Improving Patient Care: New Diagnostic Technology for Rapid Detection of Common Infectious Diarrheal Agents

Faculty

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Improving Patient Care: New Diagnostic Technology for Rapid Detection of Common Infectious Diarrheal Agents

Faculty Disclosures

Kimberle C. Chapin MD, D(ABMM), FCAP
Research requests, advisory boards:
Luminex Corporation, Becton Dickinson, Hologic Corporation

Robert Orenstein, DO, FACP, FIDSA
No financial relationships to report

Lawrence R. Schiller, MD
Speakers’ bureaus:
Forest/Ironwood Pharmaceuticals, Abbott/Abbvie Laboratories, Santarus, Takeda Pharmaceutical Company

Learning Objectives

- **Evaluate the role of new same-day single-specimen diagnostic assays** for common infectious diarrheal agents in improving time to diagnosis and facilitating treatment decisions.

- **Describe the correct use of new same-day single-specimen diagnostic assays** for common infectious diarrheal agents in clinical practice, including patient selection, specimen collection and handling, and interpretation of results.
Improving Patient Care: New Diagnostic Technology for Rapid Detection of Common Infectious Diarrheal Agents

Agenda

Welcome and Introduction
Lawrence R. Schiller, MD

The Burden of Infectious Diarrheal Disease
Robert Orenstein, DO, FACP, FIDSA

New Diagnostic Solutions for Infectious Diarrheal Disease
Kimberle C. Chapin, MD, D(ABMM), FCAP

Using New Gastrointestinal Molecular Panels in Clinical Practice
Lawrence R. Schiller, MD

Concluding Remarks
Lawrence R. Schiller, MD

Welcome and Introduction

Lawrence R. Schiller, MD
Diagnosis of Infectious Diarrhea: An Unmet Need

- Infectious diarrhea is a worldwide problem
- Approximately 80% of all causes of foodborne diarrhea go unidentified
- Absence of accurate pathogen identification often leads to inappropriate treatment
  - Antibiotics not recommended with shiga toxin-producing E coli
  - Antibiotics may prolong carriage of Salmonella, putting others at risk
- Conventional methods inadequate

References:

Conventional Diagnostic Methods

- Require multiple collection containers
- Have variable turnaround times
  - Range: 20 min to 3 wks
- Do not allow for streamlined diagnoses
- Are inconvenient for physician, patient
Stool specimen delivered to testing lab at:

10:00 AM 01/10

Specimen aliquots for:

1. Cryptosporidium/Giardia antigen test
2. Rotovirus antigen test
3. *C. difficile*; Shigatoxin enzyme immunoassay test
5. Bacterial culture (*Salmonella, Shigella, Campylobacter, STEC 0157*)

Results back:

11:00 AM 01/10

11:00 AM 01/10

01/11 to 01/12

01/12 to 01/13

STEC, shiga toxin-producing *E. coli*.

Stool specimen delivered to testing lab at:

10:00 AM 01/10

Specimen aliquots for:

5. Virology PCR send-out/Norovirus to DOH
6. Full ova and parasites
7. Other

Results back:

01/12 to 01/15

01/13 to 01/17

Variable to Variable

DOH, Department of Health; PCR, polymerase chain reaction.

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New Same-Day Single-Specimen Multiplex Assays

- In 2013 FDA cleared 2 new multiplex assays
- Allow detection of multiple pathogens with same-day turnaround on a single specimen
- Results for all pathogens returned at once

1. Luminex® xTAG® Gastrointestinal Pathogen Panel (GPP)¹
   - 11 pathogens

2. Prodesse® ProGastro® SSCS Assay²
   - 4 pathogens

Who Needs to Know About These New Multiplex Assays

- Family practice physicians
- Internal medicine physicians
- Infectious disease specialists
- Gastroenterologists
- Emergency room physicians
- Travel medicine specialists
- Nurse practitioners
- Physician assistants

All clinicians who see patients with diarrheal disease

### What Clinicians Need to Know

- Availability of these assays
- What is, is not included
- Application for use in patients with signs and symptoms of GI infection
- How to integrate assays into clinical practice

### Case Study

- A 30-year-old woman presents with diarrhea that began suddenly 3 days ago
- Diarrhea began the day after eating at a street fair
- She had consumed chicken salad, coleslaw, ice cream
- Her friend, who accompanied her to the street fair, remained well
Case Study

- Stools were fluid, bloody, occurred every 30 minutes; associated with cramps
- Mild nausea, appetite off, lost ≈5 pounds since onset
- Physical examination unremarkable except for unusually noisy bowel
  - No fever, chills
  - Vital signs normal

Case Study: Next Step?

Which of the following steps would you take next?

a. Obtain complete blood count and basic metabolic profile; call back next day if IV fluids needed
b. Send stool for bacterial culture, ova/parasite exam, Giardia antigen, Cryptosporidium antigen, Clostridium difficile testing
c. Empiric course of ciprofloxacin, metronidazole
d. Administer oral rehydration solution
The Burden of Infectious Diarrheal Disease

Robert Orenstein, DO, FACP, FIDSA

Faculty

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Associate Professor,
Mayo Clinic College of Medicine
Chair, Division of Infectious Diseases,
Mayo Clinic in Arizona,
Phoenix, AZ
Improving Patient Care: New Diagnostic Technology for Rapid Detection of Common Infectious Diarrheal Agents

Infectious Diarrhea: Etiology

- **Bacteria**
- **Viruses**
- **Parasites**

Spread through

- **Food or water**
- **Person to person**

Infectious Diarrhea: Epidemiology

- Common problem throughout developing, developed world\(^1\)
  - Burden of disease greatest in children <5 years, the immunosuppressed
- Worldwide, estimated 1.7 billion cases of diarrheal disease/year\(^1\)
- In the United States, 211–375 million episodes of diarrheal illness/year\(^2\)

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Infectious Diarrhea: United States Morbidity and Mortality

- Gastroenteritis-associated deaths doubled 1999-2007\(^1\)
  - Average 11,255 deaths/year
  - *Clostridium difficile*, *Norovirus* leading contributors
- Each year, infectious diarrhea accounts for\(^2\):
  - 73 million physician visits
  - 1.8 million hospitalizations
- Lifetime risk of being discharged from hospital with a diagnosis of gastroenteritis 1 in 8 among U.S. adults\(^3\)
- Majority of related hospitalizations\(^4\) and deaths\(^1\) in the elderly

---

Diarrheal Disease: Common Pathogens

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Most common cause of infectious diarrhea in U.S.(^{1-3})</th>
<th>68% of outbreaks, 78% of illnesses, 46% of hospitalizations, 86% of deaths in U.S. outbreak surveillance, 2009-2010(^1)</th>
<th>58% of foodborne illnesses(^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Norovirus</td>
<td>Most common pathogens detected in U.S. adults presenting to emergency room with acute gastroenteritis(^*4)</td>
<td>68% of outbreaks, 78% of illnesses, 46% of hospitalizations, 86% of deaths in U.S. outbreak surveillance, 2009-2010(^1)</td>
<td>58% of foodborne illnesses(^2)</td>
</tr>
<tr>
<td>Norovirus 16%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rotavirus 14%</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Salmonella</em> 3.5%</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^*\)Whole stool and rectal swab specimens combined

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## Diarrheal Disease: Common Pathogens

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Percentage</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Salmonella</em></td>
<td>13%</td>
<td>After norovirus, next most common pathogens in U.S. infectious diarrhea outbreaks¹</td>
</tr>
<tr>
<td><em>Shigella</em></td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td><em>STEC</em></td>
<td>4%</td>
<td></td>
</tr>
<tr>
<td><em>Clostridium difficile</em></td>
<td></td>
<td>Most significant contributor to doubling of gastroenteritis-associated mortality rate (1999-2007), followed by norovirus²</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Important community-acquired diarrheal illness, requiring treatment/interventions aimed at prevention³</td>
</tr>
</tbody>
</table>

STEC, shiga toxin-producing *E. coli*


## Diarrheal Disease: Common Pathogens

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Percentage</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Norovirus</em></td>
<td>58%</td>
<td>Leading causes of U.S. foodborne infections</td>
</tr>
<tr>
<td>Nontyphoidal <em>Salmonella</em></td>
<td>11%</td>
<td></td>
</tr>
<tr>
<td><em>Clostridium perfringens</em></td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>9%</td>
<td></td>
</tr>
<tr>
<td>Nontyphoidal <em>Salmonella</em></td>
<td>35%</td>
<td>Leading causes of U.S. foodborne infection-related hospitalizations</td>
</tr>
<tr>
<td><em>Norovirus</em></td>
<td>26%</td>
<td></td>
</tr>
<tr>
<td><em>Campylobacter</em></td>
<td>15%</td>
<td></td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td>Nontyphoidal <em>Salmonella</em></td>
<td>28%</td>
<td>Leading causes of U.S. foodborne infection-related deaths</td>
</tr>
<tr>
<td><em>Toxoplasma gondii</em></td>
<td>24%</td>
<td></td>
</tr>
<tr>
<td><em>Listeria monocytogenes</em></td>
<td>19%</td>
<td></td>
</tr>
<tr>
<td><em>Norovirus</em></td>
<td>11%</td>
<td></td>
</tr>
</tbody>
</table>

Infectious Diarrhea: The High Risk Groups

- In developed world, diarrhea often felt to be a nuisance, not a life-threatening illness
- Several groups warrant increased vigilance, more thorough diagnostic approach
  - Returning travelers
  - Individuals with foodborne illness
  - Individuals with healthcare exposures
  - Pregnant women
  - Elderly
  - Immunocompromised individuals
    - HIV
    - Transplant recipients

Travelers Diarrhea

- Affects 30%–70% of those who travel internationally
  - Risk depends on region, duration of travel
- Bacterial pathogens account for 80%–90% of cases
  - Enterotoxigenic *E. coli* (ETEC) most frequent
  - *Campylobacter jejuni*, *Shigella*, *Salmonella* next most common
- Intestinal viruses account for 5%–8% of cases
  - Noroviruses, rotavirus, astrovirus
- Protozoal pathogens account for 10% of diarrhea in longer-term travelers
  - Primarily *Giardia*

**Infectious Diarrhea: Foodborne Illness**

1 in 6 Americans will develop a foodborne illness each year¹


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**Infectious Diarrhea: Healthcare Exposures**

- *Clostridium difficile* most frequent cause¹
- Incidence, mortality, associated medical costs have reached all-time high in U.S.²
  - Over 500,000 cases annually³
  - Estimated deaths increased from 3000/year in 1999–2000 to 14,000/year in 2006–2007²
  - >90% of deaths in persons aged ≥65 years²
  - Excess associated costs estimated $5042–$7179/case; national annual estimate $897 million to $1.3 billion²
- Diagnosis remains a challenge

Infectious Diarrhea: Pregnant Women

- Pregnant women at increased risk for complications of foodborne illness, acute gastroenteritis
  - Early diagnosis critical to direct treatment
- Listeriosis affects $\approx 12/100,000$ pregnancies$^1$
  - Nearly 20-fold increase in pregnancy$^1$
  - Mild febrile illness or asymptomatic in mothers$^1$
  - Threatens fetus and newborn through direct infection of placenta and chorioamnionitis$^2$
- Salmonellosis may be associated with intrauterine infection, fetal death$^3$


Infectious Diarrhea: Immunocompromised Individuals

- Different, broader spectrum of pathogens, depending on host immune defect
- Bacterial
  - *Salmonella, Shigella, Campylobacter, Mycobacteria*
  - *Clostridium difficile*
- Viral
  - Cytomegalovirus
  - Adenovirus
  - Norovirus, Rotavirus
- Parasitic
  - *Cryptosporidium, Cytosisospora, Strongyloides*

Syndromic Management

- Patients present with a constellation of symptoms
  - NOT a microbiologic diagnosis
- Key goal to determine if antimicrobial therapy is indicated
  - Unfortunately, antimicrobials frequently prescribed in setting of unknown pathogens
- Classifying clinical syndromes allows clinician to:
  - Narrow differential diagnosis
  - Focus testing
- Symptomatology often insufficient to distinguish among potential causative agents
  - Appropriate treatment/prevention often delayed

Benefits of Rapid, Accurate Diagnostic Tools

- Limit unnecessary antimicrobial use
  - Since many diarrheal episodes self-limiting, empirical therapy could be avoided
  - Empirical fluoroquinolone may lead to development of Clostridium difficile infection, antibiotic resistance
- Allow use of earlier focused treatment
  - Some bacterial pathogens require specific targeted therapy
- Limit harmful antimicrobial use
  - In some instances antimicrobials prolong or worsen illness (e.g., Salmonella and shiga toxin-producing E coli)
- Limit unnecessary evaluation for noninfectious causes, which may be invasive and costly

Benefits of Rapid, Accurate Diagnostic Tools

- Facilitate infection control, public health action
  - Key pathogens: norovirus, *C. difficile*, shiga toxin-producing *E. coli* (STEC)
  - Identification of cluster often points to an outbreak
  - Earlier diagnosis helps prevent secondary cases
  - Improves use of isolation

Benefits must be weighed against public health limitations

Antimicrobial Stewardship

- Current era shows increasing antimicrobial resistance
- Identifying who does, does not need antimicrobials may limit their use, which could reduce emergence of further resistance
- Since norovirus is #1 infectious diarrheal agent, ability to identify this and other common viral pathogens can limit excessive antimicrobial use

Example: Patient with acute diarrheal illness treated with fluoroquinolone and/or metronidazole, after which he returns with *C. difficile* infection

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Conclusion

- Diarrheal disease is common, often mismanaged
- Though a syndromic approach is helpful, identification of cause is important for proper therapy, prevention
- Current diagnostic testing involves a patchwork of tests, which may require days to establish a diagnosis
- This may lead to excessive testing, spread of disease
- More rapid, accurate testing necessary to improve management

Panel Discussion I

Faculty
Panel Discussion Questions

- Which diarrheal pathogens are you seeing in your clinical practice? Which are being reported in your geographic region?
- What changes have you observed in diarrheal disease (occurrence, causative agents, age or type of patient, risk factors)?
- Are patients presenting quickly after onset of symptoms or are they delaying?
- What are the diagnostic challenges faced by practicing clinicians to whom these patients are presenting?

Panel Discussion Questions

- How often do you recognize rotavirus in adults?
- Which diagnostic tests do you use in your practice? What tests are available to you? What is the usual turnaround time?
- How might a more rapid diagnostic test affect your management of patients with acute diarrheal illness?
- Do you change your approach when seeing someone who is immunosuppressed, such as a patient with HIV or organ transplant?
New Diagnostic Solutions for Infectious Diarrheal Disease

Kimberle C. Chapin, MD, D(ABMM), FCAP

Algorithm for Acute Diarrhea

Reprinted from Med Clin North Am, 84(5), Schiller LR, Diarrhea, 1259-74, 2000, with permission from Elsevier.
Common Enteric Pathogens: Signs and Symptoms

<table>
<thead>
<tr>
<th>Type</th>
<th>Pathogen</th>
<th>Onset</th>
<th>Fever</th>
<th>Nausea</th>
<th>Abd Pain</th>
<th>Vomiting</th>
<th>Diarrhea watery</th>
<th>Diarrhea bloody</th>
<th>Comment / Complications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Campylobacter²</td>
<td>2–5 d</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Guilian-Barré</td>
</tr>
<tr>
<td></td>
<td>Salmonella¹</td>
<td>12 h–3 d</td>
<td>✓</td>
<td></td>
<td>✓</td>
<td></td>
<td>✓</td>
<td>✓</td>
<td>Extraintestinal, septicemia</td>
</tr>
<tr>
<td>Vignettes</td>
<td>Shigella⁴</td>
<td>1–3 d</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>HUS, life threatening</td>
</tr>
<tr>
<td>Virus</td>
<td>Norovirus¹</td>
<td>1–2 d</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Dehydration</td>
</tr>
<tr>
<td></td>
<td>Rotavirus¹</td>
<td>1–3 d</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Dehydration: young children, elderly</td>
</tr>
<tr>
<td>Toxin</td>
<td>C difficile toxin²</td>
<td>Days–months</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Nosocomial</td>
</tr>
<tr>
<td></td>
<td>STEC³</td>
<td>2–10 d</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>HUS</td>
</tr>
<tr>
<td></td>
<td>ETEC⁴</td>
<td>10 h–3 d</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Traveler’s diarrhea</td>
</tr>
<tr>
<td>Parasite</td>
<td>Giardia³</td>
<td>3–25 d</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td></td>
<td>greasy</td>
<td>✓</td>
<td>Chronic, malabsorption</td>
</tr>
<tr>
<td>Parasite</td>
<td>Cryptosporidium³</td>
<td>2–7 d</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>✓</td>
<td>Immuno-compromised</td>
</tr>
</tbody>
</table>

Common to all: diarrhea, dehydration, anorexia, period of shedding by others, variety of reservoirs.

ETEC, enterotoxigenic E. coli; HUS, hemolytic-uremic syndrome; STEC, shiga toxin-producing E. coli.

Common Diagnostic Methods for Most Common Stool Pathogens

- **Bacterial**
  - 5–7 plate set-up for **culture**
    - *Salmonella, Shigella, Campylobacter, E. coli O157*
    - 3–5 days
  - **C. difficile toxin PCR** – same day

- **Parasitic**
  - Rapid **EIA** for Giardia, Crypto
    - Confirmation with **DFA**
    - 1–2 days
  - **Ova and Parasite exam (send out)**
    - Up to 2 weeks
    - Does NOT include *Cryptosporidium*

- **Viral**
  - Rapid **EIA** rotovirus
  - Adenovirus 40/41 **shell vial RMix**
    - 2 days
  - **Norovirus PCR send-out** – 3 days

DFA, direct fluorescence antibody; EIA, enzyme immunoassay; PCR, polymerase chain reaction.

Graphics courtesy of Dr. Kimberle C. Chapin.
Additional Specialty Testing

- Clinically or epidemiologically clinicians may need to request less common pathogens
- Examples
  - Organisms identified in an outbreak situation
    - e.g., Listeria, Cyclospora
  - Unique patient history, risk factors
    - e.g., Vibrio cholera, E. histolytica

January 2013: New Device Class, 2 FDA-Cleared Multiplex GI Panels

“Gastrointestinal Pathogen Panel Multiplex Nucleic Acid-Based Assay System”
Defined as a qualitative device to detect and identify multiple GI microbial nucleic acids extracted from human stool specimens; to be used in conjunction with clinical evaluation and other laboratory findings in patients with signs and symptoms of GI infection

- Luminex® xTAG® Gastrointestinal Pathogen Panel (GPP)²
  - 11 pathogens
  - Bacterial, viral, parasitic, toxic

- Prodesse® ProGastro® SSCS Assay³
  - 4 pathogens
  - Bacterial and shiga toxins

Full Panel Vs. Ala Carte for Specific Pathogen Groups

<table>
<thead>
<tr>
<th>Luminex xTAG GPP¹</th>
<th>ProGastro SSCS²</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Campylobacter (C. jejuni, C. coli, and C. lari)</td>
<td>• Campylobacter (C. jejuni and C. coli)</td>
</tr>
<tr>
<td>• Clostridium difficile toxin</td>
<td>• Salmonella</td>
</tr>
<tr>
<td>• Cryptosporidium (C. parvum and C. hominis)</td>
<td>• Shigella</td>
</tr>
<tr>
<td>• E. coli O157</td>
<td>• Shiga toxin-producing E. coli (STEC), stx1/stx2</td>
</tr>
<tr>
<td>• Enterotoxigenic E. coli</td>
<td></td>
</tr>
<tr>
<td>• Giardia lamblia</td>
<td></td>
</tr>
<tr>
<td>• Norovirus GI/GII</td>
<td></td>
</tr>
<tr>
<td>• Rotavirus A</td>
<td></td>
</tr>
<tr>
<td>• Salmonella</td>
<td></td>
</tr>
<tr>
<td>• Shiga toxin-producing E. coli (STEC), stx1/stx2</td>
<td></td>
</tr>
<tr>
<td>• Shigella (S. boydii, S. sonnei, S. flexneri, S. dysenteriae)</td>
<td></td>
</tr>
</tbody>
</table>


Multiplex Assays in Development

- BioFire FilmArray®¹
- BD Max™ Enteric Syndromic Focus panels²
- Verigene® Enteric Pathogen Test³
- Seeplex® Diarrhea ACE Detection⁴

Improving Patient Care: New Diagnostic Technology for Rapid Detection of Common Infectious Diarrheal Agents

Luminex xTAG GPP Steps

Step 1: Extraction and Purification

Step 2: Multiplex Amplification

Step 3: Bead Hybridization and detection

Step 4: Data Acquisition/Analysis

Luminex xTAG GPP Data Capture and Analysis

MAGPIX®

Luminex®

100/200™

Graphics courtesy of Luminex Corporation.
**ProGastro SSCS Steps**

- Extraction of nucleic acids (and added internal control) from patient specimen
- Master mix with primers and probes dual-labeled with reporter dye and quencher added to PCR reaction tubes
- Amplification of targets, detection by fluorescent signal, data capture and analysis


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**ProGastro SSCS Data Capture and Analysis**

*Graphic courtesy of Hologic, Inc.*

*Cepheid SmartCycler II®*
Improving Patient Care: New Diagnostic Technology for Rapid Detection of Common Infectious Diarrheal Agents

Luminex xTAG GPP: Prospective Multicenter Trial, Retrospective Cohort

- 1610 total specimens from symptomatic patients
  - 1407 prospective specimens
    - =60% inpatient, 40% outpatient
    - 92.8% adult
    - 35% immunocompromised
  - 203 retrospective known positive samples for pathogens that were low prevalence in prospective arm
- Sensitivity and specificity (or positive and negative agreement, respectively) determined based on fraction of comparator positive or negative results relative to those obtained by xTAG GPP
- Overall, xTAG GPP showed high accuracy in detecting 90.2% of infectious gastroenteritis causative agents


Luminex xTAG GPP: Prospective, Retrospective Combined Data (N = 1610)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>Positive GPP/Comparator</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter</td>
<td>43/44</td>
<td>97.6–100</td>
<td>98.2</td>
</tr>
<tr>
<td>Salmonella</td>
<td>34/37</td>
<td>88.9–100</td>
<td>98.4</td>
</tr>
<tr>
<td>Shigella</td>
<td>22/22</td>
<td>100</td>
<td>98.5</td>
</tr>
<tr>
<td>E. coli O157</td>
<td>16/16</td>
<td>100</td>
<td>99.2</td>
</tr>
<tr>
<td>STEC</td>
<td>19/19</td>
<td>100</td>
<td>98.6</td>
</tr>
<tr>
<td>ETEC</td>
<td>40/47</td>
<td>25–97.4</td>
<td>89.8</td>
</tr>
<tr>
<td>C. difficile toxin</td>
<td>107/114</td>
<td>93.9</td>
<td>89.8</td>
</tr>
<tr>
<td>Norovirus</td>
<td>74/78</td>
<td>94.9</td>
<td>91.4</td>
</tr>
<tr>
<td>Rotavirus</td>
<td>30/30</td>
<td>100</td>
<td>99.8</td>
</tr>
<tr>
<td>Cryptosporidium</td>
<td>24/25</td>
<td>92.3–100</td>
<td>95.5</td>
</tr>
<tr>
<td>Giardia</td>
<td>19/20</td>
<td>93.7–100</td>
<td>96.7</td>
</tr>
</tbody>
</table>

Evaluation of Luminex xTAG GPP Assay in a Public Health Laboratory

- Milwaukee Health Department Laboratory
  - Evaluated GPP assay before FDA clearance with analyte-specific reagents for all pathogens currently on cleared product
  - 45 reference isolates, 254 clinical specimens
- Overall comparative performance of GPP with conventional methods:
  - Sensitivity, 94.5% (range 90%–97%)
  - Specificity, 99% (range 99%–100%)


ProGastro SSCS: Prospective Multicenter Trial (N = 1139)

<table>
<thead>
<tr>
<th>Pathogen</th>
<th># Positive (% total)</th>
<th>Sensitivity (95% CI)</th>
<th>Specificity (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Campylobacter</td>
<td>33 (2.9%)</td>
<td>83.9–100</td>
<td>98.0–99.3</td>
</tr>
<tr>
<td>Salmonella</td>
<td>30 (2.6%)</td>
<td>77.3–99.2</td>
<td>98.4–99.5</td>
</tr>
<tr>
<td>Shigella</td>
<td>21 (1.8%)</td>
<td>79.6–100</td>
<td>98.8–99.8</td>
</tr>
<tr>
<td>STEC</td>
<td>18 (1.6%)</td>
<td>70.1–100</td>
<td>98.5–99.6</td>
</tr>
</tbody>
</table>

A retrospective study of 105 known specimens showed 100% positive and negative agreement for *Salmonella, Shigella*, STEC; 96.4% positive, 93.5% negative agreement for *Campylobacter*.

CI, confidence index; STEC, shiga toxin-producing *E. coli*.

Prodesse® ProGastro™ SSCS (product insert). Waukesha, WI: Gen-Probe Prodesse, Inc; 2013
### Benefit Analysis for Implementing a New Multiplex Diagnostic Test

<table>
<thead>
<tr>
<th>Question</th>
<th>Benefit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does the test offer an improvement in patient care and management?</td>
<td>✔ More pathogens identified, one result, quicker turn-around time, single patient specimen</td>
</tr>
<tr>
<td>Does the test offer an improvement in lab workflow and efficiency of resources?</td>
<td>✔ One result that is able to address the most common pathogens rather than a web of multiple testing methods</td>
</tr>
<tr>
<td>Will the lab cost of the multiplex technology versus current methodologies be justified to implement?</td>
<td>✔ Single multiplex tests cost less than multiple methods even when all tests not ordered on every specimen; reduced tech time in testing, ordering of multiple supplies, quality control</td>
</tr>
</tbody>
</table>

### Limitations of FDA-Cleared Panels

Preset to test for all pathogens; physicians can request results for only those pathogens desired

- Absence of newly discovered relevant pathogens
- Other tests may still be needed (e.g., *Listeria*)
- No susceptibility testing
- Interpretation an educational challenge secondary to:
  - Potential false positives, false negatives, multiple pathogens in single sample
- Presumptive positive result (Luminex xTAG GPP)\(^1\)

Always check with reference lab/clinical lab for updates to these panels

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\(^1\) Luminex® [product insert]. Toronto, ON: Luminex Molecular Diagnostics, Inc.; 2013.
False Positives, False Negatives, Mixed Positives

- False positives\(^1,2\)
  - Laboratory error
  - Cross-contamination
  - Nonspecific signals
  - Colonization vs actual disease
- False negatives\(^1,2\)
  - Improper handling of specimen
  - Inhibition of amplification or too few organisms to amplify
  - Genetic variation of assay targets over time
  - Laboratory error
- Mixed positives
  - 19% for Luminex xTAG GPP\(^1\)
  - <1% for ProGastro SSCS\(^2\)


Presumptive Positive Results for Luminex xTAG GPP: FDA Requirement

- Package insert states positive results presumptive, should be confirmed\(^1\)
- Why did FDA require this?\(^2\)
  - False positive rate (0%–2.6%) in clinical study of volunteer asymptomatic subjects\(^2\)
    - Need for public health notification requirements
  - Positive predictive value <100% for any test system when low prevalence of disease
- Need for confirmatory testing/culture isolate – varies by lab
  - Does not require additional patient sample, test order
  - May be necessary according to STATE mandates that require an isolate for definition of CDC criteria for bacterial diarrheal agent
  - May not be necessary if laboratory performs in-house verification of test system

1. Luminex\(^\circledR\) [product insert]. Toronto, ON: Luminex Molecular Diagnostics, Inc.; 2013.
Improving Patient Care: New Diagnostic Technology for Rapid Detection of Common Infectious Diarrheal Agents

**Implementation: Issues for Clinicians and Labs**

- Change from traditional ordering/testing practices
- Interpretation of additional pathogen information
- Ability for labs to comply with public health needs
- Laboratories and clinicians will need to work together to establish clinical relevance of multiplex data
  - Assay performance relative to previous testing methods, patient information, and new assay results

**Isolates for Public Health Investigations**

- Current case definition for bacterial foodborne transmission requires that an isolate be identified by culture
  - Molecular analysis done to identify pathogen as part of a suspected outbreak (PulseNet)
- Each state’s public health department has different requirements
  - Some labs may have to do additional testing to comply with public health requirements
  - Laboratories implementing GI multiplex tests should discuss with their department of health prior to implementation
- CDC workgroup on culture-independent diagnostics to address issues related to implementation of such new technologies

---

Implementation:
Issues for Clinicians

- Most guidelines do not recommend testing unless dysentery or outbreak potential; therefore, clinicians will likely question the use of testing
- Most testing done by primary care physicians without GI or ID expertise
  - Will clinicians think a panel test is necessary? Only part of a panel? Or no testing performed at all and empiric RX?
  - What are the clinical benefits of knowing the pathogen?
- Discussion and experience with these assays will help address these questions

Educational Hurdles Lab Needs to Address with Clinicians

- Do clinicians know what specific GI panel is being used by lab and what it includes?
- Do clinicians know specifics about specimen collection so that test results are optimized?
- Do clinicians understand when additional test requests may be necessary?
  - Not all GI pathogens are present in any panel
Conclusion

- Multiplex technology for stool pathogens compared to current diagnostic methods:
  - Increased efficiency in laboratory workflow, decreased time to final report; 1 simultaneous report for most common pathogens
  - Increased sensitivity for detection of pathogens as well as additional pathogens not routinely available
    - e.g., viruses, toxins
  - Ability to identify pathogens more quickly in outbreak situations, aid public health objectives
  - Increased satisfaction to patient, provider, lab in diagnosis of acute GI infections

Panel Discussion II

Faculty
Panel Discussion Questions

- How quickly are these new panels being made available in clinical laboratories?
- What has been your experience in talking with clinicians whose scope of practice includes diarrheal disease? How are they responding to these new options? What kinds of questions do they have?
- How can clinicians find out where these assays can be ordered?
- What will be the impact of multiplex assays on cost to the patient?

Panel Discussion Questions

- What other tests might clinicians have to order in addition to these assays for some patients?
- Is there ever a need to mask certain pathogens from these assays? (e.g., *C difficile*)
- Is it better to keep *C difficile* as reportable even if the physician does not specifically order it?
- What does colonization mean versus true pathogen? How does the physician interpret a positive?
Using New Gastrointestinal Molecular Panels in Clinical Practice

Lawrence R. Schiller, MD

Differential Diagnosis of Acute Diarrhea

- Infectious, toxic diarrehas
- Food allergy/intolerance
- Medication reactions
- Initial presentation of chronic diarrhea
### Evaluation of Acute Diarrhea: Medical History

- Acuity/severity
- Stool characteristics
- Relation to meals/nocturnal diarrhea
- Pain/cramps
- Medications
- Urgency/incontinence
- Epidemiology

### Evaluation of Acute Diarrhea: Epidemiologic Questions

- Diet/foods consumed prior to onset
- Family/contacts with diarrhea
- Institutional setting
- Recent travel
- Water source
- Occupation
- Sexual activity
- Illicit drug use
Management of Acute Diarrhea: Traditional Paradigm

**Initial assessment**
- Toxigenic
  - Prolonged course
  - Blood in stools
  - Dehydrated

- Nontoxic
  - Short duration
  - No bleeding
  - Not tender

**Symptomatic therapy**
- Oral rehydration solution
- Antidiarrheal drugs
  - No response
  - Response

**Fluid/electrolyte replacement**

**Laboratory evaluation**
- Complete blood count
- Hemoculture
- WBC differential

- Serum chemistries
  - Electrolytes
  - Urea nitrogen
  - Creatinine

- Stool tests
  - Ova & parasite exam
  - Giardia antigen* (Campylobacter jejuni*)
  - *Clostridium difficile toxin*

- Fecal WBCs
- Positive
- Negative

- Stool culture

*In appropriate epidemiological circumstances

Reprinted from Med Clin North Am, 84(5), Schiller LR, Diarrhea, 1259-74, 2000, with permission from Elsevier.

Acute Diarrhea: Approaches to Diagnostic Work-up

Traditional Paradigm

VS.

New Paradigm
Acute Diarrhea: Approaches to Diagnostic Work-up

**Traditional Paradigm**

- Conditioned by presentation, epidemiology, toxicity\(^1,2\)
- Limited to sicker patients\(^1\) who may be candidates for antimicrobial therapy
  - Not routine due to self-limiting nature of illness, inability to get timely results, cost of multiple tests
- May be predicated on initial “predictive” test (e.g., fecal WBCs)\(^1\)
- Dependent on cultures that may not have results returned until result is irrelevant
- Protozoal causes may not be considered until diarrhea is persistent\(^1\)


Acute Diarrhea: Approaches to Diagnostic Work-up

**New Paradigm**

- Development of molecular testing will change the paradigm, particularly if reasonably priced
- Testing will occur earlier in the illness
- What is the new paradigm for diagnostic testing for pathogens in diarrhea?
Indication for New Multiplex Gastrointestinal Pathogen Assays

Indicated for use in patients with signs, symptoms of infectious GI illness\(^1,2\)

1. Luminex\textsuperscript{®} (product insert). Toronto, ON: Luminex Molecular Diagnostics, Inc.; 2013.
2. Prodesse\textsuperscript{®} ProGastro\textsuperscript{™} SSCS Assay\(^2\). Waukesha, WI: Gen-Probe Prodesse, Inc; 2013.

Candidates for Testing with New Multiplex Assays

Patients with one or more of the following signs/symptoms may have infectious diarrhea\(^1,2\)

- Acute diarrhea
- Fever
- Abdominal pain
- Bloody or watery stools
- Fecal leukocytes/increased lactoferrin
- Persistent diarrhea
- Epidemiologic exposure

Specimen Collection for New Multiplex Assays

- **Luminex xTAG GPP¹**
  - Sterile, leak-proof, wide-mouthed, preservative-free container
  - Send to testing lab under refrigeration
- **ProGastro SSCS²**
  - Cary Blair or C&S Transport Medium within 2 hours of collection
  - Send to testing laboratory
- Testing laboratory provides physician offices with collection and handling instructions

Identifying Which Multiplex Assay to Order

<table>
<thead>
<tr>
<th>Luminex xTAG GPP¹</th>
<th>ProGastro SSCS²</th>
</tr>
</thead>
<tbody>
<tr>
<td>• Campylobacter (C jejuni, C coli, C lari)</td>
<td>• Campylobacter (C jejuni, C coli)</td>
</tr>
<tr>
<td>• Clostridium difficile</td>
<td>• Salmonella</td>
</tr>
<tr>
<td>• Cryptosporidium (C parvum, C hominis)</td>
<td>• Shigella</td>
</tr>
<tr>
<td>• E coli O157</td>
<td>• STEC, stx1/stx2</td>
</tr>
<tr>
<td>• Enterotoxigenic E coli</td>
<td>• Shigella</td>
</tr>
<tr>
<td>• Giardia lamblia</td>
<td>• STEC, stx1/stx2</td>
</tr>
<tr>
<td>• Norovirus GI/GII</td>
<td>• Shigella</td>
</tr>
<tr>
<td>• Rotavirus A</td>
<td>• STEC, stx1/stx2</td>
</tr>
<tr>
<td>• Salmonella</td>
<td>• Shigella (S boydii, S sonnei, S flexneri, S dysenteriae)</td>
</tr>
<tr>
<td>• STEC, stx1/stx2</td>
<td></td>
</tr>
</tbody>
</table>

Finding a Testing Laboratory

- Check with reference lab first
- Labs often rename tests rather than use product brand names in catalogs
  - GI pathogen panel
  - Gastroenteritis panel
  - GI multiplex panel
  - GI molecular panel
- Read test details
  - Does it use the words “multiplex,” “nucleic acid testing”?  
  - Does it include desired pathogen set?

Understanding Modifications of the Multiplex Panels

- Some labs may add 1 or more single-well test or an in-lab created test to cover pathogens not included
  - Example: One lab offers the xTAG GPP 11-pathogen panel but also a 15-pathogen panel that includes original panel plus 4 additional pathogens in single-well add-on tests (Adenovirus 40/41, Entamoeba histolytica, Vibrio cholera, Yersinia enterocolitica)
- Some labs may mask results of C difficile unless test has been specifically requested
Interpreting the Multiplex Assay Results

- Always interpret results within context of full clinical evaluation, laboratory findings, epidemiologic info
- Evaluate clinical relevance of:
  - Unexpected positives
  - Mixed positives

Follow-Up Action

- Sensitivity testing
- Treatment
- Infection control/public health
  - Outbreak control
    - Isolation/hospitalization
    - School/daycare issues
    - At-home transmission control
  - Testing lab reports reportable infections to appropriate department of health
Barriers to Use of New Multiplex GI Assays

- New and unknown
- Clinicians, labs must switch from familiar ways of testing
- Not clear which labs offer these assays
- Not all pathogens as important to all clinicians and in all geographic regions
- Perception of higher cost without consideration for lower overall costs (direct, indirect)
- Capital investments by labs may block or delay adoption, which limits availability of panels
- Not clear where to put these assays in testing algorithm

Communication and Cooperation Between Clinicians and Laboratories

- A priority of the CDC’s “Clinical Laboratory Integration into Healthcare Collaborative”¹
  - To encourage clinicians to consult laboratorians for information on test ordering and interpretation
- Talk to testing laboratory staff, medical directors
- Management of infectious diarrhea facilitated when laboratorians, physicians work together

CDC, Centers for Disease Control and Prevention.

**Case Study Revisited**

- A 30-year-old woman presents with diarrhea that began suddenly 3 days ago
- Diarrhea began the day after eating at a street fair
- She had consumed chicken salad, coleslaw, ice cream
- Her friend, who accompanied her to the street fair, remained well

---

**Case Study Revisited**

- Stools fluid, bloody, occurred every 30 minutes; associated with cramps
- Mild nausea, appetite off, lost ≈5 pounds since onset
- Physical examination unremarkable except for unusually noisy bowel
  - No fever, chills
  - Vital signs normal
Case Study: Next Step?

Which of the following steps would you take next for this patient?

a. Obtain complete blood count, basic metabolic profile; call back next day if IV fluids needed
b. Send stool for bacterial culture, ova/parasite exam, *Giardia* antigen, *Cryptosporidium* antigen, *Clostridium difficile* testing
c. Empiric course of ciprofloxacin, metronidazole
d. Administer oral rehydration solution

e. Obtain stool for multiplex GI panel
Conclusions

- Acute diarrhea presents diagnostic challenges
  - Etiologic agent
  - Need for treatment

- Current approaches suboptimal because of time delays, costs in getting specific testing done

- Paradigm shift now possible because of multiplex GI panels
  - Rapid, accurate results inform treatment

- Use of multiplex panels can shorten time to diagnosis, allowing faster application of therapy and avoidance of needless testing, treatment
Panel Discussion III

Faculty

Panel Discussion Questions

- What problems are there in identifying patients who are candidates for this testing?
- How can barriers to use of these new panels be addressed?
- What do we know about the cost-effectiveness of this approach to GI pathogen testing from economic modeling?
- How can communication be encouraged and facilitated between clinicians and laborators?
Scenarios in Which Rapid Diagnostic Testing Might Be Useful

A 28-year-old Somali man presents with abdominal cramps and diarrhea of 3 days duration. He recently visited New York City and had several traditional meals prepared by cousins who recently moved to the city.

Scenarios in Which Rapid Diagnostic Testing Might Be Useful

A 68-year-old woman with chronic lymphocytic leukemia recently returned from a trip to Honduras, where she worked at a mission. She ate locally and was well until she returned home and developed loose stools. She took ciprofloxacin 500 mg BID for 3 days, but diarrhea persisted. Her physician gave her 5 days of metronidazole. She now presents with severe watery diarrhea, up to 30 loose stools/day.
**Scenarios in Which Rapid Diagnostic Testing Might Be Useful**

A 21-year-old daycare worker presents to the emergency room with 24 hours of nausea, vomiting, diarrhea. At least half her class was out ill this week with “stomach flu” and diarrhea. Yesterday she prepared Thanksgiving meals for a local shelter and now feels exhausted. She is lightheaded, tachycardic, febrile to 102°.

**Scenarios in Which Rapid Diagnostic Testing Might Be Useful**

An 88-year-old woman presents for evaluation of recurrent *C difficile* infection. She has had 8 episodes in the past year and wants to undergo fecal microbiota transplant. Her 46-year-old, healthy daughter has volunteered to be the donor.
Concluding Remarks

Lawrence R. Schiller, MD

Same-Day Single-Specimen Testing

- New multiplex panels allow for same-day single-specimen testing of common infectious diarrheal pathogens in patients with signs, symptoms of GI infection
  - Speed turnaround time
  - Facilitate management decisions
- Two multiplex GI panels cleared for use in the U.S.
  - Luminex xTAG GPP – 11 pathogens
  - ProGastro SSCS – 4 pathogens
The Clinician’s Role in Accurate and Effective Use of Multiplex Testing

- Recognize GI presentation for acute diarrhea is not specific – overlapping symptoms
- Identify patients who are candidates for these panels
- Ensure correct collection, handling of specimens
- Order appropriate test
- Interpret results within context of complete clinical picture

Clinicians and Labs Working Together

- Ask reference lab about availability of multiplex GI panels
- Talk to reference lab staff, medical directors about questions related to these panels
- Increased communication between clinicians, laboratorians will help overcome barriers to integration of these panels into clinical practice