Status of Plague Diagnostics for Vaccine Evaluation

Workshop on “Efficacy trials of Plague Vaccines: endpoints, trial design, site selection”

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Vaccine efficacy and diagnostic testing

- Measurement of efficacy
  - Preventing bubonic and pneumonic plague
    - Laboratory confirmed endpoint
Laboratory confirmation of plague

- WHO laboratory-confirmed case criteria:
  - Isolate from a clinical source identified as *Y. pestis*
    - Colony morphology and 2/4 tests positive: bacteriophage lysis, F1 detection, PCR, *Y. pestis* biochemical profile
  - 4-fold difference in F1 antibody titer between paired serum samples
    - testing of acute and convalescent samples
  - F1 antigen detection (bubo, sputum, blood) by immunochromatography
    - Endemic focus when no other confirmatory tests can be performed
Madagascar and Uganda – two study sites

35 participating clinics
2004-2007

CHAPA central lab

12 participating clinics
2004-present

Arua central lab
Suspect case definition

Rapid onset of fever of at least 38ºC, and one of the following:

• One or more buboes, defined as a tender lymph node swelling > 1cm in diameter, or;

• Clinical suspicion of pneumonic plague (e.g. prostration, cough, increased respiratory rate, hemoptysis and/or purulent sputum), or

• Clinical suspicion of plague and epidemiologic link to other cases
Diagnostic tests evaluated

- Confirmatory methods
  - Isolation from clinical source
    - Culture confirmed as *Y. pestis* by bacteriophage lysis and F1 detection (DFA)
  - Four-fold rise in anti-F1 antibody titer
    - F1 based passive hemagglutination; acute and convalescent samples
  - F1 antigen detection (bubo, sputum) by immunochromatography
    - F1 lateral flow assay (Commercial vendor)

- Presumptive method
  - PCR detection (bubo, sputum)
    - Two target real-time PCR
    - 1 chromosomal gene, 1 pMT1 gene, 2/2 = positive
Bubonic plague: Sample collection

**Acute samples**
- **Day 0**
  - Blood
  - Bubo Aspirate

**Serology**
- (Serum separator)
- Real-time PCR (K2 EDTA)
- Blood culture (pediatric bottle)

**Culture**
- (Cary-Blair swab)
  - F1 Lateral flow
  - Real-time PCR

**Convalescent Sample**
- **Day 14-21**
  - Blood
  - Serology

- Central lab
- ≤ 24 hrs ice packs
Pneumonic plague: Sample collection

Acute samples Day 0
- Blood
  - Serology (Serum separator)
  - Real time PCR (K2 EDTA)
  - Blood culture (pediatric bottle)
  - Sputum
    - Culture (Cary-Blair swab)
    - F1 Lateral flow
    - Real-time PCR

Convalescent Sample Day 14-21
- Blood
  - Serology

< 24 hrs ice packs

Central lab
Testing and processing in central lab

Blood culture bottle
- Incubate 37°C
- Subculture 24-48 hrs
  - SBA

Bubo aspirate or sputum
- Incubate 37°C
  - SBA
- Incubate 28°C
  - CIN

Bubo aspirate or sputum
- Process, aliquot
  - 200 µl
  - Score 0 to 4

Serum separator K2 EDTA blood
- Bubo aspirate
- Sputum
  - Process, aliquot
  - Store at -70°C
  - Transport to and testing at CDC
Laboratory criteria: True positive and true negative

- True positive plague cases
  - Isolation of culture (bubo, blood or sputum) and confirmation as *Y. pestis* OR
  - 4-fold change in titer between acute and convalescent serum samples

- True negative non-plague cases
  - *Y. pestis* not isolated AND
  - No change in titer between acute and convalescent serum samples
True positive and true negative cases

  - True plague positives: 34 (31 bubonic, 2 pneumonic, 1 cutaneous)
  - True negatives: 61 (47 suspect bubonic, 14 suspect pneumonic)

  - True plague positives: 84 (78 bubonic, 4 pneumonic, 2 septicemic)
  - True negatives: 75 (69 suspect bubonic, 6 suspect pneumonic)
## Sensitivity and specificity of culture in central lab

<table>
<thead>
<tr>
<th>Clinical Presentation</th>
<th>Source of isolate</th>
<th>Sensitivity (95%CI)</th>
<th>Specificity (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>All presentations</td>
<td>Bubo, sputum, blood</td>
<td>92.9% (86.0 to 96.7%)</td>
<td>100% (97.3 to 100%)</td>
</tr>
<tr>
<td>Bubonic</td>
<td>Bubo</td>
<td>90.7% (83.9 to 95.3%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Bubo + Blood</td>
<td>93.8% (87.8 to 97.5%)</td>
<td></td>
</tr>
<tr>
<td>Pneumonic</td>
<td>Sputum</td>
<td>85.7% (42.1 to 99.6%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Sputum + Blood</td>
<td>85.7% (42.1 to 99.6%)</td>
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</tr>
</tbody>
</table>
## Sensitivity and specificity of diagnostic tests

<table>
<thead>
<tr>
<th>Testing Lab</th>
<th>Test</th>
<th>Sensitivity (95%CI)</th>
<th>Specificity (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central Lab</td>
<td>Any culture</td>
<td>92.9% (86.0 to 96.7%)</td>
<td>100% (97.3 to 100%)</td>
</tr>
<tr>
<td></td>
<td>F1 lateral flow</td>
<td>90.6% (83.8 to 95.2%)</td>
<td>88.0% (82.9 to 93.9%)</td>
</tr>
<tr>
<td></td>
<td>All scores ≥1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CDC</td>
<td>Paired Serology</td>
<td>86.5% (76.6 to 93.3%)</td>
<td>100.0% (97.3% to 100.0%)</td>
</tr>
<tr>
<td></td>
<td>Real-time PCR</td>
<td>93.7% (84.5 to 98.2%)</td>
<td>100% (95.4 to 100.0%)</td>
</tr>
</tbody>
</table>
### Sensitivity and specificity of F1 lateral flow by score

<table>
<thead>
<tr>
<th>F1 Lateral flow Score</th>
<th>Sensitivity (95%CI)</th>
<th>Specificity (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 to 4</td>
<td>90.6% (83.8 to 95.2%)</td>
<td>89.3% (82.9 to 93.9%)</td>
</tr>
<tr>
<td>2 to 4</td>
<td>87.4% (80.1 to 93.0%)</td>
<td>97.7% (93.4% to 99.5%)</td>
</tr>
</tbody>
</table>
Laboratory confirmation for vaccine efficacy

- Performance of confirmatory laboratory methods under controlled collection, transport and laboratory settings
  - Central lab in country - Culture and F1 lateral flow (scored $\geq +2$)
    - Any culture (bubo, sputum, blood): Sensitivity 92.9%; Specificity 100%
    - F1 lateral flow: Sensitivity : Specificity 87.4%; Specificity 97.7%
  - CDC - Serology
    - Paired serology: Sensitivity 86.5%; Specificity 100%

- Performance of presumptive laboratory method under controlled collection, transport and laboratory settings
  - CDC - Real-time PCR
    - Real-time PCR: Sensitivity 93.7%; Specificity 100%
Collaborators:

CDC:
- Diagnostic and Reference Laboratory and Epidemiology and Surveillance Activity, Bacterial Diseases Branch, Division of Vector-Borne Diseases

Madagascar:
- Ministry of Health, Institute Pasteur

Uganda:
- Ministry of Health, Uganda Virus Research Institute

For more information, contact CDC
1-800-CDC-INFO (232-4636)

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.