Culture Independent Diagnostics: The End of Foodborne Disease Surveillance or the Beginning of a New Era

Dave Boxrud
Minnesota Department of Health
Technology Changes

- 1976-89% of photo film market
- 2012-filed bankruptcy
Overview

• Foodborne disease surveillance
• Culture independent diagnostic testing (CIDT) for foodborne disease pathogens
• Future of foodborne disease surveillance
Foodborne Illness: US

- Significant burden
- Societal cost ~$77B
- Largely preventable

1 in 6

About 1 in 6 (or 48 million) people get sick each year from contaminated food.
Current Foodborne Disease Surveillance in the US

• Outbreak detection
• Case counts (monitor trends)
• Antibiotic Susceptibility Testing
Diseases Reportable to the Minnesota Department of Health

651-201-5414 or 1-877-676-5414 24 hours a day, 7 days a week

Report Immediately by Telephone

Anthrax (Bacillus anthracis)  
Botulism (Clostridium botulinum)  
Brucellosis (Brucella spp)  
Cholera (Vibrio cholerae)  
Diphtheria (Corynebacterium diphtheriae)  
Measles (rubella)  
Meningococcal disease (Neisseria meningitidis)  
Orthopox virus  
Plague (Yersinia pestis)  
Polioviruses  
Q Fever (Coxiella burnetii)  
Rubella  
Shigellosis (Shigella flexneri)  
Tularemia (Francisella tularensis)  
Unusual or increased case incidence of any suspected infectious disease

Report Within One Working Day

Acute parotitis (Enzyma histolytica)  
Anaplasmosis (Anaplasma phagocytophilum)  
Arbovirus disease  
Borrelia burgdorferi  
Brucellosis (Brucella spp)  
Campylobacteriosis (Campylobacter spp)  
Cat scratch disease (infection caused by Bartonella spp)  
Chlamydia (Chlamydia trachomatis)  
Clostridium difficile  
Clostridium tetani  
Coccidioidomycosis  
Cryptosporidiosis (Cryptosporidium spp)  
Cyclosporiasis (Cyclospora spp)  
Dengue fever  
Ebola virus infection  
Enterovirus  
Encephalitis (caused by viral agents)  
Enterovirus (Coxsackie A)  
Escherichia coli  
Giardiasis (Giardia lamblia)  
Gonorrhea (Neisseria gonorrhoeae)  
Cryptosporidiosis (Cryptosporidium parvum)  
Diphtheria (Corynebacterium diphtheriae)  
Escherichia coli (E. coli)  
Filarial infection  
Footnotes

a Submission of clinical specimens required. Submit isolates or, if isolate is not available, submit material containing the infectious agent in the following order of preference: a patient specimen; Molecular assay on orthononucleic acid material. Call the MDH Public Health Laboratory at 651-201-4636 to report a case.

To Report a Case

For diseases that require immediate reporting call 651-201-5414 or 1-877-676-5414.

To report by mail, contact the Minnesota Department of Health at the following address: 225 East 2nd Street, St. Paul, MN 55101-5057.
Pulsed-Field Gel Electrophoresis (PFGE)
Enteric Disease Interview

• Demographics
• Symptoms/treatment
• Exposures
• Food history
Cases of *Salmonella typhimurium*, by Week of Specimen Collection, Minnesota, June-September, 1995

![Graph showing cases of *Salmonella typhimurium* by week. The graph peaks in August with a single case in September.]
PFGE Subtype Patterns of *Salmonella typhimurium*, by Week of Specimen Collection, Minnesota, June - September, 1995

Week

<table>
<thead>
<tr>
<th>June</th>
<th>July</th>
<th>Aug</th>
<th>Sept</th>
</tr>
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<tbody>
<tr>
<td>Cases</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
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<td>4</td>
<td></td>
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<td>6</td>
<td></td>
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<td>8</td>
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<td>10</td>
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<td>12</td>
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<td>14</td>
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<td>16</td>
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<td>18</td>
<td></td>
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<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>22</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

PFGE Pattern Restaurant A
PFGE Pattern Restaurant B
PFGE Pattern Restaurant C
Other PFGE Patterns
States Submit PFGE Patterns to the National Database
1993 Western States *E. coli* O157 Outbreak

- 726 cases
- 4 deaths
- outbreak detected 1993
- Recall: 150,000 patties
- 39 d

2002 Colorado *E. coli* O157:H7 Outbreak

- outbreak detected 2002
- Recall: 18,600,000 lbs (multiple dates)
- 18 d
<table>
<thead>
<tr>
<th>Year</th>
<th>Pathogen</th>
<th>Food</th>
<th>Amount recalled</th>
</tr>
</thead>
<tbody>
<tr>
<td>2011</td>
<td><em>Listeria monocytogenes</em></td>
<td>Cantaloupe</td>
<td>Unknown</td>
</tr>
<tr>
<td>2011</td>
<td><em>Salmonella</em> Heidelberg</td>
<td>Ground turkey products</td>
<td>&gt;36,000,000 lbs</td>
</tr>
<tr>
<td>2010</td>
<td><em>Salmonella</em> Enteritidis</td>
<td>Shell eggs</td>
<td>&gt;500,000,000 eggs</td>
</tr>
<tr>
<td>2010</td>
<td><em>Salmonella</em> Montevideo</td>
<td>Ready-to-eat Italian sausage products/pepper</td>
<td>&gt;1,263,754 lbs</td>
</tr>
<tr>
<td>2009</td>
<td><em>E. coli</em> O157:H7</td>
<td>Cookie dough</td>
<td>300,000 cases of product</td>
</tr>
<tr>
<td>2009</td>
<td><em>Salmonella</em> Typhimurium</td>
<td>Peanut butter/peanut products</td>
<td>&gt;3000 types of products</td>
</tr>
<tr>
<td>2008</td>
<td><em>E. coli</em> O157:H7</td>
<td>Ground beef</td>
<td>5,300,000 lbs</td>
</tr>
<tr>
<td>2007</td>
<td><em>Salmonella</em> I 4,5,12:i:-</td>
<td>Frozen pot pies</td>
<td>Millions of pot pies</td>
</tr>
<tr>
<td>2007</td>
<td><em>E. coli</em> O157:H7</td>
<td>Frozen pizza</td>
<td>5,000,000 pizzas</td>
</tr>
<tr>
<td>2007</td>
<td><em>E. coli</em> O157:H7</td>
<td>Ground beef (3 outbreaks)</td>
<td>35,400,000 lbs</td>
</tr>
<tr>
<td>2006</td>
<td><em>Salmonella</em> Tennessee</td>
<td>Peanut butter</td>
<td>326,000,000 lbs</td>
</tr>
<tr>
<td>2004</td>
<td><em>Salmonella</em> Enteritidis</td>
<td>Raw almonds</td>
<td>13,000,000 lbs</td>
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<tr>
<td>2003/'09</td>
<td><em>E. coli</em> O157:H7</td>
<td>Blade Tenderized Frozen Steak</td>
<td>865,046 lbs</td>
</tr>
<tr>
<td>2002</td>
<td><em>Listeria monocytogenes</em></td>
<td>Ready-to-eat poultry products</td>
<td>27,400,000 lbs</td>
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<tr>
<td>2002</td>
<td><em>E. coli</em> O157:H7</td>
<td>Ground beef</td>
<td>18,600,000 lbs</td>
</tr>
<tr>
<td>2000</td>
<td><em>Listeria monocytogenes</em></td>
<td>Ready-to-eat poultry products</td>
<td>16,900,000 lbs</td>
</tr>
<tr>
<td>2000</td>
<td><em>E. coli</em> O157:H7</td>
<td>Ground beef</td>
<td>1,100,000 lbs</td>
</tr>
<tr>
<td>1998</td>
<td><em>Listeria monocytogenes</em></td>
<td>Hot dogs, deli meats</td>
<td>35,000,000 lbs</td>
</tr>
<tr>
<td>1998/'08</td>
<td><em>Salmonella</em> Agona</td>
<td>Toasted oats cereal</td>
<td>&gt;3,000,000 lbs</td>
</tr>
<tr>
<td>1997</td>
<td><em>E. coli</em> O157:H7</td>
<td>Frozen ground beef</td>
<td>25,000,000 lbs</td>
</tr>
</tbody>
</table>
Industries with Improved Safety Practices
(key information provided by PulseNet-triggered investigations)

- Ready-to-eat & “ready-to cook” foods
- Peanut products
- Leafy greens
- Other vegetables
- Mellon
- Sprouts
- Flour
- Eggs
- Beef
- Tree nuts
- Poultry
- Spices
- Peanuts
- Melon
- Poultry
83 member countries from 7 national and regional PulseNet networks

- PulseNet Canada
- PulseNet USA
- PulseNet Latin America & Caribbean
- PulseNet Europe
- PulseNet Africa
- PulseNet Middle East
- PulseNet Asia Pacific

December 2011
Accurate Case Counts: Burden of Illness
Accurate Case Counts: Attribution, Understanding Trends
National Antimicrobial Resistance Monitoring System (NARMS)

http://www.fda.gov/cvm/narms_pg.html
## Selected Microbial Disease Agents Under Surveillance

<table>
<thead>
<tr>
<th>Agent</th>
<th>Public health surveillance</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella spp.</em></td>
<td>Subtype, AST</td>
</tr>
<tr>
<td>Shigatoxin-producing <em>E. coli</em></td>
<td>Subtype, AST</td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>Subtype, AST</td>
</tr>
<tr>
<td><em>Mycobacterium tuberculosis</em></td>
<td>Genotype, AST</td>
</tr>
<tr>
<td><em>Bordetella pertussis</em></td>
<td>AST</td>
</tr>
<tr>
<td><em>Neisseria meningitidis</em></td>
<td>Subtype, AST</td>
</tr>
<tr>
<td><em>Legionella pneumophila</em></td>
<td>Subtype (outbreaks)</td>
</tr>
<tr>
<td>Influenza virus</td>
<td>Serotype, AST</td>
</tr>
<tr>
<td><em>Neisseria gonorrhoea</em></td>
<td>AST</td>
</tr>
<tr>
<td>Methicillin-resistant <em>Staphylococcus aureus</em></td>
<td>Subtype (outbreaks)</td>
</tr>
<tr>
<td><em>Cryptococcus neoformans</em></td>
<td>AST</td>
</tr>
</tbody>
</table>
Antimicrobial Resistance: 1996–2010


Figure 1. Percentage of non-typhoidal *Salmonella* isolates resistant to nalidixic acid, by year, 1996–2010
“A Big Victory for Public Health”

FDA decision to withdraw the use of Baytril in poultry

In a landmark decision, U.S. Food and Drug Administration (FDA) recently ordered the removal of Baytril, an enrofloxacin, from use in domestic poultry. This decision was made despite significant opposition from the pharmaceutical industry and some members of the public and the poultry industry. Public health data, including contributions from FoodNet and the National Antimicrobial Resistance Monitoring System (NARMS), showed convincing evidence of the spread of resistant bacteria in humans associated with Baytril use. This decision is considered a significant victory for public health and the safety of consumers.

Please refer to the letter on page 3 of this issue for more detail.
Past/Present Testing

1. Patient eats contaminated food
2. Develops Illness
3. Stool Collected
4. Identification (culture)
5. Clinical diagnosis
6. Public Health
Current Foodborne Disease Surveillance in the US

- Outbreak detection- **REQUIRES ISOLATES**
- Antibiotic Susceptibility Testing- **REQUIRES ISOLATES**
- Case counts- **ACCURACY/CONSISTENCY REQUIRES ISOLATES**
CIDT and Impacts
Bacterial Culture
Rapid ("Culture-Independent"; "Non-Culture") Tests
First Generation CIDT

- **Antigen-detection tests**
  - Enzyme immunoassays (EIA) and immunochromatographic cards (lateral flow, rapid cartridge)
  - Concerns regarding accuracy and reproducibility of results

- **Non FDA-approved PCR assays**
  - Unknown validation process
Luminex launches xTAG™ Respiratory Viral Panel

Luminex Molecular Diagnostics, a division of Luminex Corporation, has launched xTAG Respiratory Viral Panel (RVP), an assay for the detection of multiple viral types and subtypes, including influenza, metapneumovirus and adenovirus. xTAG has been developed in association with a team of leading virologists and infectious disease specialists. The test can assess 12 viral targets at once and provide qualitative results in just few hours. The test has received 510(k) clearance from the US Food and Drug Administration (FDA) and CE mark for sale in Europe.

xTAG RVP was developed using LMD’s Universal Array which operates on the Luminex xMAP® system, a bioassay detection platform that can detect up to 100 different analytes simultaneously. The system uses specific nucleic acid targets to detect specific pathogens in a patient’s nasal swab. The test will allow for rapid diagnosis of viral and bacterial infections. The system uses an array of probes to detect the presence of specific pathogens. The results are then analyzed using Luminex xMAP® technology, allowing for the detection of multiple pathogens in a single test.

Test for up to 15 GI bacteria, viruses, and parasites in 5 hours
• 4 bacterial pathogens in 4 hours
• Campylobacter
• Salmonella
• Shigella
• Shiga toxin 1 and 2
Outbreaks of Foodborne Illnesses Are Becoming Harder to Detect

New diagnostic tests inadvertently undercut surveillance abilities of public health officials

By Clare Leschin-Hoar

NEW TESTS COULD HAMPER FOOD OUTBREAK DETECTION

By MARY CLARE JALONICK and LAURAN NEERGAARD — Dec. 11 2:58 PM EST
Demise of GC Culture

- Rapid (hours)
- Urine specimen (vs urethral swab)
- Includes *Chlamydia trachomatis*
- High sensitivity/specificity
- No susceptibility data
- Specimen incompatible with culture
- Expensive
### Rapid / Culture-Independent Tests versus Culture

<table>
<thead>
<tr>
<th></th>
<th>Culture</th>
<th>Rapid/culture-independent tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Speed</strong></td>
<td>Slow</td>
<td>Fast</td>
</tr>
<tr>
<td><strong>Infrastructure needed</strong></td>
<td>Significant</td>
<td>Minimal</td>
</tr>
<tr>
<td><strong>Expertise required</strong></td>
<td>Significant</td>
<td>Minimal</td>
</tr>
<tr>
<td><strong>Labor cost</strong></td>
<td>High</td>
<td>Low</td>
</tr>
<tr>
<td><strong>Cost of materials</strong></td>
<td>Low</td>
<td>High</td>
</tr>
</tbody>
</table>
## Rapid / Culture-Independent Tests versus Culture

<table>
<thead>
<tr>
<th></th>
<th>Culture or standard tests (e.g. microscopy)</th>
<th>Rapid/culture independent tests</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sensitivity</strong></td>
<td>Gold standard</td>
<td>Low to high</td>
</tr>
<tr>
<td><strong>Specificity</strong></td>
<td>High</td>
<td>Low to high, almost always different</td>
</tr>
<tr>
<td><strong>Interpretation of positive findings</strong></td>
<td>Usually straightforward</td>
<td>Significant issues</td>
</tr>
<tr>
<td><strong>Range of pathogens detected</strong></td>
<td>All pathogens allowed by growth or test conditions</td>
<td>Limited to specific pathogen tested</td>
</tr>
<tr>
<td><strong>Allows for susceptibility testing &amp; genotyping?</strong></td>
<td>Yes</td>
<td>Generally no</td>
</tr>
</tbody>
</table>
Impacts of CID

• Patient Management
  • Need high sensitivity/specificity
  • More rapid treatment
  • Less expertise required
Impacts: Public Health Programs

- Surveillance requiring accurate case counts
- Isolate-requiring programs
**Solutions:** Accurate Case Counts

- Understand extent of issue
- Study test performance
- Redefine case definitions
- Sentinel reflex culturing?
## Crypto Assays Used by Clinical Laboratories, FoodNet Lab Surveys*

<table>
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<tr>
<th>Assay</th>
<th>2000(^a)</th>
<th>2012(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staining</td>
<td>68.9%</td>
<td>23%</td>
</tr>
<tr>
<td>DFA</td>
<td>23%</td>
<td>19%</td>
</tr>
<tr>
<td>EIA</td>
<td>17.3%</td>
<td>2%</td>
</tr>
<tr>
<td><strong>Rapid Assay (lateral flow)</strong></td>
<td>0</td>
<td>75%</td>
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</tbody>
</table>

*Some labs used more than 1 assay for diagnosing cryptosporidium

\(^a\)All FoodNet sites

\(^b\)MN clinical labs only
The overall PPV was 56% for rapid assays versus 97% for nonrapid assays; clinicians and laboratorians need to be aware of the low PPV of rapid assays when diagnosing cryptosporiosis.
Number of Cryptosporidium Cases in Minnesota, 2000-2011

Year of Specimen Collection

Number of Cases
Impacts: Public Health Programs

- Surveillance requiring accurate case counts

- Isolate-requiring programs
  - Subtype-based tracking programs
  - Susceptibility monitoring
  - Subtype-based attribution studies
Past/Present Testing

- Patient eats contaminated food
- Develops Illness
- Stool Collected
- Identification (culture)

Clinical diagnosis
Public Health
Testing with CIDT

Patient eats contaminated food

Develops Illness

Stool Collected

Identification (CIDT)

Identification (culture)

Clinical diagnosis

Public Health

?
Current Foodborne Disease Systems
General Strategies To Address Issue

- **Short-term**: Preserve isolates
- **Longer-term**: Develop culture-independent pathogen characterization methods
Short Term: Preserve Isolates

• Work with medical industry to make new tests compatible with public health needs
• Change criteria for medical device licensure?
• Modify State reporting rules
Submission of clinical materials required. Submit isolates or, if an isolate is not available, submit material containing the infectious agent in the following order of preference: a patient specimen; nucleic acid; or other laboratory material. Call the MDH Public Health Laboratory at 651-201-4953 for instructions.
Short Term: Preserve Isolates

• Work with medical industry to make new tests compatible with public health needs
• Change criteria for medical device licensure?
• Modify State reporting rules
• Develop isolate recovery capacity for PHLs*
• Sentinel susceptibility surveillance*
• Modify reimbursement?

*Requires new $$$
Long Term: Develop Culture Independent Characterization Methods

- Targeted sequencing/detection
- Single cell isolation and sequencing
- Metagenomics
Targeted Detection/Sequencing

characterize

Variable region
Single Cell Isolation and Sequencing

• Isolate single cells by micromanipulation or flow cytometry

• Single cell analysis by sequencing or microarrays.
Random Shotgun Metagenomics

Clinical Sample  Total Host & Microbial NA  Random Fragment Sequencing
Metagenomic Approaches

• Sequence selected targets (e.g. 16S, 18S rRNA)

• “Deep sequencing” (all genetic material in sample)
  • Assemble and identify contigs
  • Extract and analyze sequences of interest
Bacteria in Human Stool

Up to $10^{11}$ bacteria/ml

Bacteroides fragilis
Bacteroides vulgatus
Bacteroides eggerthii
Bacteroides sp. (B. fragilis)
Bacteroides sp. (B. thetaiotaomicron)
Bacteroides sp. (B. vulgatus)
Bacteroides sp. (B. eggerthii)
Bacteroides sp. (B. uniformis)
Cytophaga xylanolytica
Bacteroides distasonis
Bacteroides sp. (B. distasonis)
Clostridium oroticum
Clostridium sp. (C. nexile)
Ruminococcus hansenii
Ruminococcus productus
Eubacterium ventriosum
Clostridium sp. (C. clostridiiforme)
Clostridium histolyticum
Clostridium sp. (C. beijerinckii)
Clostridium sp. (C. butyricum)
Clostridium sp. (C. perfringens)
Clostridium putrificum
Clostridium sp. (C. cadaveris)
Clostridium difficile
Eubacterium tenue
Clostridium bifermentans
Clostridium sp. (C. sordellii)
Peptostreptococcus (P. anaerobius)
Fusobacterium nucleatum
Eubacterium plautii
Eubacterium sp. (E. cylindroides)
Streptococcus sanguis
Streptococcus oralis
Streptococcus intermedius
Lactococcus lactis subsp. cremoris
Streptococcus sp. (S. mitis)
Leuconostoc lactis
Streptococcus sp. (S. bovis)
Streptococcus sp. (S. equi subsp. equi)
Streptococcus mutans
Streptococcus sp. (S. sanguis)
Streptococcus sp. (S. salivarius)
Streptococcus sp. (S. equinus)
Streptococcus sp. (S. pyogenes)
Enterococcus faecalis
Enterococcus gallinarum
Lactobacillus acidophilus
Weissella kandleri
Lactobacillus fermentum
Vagococcus fluvialis
Bifidobacterium infantis
Bifidobacterium dentium
Bifidobacterium sp. (B. longum)
Bifidobacterium adolescentis
Bifidobacterium pseudolongum
Escherichia coli
Carnobacterium divergens
Lactobacillus maltaromaticus
Salmonella sp. (S. typhi)
Enterobacter sp. (E. aerogenes)
Serratia sp. (S. marcescens)
Proteus sp. (P. vulgaris)
Klebsiella sp. (K. pneumoniae)
The ‘Stool’ Problem

- Stool background
- Bacteria ($\sim 10^{11} / g$; 500–1000 species per person; 10,000 – 40,000 total predicted species)
- Viruses, parasites, fungi
- Human DNA
- Food DNA
- Unfavorable commensal to pathogen ratio
- Sequences for most microbes not available
- Many pathogens are similar to commensals
Why Shotgun Metagenomics? (for clinical diagnostics and public health)

• New tool for culture-independent pathogen characterization

• Potentially ultimate tool for pathogen discovery, understanding microbial relationships
Etiology of Acute Gastroenteritis is the US

Calculated from:
What’s Driving Metagonomics
Why study the Microbiome?

“Evidence is building that this resident community of microbes, called the microbiome, plays a major role in health and disease”

-Michael Balter; Science 336: June 8, 2012
Advantage of Public Health CIDTs: Speed

1. Patient Eats Contaminated Food
2. Stool Sample Collected
3. Public Health Laboratory Receives Sample
4. Patient Becomes Ill
5. Contact with health care system: 1 – 5 days
6. Diagnosis: 1 – 3 days
7. Shipping: 0 – 7 days
8. Serotyping & DNA fingerprinting: 2 – 10 days
9. Salmonella Identified
10. Case Confirmed as Part of Outbreak

1 day

3 days

1 – 5 days

1 – 3 days

0 – 7 days

2 – 10 days
Advantages of Public Health CIDTs: Finely-Tuned Case Definitions
Advantages of Public Health CIDTs:
Better Understanding of Disease

- Pathogen discovery
- Microbial interactions
- Host factors
- Exposures?
Reference Genomics Database Projects

- CDC STEC genomics project
- 100,000 Pathogen Genome Collaborative
- International genomics initiative (not yet named)
- Human Microbiome Project
Outbreak Detection in an Isolate-Free World: Method Development

Specimens → Deep metagenomics (or other CID method, e.g. targeted sequencing, single-cell sequencing)

Isolates → Genomics infrastructure → Data mining → Whole genome reference library

Target discovery → Sequence Identification
Metagenomics: Issues

- Sensitivity
- Specificity/gene linkage
- Cost of data processing, handling, storage, analysis
- Ethical problems

* One sample may require hundreds of gigabytes of data storage
## Game Changing Advances

<table>
<thead>
<tr>
<th>Advance</th>
<th>Likelihood</th>
</tr>
</thead>
<tbody>
<tr>
<td>Much cheaper sequencing</td>
<td>+++</td>
</tr>
<tr>
<td>Much longer read length (low, unbiased error rate)</td>
<td>+++++++</td>
</tr>
<tr>
<td>Better metagenomic-specific software, pipelines</td>
<td>+++++++</td>
</tr>
<tr>
<td>Greater processing power, bandwidth, cloud computing</td>
<td>++++</td>
</tr>
<tr>
<td>Better “clutter mitigation” strategies</td>
<td>+++</td>
</tr>
</tbody>
</table>
Summary: Culture Independent Diagnostics

- High probability, high impact issue
- Risks of inaction and benefits of change are significant
Acknowledgements

John Besser
Enteric Diseases Laboratory Branch
CDC/NCEZID/DFWED