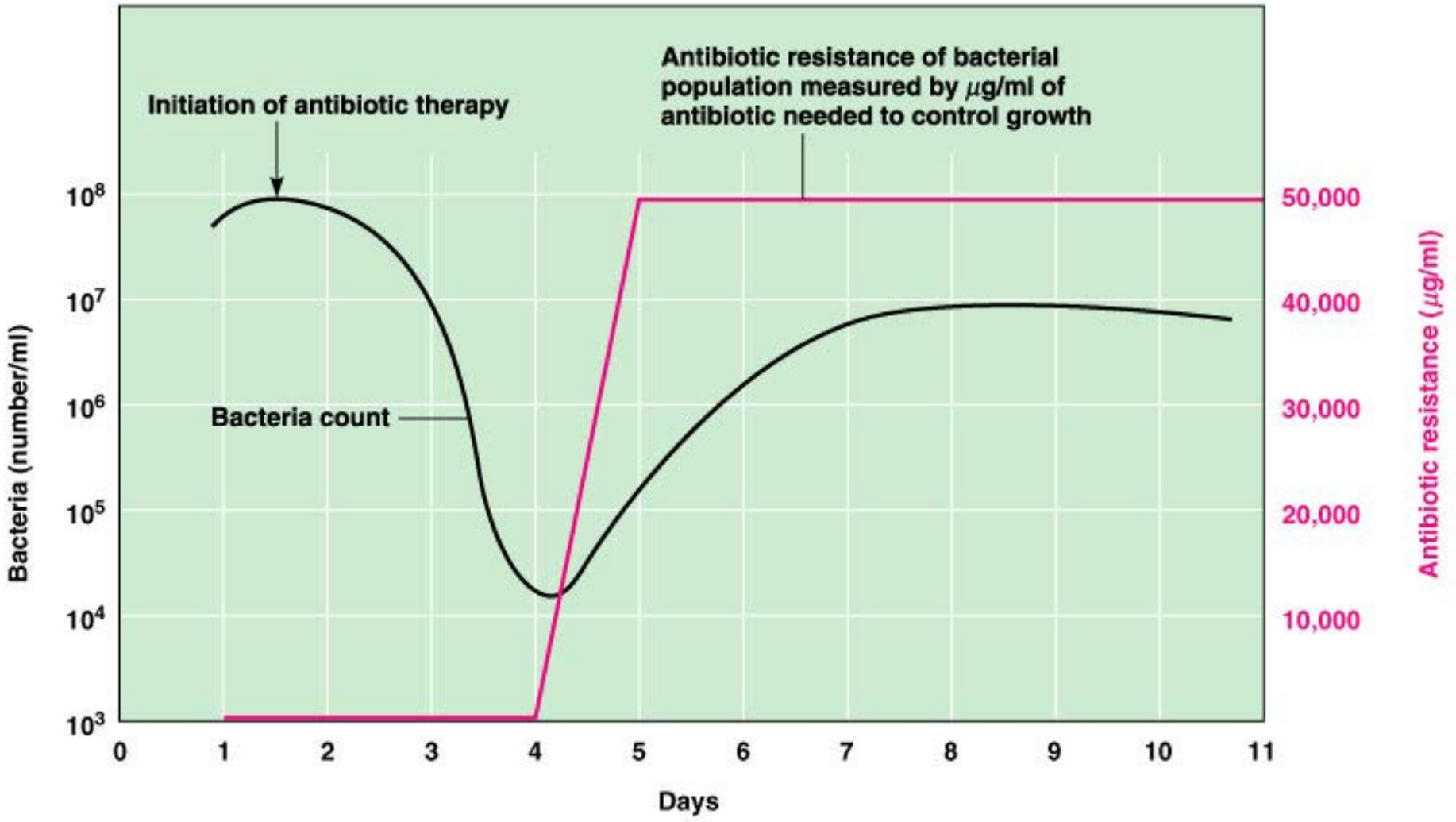
Source adapted from New York Times (2010) Rising Threat of Infections Unfazed by Antibiotics. [Online]. Available at: http://www.nytimes.com/imagepages/2010/02/27/business/27germ_graphic.html

“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.”

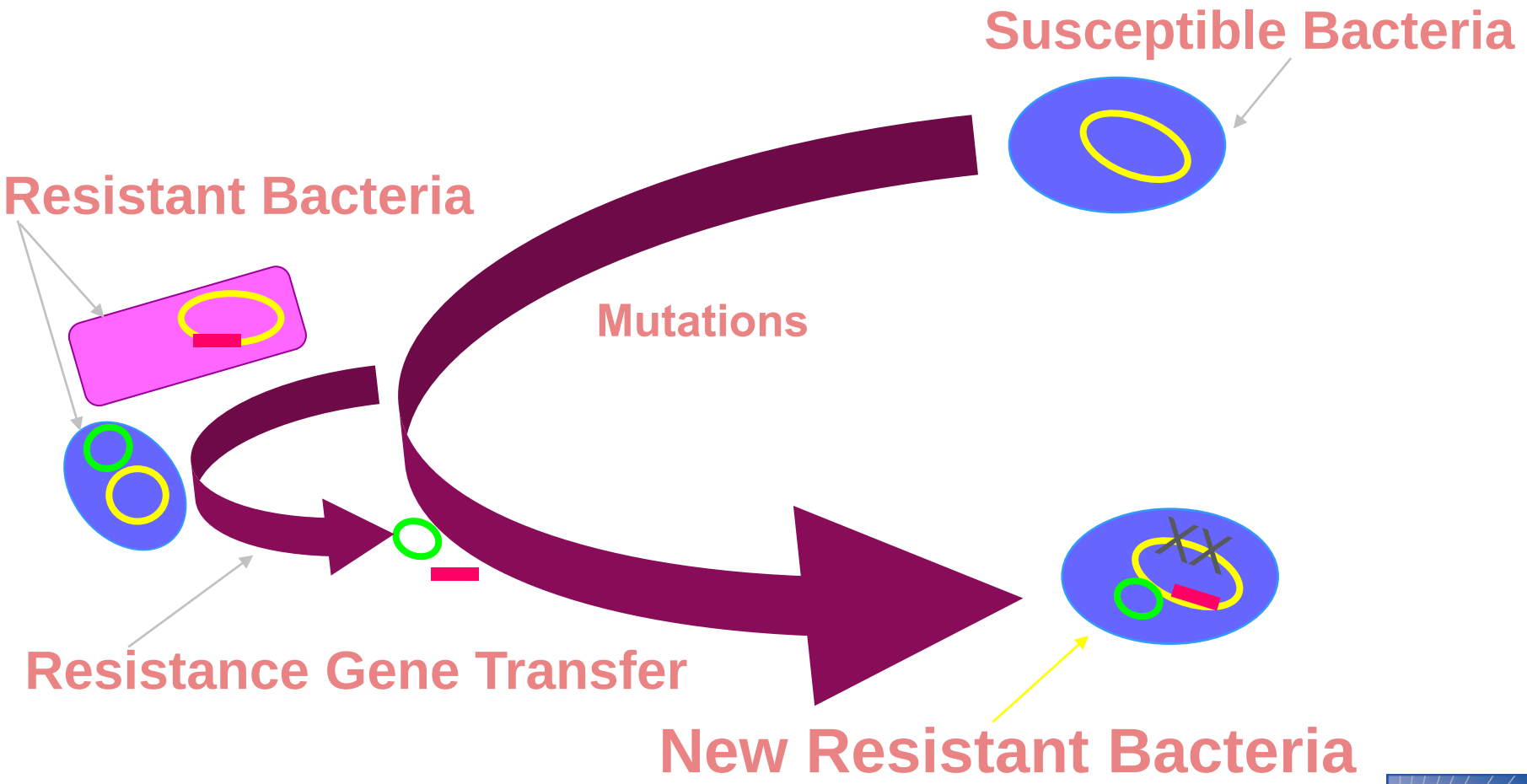
Margaret Chan, WHO Director General

Test Target Treat model

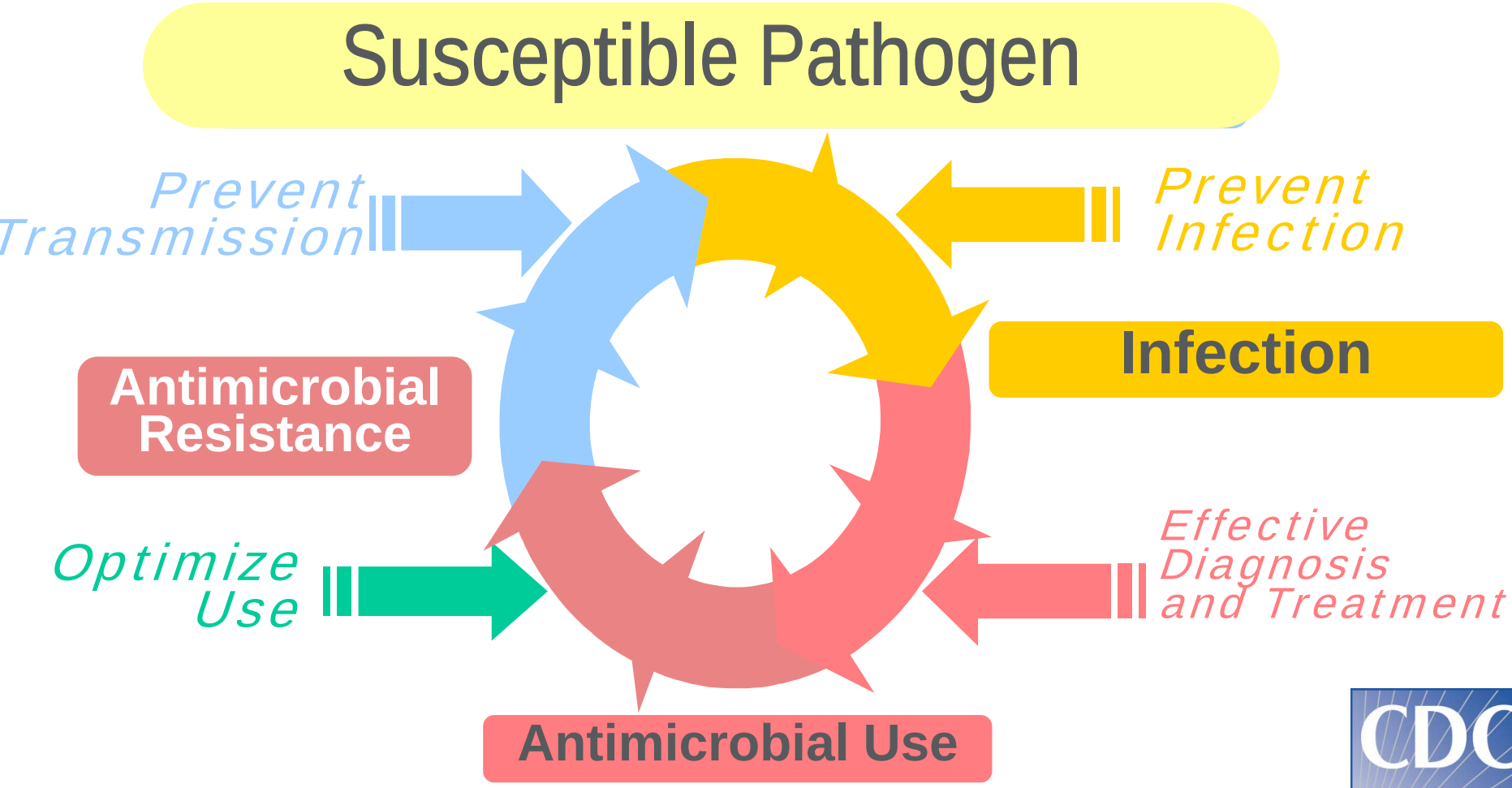
ANTIBIOTIC RESISTANCE



EMERGENCE OF ANTIMICROBIAL RESISTANCE



ANTIMICROBIAL RESISTANCE: KEY PREVENTION STRATEGIES



What percent of antibiotics made in this country goes into animal feed?

What percent of antibiotics made in this country goes into animal feed?

80%

Study on CAP Patients and Therapy

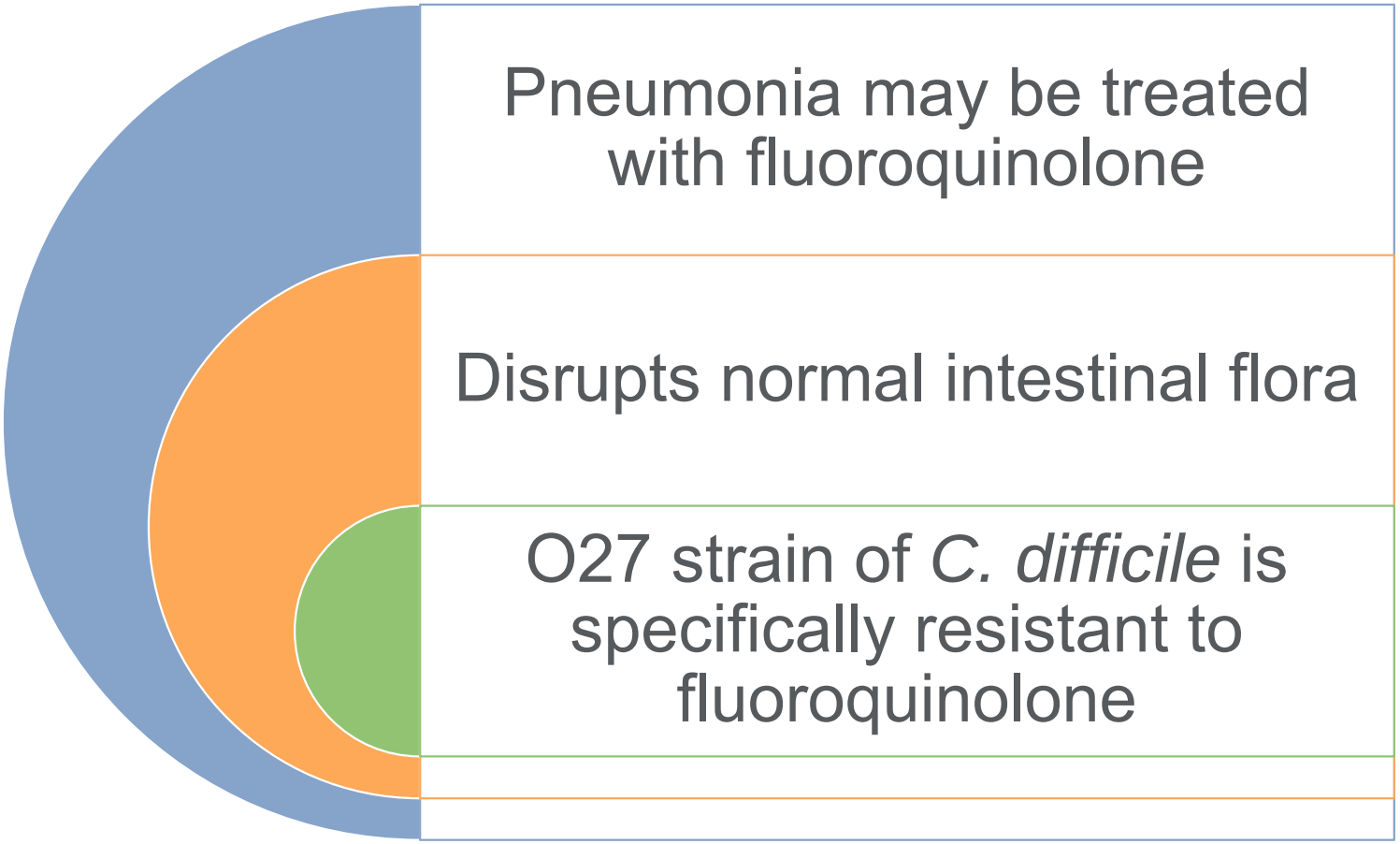
Retrospective study on 175 CAP patients in New York

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

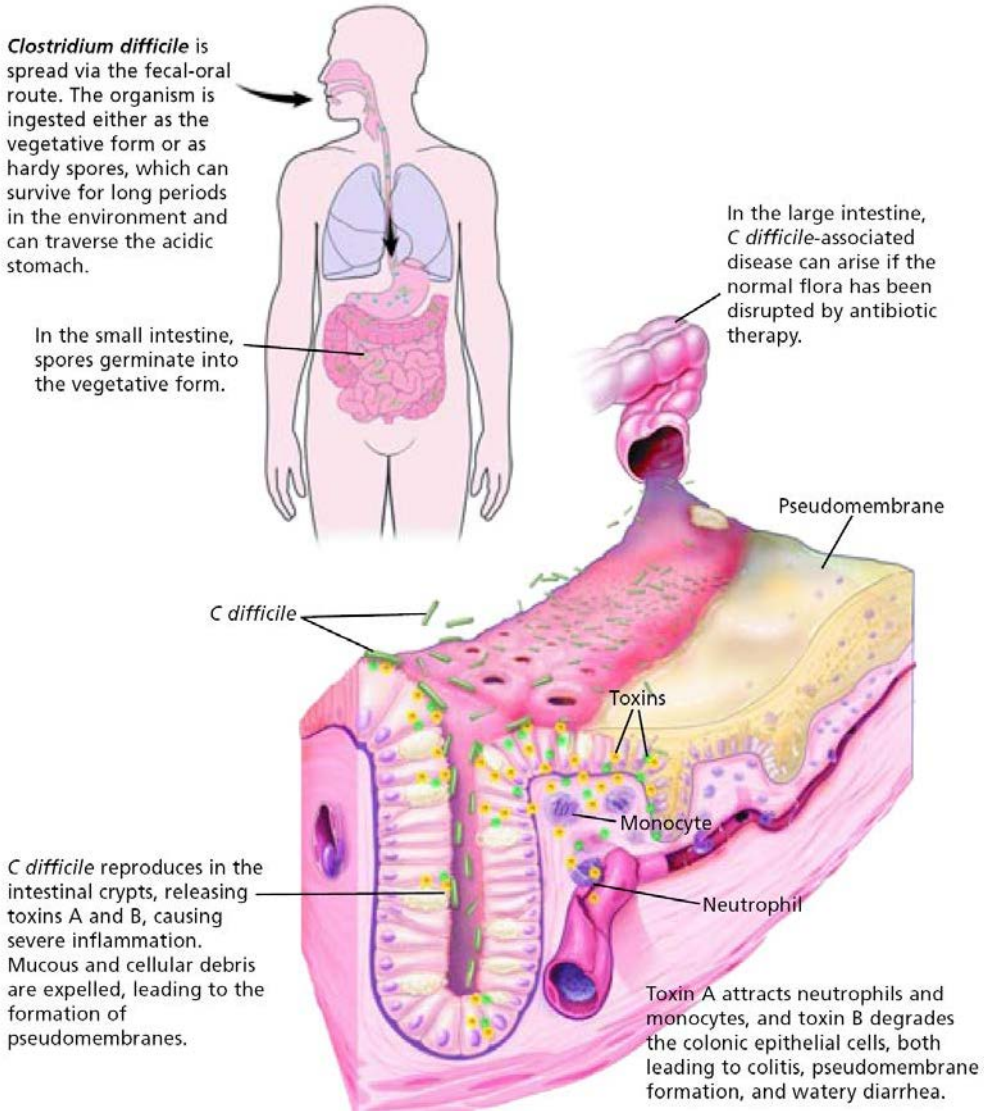
Rate of multidrug resistant organism detected within 90 days

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

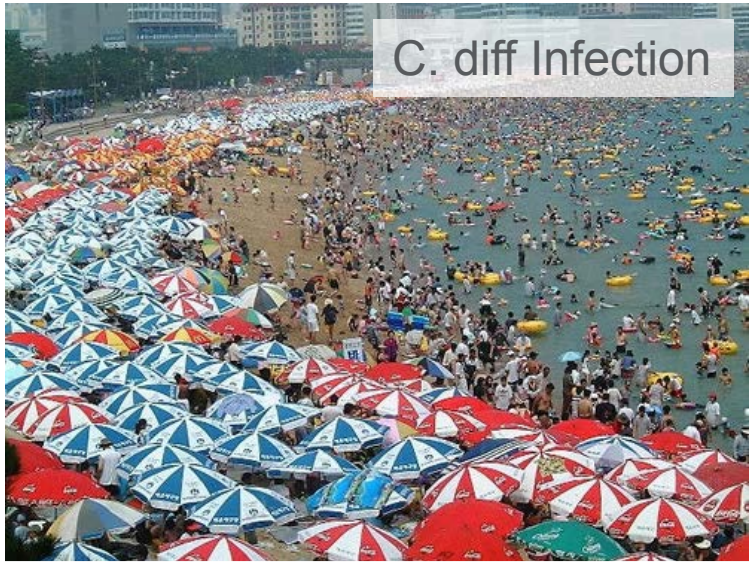
Misuse of Antibiotics Can Lead to Other Medical Issues



Pathogenesis of CDAD



Antibiotic-Associated Diarrhea:
Life's a Beach with *C. difficile*



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

Advantages of Rapid Testing for Infectious Diseases

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Molecular Mechanisms

Pros and Cons of Molecular

Pros	Cons
Good for pathogens that you only have when you are sick	May only be a screen for bacteria/viruses that people may normally carry
• Influenza	• *Clostridium difficile, S. pneumoniae*
Good for living things which would have RNA/DNA	Bad for non living things
	• Protein, DOA
Good to see if active infection & can test where the infection is	Bad for past infection
• Not things like sepsis	• Want test that detects antibody

Molecular Tests on the Market

PCR – Polymerase Chain Reaction

- Rely on the ability to amplify due to temperature cycling
- Many traditional molecular companies
- Alere q - Competitive Reporter Amplification
- 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)
- Alere i – NEAR / RPA (Nicking enzyme amplification rxn/ Recombinase polymerase amplification)

PCR Cycle

Double-stranded DNA

95° Denaturation

Heating separates strands

57° Annealing

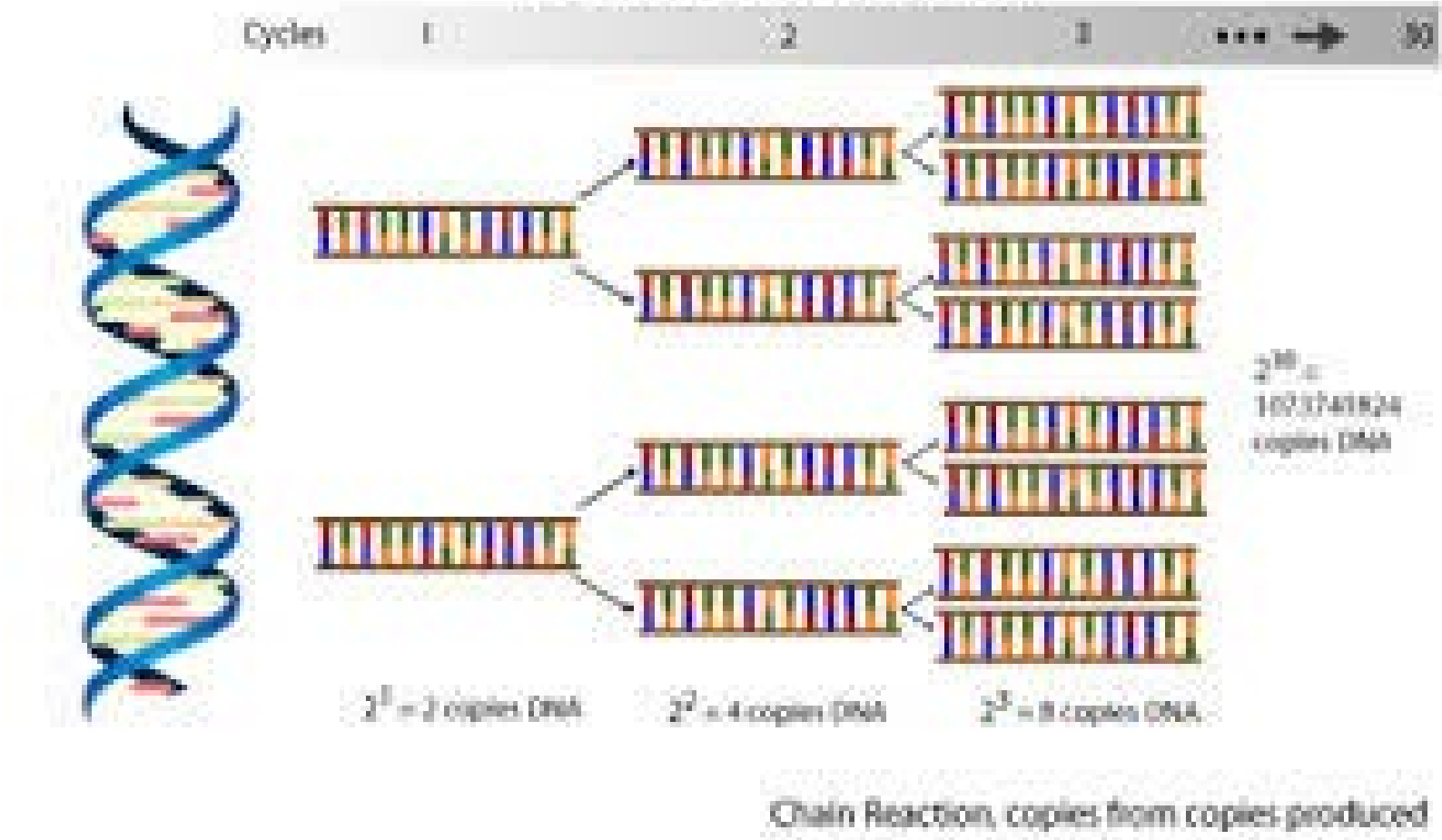
Primers Bind to target sequences

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

Not Yet Available

- 75 minutes to results
  - 2 min hands on time
- Broad molecular menu
- Multiple Versions

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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TMA : Transcription mediated amplification

Illumigene – Meridian Bioscience

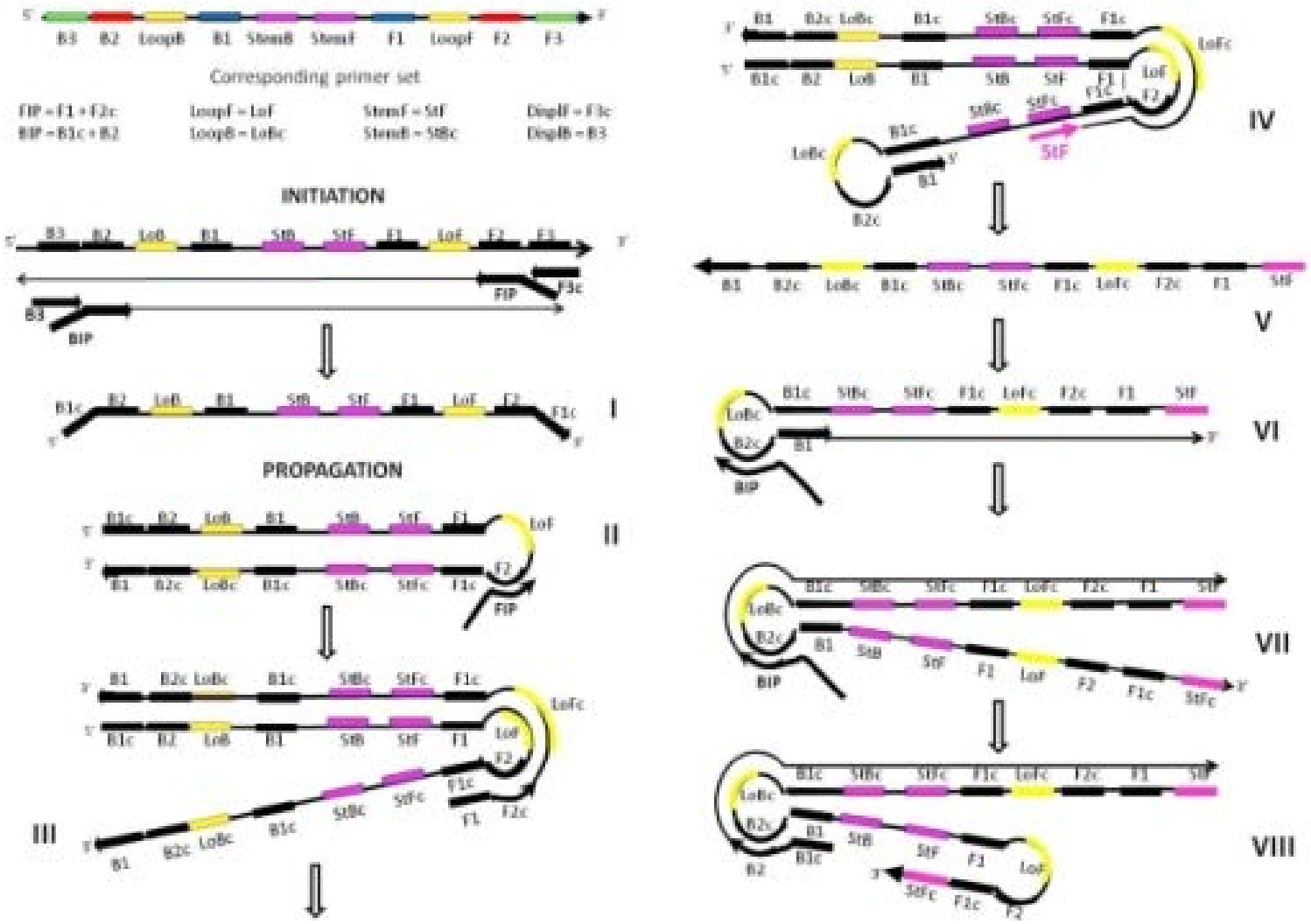
< 60 minutes to results

- Including heat pretreatment step

< 2 minutes hands on time

Small footprint (8.5” x 11”)

Loop Mediated Isothermal Amplification (LAMP)



LIAT - Lab In a Tube

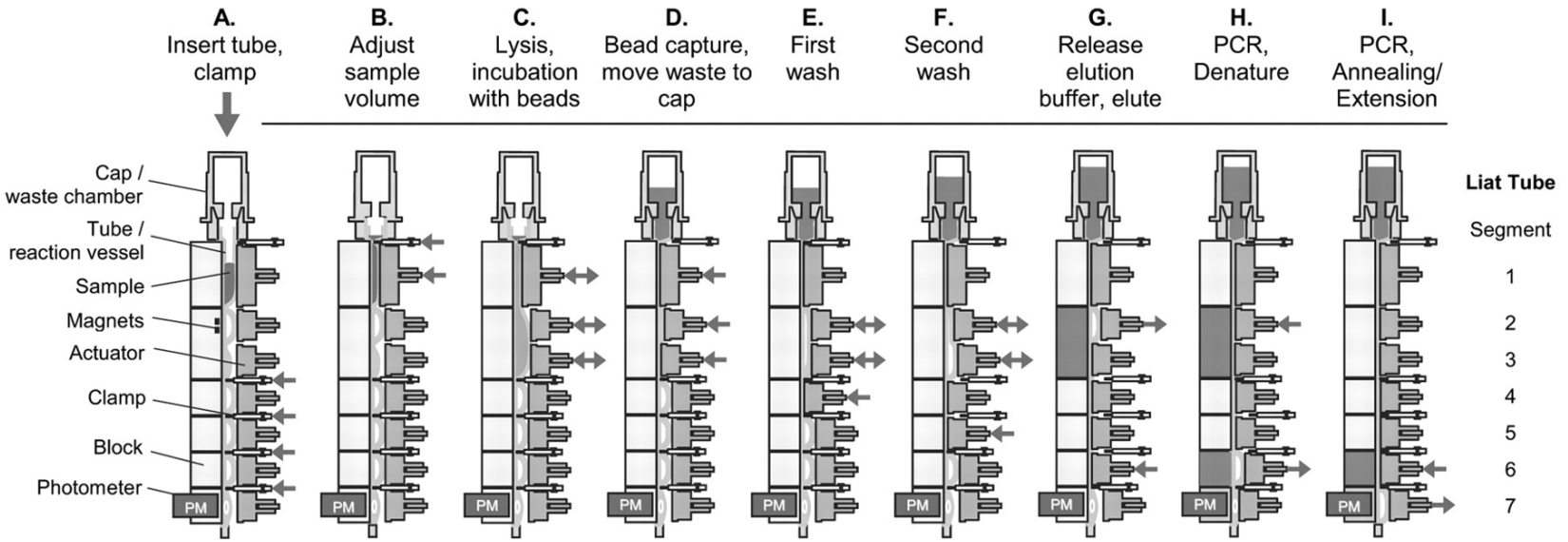
20 minutes to results
Flu

15 minutes to results
Strep A

Footprint 4.5 x 9.5 x 7.5

Weight 8.3 lbs

Sample processing in the Liat Tube.

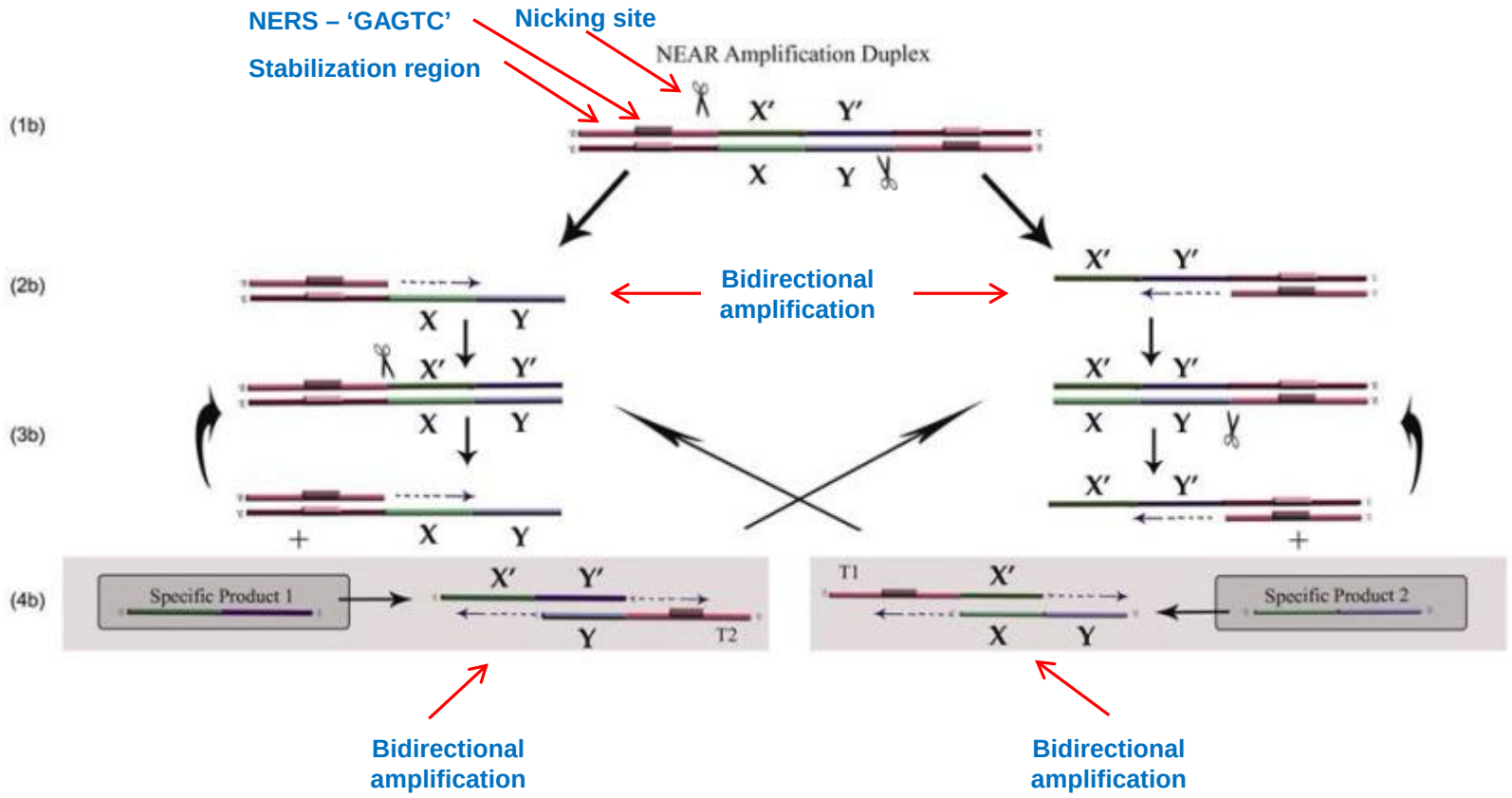


Sultan Tanriverdi et al. J Infect Dis. 2010;201:S52-S58

Alere™ i

- < 15 minutes to results
- < 2 minutes hands on time
- Small footprint (8.15” W x 5.71” H x 7.64” D)
- 1.4 lbs / 3 kg
- 2 approved tests – Flu A/B, GAS

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

Applying Molecular Technologies to Influenza

Influenza A&B

Can have mortality

- Especially in the young & old

Can lead to complications

- Pneumonia primarily from *S. pneumoniae*

Influenza mutates so the population can get influenza multiple times

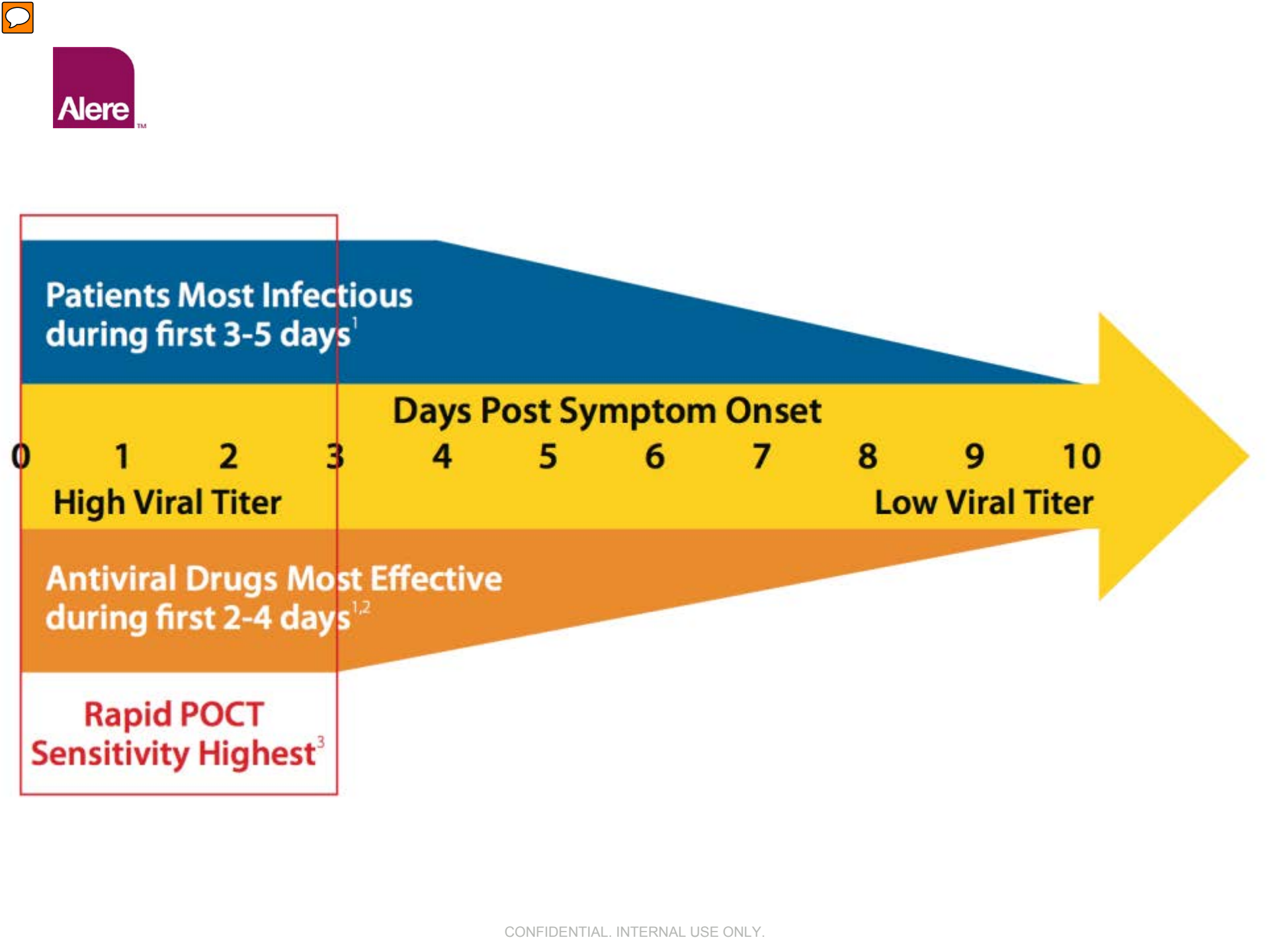
History

Hippocrates described flu back in the 5th century.

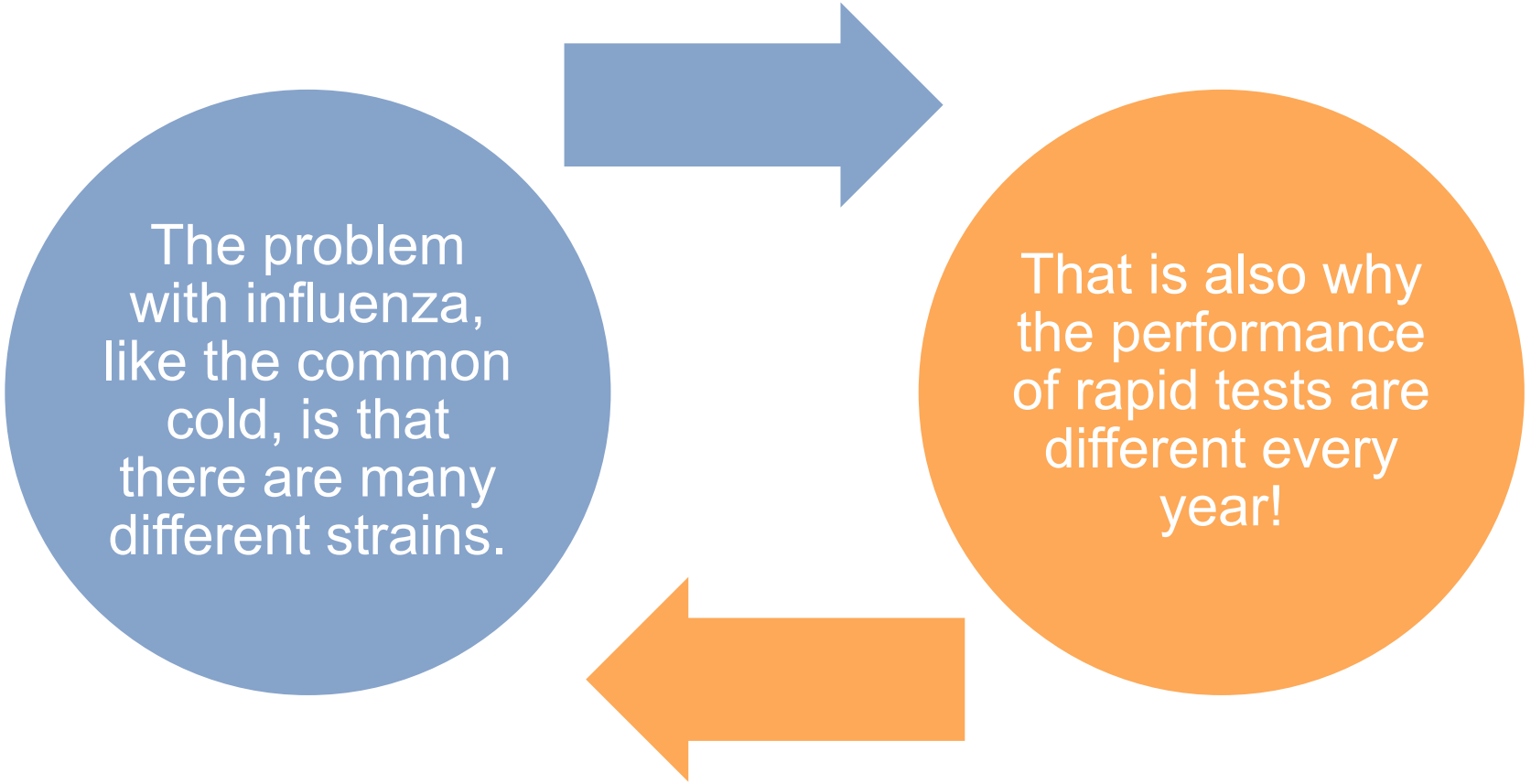
Columbus brought a devastating flu on his second voyage to the new world.

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

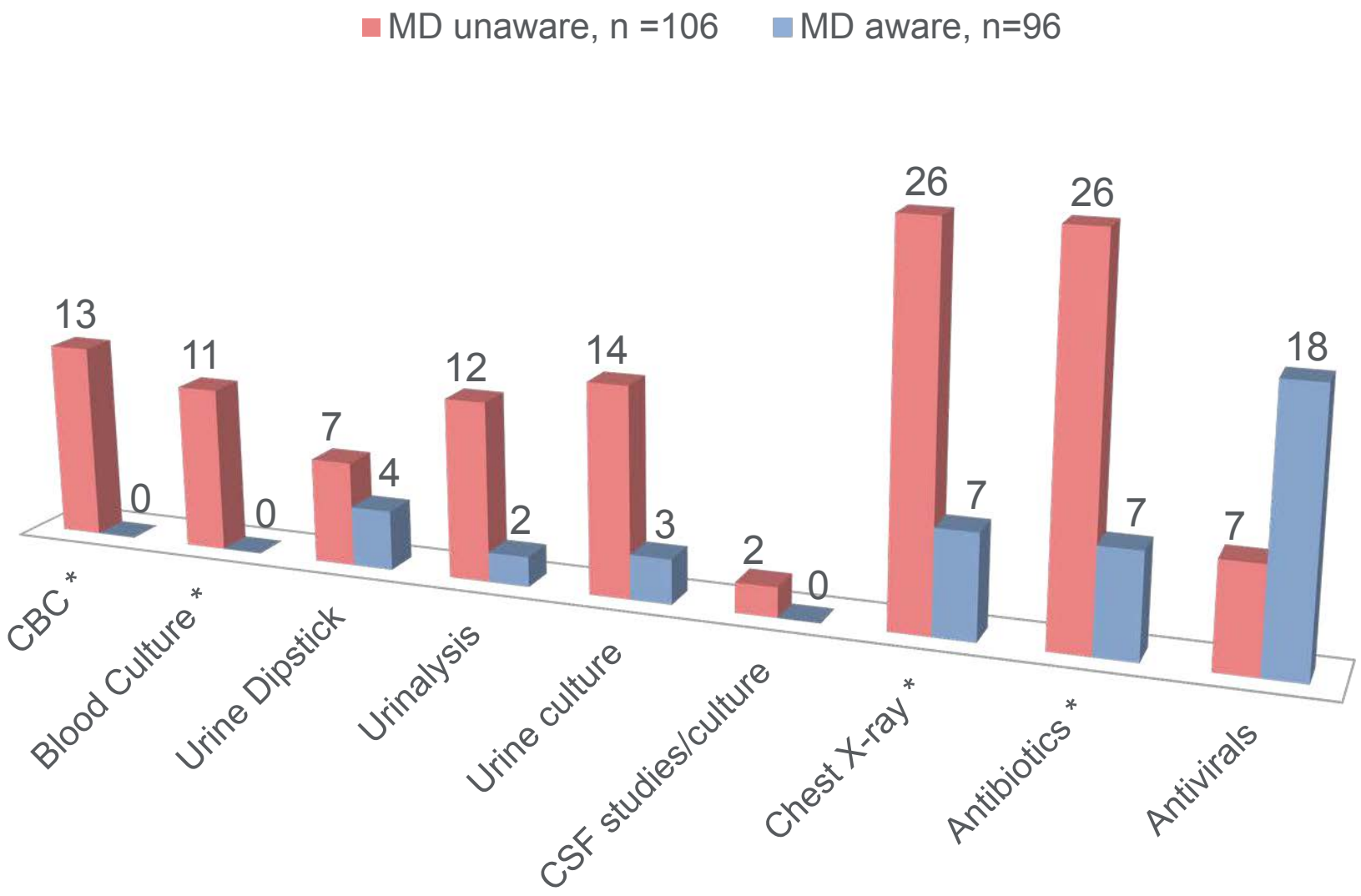
- 50 to 100 million people were killed (3-6% of the world's population!)
- Another 500 million were infected (1/3rd of the world's population)



Aren’t you supposed to build immunity to influenza?



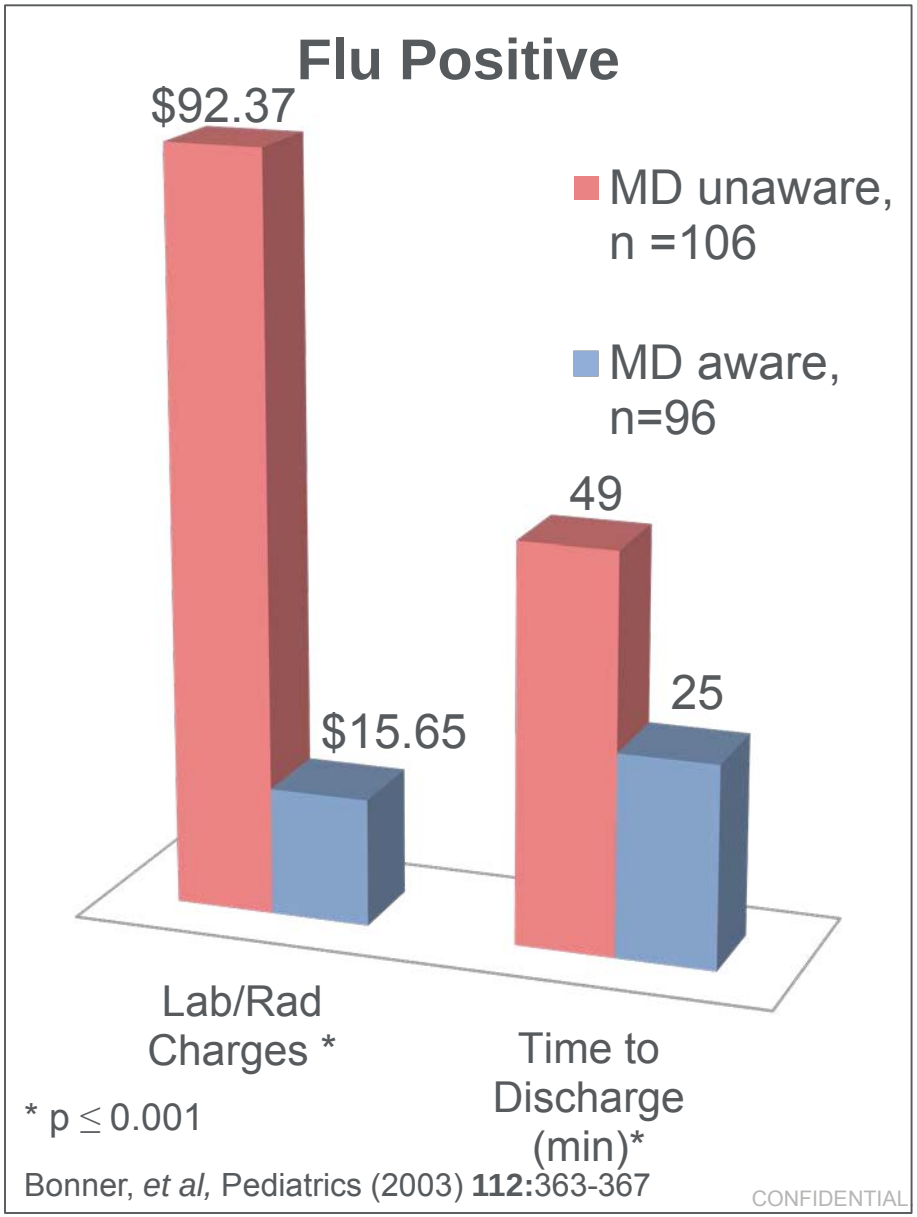
Results – Flu Positive

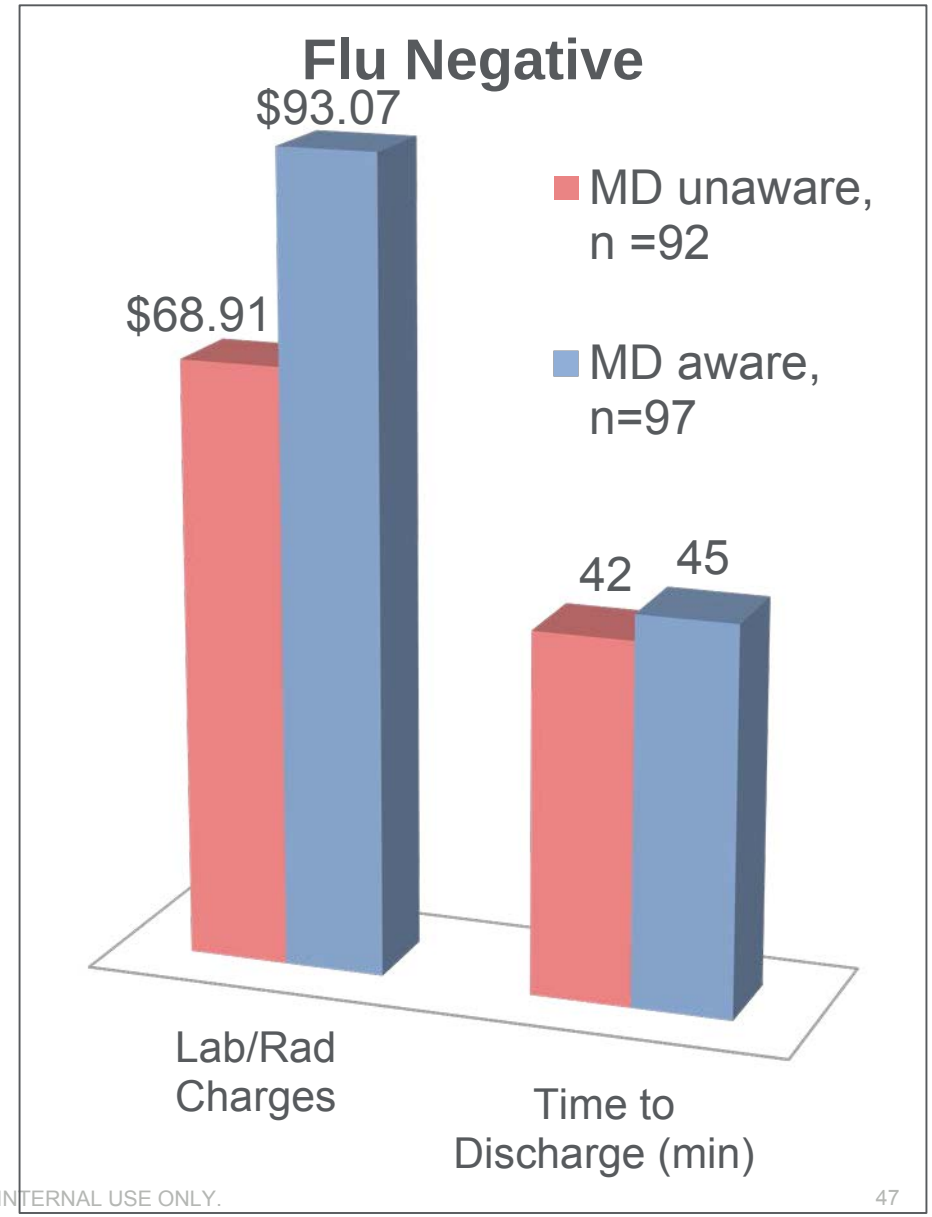


Bonner, *et al,* Pediatrics (2003) **112:**363-367

* - $p \leq 0.001$

Key Operational Metrics





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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 days of symptoms since that is when viral titers are highest and antiviral therapy is effective

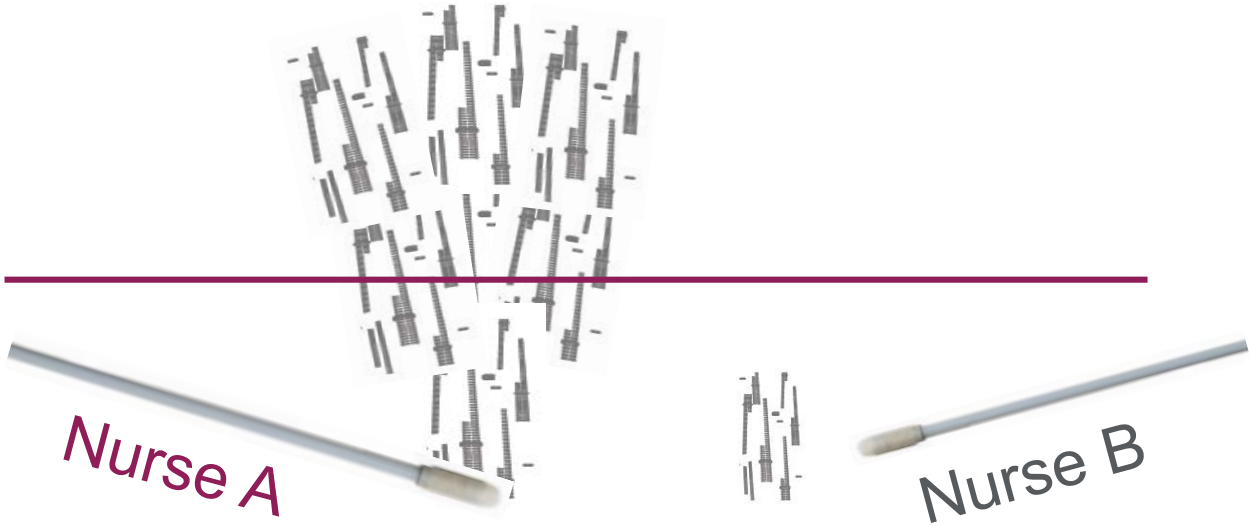
Testing can be done immediately with rapids or sample placed in transport media

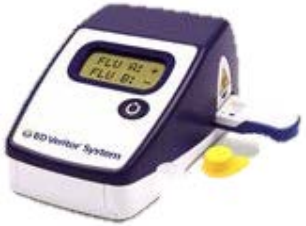
- Infectivity is maintained up to 5 days when stored @ 4-8°C
- If the sample cannot be evaluated in this time period, the sample should be frozen @ -70°C.

The Power of Sample Amplification

Amplify the sample up to 1 trillion times! Without amplification, a positive test might not be detected.

Detection threshold



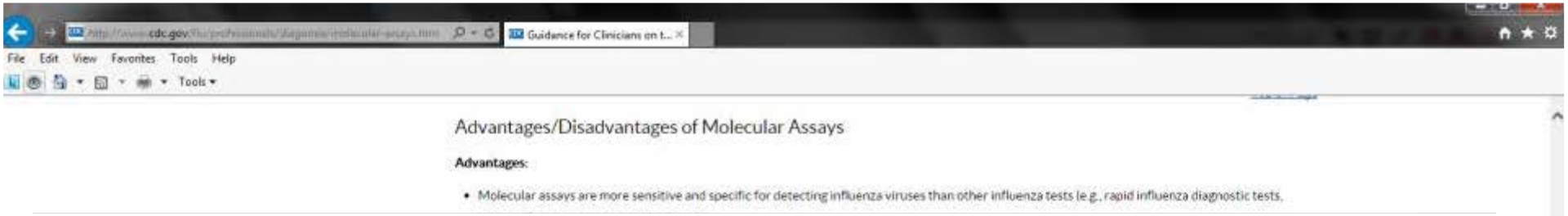


Amplified
Flu+ Sample

Not Amplified
Flu+ Sample

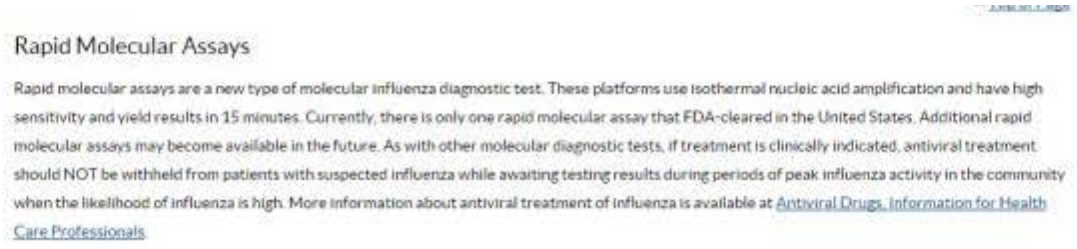
CDC Website Creates a New Diagnostic Category

http://www.cdc.gov/flu/professionals/diagnosis/molecular-assays.htm



Rapid Molecular Assays

Rapid molecular assays are a new type of molecular influenza diagnostic test. These platforms use isothermal nucleic acid amplification and have high sensitivity and yield results in 15 minutes. Currently, there is only one rapid molecular assay that FDA-cleared in the United States. Additional rapid molecular assays may become available in the future. As with other molecular diagnostic tests, if treatment is clinically indicated, antiviral treatment should NOT be withheld from patients with suspected influenza while awaiting testing results during periods of peak influenza activity in the community when the likelihood of influenza is high. More information about antiviral treatment of influenza is available at Antiviral Drugs, Information for Health Care Professionals.



Why Test

Knowledge of a positive test has been shown to

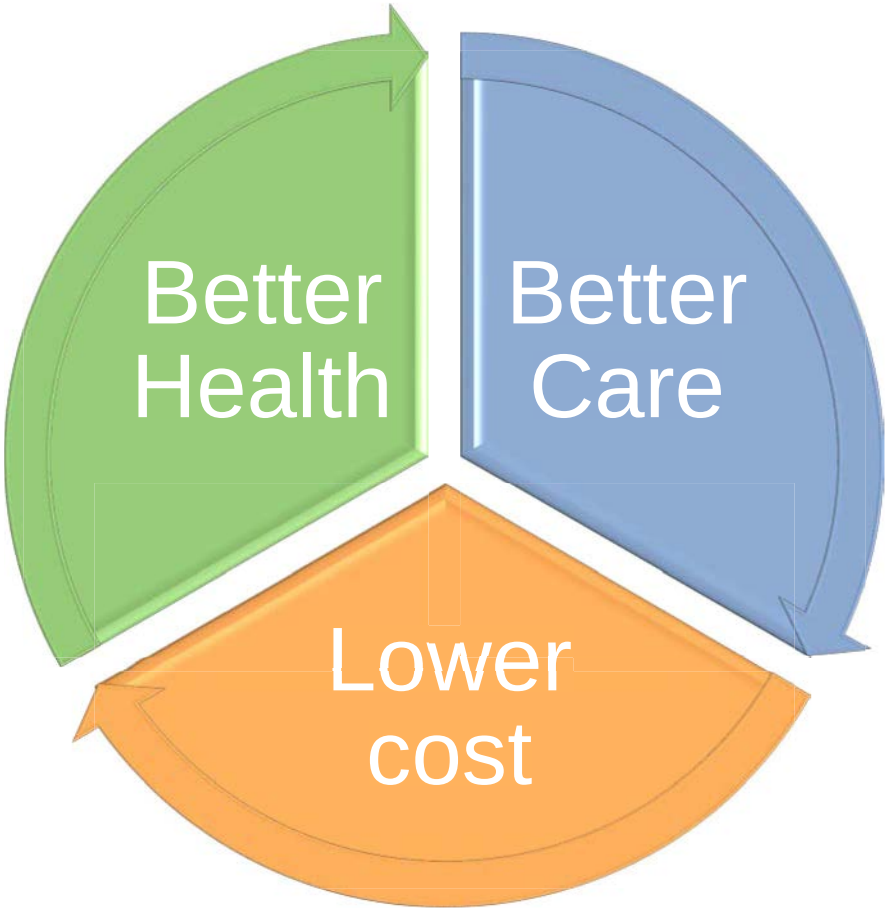
- Limit unnecessary antibiotic use
- Limit unnecessary diagnostic procedures
- Increase the appropriate use of antivirals

Help form decisions to undertake appropriate infection-control measures

Technology Comparison

	IMMUNOASSAY		MOLECULAR	
	RAPIDS	LAT FLOW READERS	PCR	Rapid
FAST	✓	✓		✓
CONVENIENT	✓	✓		✓
POC-FRIENDLY	✓	✓		✓
ACTIONABLE RESULTS	✓	✓		✓
REMOVES SUBJECTIVITY		✓	✓	✓
CONNECTED		✓	✓	✓
EXCELLENT PERFORMANCE			✓	✓

Healthcare's "Triple Aim"



Better Health (Clinical)

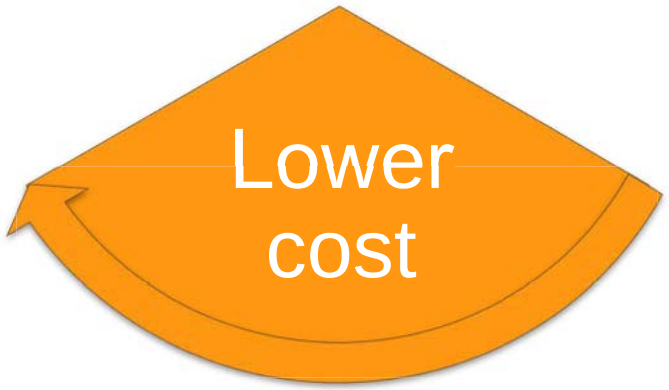
- Detect more true positives than gold standard
- Increased confidence in diagnosis may lead to better directed therapy

Better Care (Operational)

- Confidently make appropriate clinical decisions sooner
- Molecular results in the time of a rapid assay
- Actionable results at the point of care

Lower Cost (Economic)

- Limit number of cultures being done
- Reduce follow-up burden on staff
- More rapid discharge/treatment decision compared to traditional testing



What Would Point-of-Care Molecular Mean For?

- Strep A?
- RSV?
- MRSA screening?
- CRE screening?
- *C. difficile* screening?
- *Gonorrhea*/*Chlamydia*?
- Norovirus?
- Walking pneumonia?

Conclusions

Molecular assays have had superior performance in microbiology over current assays.

Newer technologies will allow faster results that may affect antibiotic prescribing.

Directed therapy can prolong the effectiveness for broad spectrum antibiotics

Discussion