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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

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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

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DPP Typhoid Assay

- The Dual Path Platform® (DPP) Typhoid assay (Chembio) is a novel, point-of-care multiplex immunochromatographic
- 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

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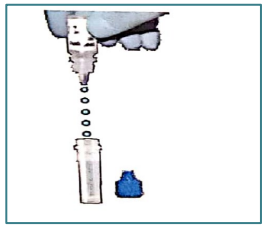
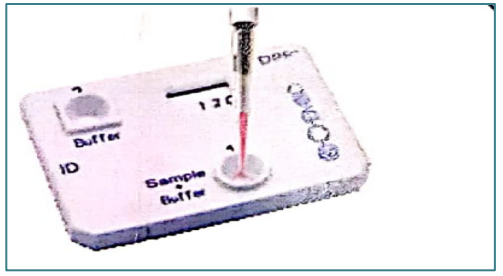
Assay Procedure

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



5 Drops (150ul) of Typhoid Buffer into well 2 & wait for 10 mins

Read results between 10-15 mins

Recommended Cut-offs as per manufacturer

Antigen	Reactive	Non-Reactive
LPS	≥ 20	< 20
HLyE	≥ 14	< 14

Endpoints for Assay Evaluation

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

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Results

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

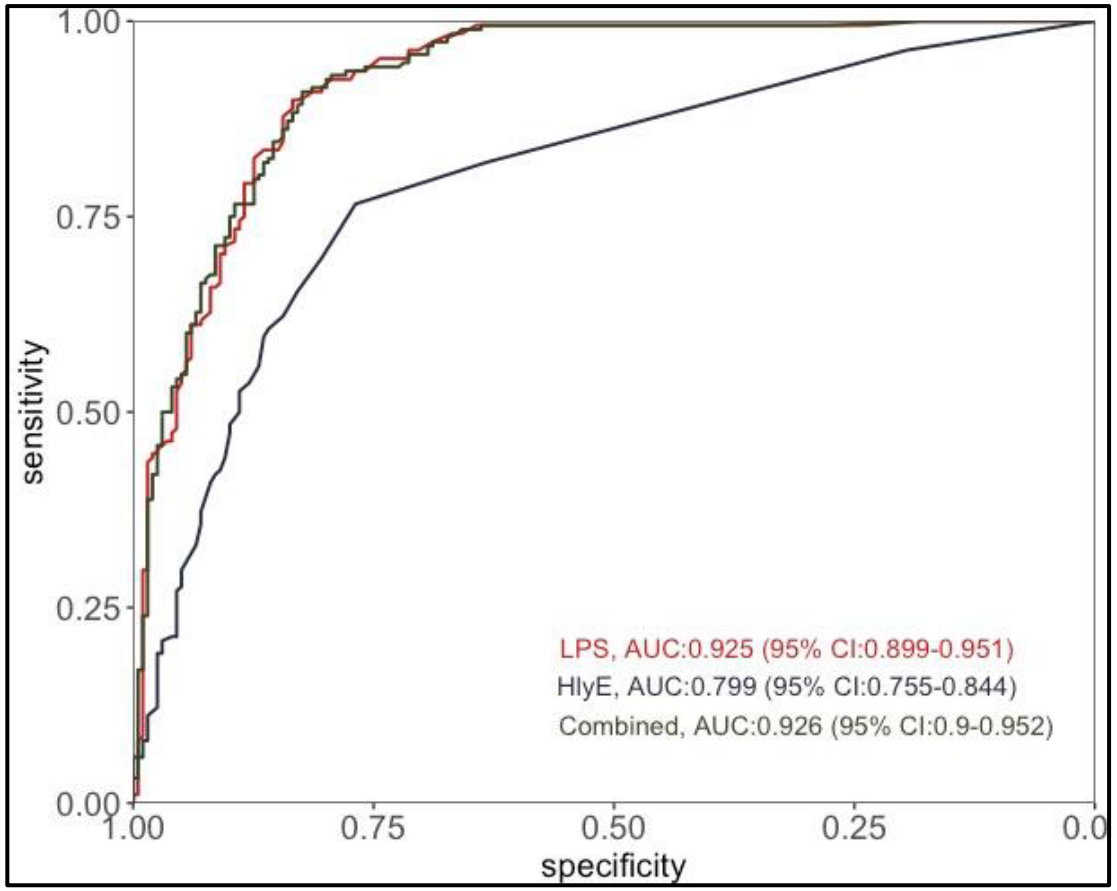
Diagnostic Test	Sensitivity	Specificity
DPP Typhoid Assay	97.8%	65.3%

Antigen	Sensitivity	Specificity
LPS	97.8 %	47.6 %
HlyE	67.8 %	90.0 %

Cont..

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

- **ROC curve for the DPP Typhoid assay**



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Cont..

- **Latent class modeling results: sensitivity and specificity for various typhoid diagnostic tests**

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

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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

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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

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Thank You