**Bordetella pertussis**: Resurgence of an Old Disease

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Faculty Disclosure

The Association of Public Health Laboratories adheres to established standards regarding industry support of continuing education for healthcare professionals. The following disclosures of personal financial relationships with commercial interests within the last 12 months as relative to this presentation have been made by the speaker(s):

Jim Dunn, PhD, D(ABMM) - Nothing to disclose.
Objectives

• Describe the current epidemiology of pertussis infections in the U.S.

• Discuss the most appropriate test methods for diagnosis of pertussis.

• Explain the public health importance of rapidly and accurately identifying cases of pertussis.
Pertussis

• Highly contagious bacterial infection
  ➢ 80% secondary attack rate among susceptible household contacts

• Spread easily via aerosolized droplets from coughing or sneezing

• Reservoir: untreated, symptomatic persons
  ➢ particularly adolescents & adults

• Immunity from natural infection is NOT lifelong
Pertussis

• Affects all ages – young infants most vulnerable

• Typical Symptoms
  • coryza (no pharyngitis)  • post-tussive vomiting
  • lack of fever  • post-tussive whoop
  • paroxysmal cough  • no systemic illness
Pertussis: from Latin meaning “intense cough”
# Pertussis Diagnosis

<table>
<thead>
<tr>
<th>Test</th>
<th>Sens</th>
<th>Spec</th>
<th>Optimal Timing</th>
<th>Advantage</th>
<th>Disadvantage</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture</td>
<td>12-60%</td>
<td>~100%</td>
<td>&lt;2 wks onset</td>
<td>specificity</td>
<td>sensitivity &amp; TAT</td>
</tr>
<tr>
<td>PCR</td>
<td>70-99%</td>
<td>86-100%</td>
<td>&lt;4 wks cough</td>
<td>rapid &amp; sensitive</td>
<td>2 FDA-app, false pos</td>
</tr>
<tr>
<td>DFA</td>
<td>11-68%</td>
<td>76-99%</td>
<td>&lt;2 wks onset</td>
<td>rapid</td>
<td>sensitivity &amp; specificity</td>
</tr>
<tr>
<td>Paired Sera</td>
<td>90-92%</td>
<td>72-100%</td>
<td>Onset &amp; 4-6 wks</td>
<td>sensitivity</td>
<td>No FDA-app, too long, vaccination</td>
</tr>
</tbody>
</table>

CSTE Case Definition

• Clinical Case Definition
  • Cough ≥2 wks & at least 1 symptom: paroxysms, whoop, posttussive vomiting

• Case Definition
  ➢ Confirmed Cases
    • Culture Positive
    • Clinical Case + PCR Positive
    • Clinical Case + Epi-linked to confirmed case
  ➢ Probable Case
    • Only meets the clinical case definition
Molecular Diagnostics


CDC. MMWR 2007;56:837.
Duration of disease at time of diagnosis, age of patient, and PCR positivity

More likely PCR pos:
- Younger age
- Earlier in course of disease

van der Zee et al. J Infect Dis 1996;174:89
Pertussis Real-Time PCR

• Target Sequence
  - *IS481* - 50 to >200 copies per genome
  - 114 bp amplicon
  - cross-reacts w/ *B. holmesii, B. bronchiseptica*
**Bordetella holmesii**

- 1st reported case 1983 – bacteremia in asplenia
- CDC NO-2 (non-oxidizer group 2) until 1995
- found in 0 to 29% of NP specimens from patients with pertussis-like illness in several countries
- rare diseases: pneumonia, endocarditis, meningitis, septic arthritis
- in mice, whole cell oracellular Bp vaccines do not protect against *B. holmesii* infection

Pittet et al. Lancet Infect Dis 2014;14:510
# Insertion Sequence Targets

<table>
<thead>
<tr>
<th>Insertion Sequence</th>
<th><em>B. pertussis</em></th>
<th><em>B. parapertussis</em></th>
<th><em>B. holmesii</em></th>
<th><em>B. bronchiseptica</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>IS481</td>
<td>+/&gt;50</td>
<td>--</td>
<td>+/8-10</td>
<td>rare</td>
</tr>
<tr>
<td>IS1001</td>
<td>--</td>
<td>+/~20</td>
<td>--</td>
<td>rare</td>
</tr>
<tr>
<td>hIS1001</td>
<td>--</td>
<td>--</td>
<td>+/3-5</td>
<td>--</td>
</tr>
<tr>
<td>IS1002</td>
<td>+/4-8</td>
<td>+/9</td>
<td>--</td>
<td>rare</td>
</tr>
</tbody>
</table>
Pertussis Toxin Target

• Single-copy gene target
  – Promoter region – \( ptxP \)
  – Toxin subunit 1/A – \( ptxS1/ptxA \)

• Present in:
  – \( B. \) pertussis
  – \( B. \) parapertussis
  – \( B. \) bronchiseptica (64% of human-derived isolates)

• Post-amplification melt analysis or multiplex to differentiate species

# CDC Multi-Target PCR Approach

<table>
<thead>
<tr>
<th>Species</th>
<th>ptxS1</th>
<th>IS481</th>
<th>hIS1001</th>
<th>pIS1001</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. pertussis</em></td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td><em>B. parapertussis</em></td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td><em>B. holmesii</em></td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Illumigene Pertussis

- IS481 target
- LAMP
- 1 hr
- LOD = 3,265 cfu/mL
  (1.48 cfu/rxn)

FilmArray Respiratory Panel

- PtxP target
- PCR
- 1 hr
- LOD = 4,000 cfu/mL
  (~450 cfu/rxn)
Illumigene Pertussis

1. Sample Collection: Collect nasopharyngeal specimens in accordance with institutional procedures for the collection of clinical specimens for Bordetella pertussis infection.

2. Insert swab into Sample Buffer Tube. Cut swab shaft to fill, re-cap tube and vortex for 45 seconds. Remove swab.

3. Transfer 50 µl from Sample Buffer Tube to Assay Control Tube, Vortex for 10 seconds.

4. Heat the Assay Control Tube at 95°C for 10 minutes. Vortex for 10 seconds.

5. Using a new pipette tip for each, transfer 50 µl of the heat-treated sample to the TEST and CONTROL chambers of the illumigene<sup>®</sup> Test Device.

6. Add 1 drop of Mineral Oil to the TEST and CONTROL chambers.

7. Close and fasten the latch securely. Gently tap device to remove air bubbles trapped on the bottom of the tubes. Carefully examine the reaction tubes to ensure that there are no air bubbles left in the tube.

8. Insert illumigene<sup>®</sup> Test Device into the illumigene<sup>®</sup> 10<sup>™</sup> and initiate amplification reaction and detection.

Sensitivity

87.8%

Specificity

97.8%
FilmArray Respiratory Panel

<table>
<thead>
<tr>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>94-100%</td>
<td>99.9%</td>
</tr>
</tbody>
</table>

• FilmArray Panel including *B. pertussis*
  • compared to LDT PCR and/or culture from retrospectively positive NP swab specimens
  • 38/39 (97%) positive by FilmArray
  • noted cross-reactivity: *B. bronchiseptica* & *B. parapertussis* since both have PtxP

• Illumigene Pertussis
  • prospectively collected NP swabs (n=94)
  • compared to *B. pertussis* PCR (ASR, Cepheid)
  • sens = 94.4% (17 of 18) and spec = 98.6% (73 of 74)
PH Importance of Culture

• Important if outbreak is suspected

• Isolation confirms pertussis (100% specific)
  ➢ Other pathogens w/ similar clinical presentation
  ➢ Co-infections can occur (e.g. RSV)
  ➢ Can identify other *Bordetella* species

• Needed for susceptibility testing and typing
Post-Treatment

Unvaccinated Infants < 3 Months of Age

- Culture
  - may be positive up to 5-7 days after start of therapy

- PCR
  - positive up to at least 10-13 days after start of therapy
  - No clear association with duration of cough
Pertussis: the last 100 years

• Early 1900’s: 1 death per 10 cases
• 1922: made a notifiable disease
• 1934: >265,000 cases
• 1940: whole cell vaccine available
• 1943: AAP recommends pertussis vaccine
• 1976: 1,010 reported cases
Pertussis: the last 100 years

• 1990’s: acellular vaccine replaces whole cell
• 2000’s: emergence of disease among older children & adolescents (most vaccinated)
• 2005: recommendation for Tdap at age 11 or 12
• 2012: 48,000 reported cases
• 2013: 29,000 reported cases
Pertussis cases by year – United States, 1922–2012

Pertussis incidence (per 100,000 population) – U.S., 1981-2011

- DTaP 4th, 5th doses
- DTaP all doses
- Tdap booster
Reported pertussis incidence (per 100,000 population) by year and age group—United States, 1990–2012
Recent Pertussis Outbreaks

- 2010, California: >9,000 cases, 800 hospitalizations, 10 deaths (all <3 months of age)
- 2012, Wisconsin: >6,400 cases
- 2012, Washington: 2,500 cases

In all outbreaks, high rates of disease in fully vaccinated children
Pertussis – Tarrant County (TX), 2013

Tarrant pop. ~2 million
Tarrant Pertussis by Month for 2013

Total = 700 cases
2013 Pertussis, Age Specific Rates

Overall case rate: 36.9
Report date: Mar 27, 2014
## 2013 Pertussis Isolates

<table>
<thead>
<tr>
<th>Age</th>
<th>Collect</th>
<th>Whoop</th>
<th>Parox</th>
<th>Vom</th>
<th>Apnea</th>
<th>Hosp</th>
<th>Cough dur</th>
<th>Vacc</th>
<th>Eryth/Azith</th>
<th>PFGE</th>
<th>Pert def</th>
</tr>
</thead>
<tbody>
<tr>
<td>13 mo</td>
<td>July 7</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>22 d</td>
<td>UTD-3</td>
<td>S</td>
<td>CDC010</td>
<td>N</td>
</tr>
<tr>
<td>19 mo</td>
<td>July 9</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>unk</td>
<td>2</td>
<td>S</td>
<td>CDC253</td>
<td>Y</td>
</tr>
<tr>
<td>12 mo</td>
<td>June 28</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>19 d</td>
<td>2</td>
<td>S</td>
<td>CDC002</td>
<td>N</td>
</tr>
<tr>
<td>4 mo</td>
<td>July 15</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>16 d</td>
<td>UTD-2</td>
<td>S</td>
<td>CDC260</td>
<td>Y</td>
</tr>
<tr>
<td>23 mo</td>
<td>July 31</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>17 d</td>
<td>UTD-4</td>
<td>S</td>
<td>CDC237</td>
<td>Y</td>
</tr>
<tr>
<td>2 mo</td>
<td>July 12</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>unk</td>
<td>23 d</td>
<td>underage</td>
<td>CDC265</td>
<td>Y</td>
</tr>
<tr>
<td>15 mo</td>
<td>June 19</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>20 d</td>
<td>3</td>
<td>S</td>
<td>CDC377</td>
<td>Y</td>
</tr>
<tr>
<td>3 mo</td>
<td>June 24</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>30 d</td>
<td>UTD-1</td>
<td>S</td>
<td>CDC253</td>
<td>Y</td>
</tr>
<tr>
<td>13 yr</td>
<td>June 8</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>21 d</td>
<td>1</td>
<td>S</td>
<td>CDC082</td>
<td>Y</td>
</tr>
<tr>
<td>9 mo</td>
<td>June 19</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>25 d</td>
<td>unknown</td>
<td>S</td>
<td>CDC253</td>
<td>Y</td>
</tr>
<tr>
<td>10 mo</td>
<td>July 18</td>
<td>Y</td>
<td>N</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>16 d</td>
<td>UTD-3</td>
<td>S</td>
<td>CDC378</td>
<td>Y</td>
</tr>
<tr>
<td>2 d</td>
<td>June 11</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Y</td>
<td>40 d</td>
<td>underage</td>
<td>S</td>
<td>CDC379</td>
<td>Y</td>
</tr>
<tr>
<td>11 yr</td>
<td>June 17</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>20 d</td>
<td>UTD-5</td>
<td>S</td>
<td>CDC036</td>
<td>N</td>
</tr>
<tr>
<td>3 yr</td>
<td>July 15</td>
<td>N</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>30 d</td>
<td>UTD-4</td>
<td>S</td>
<td>CDC010</td>
<td>N</td>
</tr>
<tr>
<td>6 mo</td>
<td>July 12</td>
<td>Y</td>
<td>Y</td>
<td>Y</td>
<td>N</td>
<td>N</td>
<td>17 d</td>
<td>UTD-2</td>
<td>S</td>
<td>CDC002</td>
<td>N</td>
</tr>
</tbody>
</table>

- **53%**
- **87%**
- **67%**
- **53%**
- **14%**
- avg 22 d
- **67%**
- **67%**
### Recommended Immunization Schedule - 2015

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>2 mo</th>
<th>4 mo</th>
<th>6 mo</th>
<th>15 mo</th>
<th>18 mo</th>
<th>4-6 yrs</th>
<th>11-12 yrs</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTaP</td>
<td>1&lt;sup&gt;st&lt;/sup&gt; dose</td>
<td>2&lt;sup&gt;nd&lt;/sup&gt; dose</td>
<td>3 dose</td>
<td>&lt; -- 4&lt;sup&gt;th&lt;/sup&gt; dose -- &gt;</td>
<td>5&lt;sup&gt;th&lt;/sup&gt; dose</td>
<td>Tdap</td>
<td></td>
</tr>
</tbody>
</table>

### Estimated vaccination coverage among children 19-35 months – U.S., 2009-2013

<table>
<thead>
<tr>
<th>DTaP</th>
<th>2009</th>
<th>2010</th>
<th>2011</th>
<th>2012</th>
<th>2013</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥ 3 doses</td>
<td>95.0%</td>
<td>95.0%</td>
<td>95.5%</td>
<td>94.3%</td>
<td>94.1%</td>
</tr>
<tr>
<td>≥ 4 doses</td>
<td>83.9%</td>
<td>84.4%</td>
<td>84.6%</td>
<td>82.5%</td>
<td>83.1%</td>
</tr>
</tbody>
</table>

CDC. MMWR 2014;64:741
Why is Adaptive Immunity Short-Lived?

- Mixed Th1/Th2 response (acellular) vs. mostly Th1 (whole cell or natural infection)
- Potentially missing important antigens
- Insufficient concentration or balance of antigens
- Poor match between vaccine Ags and current circulating strains
  - Allelic variation of current strains vs. vaccine strains
## Pertussis Acellular Vaccines

### Antigenic Composition (µg/dose)

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Name</th>
<th>Licensed</th>
<th>PT</th>
<th>FHA</th>
<th>PRN</th>
<th>FIM 2/3</th>
</tr>
</thead>
<tbody>
<tr>
<td>DTaP</td>
<td>Pediarix</td>
<td>2002</td>
<td>25.0</td>
<td>25.0</td>
<td>8.0</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Daptacel</td>
<td>2002</td>
<td>10.0</td>
<td>5.0</td>
<td>3.0</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Adacel</td>
<td>2005</td>
<td>2.5</td>
<td>5.0</td>
<td>3.0</td>
<td>5.0</td>
</tr>
<tr>
<td></td>
<td>Boostrix</td>
<td>2005</td>
<td>8.0</td>
<td>8.0</td>
<td>2.5</td>
<td>-</td>
</tr>
</tbody>
</table>

PT = pertussis toxin  
FHA = filamentous hemagglutinin  
PRN = pertactin  
FIM = fimbriae
Evolution of *B. pertussis* Strains

- whole genome sequencing of 343 strains from 19 countries isolated between 1920 and 2010, including vaccine strains
- lineages and antigenic genotypes of vaccine strains are not commonly seen in recent isolates
- changes in genes in acellular vaccine components started occurring after introduction of whole cell vaccine but before the switch to acellular

Bart et al. mBio 2014;5:e01074-14
Pertactin-Deficient *B. pertussis*

- Recent emergence since 2009-10 of *B. pertussis* isolates lacking pertactin protein
  - Component of acellular vaccine
  - Clinically does not appear to alter disease severity
  - Not clear if it’s due to vaccine selection pressure
  - Fully vaccinated case-patients have 2 to 4-fold greater odds of having PRN-negative *B. pertussis* strain compared to those unvaccinated

Pertactin-Deficient *B. pertussis*

Australia

United States

Lam et al. Emerg Infect Dis 2014;20:626
Vaccine Effectiveness (VE) of Tdap in Adolescents

- during statewide outbreak in Wisconsin, 2012
- Tdap VE decreased with increasing time since receipt
- increased time since receipt associated with increased risk of pertussis infection

<table>
<thead>
<tr>
<th>Year of Tdap Receipt</th>
<th>Estimated VE, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No Tdap received</td>
<td>Reference</td>
</tr>
<tr>
<td>Any Tdap brand</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>75.3 (55.2–86.5)</td>
</tr>
<tr>
<td>2011</td>
<td>68.2 (60.9–74.1)</td>
</tr>
<tr>
<td>2010</td>
<td>34.5 (19.9–46.4)</td>
</tr>
<tr>
<td>2009/2008</td>
<td>11.9 (−11.1 to 30.1)</td>
</tr>
</tbody>
</table>

By known Tdap brand:

Adacel
| 2012 | 61.8 (21.5–81.4) |
| 2011 | 59.4 (47.9–68.4) |
| 2010 | 14.0 (−9.4 to 32.4) |
| 2009/2008 | −1.8 (−34.0 to 22.7) |

Boostrix
| 2012 | 90.7 (62.4–97.7) |
| 2011 | 79.6 (71.8–85.2) |
| 2010 | 53.4 (39.2–64.3) |
| 2009/2008 | 30.5 (6.2–48.5) |

Koopke et al. J Infect Dis 2014;210:942
Summary

• pertussis is a highly contagious respiratory infection that has re-emerged in recent years due to waning immunity, changes in VE, and/or changes in circulating strains

• the specific diagnosis of pertussis is best accomplished by multi-target PCR to rule out other *Bordetella* species

• in outbreak situations, culture is recommended for confirmation and characterization of isolates
QUESTIONS?