Overview of CIDT – Challenges and Opportunities

Peter Gerner-Smidt, MD, DSc
Enteric Diseases Laboratory Branch
InFORM II
Phoenix, AZ, 19 November 2015
CIDT’s

- Enzyme immunoassays
- Molecular analytical panels
July 28, 2015

**World's Most Portable Molecular Diagnostics System Unveiled at AACC**

**GeneXpert Omni to Further Decentralize Critical TB, Virology and Ebola Tests**

SUNNYVALE, Calif. and GENEVA, July 28, 2015 /PRNewswire/ -- Cepheid (Nasdaq: CPHD) and FIND today unveiled the GeneXpert® Omni, the world's most portable molecular diagnostics system enabling unprecedented access to accurate, fast and potentially life-saving diagnosis for patients suspected of TB, HIV and Ebola in even the most remote areas of the world.
CIDT Opportunities & Challenges

- Patient care
- Accurate case counting
- Maintaining isolate-based surveillance
CIDT’s are developed to aid clinicians

- Fast diagnosis to guide treatment
CIDT Challenges To Clinicians

• **EIA’s**
  - Developed to detect one pathogen at a time
  - Sensitivity and specificity issues

• **Molecular diagnostic panels**
  - Up to 22 bacteria, virus, parasites
  - Sensitivity issues
    - Stx2f not detected
    - Norovirus targets change
      - Even though it works today, it might not tomorrow
  - Specificity issues
    - Are they detecting what they claim?
      - EPEC, EAEC, ETEC
  - How to interpret mixed infections?
Opportunities Of CIDT’s To Public Health

• Better case counting of pathogens rarely looked for by traditional methods
  • *Yersinia*, DEC, *Plesiomonas*, Vibrio, virus, parasites

**But………..**

• Panels vary widely in pathogens they diagnose
  • Both the number of pathogens and the targets used
• Suffer from sensitivity and specificity issues
• Critical for public health to know what CIDT was used to diagnose each patient
CIDT’s Are A Threat To Laboratory Surveillance Because We Lose The Cultures Critically Important For Our Surveillance
Laboratory Strategy to Meet The Challenge of Culture
Independent Diagnostic Methods (CIDT)

1. Preserve cultures
   - Surveillance by current methods (serotyping, AST, PFGE, MLVA etc.)

2. Prepare for the future working on pure cultures
   - Surveillance by whole genome sequencing (WGS)
     - 250 STEC genomes study
     - Bigs.db

3. Metagenomics
   - No cultures
     - 250 STEC genomes study
     - Applied Maths, DNASTAR
     - Real-time WGS surveillance of listeriosis
     - Global Microbial Identifier (GMI)

Surveillance and diagnostics by metagenomics

CIDT Working groups:
1. Overarching WG
2. Regulatory WG
3. Isolate recovery WG
4. PHL/Funding/Best Practices WG
5. CIDT panel evaluation WG
6. Industry WG

Retrospective WGS studies:
- V. cholerae, Salmonella, Listeria, E. coli, Campylobacter

Communication:
- White papers, Meetings
- Presentations, Publications

100k Foodborne pathogens sequencing study
LRN metagenomics study

Proof of concept
Regulatory Workgroup

• Charter: Identify barriers and make recommendations/develop strategies to assure continued flow of specimens and isolates to public health

• Members:
  • CDC, APHL, ASM, FDA (Microbiology Devices), AdvaMed, Joint Commission, CAP, IDSA

• Issues discussed:
  • Laboratory regulation
  • Test regulation
  • Test coding, coverage, reimbursement & compliance
  • Case reporting rules, state isolate/specimen submission requirement
  • Diagnostic test development
Isolate Recovery

- The Culture Preservation Workgroup:
  - Public Health Labs (CO, IA, LA County (CA), MN, and TN), APHL, and CDC
- Generate data to formulate recommendations for the efficient recovery of *Salmonella* and STEC (Shiga toxin-producing *E. coli*) from CIDT-positive specimens.
  - Media Study
  - Seeded Stool Study
Culture Preservation Study Final Steps

- **CIDT Steering Committee meeting for Oct 2015**
  - Review results of CIDT media and stool studies
  - Develop *science based* recommendations for recovery of STEC and *Salmonella* isolates

- **Disseminate results to scientific community**
  - INFORM meeting (Nov 2015)
  - MMWR guidance document

- **Bio-stability of specimens for metagenomics analyses**
The Promise Of Metagenomics

- More pathogens will be detected
- More paradigm shifts are possible/likely:
  - Pathogen interaction/complementation
    - Virulence factors from different pathogens may interact and thereby enhance/ reduce the virulence of one or both pathogens
  - Virulence complementation/enhancement/inhibition by normal flora
    - Virulence factors in a commensal could complement the virulence gene repertoire of a pathogen
    - A commensal may compete for receptors for a pathogen thereby rendering it less pathogenic
  - Host ~ Pathogen associations
    - The host genotype may be determined
    - Non-secretors and resistance to norovirus infection
- But technology, bioinformatics and ethics are not there, yet
Three Public Health Approaches To Metagenomics

- Amplicon sequencing
- “Shotgun” metagenomics
- Single-cell sorting and sequencing
Development of Organism-specific Strain Markers Through Amplicon Sequencing

1. Identify suitable markers:
   - Pathogen-specific strain Markers
   - Virulence, Resistance, Serotype Markers
   - Variable region
   - Conserved primer sites
   - Characterize
   - Establish linkage

2. Test markers against stool metagenomic data sets
3. Conduct field trial
Metagenomics By Shotgun Sequencing
Phylogeny of isolates and metagenomes

(Across 2.1 M sites out of 4.4 M)

5 SNPs per 100k bp
## Metagenomics: Limiting Factors

<table>
<thead>
<tr>
<th>Factor</th>
<th>Limitation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Amplicon Sequencing</strong></td>
<td>Yes</td>
</tr>
<tr>
<td><strong>Shotgun</strong></td>
<td>No</td>
</tr>
<tr>
<td>Need for <em>a priori</em> hypothesis</td>
<td>Yes</td>
</tr>
<tr>
<td>Cost</td>
<td>No</td>
</tr>
<tr>
<td>Sequencing read length (and error rate)</td>
<td>No</td>
</tr>
<tr>
<td>Metagenomic-specific software, pipelines</td>
<td>No</td>
</tr>
<tr>
<td>Computing processing power, bandwidth</td>
<td>No</td>
</tr>
<tr>
<td>Signal to noise</td>
<td>No</td>
</tr>
<tr>
<td><strong>Need for <em>a priori</em> hypothesis</strong></td>
<td>Yes</td>
</tr>
<tr>
<td>Cost</td>
<td>No</td>
</tr>
<tr>
<td>Sequencing read length (and error rate)</td>
<td>No</td>
</tr>
<tr>
<td>Metagenomic-specific software, pipelines</td>
<td>No</td>
</tr>
<tr>
<td>Computing processing power, bandwidth</td>
<td>No</td>
</tr>
<tr>
<td>Signal to noise</td>
<td>No</td>
</tr>
</tbody>
</table>
Metagenomics: Timeline

- Outbreaks of undetermined etiology: 2016\(^1\) – 2018
- PulseNet: 2019\(^2\) (per John) – 2025 (per Peter)

\(^1\) With limitations
\(^2\) Assuming key technological advancements
Acknowledgements

Colleagues in EDLB & Office of Advanced Molecular Detection
University of Georgia: X. Deng
Center for Genomic Epidemiology, DTU
University of Oxford, M. Maiden

Disclaimers:
“The findings and conclusions in this presentation are those of the author and do not necessarily represent the official position of the Centers for Disease Control and Prevention”

“Use of trade names is for identification only and does not imply endorsement by the Centers for Disease Control and Prevention or by the U.S. Department of Health and Human Services.”