Typhoid Diagnostics for Measuring Disease Burden

Bangladesh Study

Funded by:
To develop and validate a pilot molecular assay for the detection and identification of *Salmonella* directly in blood from patients with suspected enteric fever (typhoid) aiming at monitoring disease burden in developing countries

- *Salmonella typhi*
- *Salmonella paratyphiA*
- *Salmonella spp.*
Typhoid consortium

Bill & Melinda Gates Foundation

Fondation Merieux

IP
FTD
CHRF
Kemri-CDC
Evaluation of the primers/probes on characterized strains:

- 90 *Salmonella* Typhi
- 33 *Salmonella* ParatyphiA
- 16 *Salmonella* spp. (non-Typhi strains)
- 39 other bacterial species

→ Very high specificity
Optimization of the sample preparation

- Preliminary results on 1ml of blood clinical samples showed low sensitivity.

- Modifications of the sample preparation method were implemented to increase the sensitivity:
  - Increase the volume of blood from 1 ml to 5ml
  - Use of a pre-enrichment media including 10% oxgall
  - Optimization of the pre-enrichment time: 5 hours at 37°C

- The whole assay gives a result in 8 hours
M&M: RT-Multiplex PCR

1. Blood samples from Patients with suspected typhoid fever
2. Nucleic acid extraction and purification from whole blood lysis
3. 5 hours pre-enrichment
4. Real-time Multiplex PCR:
   - S.Typhi
   - S.Paratyphi
   - S.spp
   - IC
5. Positive control pool
6. Negative control
FTD Enteric Fever Kit

FTD Enteric fever

Multiplex RT-PCR for the detection of Salmonella spp., Salmonella typhi
and Salmonella paratyphi

Fast-track Diagnostics Luxembourg s.à.r.l.
38, rue Holz, ZAC, Leopolda, L-6131, Luxembourg
Phone: +352 790 290-329 / Fax: +352 790 290-314
email: ctm@fast-trackdiagnostics.com / www.fast-trackdiagnostics.com
First evaluation in Dhaka, Bangladesh
CHRF
Inclusion criteria

- Patients with fever conditions > 38°C
- Volume of 5ml of blood for the PCR
- Volume of 3-4 ml for blood culture
First evaluation on febrile patients (n=206)

3-4 ml blood sample

Blood culture

Microbiological identification

5ml blood sample
+ 5ml TSB-oxgall

5 hours pre-enrichment culture

Nucleic acid extraction
+ RT-PCR
The assay allows an increase of laboratory confirmed cases of Typhoid fever of >50% in this study.

Results indicate an significant increase of sensitivity vs blood culture (chi2(1) = 13.58   p = 0.0002)

Sensitivity of PCR was 93.6% (82.4-97.8), sensitivity of culture was 61.7% (47.3-74.2), their specificity were 100% (data from 20 controls)

3 blood culture positive cases were missed by PCR
Conclusion of the first study

- Extension to other sites in Bangladesh including urban slums
- Increase sample size
- Decrease blood sample volume to 3ml or less
- Discrepancy analysis
Second evaluation in Dhaka, Bangladesh

4 study sites

including two Dhaka slums

3ml of blood
Study protocol

First patient included: 09/01/2015

As per 04/27/2015: 601 patients included (61 blood culture positive)
Study protocol

Collection sites
- Children
  - Shishu Hospital

Laboratories
- CHRF
  - LPE-Fondation Mérieux
    - icddr, b ideSHI
  - Shours pre-enrichment + Freezing

Typhoid tests
- Blood culture
- TPTest (Performed at ideSHI)
- RT-PCR
  - Shours pre-enrichment + Freezing
  - TPTest
  - Blood culture
<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Blood culture</th>
<th>PCR</th>
<th>TPTest</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>18+</td>
<td>5 ml</td>
<td>3 ml</td>
<td>1 ml</td>
<td>9 ml</td>
</tr>
<tr>
<td>5-17</td>
<td>3-5 ml</td>
<td>3 ml</td>
<td>1 ml</td>
<td>7-9 ml</td>
</tr>
<tr>
<td>2-4</td>
<td>3 ml</td>
<td>3 ml</td>
<td>-</td>
<td>6 ml</td>
</tr>
<tr>
<td>0-2</td>
<td>Excluded</td>
<td>Excluded</td>
<td>Excluded</td>
<td>Excluded</td>
</tr>
</tbody>
</table>
### Preliminary results (n=350)

<table>
<thead>
<tr>
<th></th>
<th>Blood culture +</th>
<th>Blood culture -</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCR +</td>
<td>41</td>
<td>49</td>
<td>90</td>
</tr>
<tr>
<td>PCR -</td>
<td>3</td>
<td>257</td>
<td>260</td>
</tr>
<tr>
<td>Total</td>
<td>44</td>
<td>306</td>
<td>350</td>
</tr>
</tbody>
</table>

- **Definition true positive**: blood culture and/or PCR positive
- **Sensitivity blood culture**: $\frac{44}{93} = 47.3\%$
- **Sensitivity PCR**: $\frac{90}{93} = 96.8\%$
- **100% match identification (S. Typhi and S. ParatyphiA)**
Study extension and evaluation in Africa

Funder: BMGF
To evaluate the performance of our Typhoid RT-PCR assay against blood culture in febrile patients in 4 African countries

Proposed study sites:

- Malawi (R.Heyderman)
- Burkina Faso (F.Marks)
- Ghana (F.Marks)
- Cameroun (G.Vernet)*
Objectives & Study sites

- Inclusion period 1 year

- 1000 cases included (100 expected blood culture positive) / site and 100-200 controls
Objectives & Study sites

- Patients with fever condition
  - Malaria test
  - Patients with fever condition
    - Malaria negative
      - Blood culture
      - Molecular assay
Inclusion criteria:

- Fever (≥38°C) for ≥ 5 days
- Age > 3 months old
- Accompanied by a written informed consent from the patient (≥18yo) or parental/guardian (<18yo)
Exclusion criteria

- Recent history of a laboratory confirmed enteric fever diagnosis
- Malaria test positive (RDT or blood film)
- Age <3 months old
## Objectives & Study sites

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Blood culture</th>
<th>PCR</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>18+</td>
<td>5 ml</td>
<td>3 ml</td>
<td>8 ml</td>
</tr>
<tr>
<td>5-17</td>
<td>3-5 ml</td>
<td>3 ml</td>
<td>6-8 ml</td>
</tr>
<tr>
<td>2-4</td>
<td>2-3 ml</td>
<td>2-3 ml</td>
<td>4-6 ml</td>
</tr>
<tr>
<td>3mths-2yo</td>
<td>1-2ml</td>
<td>1-2 ml</td>
<td>2-4 ml</td>
</tr>
</tbody>
</table>
Objectives & Study sites

Enrolment sites

**Children + Adults**
- Malawi
- Burkina Faso
- Ghana
- Cameroun

Laboratories
- Malawi-Liverpool-Wellcome Trust
- Schiffra Hospital Laboratory
- Kumasi Centre for Collaborative Research in Tropical Medicine
- Centre Pasteur Cameroun

Typhoid tests
- Blood culture
- Molecular assay

Project coordination: LPE-Fondation Mérieux
Training/logistic Support: LPE-Fondation Mérieux
Data validation: LPE-Fondation Mérieux
Asian typhoid surveillance

Funder: BMGF
Objectives

- To include the PCR assay in laboratories involved in the Surveillance for Enteric fever in Asia Project (SEAP)

- Countries involved: Nepal, Bangladesh, Pakistan, India, Indonesia

- **Role of Fondation Mérieux:**
  - Technology transfer
  - Training
  - Logistic
  - Support

- **Starting date:** End of 2015