Evaluation of a point-of-care multiplex immunochromatographic assay (DPP Typhoid assay) for the diagnosis of typhoid

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Outline

- Background
- Aims & Objectives
- Methods
- Results
- Study Limitations
- Conclusion
Introduction

- *Salmonella enterica* Typhi is a gram-negative bacterium causing Typhoid Fever affecting an estimated 9 million people yearly resulting in up to 110,000 deaths

- Blood Culture remains the gold-standard diagnostic procedure for *S. Typhi* diagnosis, however several factors limit the use that is cost, requiring skilled staff to perform, specific infrastructure & long wait for the results

- Several rapid diagnostic tests (RDTs) have been adopted for use in point-of-care settings with poor-to-moderate sensitivity and specificity

- Effective, reliable and rapid point-of-care diagnostic tests are needed with good sensitivity & specificity
DPP Typhoid Assay

- The Dual Path Platform® (DPP) Typhoid assay (Chembio) is a novel, point-of-care multiplex immunochromatographic assay.
- It detects IgA antibodies for lipopolysaccharide (LPS) and hemolysin E (HlyE) antigens.

Aims & Objectives

- To Evaluate the Sensitivity and Specificity of the DPP Typhoid Assay
- To investigate the accuracy of DPP Typhoid Assay in relation to available Typhoid RDTs.
Methods

Study Design
Retrospective Observational

Samples (n=385)
Frozen Serum from previous typhoid study (Oct-2020- July 2021)

S.Typhi Pos = 186
S.Typhi Neg = 199

Samples tested using DPP Typhoid Assay as per manufacturer’s instructions

Assay results were analyzed using the Chembio DPP Micro Reader II
**Assay Procedure**

5 Drops (150ul) of Typhoid Buffer into sample vial

10ul sample (serum/plasma) into sample vial+mix

100ul (sample+buffer mix) into well 1 + wait for 5 mins

Read results between 10-15 mins

5 Drops (150ul) of Typhoid Buffer into well 2 & wait for 10 mins
Recommended Cut-offs as per manufacturer

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Reactive</th>
<th>Non-Reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>LPS</td>
<td>≥ 20</td>
<td>&lt; 20</td>
</tr>
<tr>
<td>HLyE</td>
<td>≥ 14</td>
<td>&lt; 14</td>
</tr>
</tbody>
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Endpoints for Assay Evaluation

- Primary: sensitivity and specificity of the DPP Typhoid assay
- Secondary: Accuracy, Invalidity rate
Results

- Sensitivity and specificity of the DPP Typhoid assay using manufacturer’s threshold stratified by antigen (ITT population)

<table>
<thead>
<tr>
<th>Diagnostic Test</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>DPP Typhoid Assay</td>
<td>97.8%</td>
<td>65.3%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Sensitivity</th>
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<tbody>
<tr>
<td>LPS</td>
<td>97.8 %</td>
<td>47.6 %</td>
</tr>
<tr>
<td>HlyE</td>
<td>67.8 %</td>
<td>90.0 %</td>
</tr>
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Cont..

- **Youden’s optimal threshold**
  - Sensitivity: 91.0%
  - Specificity: 82.0%

- **ROC curve for the DPP Typhoid assay**
### Latent class modeling results: sensitivity and specificity for various typhoid diagnostic tests

<table>
<thead>
<tr>
<th>Diagnostic test*</th>
<th>Sensitivity, % (95% CI)</th>
<th>Specificity, % (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood culture</td>
<td>80.4 (75.6–84.8)</td>
<td>100 (100–100)</td>
</tr>
<tr>
<td>CTK IgG</td>
<td>82.5 (76.9–87.3)</td>
<td>63.5 (56.2–70.3)</td>
</tr>
<tr>
<td>DPP (LPS)</td>
<td>89.7 (85–93)</td>
<td>90.4 (85.3–94.4)</td>
</tr>
<tr>
<td>Enterocheck</td>
<td>71.7 (65.1–77.4)</td>
<td>96.9 (93.5–98.9)</td>
</tr>
<tr>
<td>SD IgM</td>
<td>18.3 (13.5–24)</td>
<td>99.6 (97–100)</td>
</tr>
<tr>
<td>Spectrum IgM</td>
<td>57 (50.2–63.6)</td>
<td>78.1 (71.5–83.8)</td>
</tr>
<tr>
<td>TestIt</td>
<td>58.9 (52.1–65.5)</td>
<td>99.4 (97.4–100)</td>
</tr>
<tr>
<td>Tubex</td>
<td>61.3 (54.5–67.7)</td>
<td>97.1 (93.7–99.1)</td>
</tr>
<tr>
<td>Typhidot IgM</td>
<td>34.6 (28.4–41.3)</td>
<td>95.4 (91.5–97.9)</td>
</tr>
<tr>
<td>Widal test</td>
<td>49.9 (43.2–56.7)</td>
<td>79.8 (73.4–85.4)</td>
</tr>
</tbody>
</table>
Study Limitations

- Used frozen serum samples collected from a single geographical area.
- Further studies to ensure accuracy and comparability across various sample types and in different endemic settings.
- Further testing is required to provide insights on the impact of testing kit adjustment on assay performance.
- Cross-reactivity with different febrile illnesses with same presenting enteric fever were not evaluated.
Conclusion

- High diagnostic accuracy for typhoid and for the presence of the individual assay antigens (LPS and HlyE)
- Sensitivity and specificity of the DPP Typhoid assay compared favorably with other typhoid diagnostic tests
- The Threshold needs to be adjusted
Our Team

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Thank You