CDC PUBLIC HEALTH GRAND ROUNDS

Changes in Clinical Diagnostics and Tracking Infectious Diseases

October 18, 2016

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

The Impact of Culture-independent Diagnostic Testing in Foodborne Diseases

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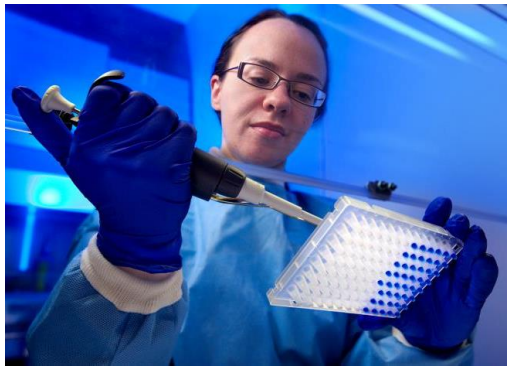
U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

Diagnostic Methods Through Time

1860s:
Culture-based tests
Invented by French scientist Louis Pasteur, a.k.a., the “father of microbiology”

1980s-90s:
Antigen-based tests
Detect antigens specific to pathogen type



2000s:
Polymerase Chain Reaction (PCR) tests
Detect short genetic sequences specific to pathogen type

2010s:
Multiplex PCR panels
Use PCR to detect one or multiple pathogens simultaneously, often designed for disease syndromes, can detect viral pathogens

Culture-independent Diagnostic Tests

Number and Types of Culture-independent Diagnostic Tests Are Increasing

2011

Antigen-based tests (FDA cleared)

- 3 tests for Campylobacter
- 2 tests for STEC

2016

Antigen-based tests (FDA cleared)

- 3 tests for Campylobacter
- 5 tests for STEC

Laboratory-developed tests (not FDA cleared)

- Molecular detection (PCR) tests for single or multiple pathogens

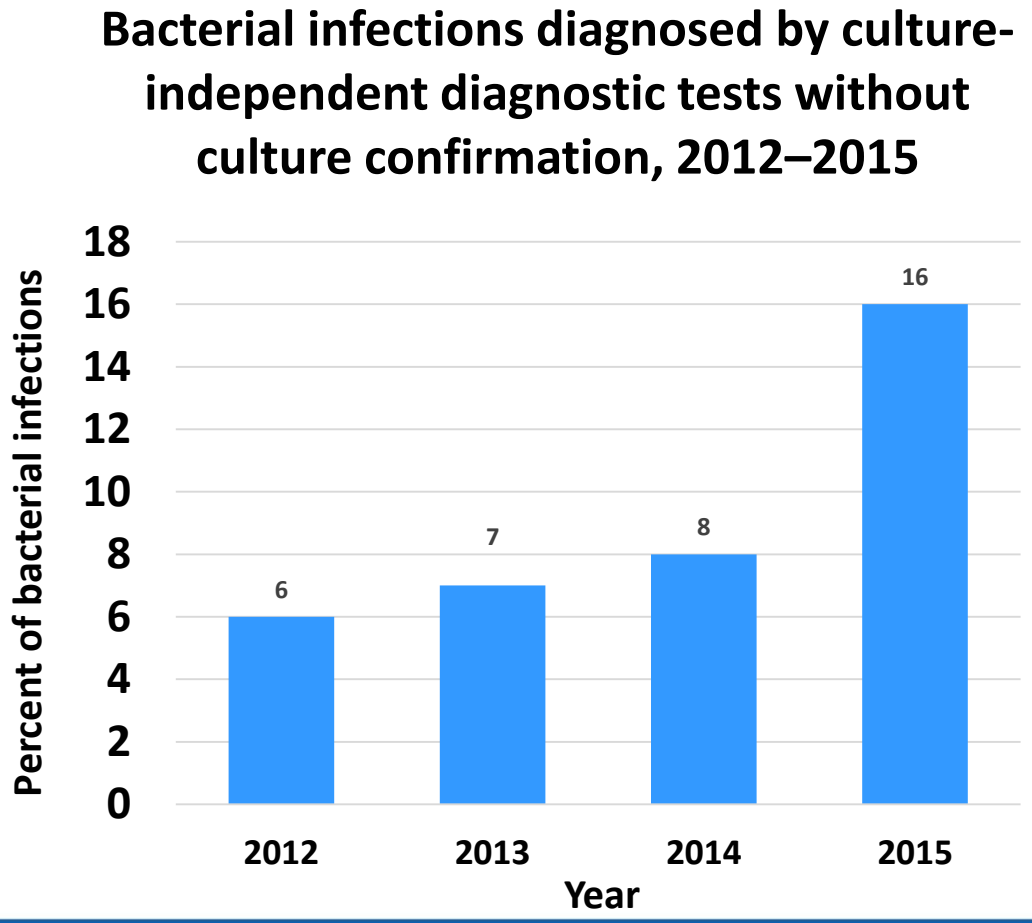
Multiplex PCR panels (FDA cleared)

- Luminex
- Nanosphere
- ProGastro SSCS
- BD Max
- BioFire

STEC: Shiga toxin-producing *E. coli*

Names of products are provided for identification purposes only and do not imply any endorsement by the CDC

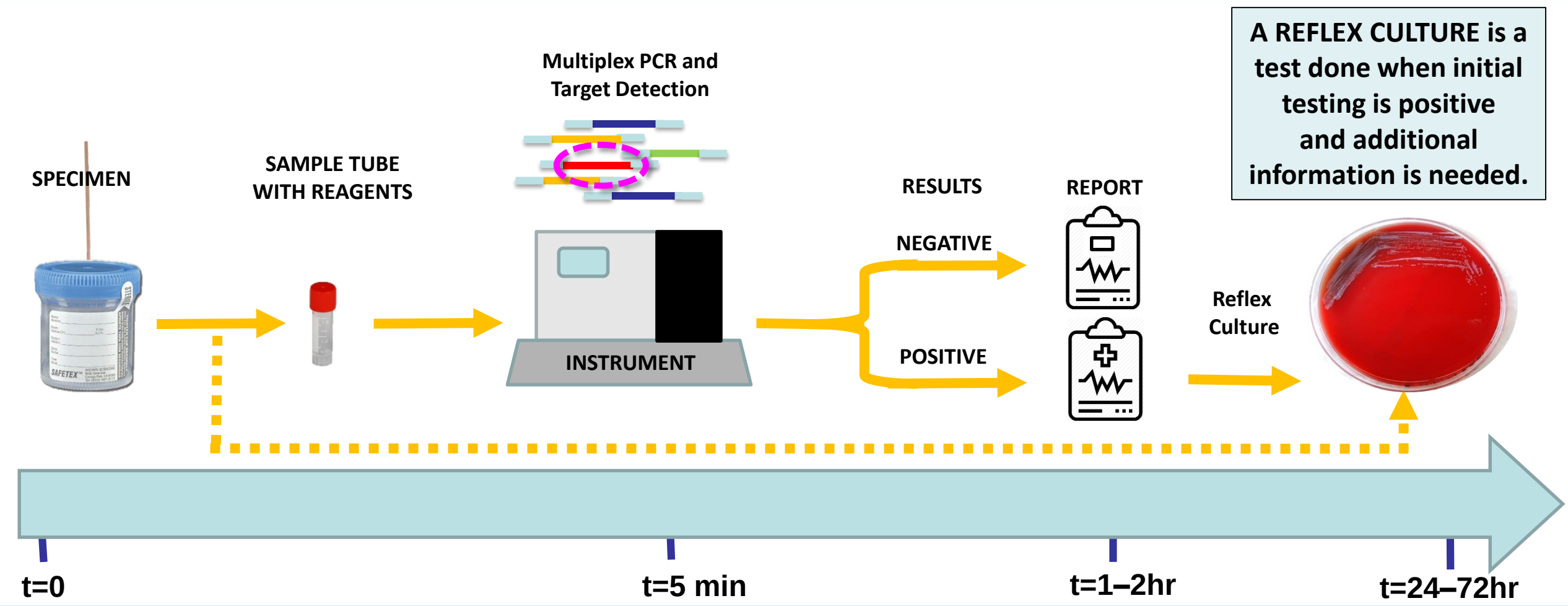
Use of Culture-independent Diagnostic Tests (CIDT) Is Increasing



- **For diagnosing enteric infections, increases in CIDT use show**
  - Uptake varies by pathogen
  - Growing use of multiplex PCR panels
- **For surveillance and tracking, increases in CIDT impacts trends**
  - Increased incidence of *Cryptosporidium* and non-O157 Shiga toxin-producing *E. coli* (STEC) might be due to increased use of CIDTs

April 2016 FoodNet MMWR
2015 – updated since April 2016 MMWR to include most current data, not yet published

Multiplex PCR Panels – Generic Workflow



The Benefits of Using CIDT for Diagnosis

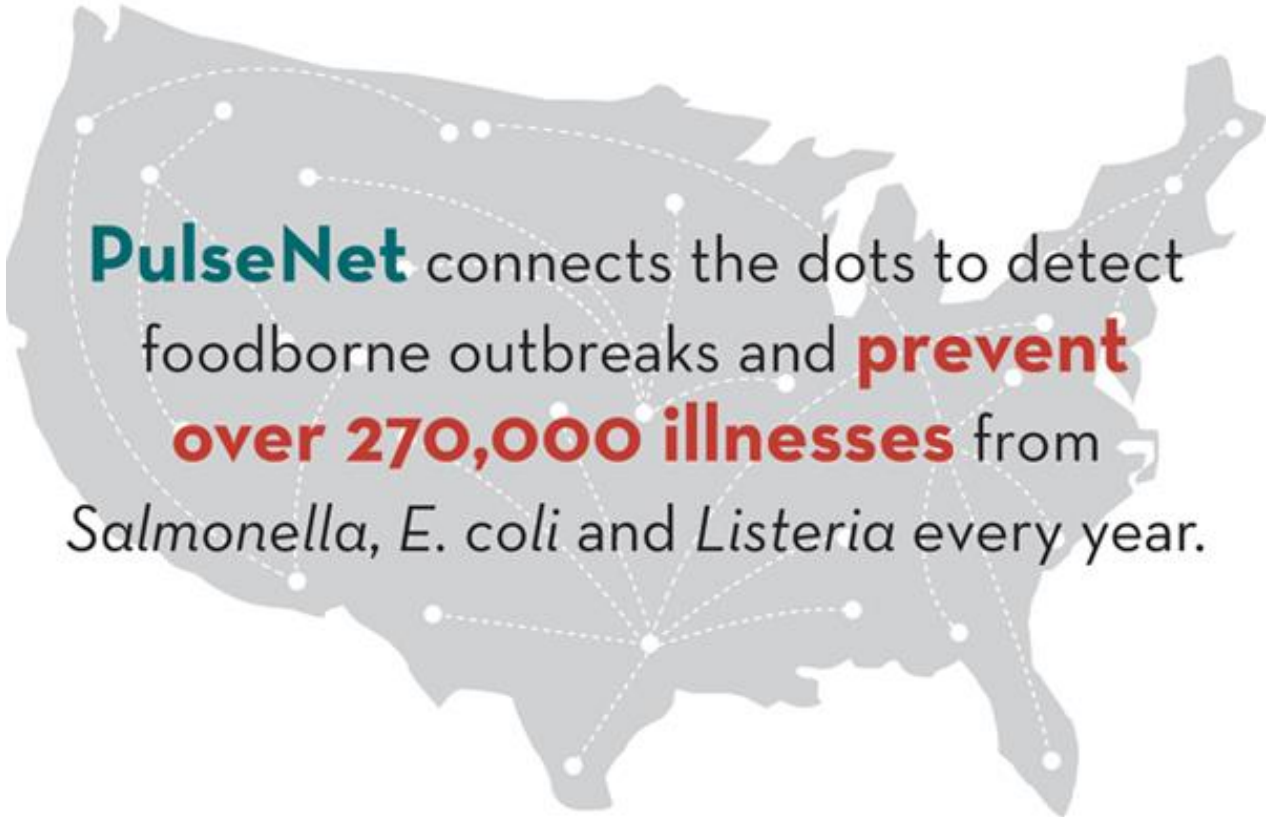
- Faster results
- Targeted treatment
- Single test can detect or rule out multiple pathogens (e.g., viruses, parasites, and bacteria)
- Likely more sensitive than culture
- Faster information for local public health action

CIDT Do Not Provide Isolates Nor Characterize Pathogens

- **CIDT do not provide isolates**
- **Reflex cultures needed to characterize the pathogen**
  - Antimicrobial susceptibility
    - Tailor treatment
    - Track resistance trends
  - Virulence factors
  - Serotype
  - Genotype (i.e., DNA fingerprints)
    - Identify outbreaks

Why is Pathogen Characterization Important for Food Safety?

- **PulseNet connects cases to identify outbreaks**
- **Detailed DNA fingerprints facilitate outbreak detection**
  - DNA analyses with whole genome sequencing technology require cultured isolates
- **Each year, 48 million people get sick, 128,000 are hospitalized and 3,000 die from foodborne diseases**

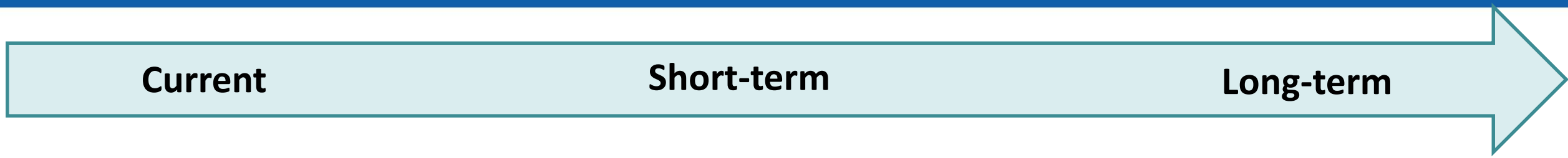


Other Drawbacks of CIDT: Positive Results Can Be Difficult to Interpret

- **DNA from dead microbes can produce a positive result**
  - Clinicians may not know if patient is still contagious
  - Unclear if it's safe for patient to return to work or day care
- **A single test may detect multiple pathogens, some of which may not be causing illness**
  - One study found that over 30% of positive tests detected more than one enteric pathogen

Buss SN, Leber A, Chapin K, et al. 2015. Multicenter evaluation of the BioFire FilmArray Gastrointestinal Panel for etiologic diagnosis of infectious gastroenteritis. J Clin Microbiol 53:915–925

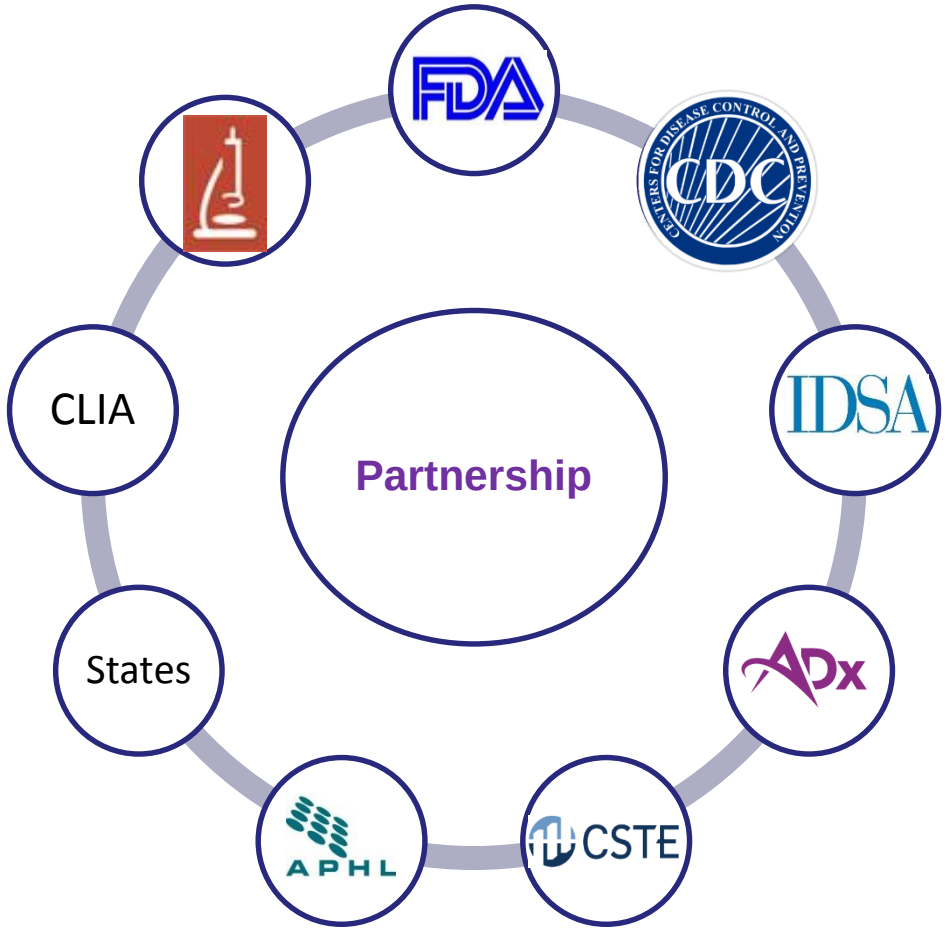
Strategies to Meet the Surveillance Challenge of CIDT



Culture-based	Whole Genome Sequencing	Metagenomics
Requirement 1. Use of reflex culture to obtain isolates	**Requirements** 1. Continued use of reflex culture 2. Development of large genome database	**Requirements** 1. Identify subtyping targets for amplicon sequencing 2. Refine shotgun metagenomics methods

Building a Broad Set of Partnerships

- **Maintain access to cultures in short term and work toward the future of CIDTs**
  - Building the coalition
  - Raising awareness
  - Publishing information
  - Tracking progress
  - Adapting surveillance methods



ADX: AdvaMedDx
APHL: Association of Public Health Laboratories
ASM: American Society for Microbiology

CLIA: Clinical Laboratory Improvement Amendments
CSTE: Council of State and Territorial Epidemiologists
IDSA: Infectious Diseases Society of America

Managing New Diagnostic Tests in Colorado

Alicia Cronquist, RN, MPH

Foodborne Disease Program Manager

Communicable Disease Branch

Colorado Department of Public Health and Environment

Impact of CIDT on Surveillance and Isolate Recovery in Colorado

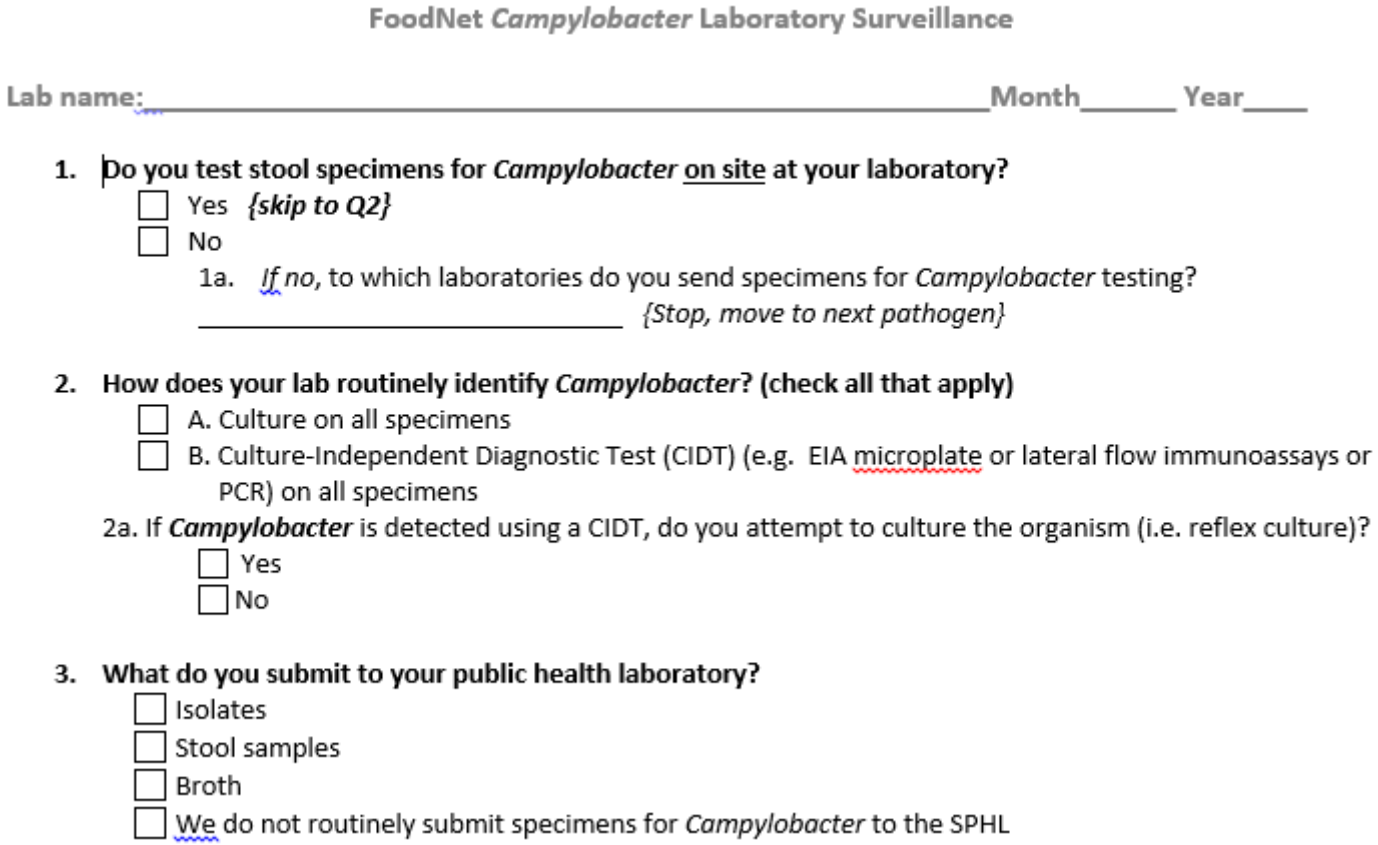
- **Since 2013, 15 labs use CIDT (e.g., multiplex PCR testing)**
  - So far in 2016, 40% of bacterial enteric cases reported were tested using PCR (N=537)
    - For *Campylobacter, Salmonella, Shigella, STEC, Vibrio, Yersinia*
  - Reflex culture performed for 89% of the *Salmonella*, *Shigella* and STEC tested with PCR
- **Impact on surveillance in Colorado**
  - Ensure accurate case reporting
  - Facilitate isolate recovery
  - Adapt public health practice to new type of 'cases' being reported
    - Previously only culture-confirmed reports were considered 'cases'
    - 'Probable case' definitions include CIDT-positive results

STEC: Shiga toxin-producing *E. coli*

Unpublished data, Colorado Department of Public Health and Environment

Accurate Case Reporting: Understanding Which Tests Are Used and By Whom

- **Routine survey of laboratory methods**
  - Established in 2009
  - Twice per year in FoodNet catchment area (Denver metropolitan area)
  - Once per year in rest of state
  - Labor intensive



FoodNet *Campylobacter* Laboratory Surveillance

Lab name:_____Month_____ Year____

1. Do you test stool specimens for *Campylobacter* on site at your laboratory?
   - ☐ Yes *{skip to Q2}*
   - ☐ No
     - 1a. *If no*, to which laboratories do you send specimens for *Campylobacter* testing?
       _____ *{Stop, move to next pathogen}*

2. How does your lab routinely identify *Campylobacter*? (check all that apply)
   - ☐ A. Culture on all specimens
   - ☐ B. Culture-Independent Diagnostic Test (CIDT) (e.g. EIA microplate or lateral flow immunoassays or PCR) on all specimens

   2a. If ***Campylobacter*** is detected using a CIDT, do you attempt to culture the organism (i.e. reflex culture)?
   - ☐ Yes
   - ☐ No

3. What do you submit to your public health laboratory?
   - ☐ Isolates
   - ☐ Stool samples
   - ☐ Broth
   - ☐ We do not routinely submit specimens for *Campylobacter* to the SPHL

Accurate Case Reporting: Collecting the Right Information

- **Modify disease surveillance database to capture data from new tests**
  - Collaborate with IT department
- **Ensure correct reporting of CIDT results**
  - Change settings so electronic laboratory reporting (ELR) data flow correctly
  - Correct test names in printed case reports sent to public health
    - Reporting "culture" for *Salmonella* when results were from CIDT
  - Address human error in interpreting multiplex panel results
    - Disease and test names sound alike and can be confusing
      e.g., Shigella, Shiga toxin-producing *E. coli* (STEC), *Plesiomonas shigelloides*

Accurate Case Reporting: Outreach Is Important

- **Education and communication are key**
- **Create guidance documents**
- **Hold frequent meetings with stakeholders**
  - Infection preventionists (e.g., hospital epidemiologists)
  - Laboratories
  - Local public health partners

Isolate Recovery at Clinical Laboratory Is Preferred

- **Where is reflex culture performed?**
  - Hybrid approach in Colorado
- **Isolation at clinical laboratory is preferred**
  - Faster results
  - Less concern about transit of raw specimens
  - Susceptibility results available for patient care
- **Outreach to clinical laboratories that adopt CIDT**
  - Request reflex cultures for *Salmonella, Shigella* and *Vibrio*
  - Review isolate submission protocols

Isolate Recovery at the State Public Health Laboratory (SPHL)

- **Clinical material sent from laboratory to SPHL**
  - Isolate recovery done at SPHL
- **Determine resources**
  - Select priority pathogens: STEC, *Salmonella*, *Shigella*, *Vibrio*
  - Seek additional funding for culture
- **Review and modify Board of Health reporting regulations and submission requirements**
  - "Isolates or clinical material" of selected pathogens
  - Required, no longer voluntary

STEC: Shiga toxin-producing *E. coli*

Facilitating Specimen Submissions to the State Public Health Laboratory (SPHL)

- **Facilitate rapid delivery to SPHL**
  - Courier service to ensure regular service where needed
- **Provide transport media**
- **Written guidance based on new APHL studies**
- **Continuous improvement and education**
  - Work with laboratories when specimen sent incorrectly

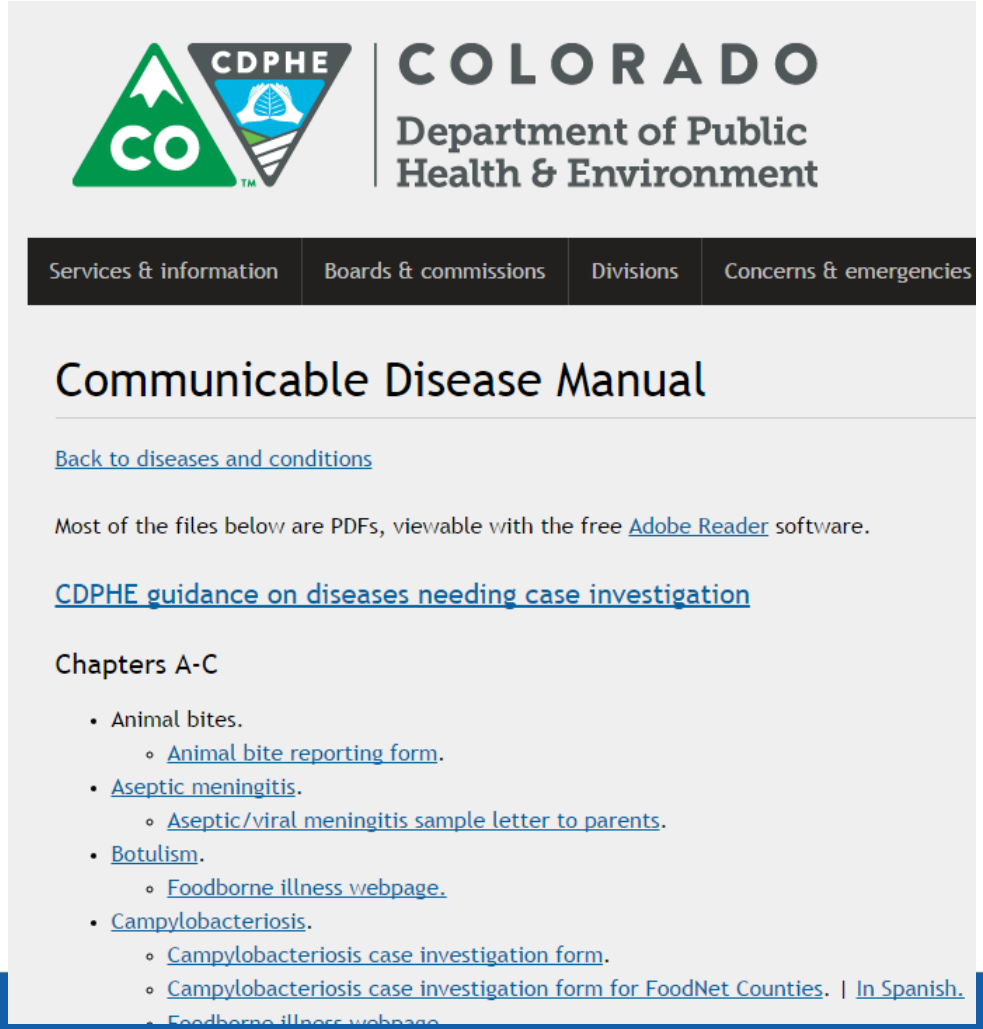
APHL: Association of Public Health Laboratories

Adapting Public Health Practice

- **Increase in case reports with less certainty about each one**
- **Some data received more quickly, but lag time increased for others**
  - Subtyping data is delayed
- **Implement new case definitions**
  - Collect more detailed test data
  - Capture pertinent negative results (e.g., PCR positive but culture negative)
- **Train staff to appropriately assign case status**
  - Create new algorithms and guidance documents

Adapting Public Health Practice: Case Investigation

- **Establish and evaluate guidance**
- **Prioritize which cases should be investigated**
  - Consider local resources
  - Priority based on disease and test results
- **Timing of case investigation**
  - If public health will investigate, don't wait for culture results
  - Other jurisdictions might make different decisions



Adapting Public Health Practice: Exclusions and Treatment

- **Worker and childcare exclusion or restriction for PCR-positive results**
  - Treating PCR-positive results like culture
  - Follow up testing is often done at SPHL at no charge
- **Handling patients CIDT positive for 2 or more reportable conditions**
  - Treatment and disease control decisions
  - Choose control measures for pathogen with greatest risk of transmission
  - Use the most comprehensive pathogen-specific questionnaire

SPHL = State public health laboratory

Areas of State Action in Response to CIDT

- Accurate case reporting
- Isolate recovery
- Adapting public health practice to new type of cases being reported

- Assessment of resources
- Prioritization for detecting disease and mitigating risk
- Frequent communication with partners

Thank You

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Advancing Diagnostic Innovations and Public Health Needs

Brad Spring

Vice President, Regulatory Affairs & Compliance

BD Life Sciences (representing AdvaMedDx)

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

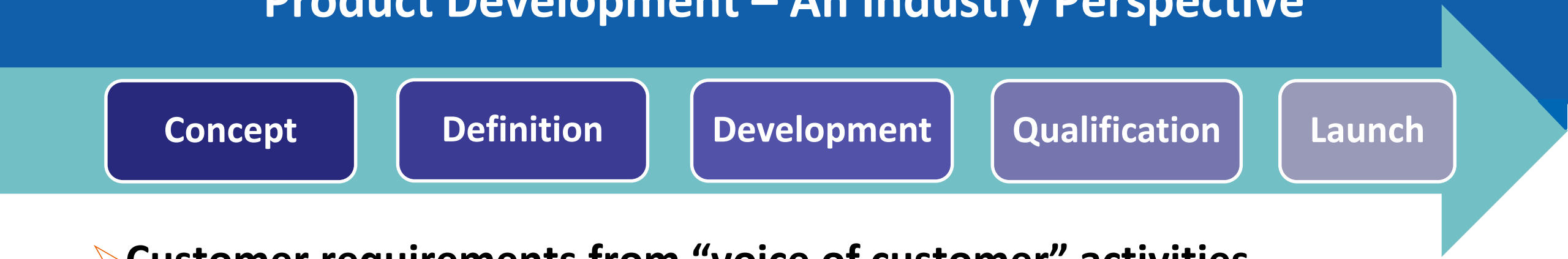
Advantages and Challenges

- New multiplexed diagnostic tests offer great benefit to physicians, patients, and laboratories
- High sensitivity and more rapid results as compared to conventional culture
- However, these new tests may hinder the ability to preserve viable organisms needed for public health related activities

We support continuing partnerships to ensure the availability of organisms for surveillance and susceptibility testing

ADX: AdvaMedDx
APHL: Association of Public Health Laboratories
CSTE: Council of State and Territorial Epidemiologists

Product Development – An Industry Perspective



- **Customer requirements from "voice of customer" activities are gathered during concept and definition phases**
  - Customer "must haves" and other requirements are documented through interviews with lab personnel, clinicians, administrators and other key stakeholders
  - Requirements are translated into specification
  - Technology solutions are chosen to meet specifications
  - Conflicting requirements can create challenges
    - e.g., cell lysis required for testing while preserving a viable organism

Opportunities for Improvement in Product Development

- **Ensure engagement with public health laboratories in "voice of customer" activities**
  - Understand and incorporate public health needs into the product development process
- **Encourage incorporation of future public health needs for access to needed specimens in the event that a notifiable pathogen is detected**
- **Manage conflicting product requirements**

Opportunities for Improvement in Product Development

- **Future technology trends may align better with public health needs**
  - e.g., metagenomics, proteomics, and next generation sequencing, including whole genome sequencing
- **Work with clinical labs that develop their own tests**

Opportunities to Improve Collection and Preservation of Isolates

- **Laboratories are required to follow manufacturers' instructions according to the Clinical Laboratory Improvement Amendments (CLIA)**
  - AdvaMedDx members support providing APHL recommendations to clinical labs
  - Information should reinforce the need to preserve isolates or clinical materials for submission to the appropriate public health laboratory
    - <u>Precaution related to Public Health Reporting</u>:
      Laboratories must follow state and/or local rules pertaining to reportable pathogens and should consult their local and/or state public health laboratories for isolate and/or clinical sample submission guidelines

www.aphl.org
www.cms.gov
www.fda.gov

Opportunities to Continue Collaborations

- **Continue to work with public health laboratories, manufacturers, and appropriate Federal agencies to discuss status of efforts and explore additional measures to aid surveillance efforts**
- **Educational outreach with key constituency meetings**
  - Public health labs, microbiology groups, and industry meetings
  - Manufacturers can assist by distributing education material and holding in-service training

Opportunities to Better Understand the CIDT Landscape

Provide informational resources

- FDA could, for example, post a list of approved or cleared molecular diagnostics on the FDA website
  - This will serve as a helpful resource on new molecular multi-analyte gastrointestinal (GI) disease agent detection panel devices that are cleared or approved with a one-stop shop for understanding how specimens are processed

Next Steps: Direct-from-specimen Testing to Characterize Pathogens

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Division of Foodborne, Waterborne, and Environmental Diseases
National Center for Emerging Zoonotic and Infectious Diseases

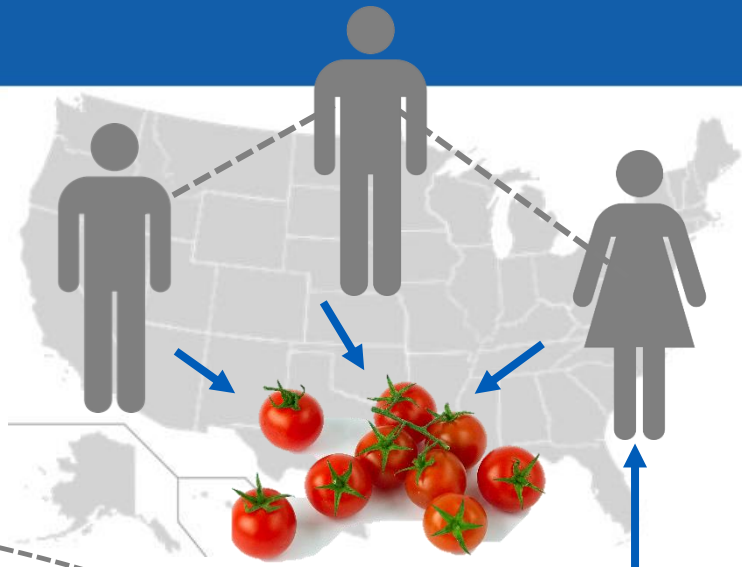
U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

Why Develop Direct-from-specimen Tests to Characterize Pathogens?

- **Specimen compatibility with commercial systems**
  - Even if biologically inactivated
- **Reduced time to actionable results**

Direct-from-specimen Tests Reduce Time to Actionable Results

- More outbreaks solved more quickly
- More illnesses prevented



Patient Eats Contaminated Food

Onset of Illness 1–3 days

Patient Becomes Ill

Contact with health care system 1–5 days

Stool Sample Collected

Diagnosis 1–3 days

***Salmonella* Identified**

Shipping 0–7 days

Public Health Laboratory Receives Sample

Serotyping and DNA fingerprinting 2–10 days

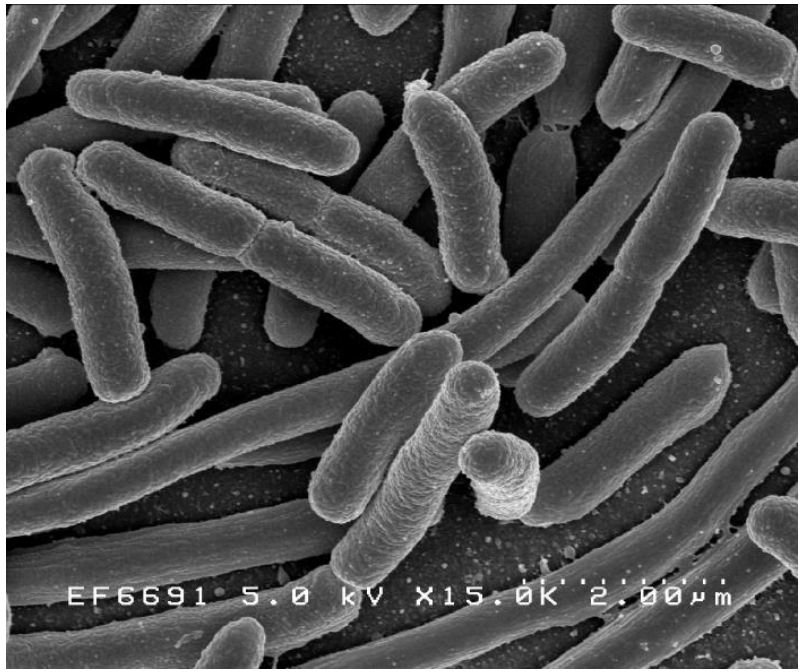
Case Confirmed as Part of Outbreak

Food Vehicle Identified

Opportunity to reduce reporting time

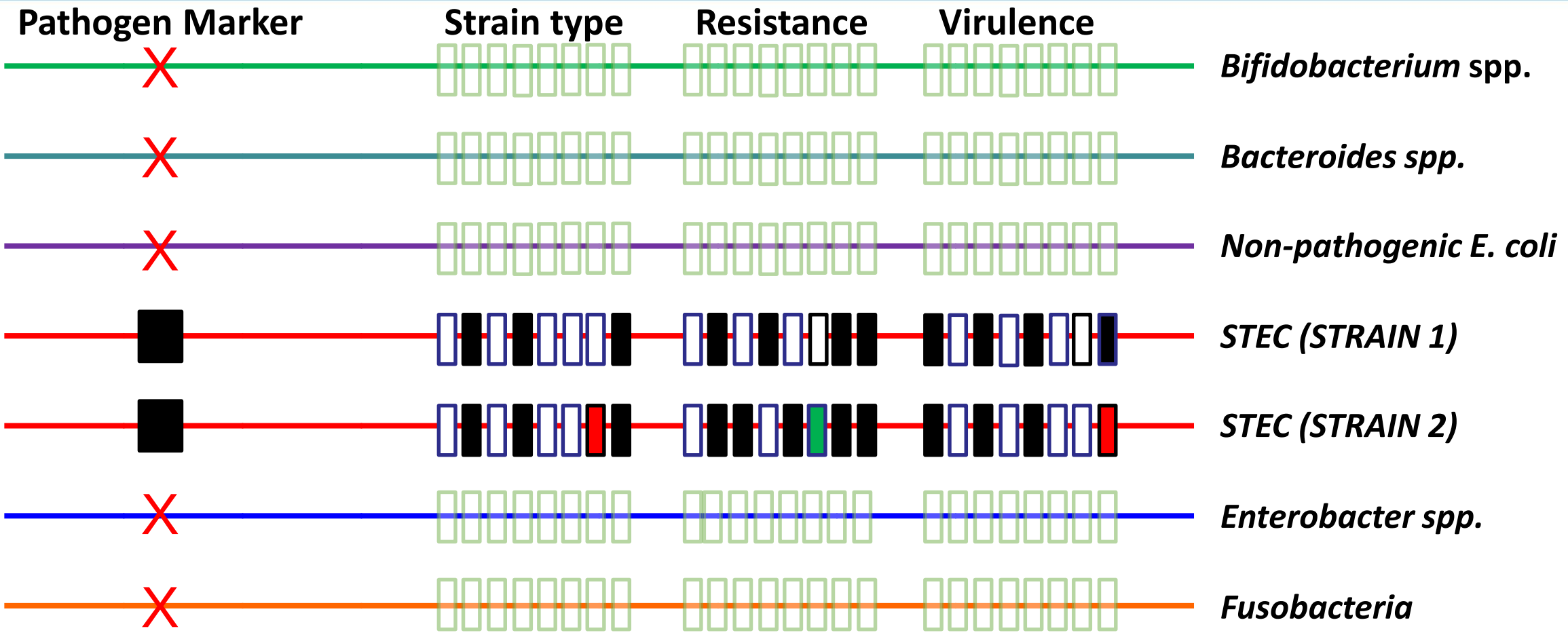
Stool Is a Complex Environment

- **Stool contains a variety of DNA and RNA**
  - Human
  - Food (consumed plant and animal material)
  - Bacteria, parasites, viruses, fungi
    - Average number of microbial species per person: 1,000
    - Microbial load: ~100 billion organisms per gram of stool
- **Some pathogens are genetically similar to commensal flora (i.e., other organisms normally found in the stool)**
  - e.g., *Salmonella,* Shiga toxin-producing *E. coli*

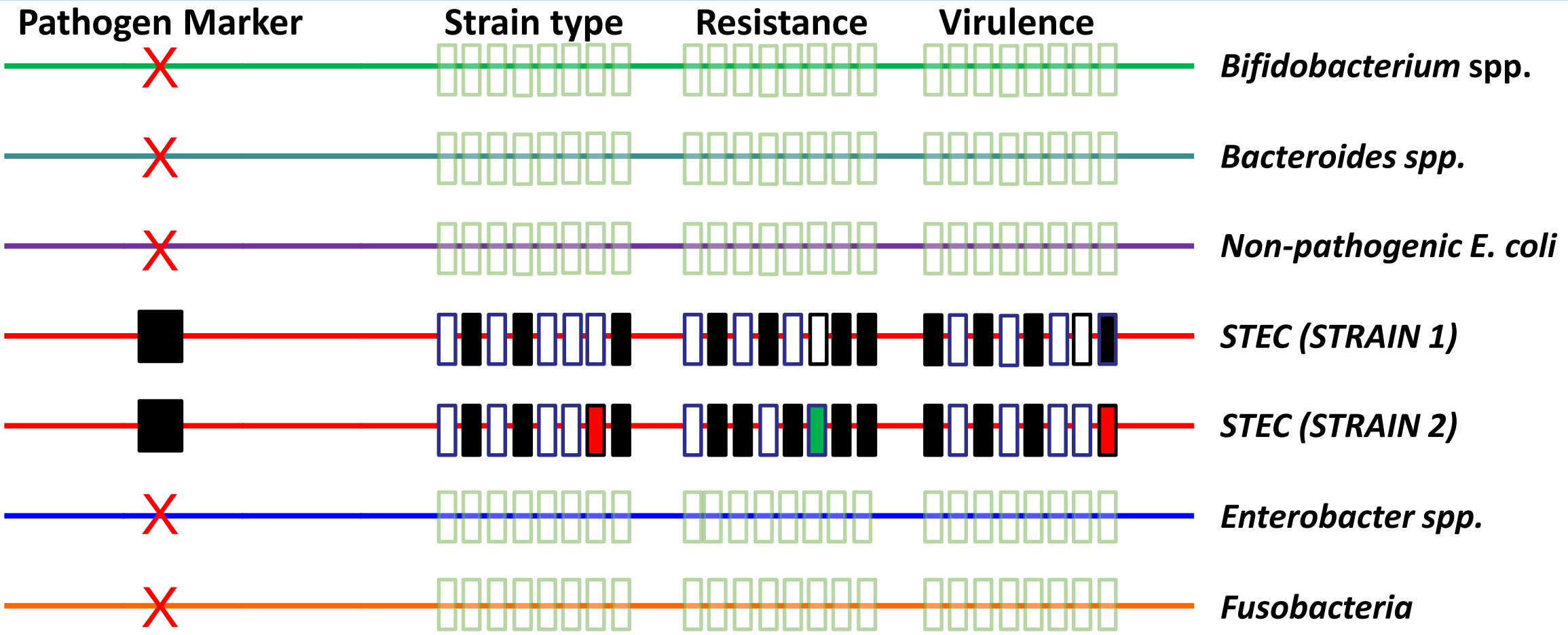


Science 336:a8 1246-1247
Lepage P, Leclerc MC, Joossens M, et al. A metagenomic insight into our gut's microbiome. Gut. 2012 Apr 23

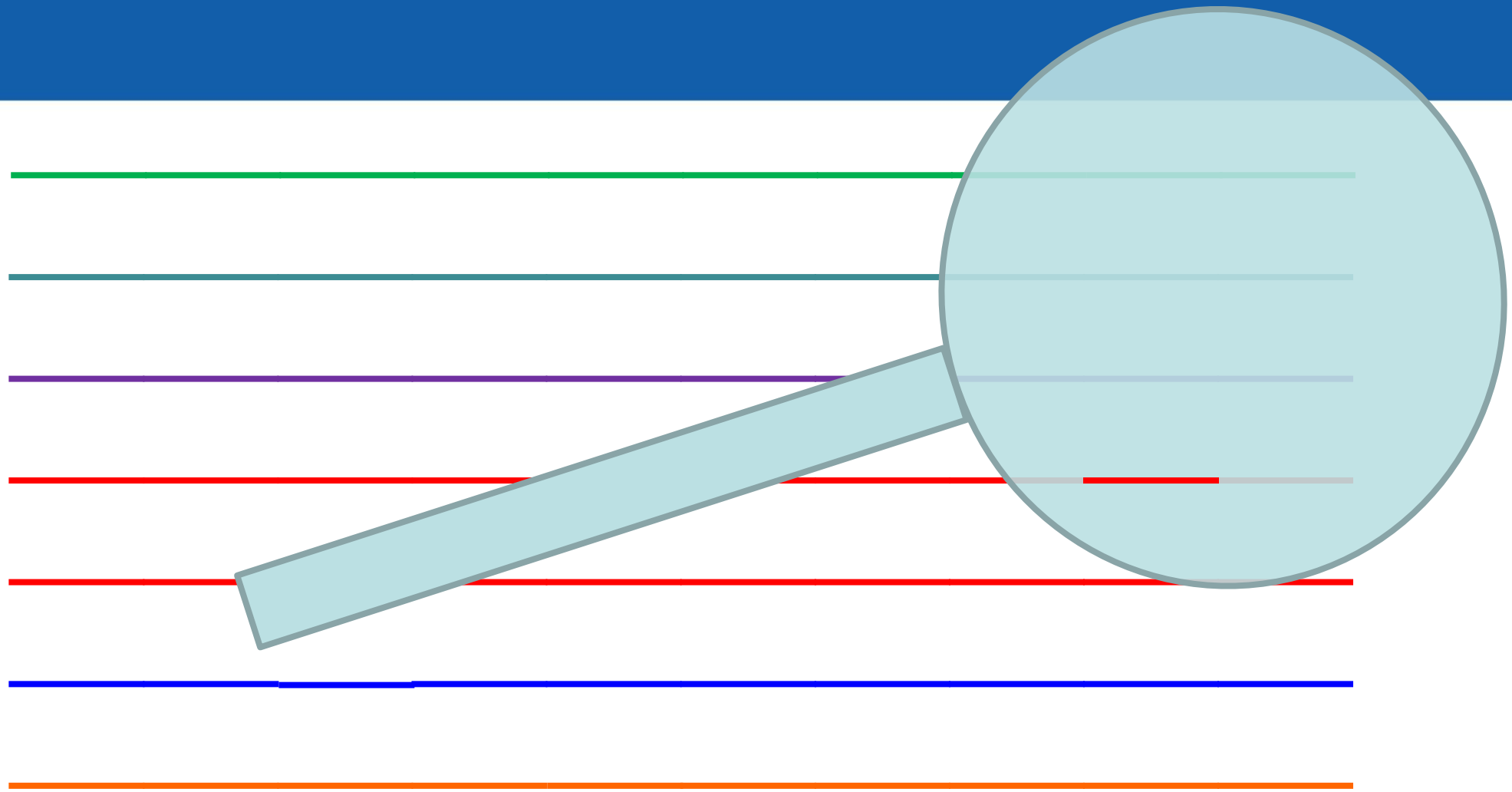
Direct-from-stool Pathogen Characterization



Direct-from-stool Pathogen Characterization



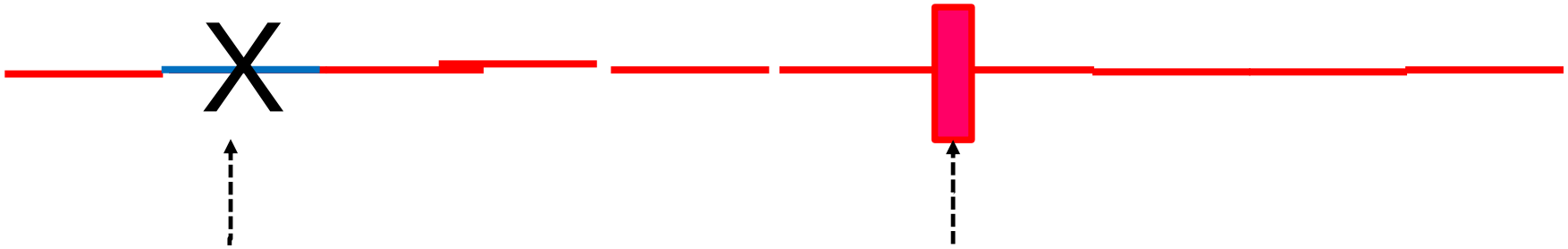
Challenges to Direct-from-stool Pathogen Characterization



SIGNAL-TO-NOISE
Finding pathogen DNA among millions of DNA segments in a sample

PHASING
Distinguishing pathogen DNA from closely related organisms' DNA

Challenges to Direct-from-stool Pathogen Characterization



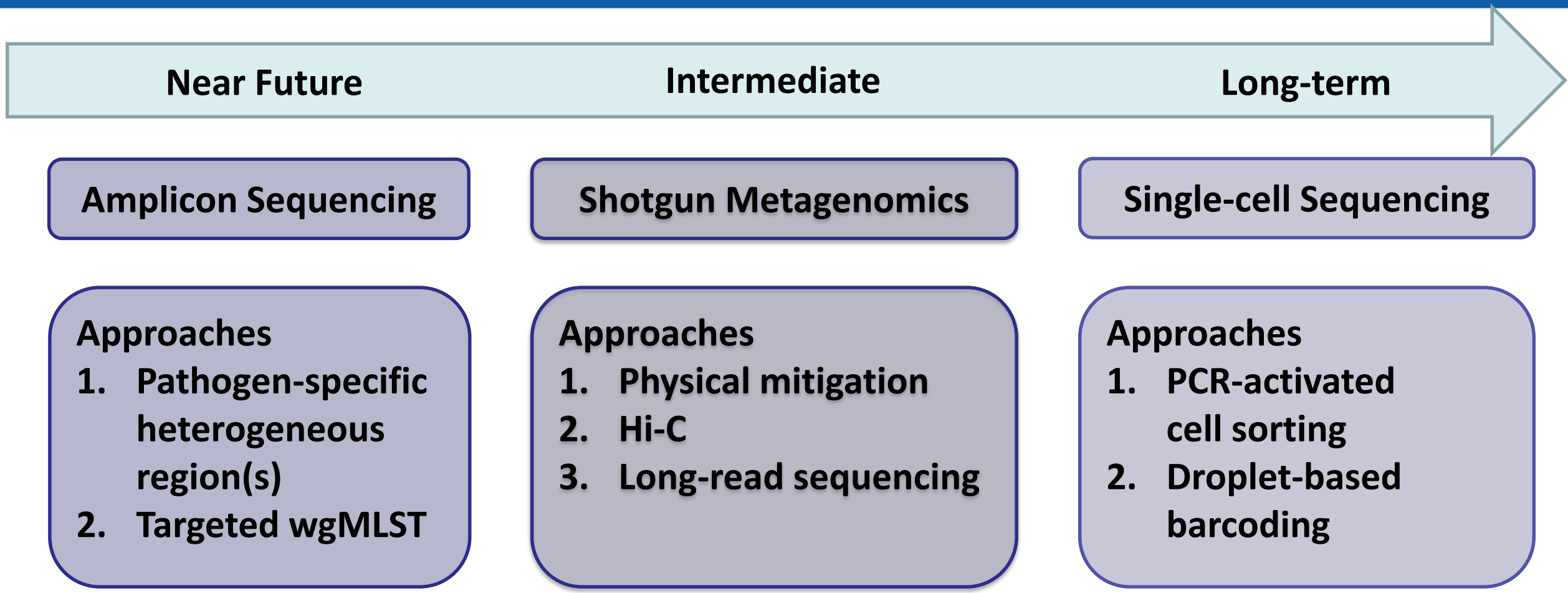
Genetic material shared by commensal flora

Pathogen specific marker

SIGNAL-TO-NOISE
Finding pathogen DNA among millions of DNA segments in a sample

PHASING
Distinguishing pathogen DNA from closely related organisms' DNA

Future Approaches to Pathogen Characterization Using Direct-from-stool Tests



wgMLST: Whole genome multilocus sequence typing
Hi-C: Variant of chromosome conformation capture technique

Future Approaches to Pathogen Characterization Using Direct-from-stool Tests

Near Future	Intermediate	Long-term
Amplicon Sequencing	Shotgun Metagenomics	Single-cell Sequencing
Approaches 1. **Pathogen-specific heterogeneous region(s)** 2. **Targeted wgMLST**	Approaches 1. Physical mitigation 2. Hi-C 3. Long-read sequencing	Approaches 1. PCR-activated cell sorting 2. Droplet-based barcoding

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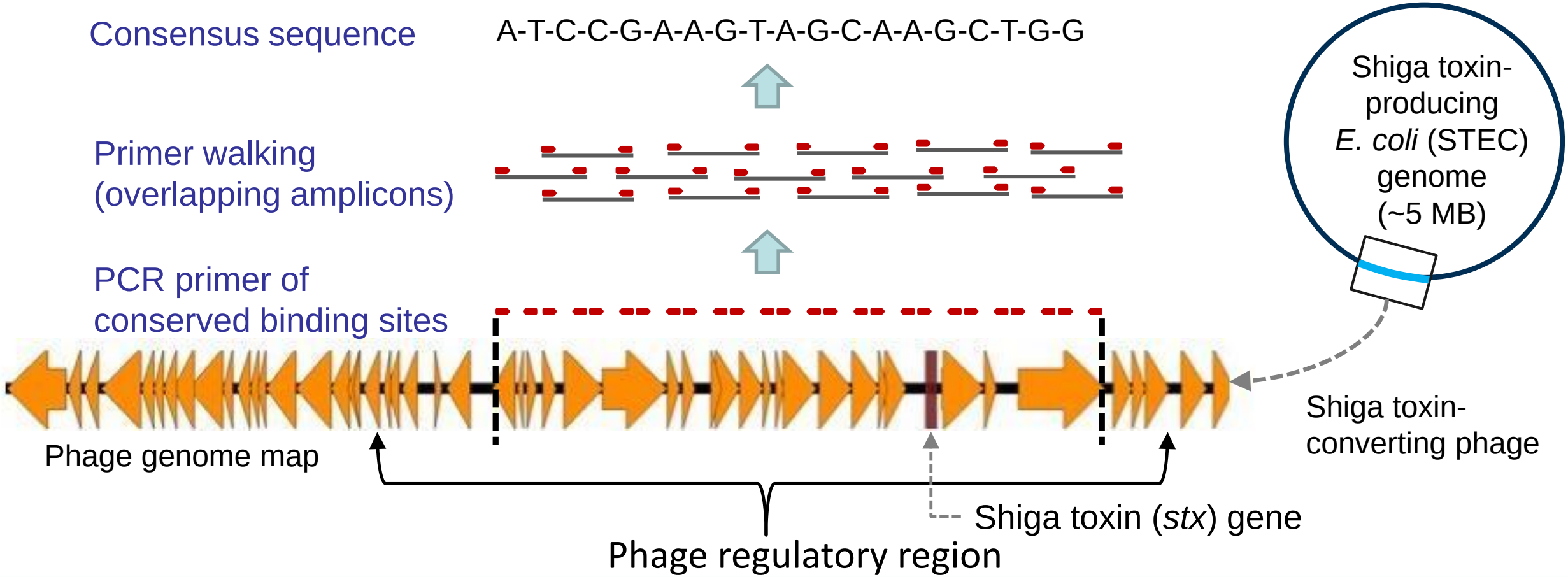
Amplicon Sequencing: Heterogeneous Region of *Escherichia coli* O157:H7 Sakai



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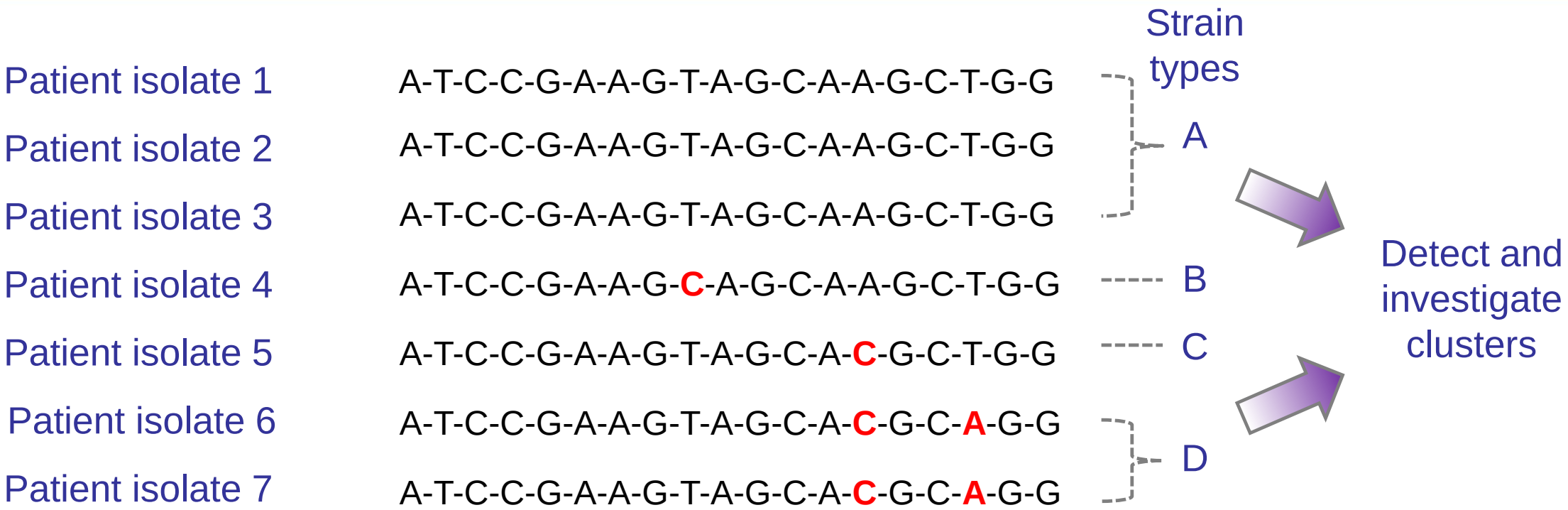
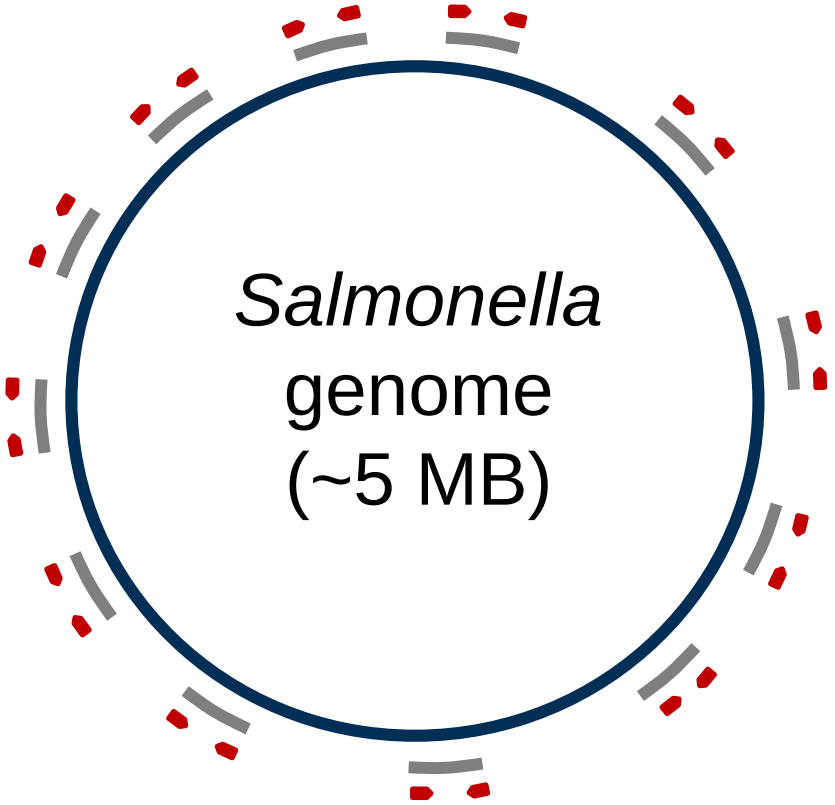


Second Approach to Amplicon Sequencing: Targeted wgMLST of *Salmonella spp.*



- **Pipeline of different processes to identify suitable targets**
  - Identify homologous genes
  - Create primers capturing variation
  - Test primers for specificity
  - Test subtyping resolution

wgMLST: whole genome multilocus sequence typing

Future Approaches to Pathogen Characterization Using Direct-from-stool Tests

Near Future	Intermediate	Long-term
Amplicon Sequencing	**Shotgun Metagenomics**	Single-cell Sequencing
Approaches 1. Pathogen-specific heterogeneous region(s) 2. Targeted wgMLST	**Approaches** **1. Physical mitigation** **2. Hi-C** **3. Long-read sequencing**	Approaches 1. PCR-activated cell sorting 2. Droplet-based barcoding

wgMLST: Whole genome multilocus sequence typing
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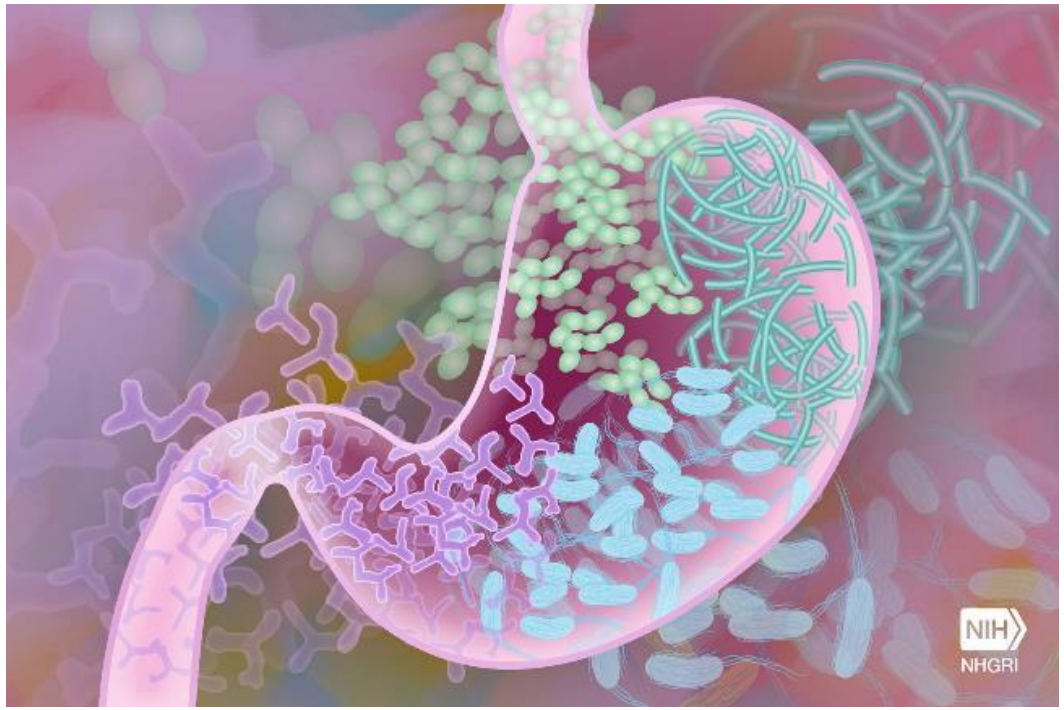
Shotgun Metagenomics

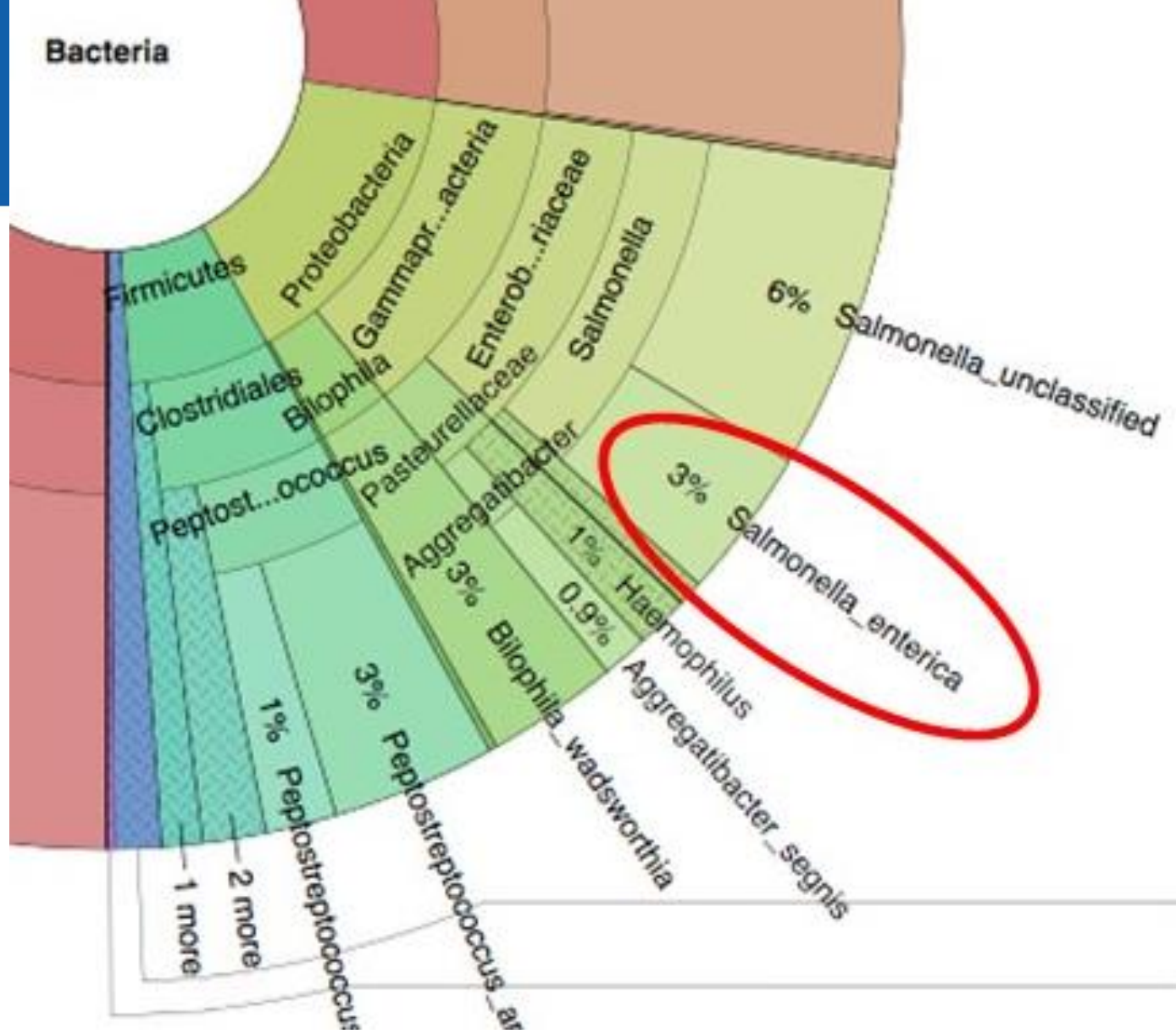
- Unbiased sequencing of nucleic acids recovered directly from an environment, such as a stool or sputum
- Widely used for characterizing microbiomes



A microbiome is a community of commensal, symbiotic and pathogenic microorganisms that live in an area of the body

Shotgun Metagenomics

- **Detect pathogens directly in stool**
- **Current capability**
  - Differentiate strains
- **Current limitations**
  - Insensitive
  - Expensive
  - Long turnaround time
  - Large data computing and storage demands



Partial Krona plot from patient specimen,
Outbreak of *Salmonella* Heidelberg

Strategies to Making Shotgun Metagenomics Practical for Public Health

Many direct-from-specimen approaches are being explored to improve signal-to-noise, phasing, cost, and data volume!

Specimen

NUCLEIC ACID EXTRACTION

DNA
RNA
TNA

POST-EXTRACTION AND LIBRARY CONSTRUCTION

LIBRARY

SEQUENCING

Sequencer

EXAMPLES

1. Differential cell lysis
2. Separation/Pulldown
3. Formaldehyde cross-linking

EXAMPLES

1. Nucleases (RNAse/DNAse)
2. Bind/degrade CpG methylated DNA
3. Preferential separation

EXAMPLES

1. Long read technology
2. Multiplexing and pooling
3. Bioinformatics binning strategies

TNA: Threose nucleic acid

Future Approaches to Pathogen Characterization Using Direct-from-stool Tests

Near Future	Intermediate	Long-term
Amplicon Sequencing	Shotgun Metagenomics	**Single-cell Sequencing**
Approaches 1. Pathogen-specific heterogeneous region(s) 2. Targeted wgMLST	Approaches 1. Physical mitigation 2. Hi-C 3. Long-read sequencing	**Approaches** **1. PCR-activated cell sorting** **2. Droplet-based barcoding**

wgMLST: Whole genome multilocus sequence typing
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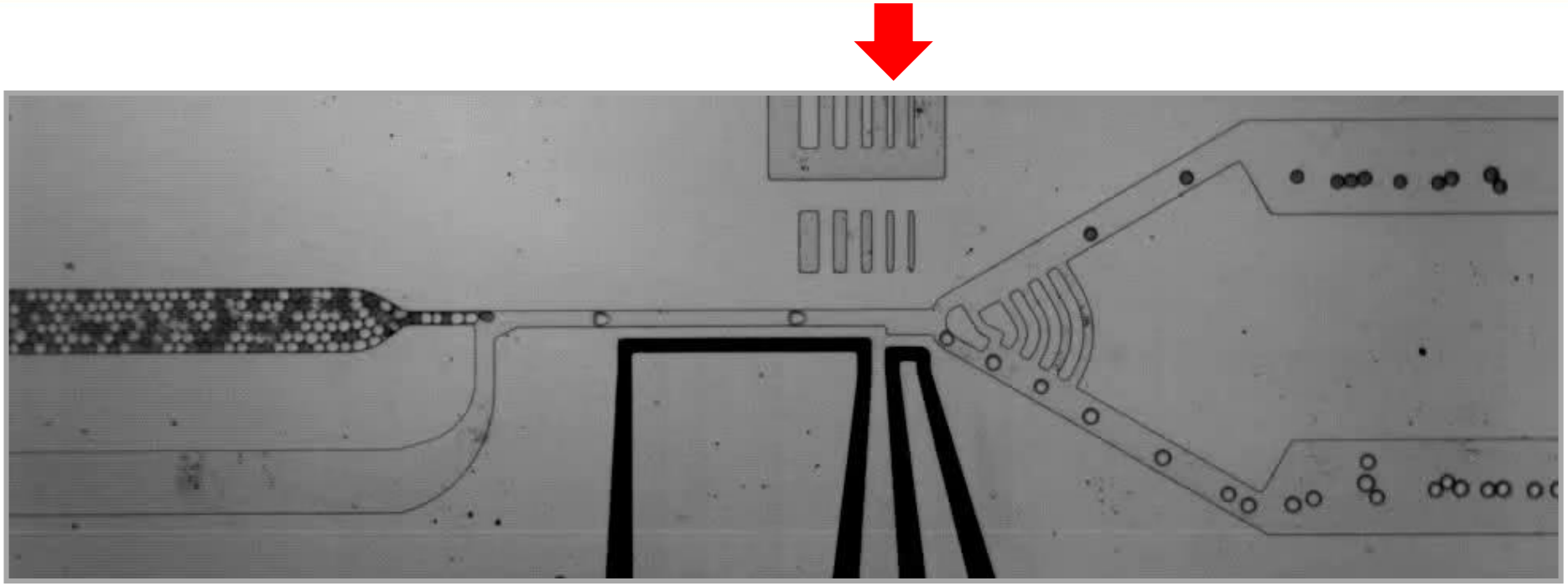
PCR-Activated Cell Sorting and Single Cell Sequencing



Pathogen cells

Non-pathogen cells

Sorts individual cells based on selected characteristics, using drops with optical probe

from Dr. David Weitz, Harvard University

Development of Direct-from-specimen Pathogen Characterization Assays

- Increase compatibility between CIDTs and public health needs
- Current technological limitations are likely to be overcome with research effort from multiple partners
- Make PulseNet more efficient and more effective

To Keep Everyone Healthy, Pursue a Path that Benefits Patient Care and Public Health

- **Technology for clinical diagnoses will continue to advance**
- **Public health continues to adapt surveillance efforts**
  - Modifying case definitions
  - Encouraging reflex culture
  - Coordinating efforts with the medical device industry
- **Advancing technology for public health can make our lives safer**
- **The solution is working together to develop better diagnostic tests to benefit patient care and public health**

Changes in Clinical Diagnostics and Tracking Infectious Diseases

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