Enteric fever and Diagnostic Challenges

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Diagnosis of enteric fever

- Undiagnosed and maltreated cases of enteric fever may result in severe complications and increased morbidity.
- The development of a reliable and rapid diagnostic methods for enteric fever is urgently needed.
The lifestyle of *Salmonella* Typhi in the human host and implications for diagnostics

http://www.biomedcentral.com/1471-2334/10/45/figure/F2
Baker et al. *BMC Infectious Diseases* 2010
Diagnostics tests available for enteric fever

**Bone marrow-**
Culture- this invasive procedure is not routinely used

**Blood-**
Culture: sensitivity 20-70%, takes 3-7 days
PCR: non-specific amplification and contamination

**Serum/ Plasma-**
Widal test: less specificity in endemic areas
Rapid Immunological tests:
  Tubex - 60% sensitive and 58% specific
  Typhidot- 67% sensitive and 54% specific

**Stool-**
Poor sensitivity at the acute phase of disease

**Urine-**
Culture- Sensitivity very low
  PCR- report of flagellin gene (*fliC*) for diagnosis of S. Typhi
Mucosal response and surrogate markers of protective immunity

- Activated mucosal lymphocytes migrate from intestinal tissue and circulate within peripheral blood before re-homing to mucosal tissues.

- This migration peaks around 5-7 days after intestinal infection.

- The immune response can be measured in peripheral blood mononuclear cells (PBMC) in lymphocyte secretions and responses measured using enzyme immunoassays.
Activated mucosal lymphocytes migrate from intestine to the circulation and best depicts a mucosal response and a RECENT exposure.

Blood (1 ml)

Incubation of cells at 37°C for 18-48 hours

Differential centrifugation

Kinetic ELISA readout

>10 ELISA Unit is taken as positive
S. Typhi specific IgA antibody responses in secretions of lymphocytes

Sheikh et al. 2009

Patients with febrile illnesses
Gr-I, blood culture positive;
GrII, fourfold change in Widal
GrIII, Widal titer of 320
GrIV, negative culture and Widal but an anti-serovar Typhi IgA titer of >10 ELISA units in the assay
GrV, all assays negative; OF, other febrile illness; HC, healthy controls

Other febrile illnesses
Dengue, Leptospirosis
Kala azar, TB, others
This method is also useful for diagnosis of patients with *S. Paratyphi* infections.

**TPTest (Typhoid and Paratyphoid test)**

**TPTest results in study participants**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>No. of individuals</th>
<th>TPTTest Positive</th>
<th>TPTTest Negative</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with <em>S. Typhi</em> bacteremia</td>
<td>27</td>
<td>27</td>
<td>0</td>
</tr>
<tr>
<td>Patients with <em>S. Paratyphi A</em> bacteremia</td>
<td>12</td>
<td>12</td>
<td>0</td>
</tr>
<tr>
<td>Clinically suspected enteric fever but blood culture negative</td>
<td>204</td>
<td>44</td>
<td>160</td>
</tr>
</tbody>
</table>

The TPTTest was also tested in patients with confirmed illness other than enteric fever. The sensitivity and specificity of the TPTTest are **100% and 91%** respectively.
Patients with suspected enteric fever have been recruited from different field areas:

- Kamalapur field site
- icddr,b hospital
- Mirpur field site

Based on the reproducibility of the test, the TPTest was introduced at the Clinical Diagnostic Services of the icddr,b from May 2012. This was based on results of over 1000 specimens tested in febrile patients and healthy controls.

Utilizing the TPTest we can detect 60% of cases within 14-18 hours of receiving a specimen and the remaining at around 48 h of culture of cells.
No difference was found at the acute stage of disease among young children, older children and adults showing the utility of the TPTest in all age groups.

The method can be used even in young children for detection of enteric fever using 1 ml of blood only.
Technology Transfer to the Microbiology Department of the Dhaka Medical College and Hospital (DMCH)

From the Outpatient Department of DMCH

- 152 suspected enteric fever patients enrolled

- S. Typhi and S. paratyphi A isolated from 22 and 7 patients respectively
  - all were positive by PTTest

- Additional 38 patients were detected by PTTest in patients negative by culture
Simplification of the TPTest for use in laboratories lacking facilities

Our existing method uses the following:

- Density gradient centrifugation on ficoll-hypaque for separation of peripheral blood lymphocytes
- 37°C incubator with a constant 5% CO₂ supply
- ELISA reader for readouts

We are working on procedures to make the TPTest simpler

- Simple cell separation procedure i.e. buffy coat preparation or RBC lysis
- Incubation of cells at 37°C without CO₂ incubation
- Immunodot blot assay for detection of the antibody response in cell culture secretions
Comparison of TPTest results using leukocytes recovered by various techniques

No significant differences in antibody responses were seen when cells were separated by different methods

Farhana, Shahnaz et al. ongoing studies
Comparison of TPTest results using peripheral blood mononuclear cells incubated at 37ºC in presence or absence of supplemental CO₂

No significant difference in antibody response was found
ELISA and immunodot blot approaches

Immunodot blot sensitivity ≥16 ELISA units
Initiated development of lateral flow device for rapid test with industrial partner

Biotechnology Derived Product Facility (BDPF), Incepta Pharmaceuticals Ltd

Dispenser

Strip Cutter

Immunochromatography strip test/ lateral flow test
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