Development and Challenges faced in Optimization of Sensitive Diagnostic Tests for Typhoid fever

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Disease burden of enteric fever in Bangladesh

Studies in urban slums in Dhaka indicate that the incidence of typhoid fever for all age groups is 3.9 episodes/1000 person-years (Brooks *et al*., 2005)

A significantly higher proportion of cases were found in patients younger than 5 years of age. The highest rate of isolation was in the second year of life, among them >70% of isolates were from children aged 9 to 24 months (Saha *et al*., 2001)

The incidence of typhoid fever was shown to be 2.0 episodes/1000 person-years, with a higher incidence in children aged < 5 years (10.5/1000 person-years) than in older persons (0.9/1000 person-years) [Naheed *et al*., 2010]

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Existing methods for disgnosis of enteric fever

For measurement of true disease burden, sensitive diagnostic methods plays a crucial role and the timing of the testing is also important

Blood or Bone marrow culture, PCR- Week 1
Serological assays- Week 2
Stool antigen- Week 2
Urine- Week 4

Testing for typhoid fever is not always sought early on and often antibiotics are taken before diagnosis is carried out. There are limitations to existing methods of diagnosis

Mogasale *et al. Ann Clin Microbiol Antimicrob (2016) 15:32*
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Annals of Clinical Microbiology and Antimicrobials

REVIEW Open Access

What proportion of *Salmonella* Typhi cases are detected by blood culture? A systematic literature review

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Abstract

Blood culture is often used in definitive diagnosis of typhoid fever while, bone marrow culture has a greater sensitivity and considered reference standard. The sensitivity of blood culture measured against bone marrow culture results in measurement bias because both tests are not fully sensitive. Here we propose a combination of the two cultures as a reference to define true positive *S.* Typhi cases. Based on a systematic literature review, we identified ten papers that had performed blood and bone marrow culture for *S.* Typhi in same subjects. We estimated the weighted mean of proportion of cases detected by culture measured against true *S.* Typhi positive cases using a random effects model. Of 529 true positive *S.* Typhi cases, 61 % (95 % CI 52–70 %) and 96 % (95 % CI 93–99 %) were detected by blood and bone marrow cultures respectively. Blood culture sensitivity was 66 % (95 % CI 56–75 %) when compared with

Blood culture

Blood culture is ~40 to 60% sensitive. Various factors contribute to low sensitivity

Bacteria mostly remain intracellular (median- 0.5 CFU/ml blood); large volume of blood is a critical factor

The efficacy of blood culture decreases with the duration of illness

Use of antibiotics reduces the sensitivity of blood culture

Requires at least 3-5 days for diagnostic results

Real Time Multiplex PCR has been used and being optimized for detection of *S.*Typhi in blood

Tennant et al. 2015
Stephane Pouzal et al. presentation at CAT on 4th April

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Culture plus PCR in Typhoid challenge models

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Blood culture-PCR to optimise typhoid fever diagnosis after controlled human infection identifies frequent asymptomatic cases and evidence of primary bacteraemia

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Commercially available diagnostic kits (Tubex, Typhidot)

None of these assays have reached widespread use because of lack of sensitivity and specificity

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Diagnostic Microbiology and Infectious Disease 61 (2008) 381 – 386

DIAGNOSTIC MICROBIOLOGY AND INFECTIOUS DISEASE

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Clinical value of Tubex™ and Typhidot® rapid diagnostic tests for typhoid fever in an urban community clinic in Bangladesh

Aliya Naheed[a,*], Pavani K. Ram[b,c], W. Abdullah Brooks[a], Eric D. Mintz[b], Md. Anowar Hossain[a], Michele M. Parsons[b], Stephen P. Luby[a,b], Robert F. Breiman[a,d]

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Received 17 November 2007; accepted 28 March 2008

Abstract

Tubex™ and Typhidot®, rapid tests for typhoid fever, performed well in evaluations conducted in hospital settings among patients with culture-confirmed typhoid fever. We evaluated these tests in a community clinic in Bangladesh. Blood samples were obtained from 867 febrile patients for culture, Typhidot® and Tubex™ tests. Considering the 43 blood culture-confirmed cases of typhoid fever as typhoid positive and the 24 other confirmed bacteremia cases as typhoid negative, Tubex™ was 60% sensitive and 58% specific, with 90% positive and 58% negative predictive values (NPVs); Typhidot® was 67% sensitive and 54% specific, with 85% positive and 81% NPVs. When blood

The value of Tubex and Typhidot tests for typhoid fever diagnosis in a community clinic in urban Bangladesh was low

What are the attributes for a suitable test for diagnosis and for determining burden of enteric fever?

i. Can be tested using low volumes of blood (1-2 ml) for use in infants and young children

ii. Broad window of testing time after onset of disease

iv. Non-interference by antibiotic use prior to testing

v. No effect of pre-existing antibodies as in endemic areas to S. Typhi/S. Paratyphi

vi. Relatively rapid so as to be useful for patient treatment (~ 12-24 hours)

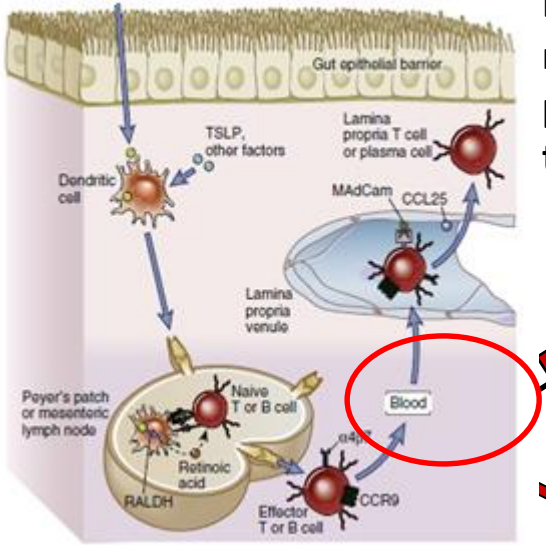
vii. Easily adaptable to laboratories in developing country settings

viii. Key antigens incorporated for specific diagnostic analysis

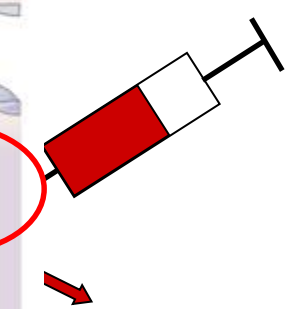
Use of antibodies in lymphocyte secretions as a measure of recent exposure as opposed to plasma/serum for diagnosis of enteric fever

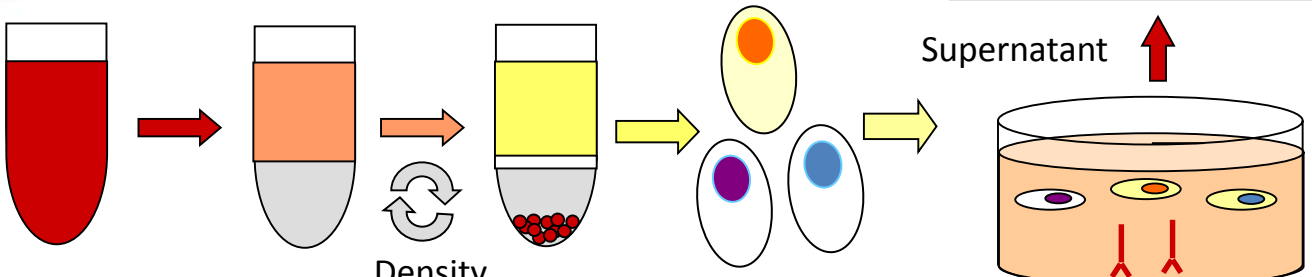
Results within 14-36 hrs of processing → **TPTEST**

Exposure to pathogen in the gut



B cells activated in the mucosa migrate via peripheral blood back to the mucosa





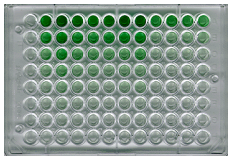
Density gradient centrifugation

PBMCs (Peripheral Blood Mononuclear Cells)

Supernatant

ALS
Antibodies in Lymphocyte Supernatants

ELISA



Antigen specific Antibody response due to a recent exposure

Assessment of the lymphocyte secreted IgA antibodies to diagnose Typhoid fever

CLINICAL AND VACCINE IMMUNOLOGY, Nov. 2009, p. 1587–1594 Vol. 16, No. 11
1556-6811/09/$12.00 doi:10.1128/CVI.00311-09

Salmonella enterica Serovar Typhi-Specific Immunoglobulin A Antibody Responses in Plasma and Antibody in Lymphocyte Supernatant Specimens in Bangladeshi Patients with Suspected Typhoid Fever▽

Alaullah Sheikh,[1]† M. Saruar I
Dilruba Ahmed,[1] K.
Mian Mashhud
Stephen
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International Centre for Diarrhoeal Disease
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Medicine, Harvard Medical School
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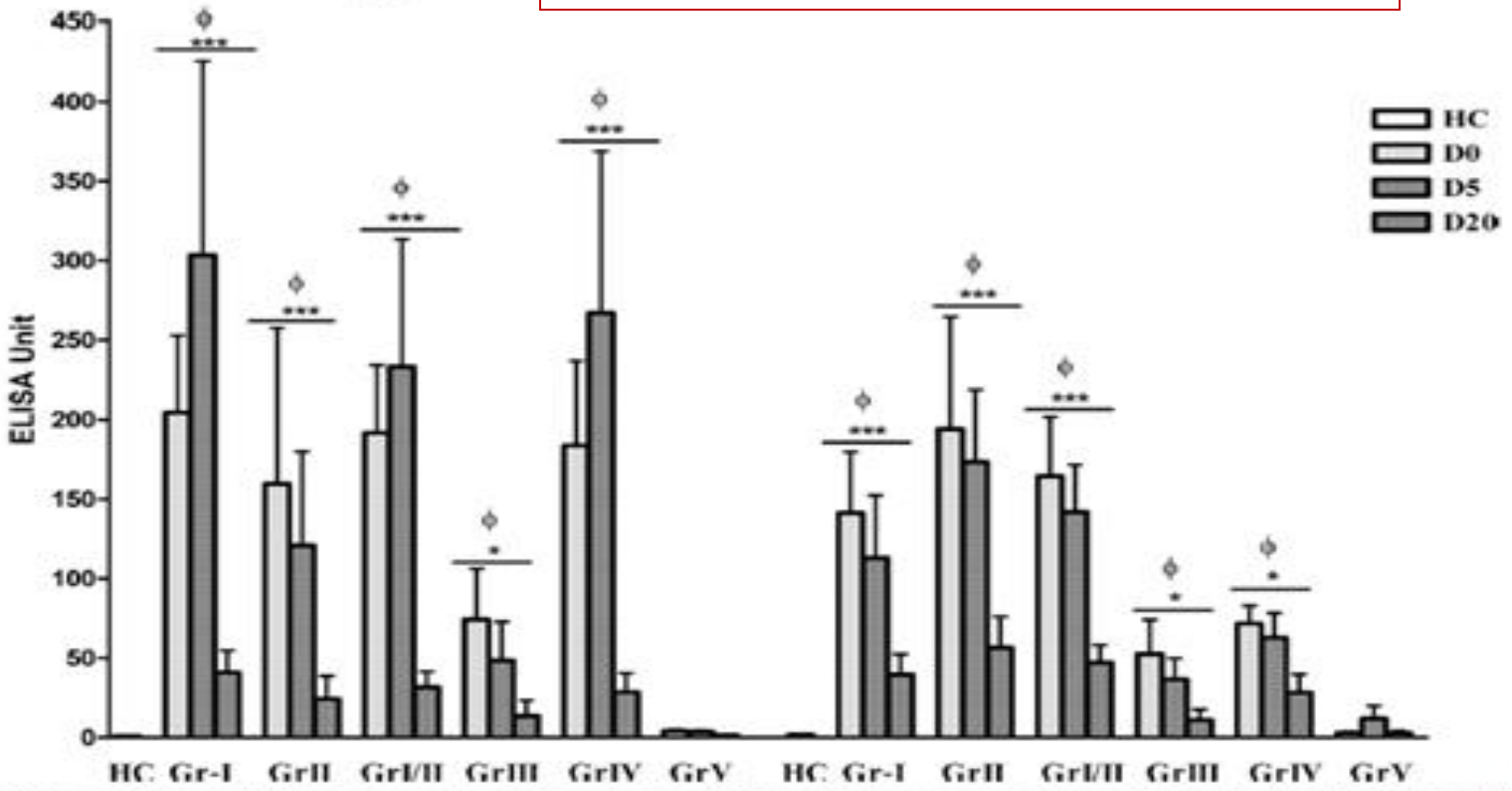
Sensitivity of 100% and Specificity of 78-97% for diagnosis of typhoid and paratyphoid fever



. MP- and WC-specific IgA responses in ALS specimens of different study groups on different study days (see Fig. 1 legend f
ic means with the SEM are shown for day 0 (D0), day 5 (D5), and day 20 (D20). Statistical difference compared to healthy con
01; **, $P < 0.005$; *, $P < 0.05$. Statistical difference compared to GrV: ϕ, $P < 0.05$.

Response in lymphocyte secretion from bacteremic young children

PLOS | NEGLECTED TROPICAL DISEASES

RESEARCH ARTICLE

Typhoid Fever in Young Children in Bangladesh: Clinical Findings, Antibiotic Susceptibility Pattern and Immune Responses

Farhana Khanam[1], Md. Abu Sayeed[1], F
Dilruba Ahmed[1], Doli Goswami[1], Md. L
B. Calderwood[3,4], Richelle C. Charles[3,
Firdausi Qadri[1]*

Using low volumes of blood-1 ml

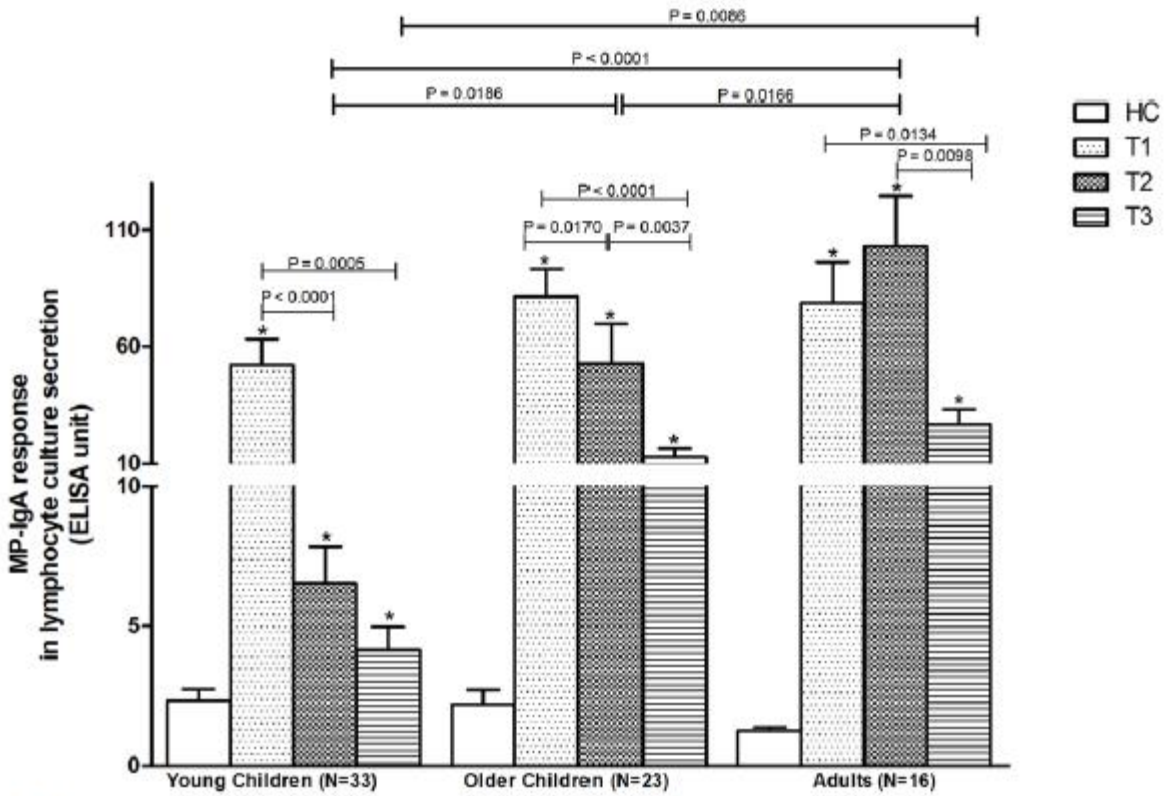


Fig 1. MP-IgA responses in lymphocyte culture secretion in patients with *S.* Typhi bacteremia. Mean with standard error of mean (SEM) are shown for T1 (at day of enrolment), T2 (at early convalescence: 7–10 days after enrolment) and T3 (at late convalescence: 21–28 days after enrolment). Statistical difference between patients and age-matched healthy control (HC): *. MP: *S.* Typhi membrane preparation.

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Bayesian Latent class modeling was used for comparing TPTest with other diagnostic tests

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RESEARCH ARTICLE

Comparison of the Performance of the TPTest, Tubex, Typhidot and Widal Immunodiagnostic Assays and Blood Cultures in Detecting Patients with Typhoid Fever in Bangladesh, Including Using a Bayesian Latent Class Modeling Approach

Kamrul Islam[1], Md. Abu Sayeed[1], Emran Hossen[1], Farhana Khanam[1], Richelle C. Charles[2,3], Jason Andrews[4], Edward T. Ryan[2,3,5]*, Firdausi Qadri[1]*

Table 3. Estimated sensitivity and specificity of six diagnostic tests for enteric fever in 92 febrile patients (95% confidence intervals shown in parenthesis), under a Bayesian latent class modeling approach.

Test	Sensitivity	Specificity
Culture	51.8% (41.2–62.9%)	100%
TPTest	96.0% (87.1%-99.8%)	96.6% (90.7%-99.2%)
Tubex	60.2% (49.3%-71.2%)	89.9% (79.6%-96.8%)
Typhidot	59.6% (50.1%-69.3%)	80.0% (67.7%-89.7%)
Widal $\geq$ 1:320	14.9% (7.6%-24.7%)	86.3% (75.7%-93.6%)
Widal 4 fold titer rise	12.6% (6.2%-22.0%)	100.0% (99.9%-100.0%)

Jason Andrews
Stanford University

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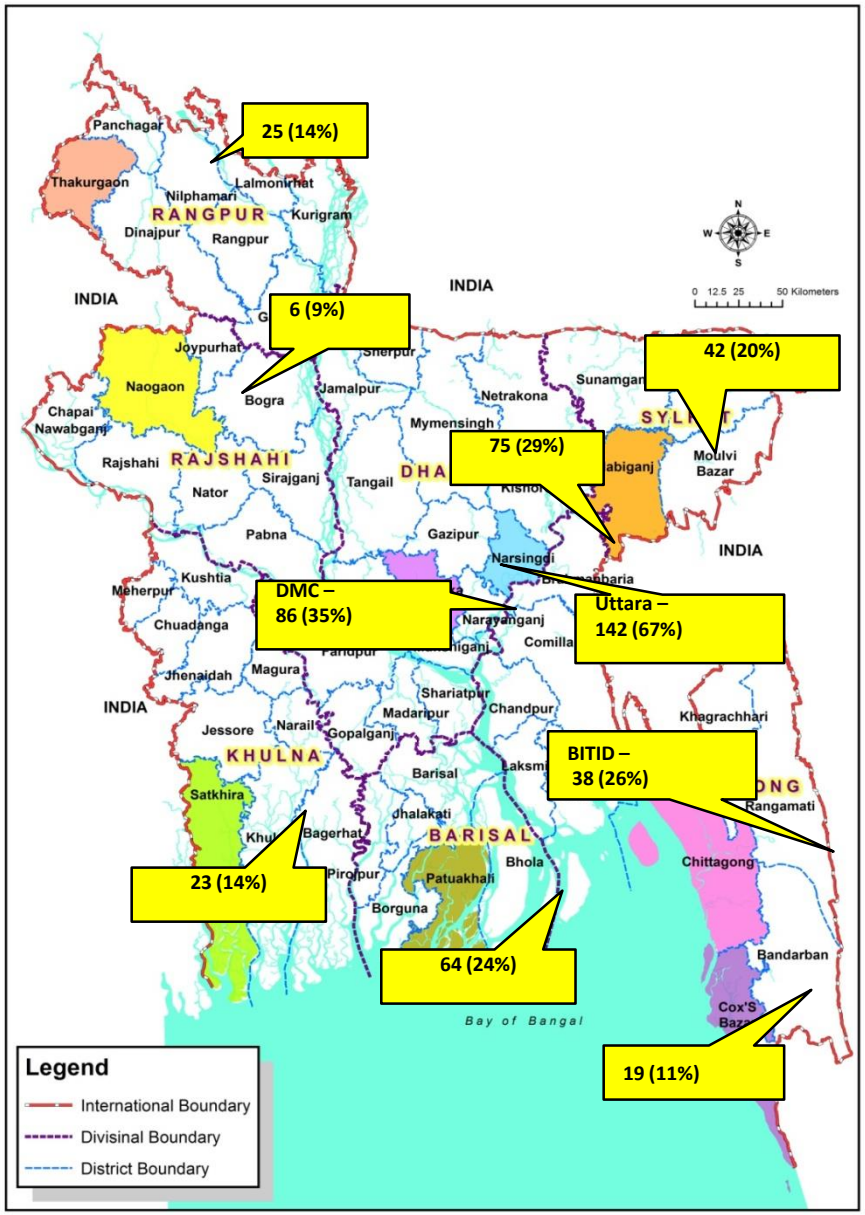
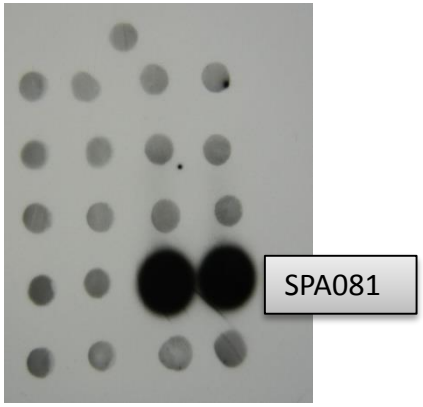
Enteric fever surveillance is being carried out using TPTEST in field sites in Bangladesh



Identification of novel antigens



Using a high throughput immunoscreening technique- SCOTS, IVIAT, microarray techniques- subsets of immunogenic *S.*Typhi, *S.* Paratyphi A proteins have been identified using sera or ALS from patients as probes

35 proteins in *S.*Typhi and
20 proteins in *S.* Paratyphi A infections

Immunoproteomic analysis and Mass Spectrometry- 57 proteins detected

Important antigens undergoing evaluation include- LPS, HlyE, YncE, and CdtB and others as single antigen or combined testing

Richelle Charles, Ed Ryan et al. ongoing studies

OPEN ACCESS Freely available online

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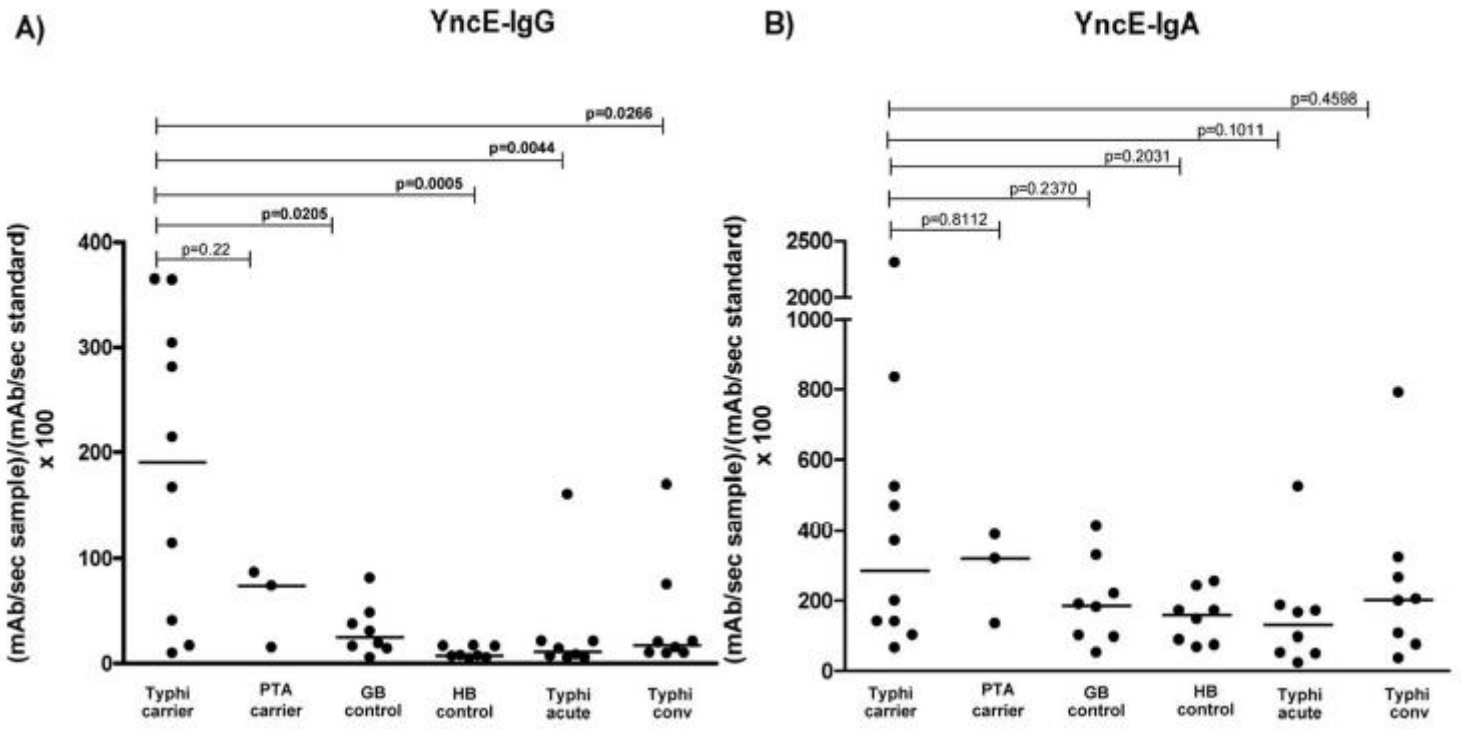
Identification of Immunogenic *Salmonella enterica* Serotype Typhi Antigens Expressed in Chronic Biliary Carriers of *S.* Typhi in Kathmandu, Nepal

Richelle C. Charles[1,2*], Tania Sultana[1,3], Mohammad Murshid Alam[3], Yanan Yu[1], Ying Wu-Freeman[1], Meagan Kelly Bufano[1], Sean M. Rollins[1,2,4], Lillian Tsai[1], Jason B. Harris[1,2], Regina C. LaRocque[1,2], Daniel T. Leung[1,2,3], W. Abdullah Brooks[3], Tran Vu Thieu Nga[5], Sabina Dongol[6], Buddha Basnyat[6], Stephen B. Calderwood[1,2,7], Jeremy Farrar[5,8], Farhana Khanam[3], John S. Gunn[9], Firdausi Qadri[3¶], Stephen Baker[5,8,10¶], Edward T. Ryan[1,2,11¶]

Characterization of anti- YncE (STY1479) immune responses



YncE and other antigens may lead to development of an improved diagnostic assay and better understanding of the survival of *S.* Typhi within the biliary tract of carriers

Conversion of TPTEST to a lateral flow device platform

AMERICAN SOCIETY FOR MICROBIOLOGY | Clinical and Vaccine Immunology

Development of a Simple, Peripheral-Blood-Based Lateral-Flow Dipstick Assay for Accurate Detection of Patients with Enteric Fever

Iqbal Hassan Khan,[a] M. Abu Sayeed,[b] Nishat Sultana,[a] Kamrul Islam,[b] Jakia Amin,[a] M. Omar Faruk,[b] Umama Khan,[a] Farhana Khanam,[b] Edward T. Ryan,[c,d,e] Firdausi Qadri[b]

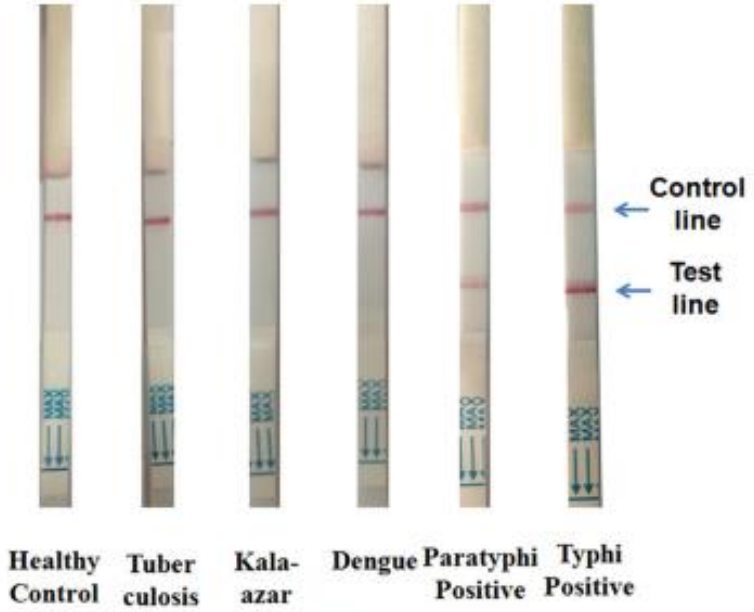


FIG 4 Dipsticks detecting *S.* Typhi LPS-specific IgG in different samples. Both the control line and test line appear in the positive sample. The absence of the test line in the presence of the control line indicates a negative sample.

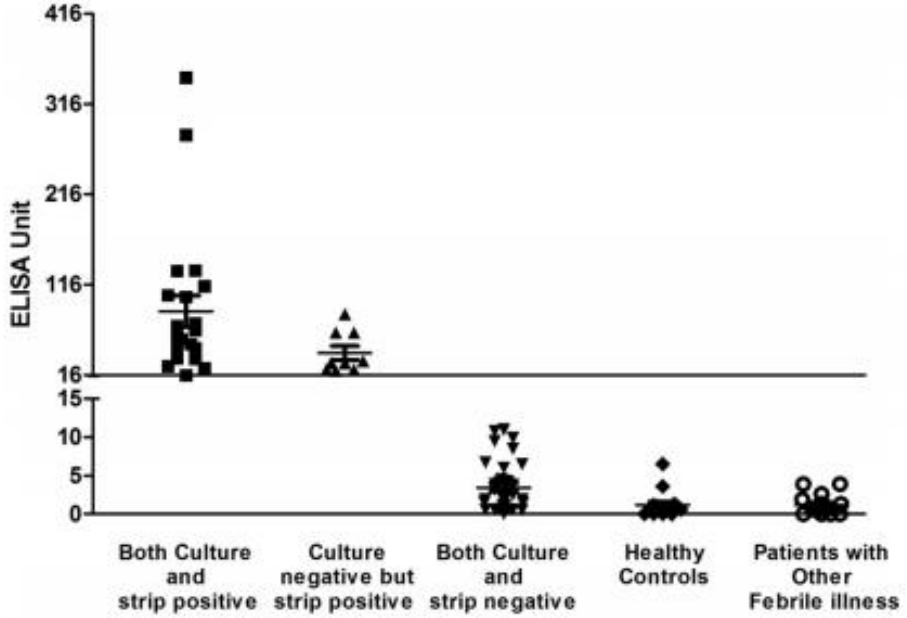
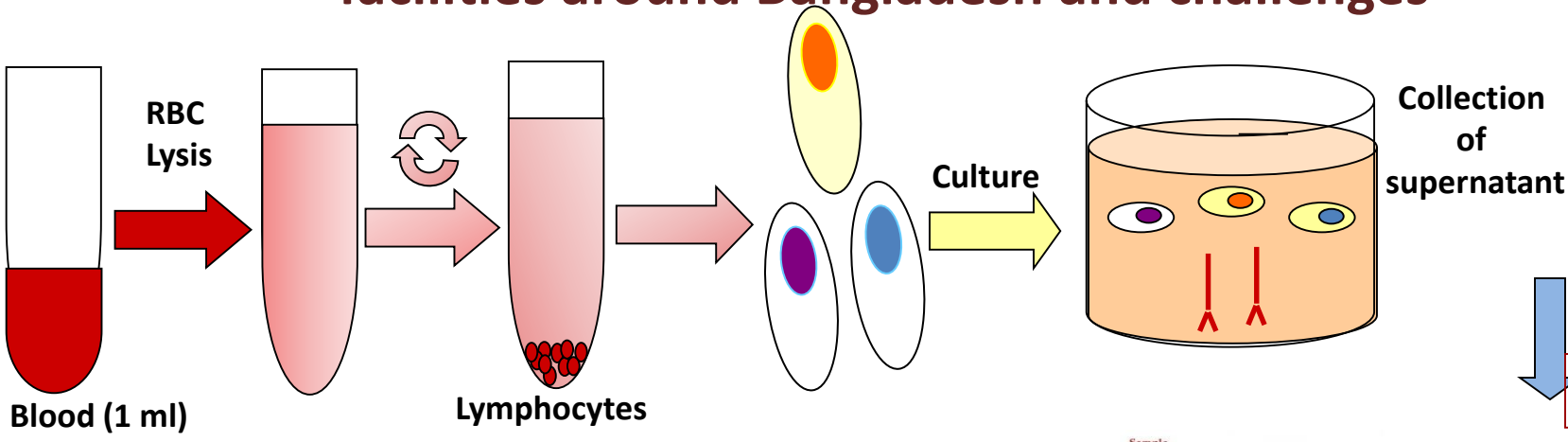
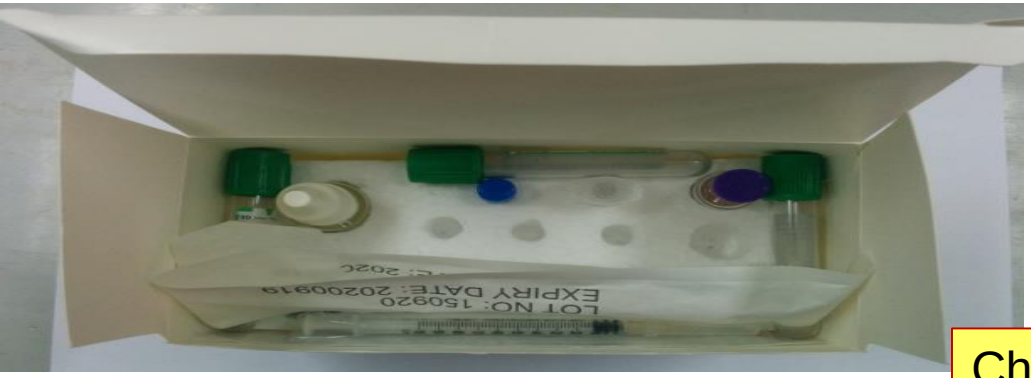


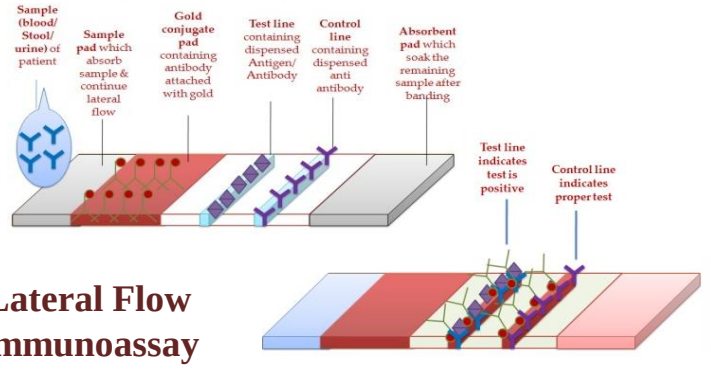
FIG 5 *S.* Typhi LPS-specific IgG responses in lymphocyte culture supernatant prepared from different groups. The dipstick was positive for specimens with responses of ≥16 ELISA units.

Sensitivity of 98% compared to blood culture results and a specificity that ranged from 78 to 100% depending on the definition of a true negative

Diagnostic Kit for Typhoid Fever- Typhokit for use in diagnostic facilities around Bangladesh and challenges







Lateral Flow Immunoassay

Changes made from TPTEST to TYPHOKIT

1. RBC lysis instead of Ficoll
2. Incubator without CO2
3. Tubes instead of cell culture plates
4. Modification of antigen

Typhokit is being tested in Different Diagnostic Laboratories

Instruments are used for making the kits

FURTHER OPTIMIZATION ONGOING BASED ON FEEDBACK FROM DIAGNOSTIC LABORATORIES IN BANGLADESH

Conclusions

- The TPTest shows good sensitivity and specificity and can be used as a diagnostic tool for complementing blood culture tests/ qPCR
- New antigens need to be evaluated for incorporation in the diagnostic assays
- Large scale production and wider field testing of Typhokit will be needed
- These methodologies can be used as a useful tool for evaluation of burden of disease as well as for testing effectiveness of TCVs in the near future

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