Typhoid Diagnostics for Measuring Disease Burden

Bangladesh Study

Funded by:

BILL & MELINDA GATES foundation

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Primary objective

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.*

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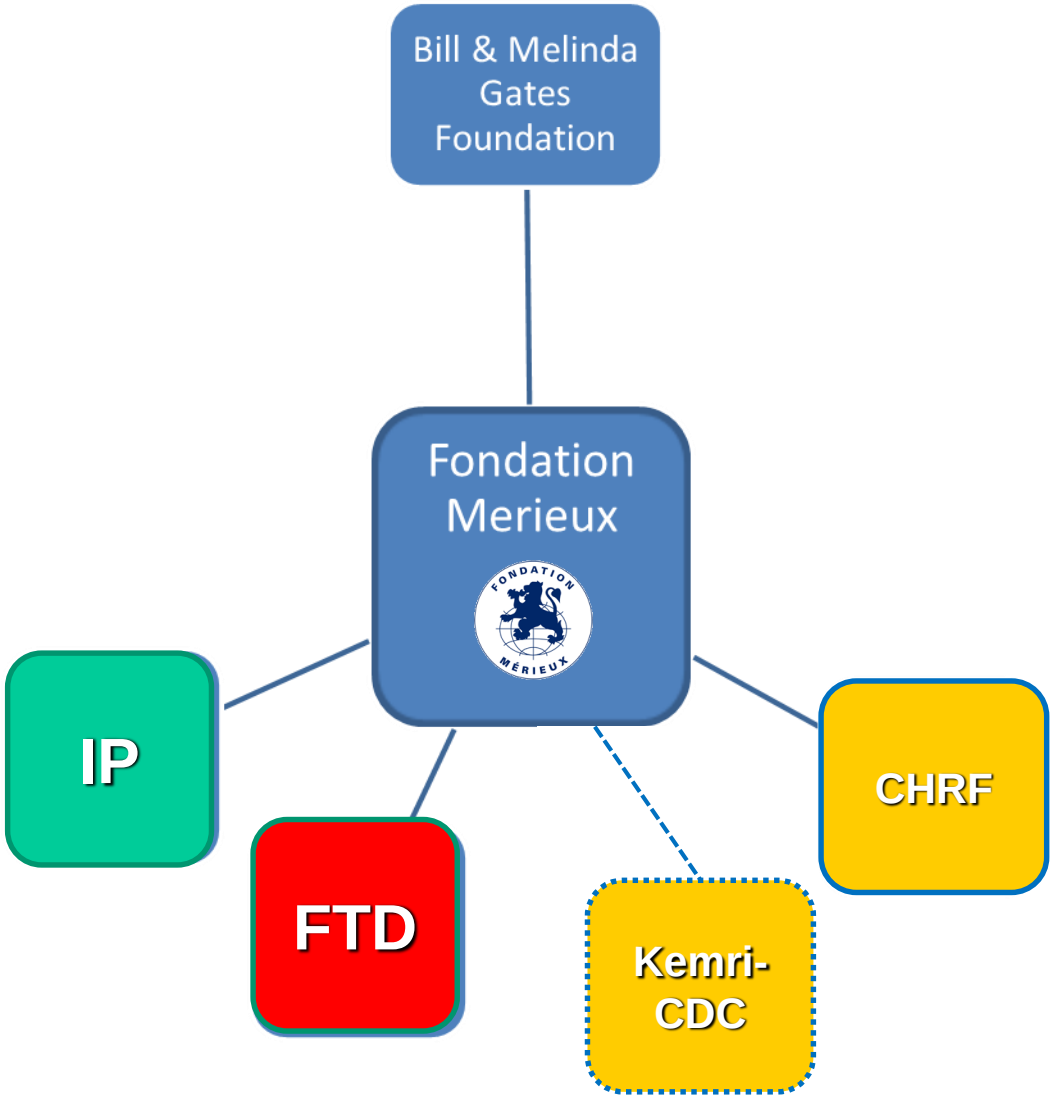
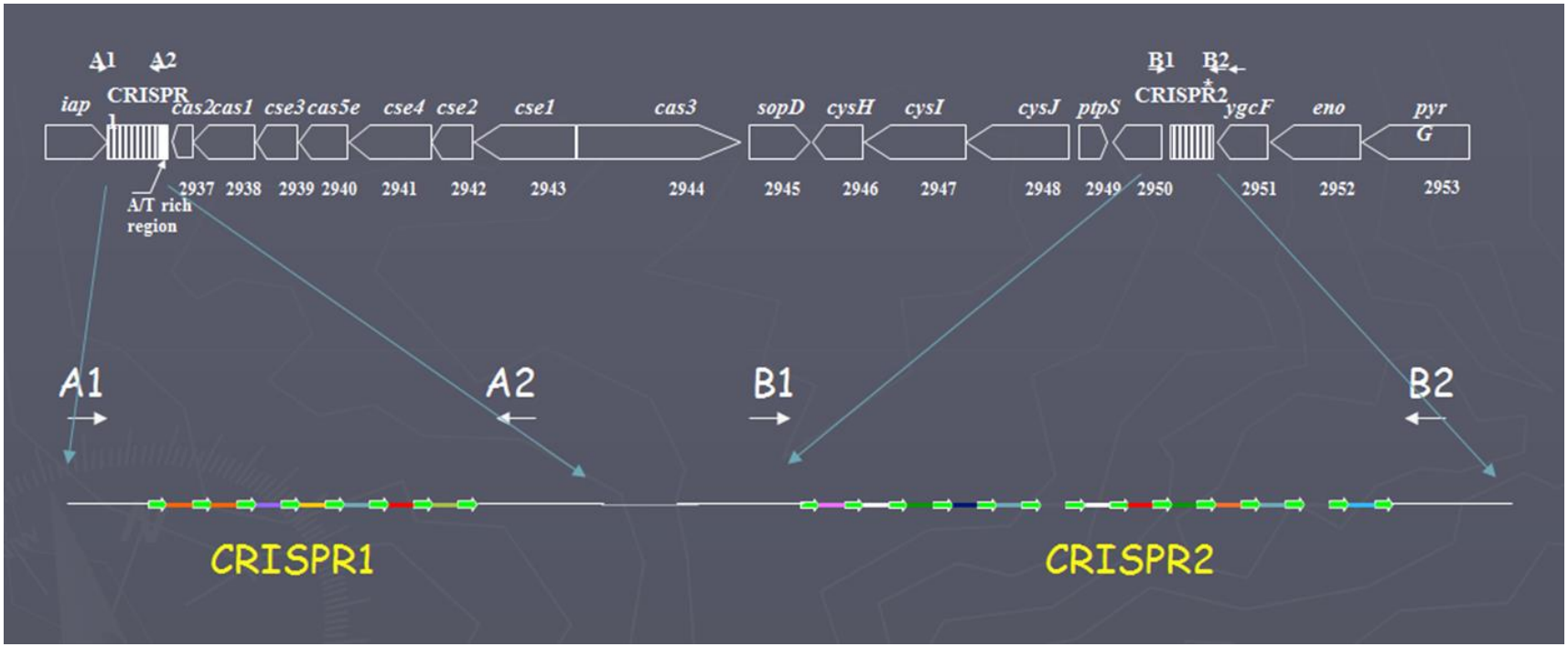
Typhoid consortium



Design of the PCR



Evaluation of the primers/probes on characterized strains:

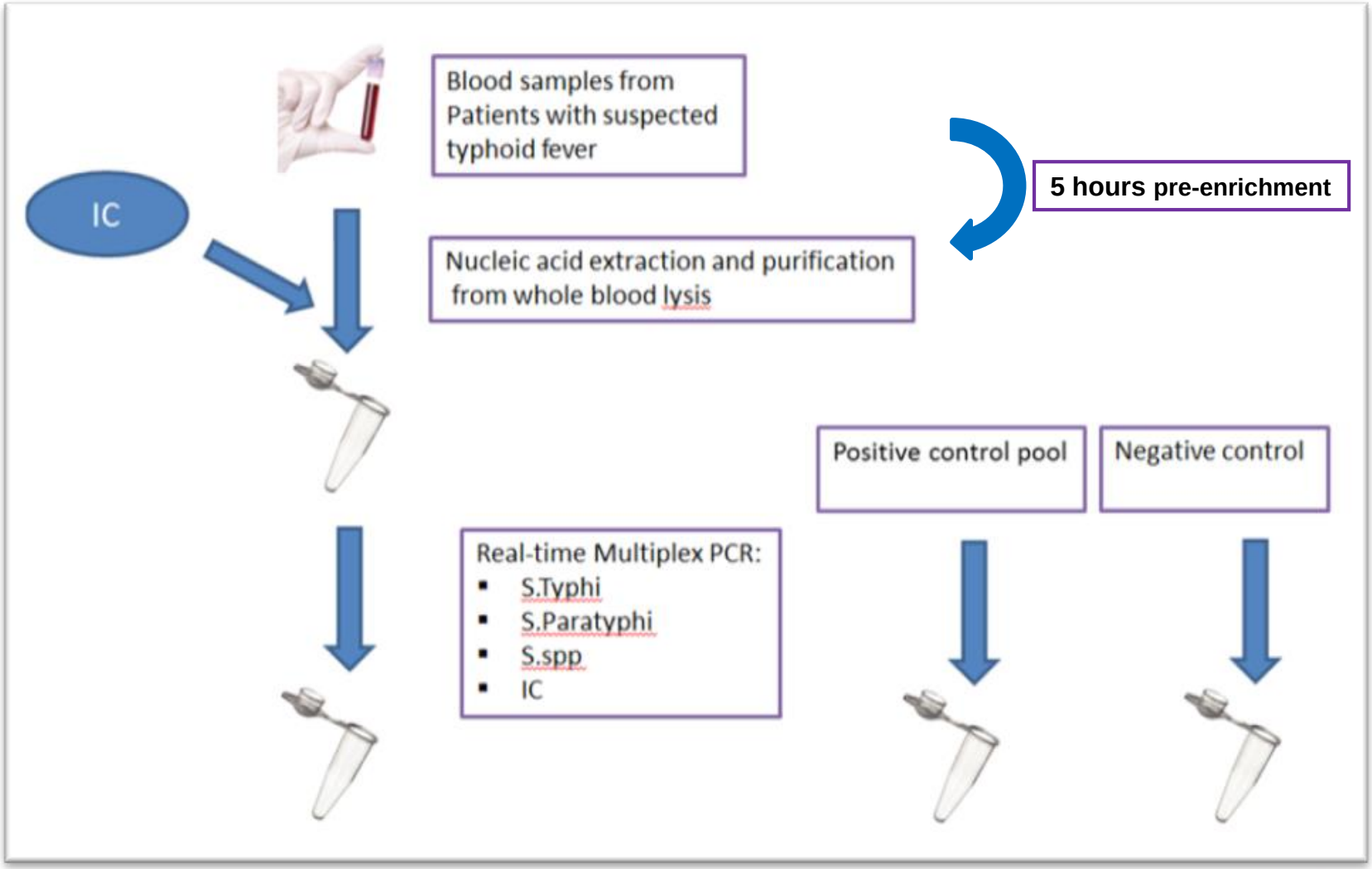
- 90 *Salmonella* Typhi
- 33 *Salmonella* ParatyphiA
- 16 *Salmonella* spp. (non-Typhi strains)
- 39 other bacterial species

Very high specificity

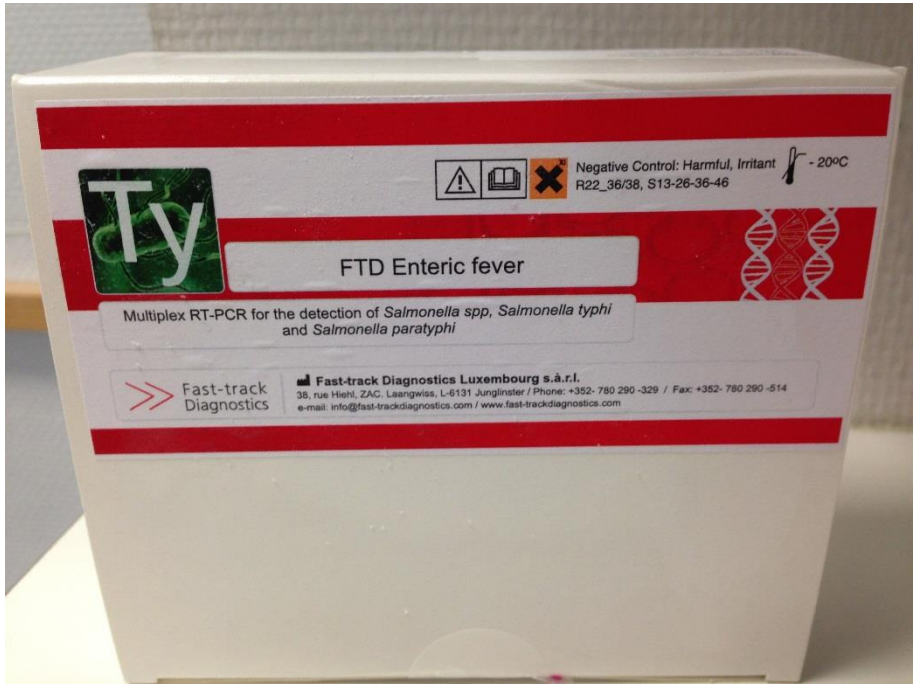
Optimization of the sample preparation

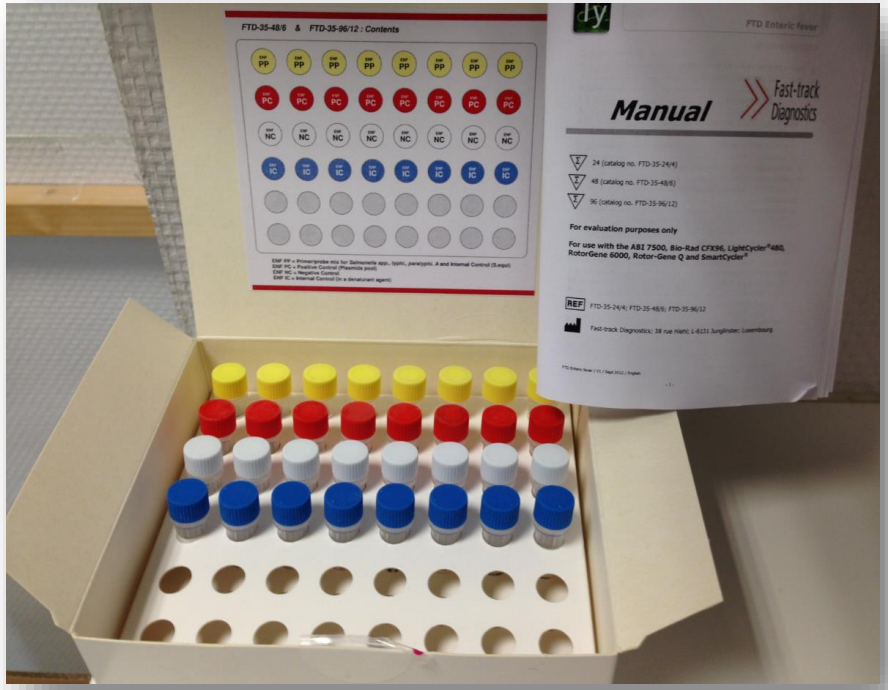
- Preliminary results on 1ml of blood clinical samples showed low sensitivity.
- Modifications of the sample preparation method were implemented to increase the sensitivity:
  - Increase the volume of blood from 1 ml to 5ml
  - Use of a pre-enrichment media including 10% oxgall
  - Optimization of the pre-enrichment time: 5 hours at 37° C
- The whole assay gives a result in 8 hours

M&M: RT-Multiplex PCR



FTD Enteric Fever Kit





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First evaluation in Dhaka, Bangladesh
CHRF

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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

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Results PCR vs bloodculture

n=206	Blood Culture positive	Blood Culture negative	Totals
qPCR positive	26	18	44
qPCR negative	3	159	162
Total	29	177	206

- 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

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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

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Second evaluation in Dhaka, Bangladesh

4 study sites

including two Dhaka slums

3ml of blood

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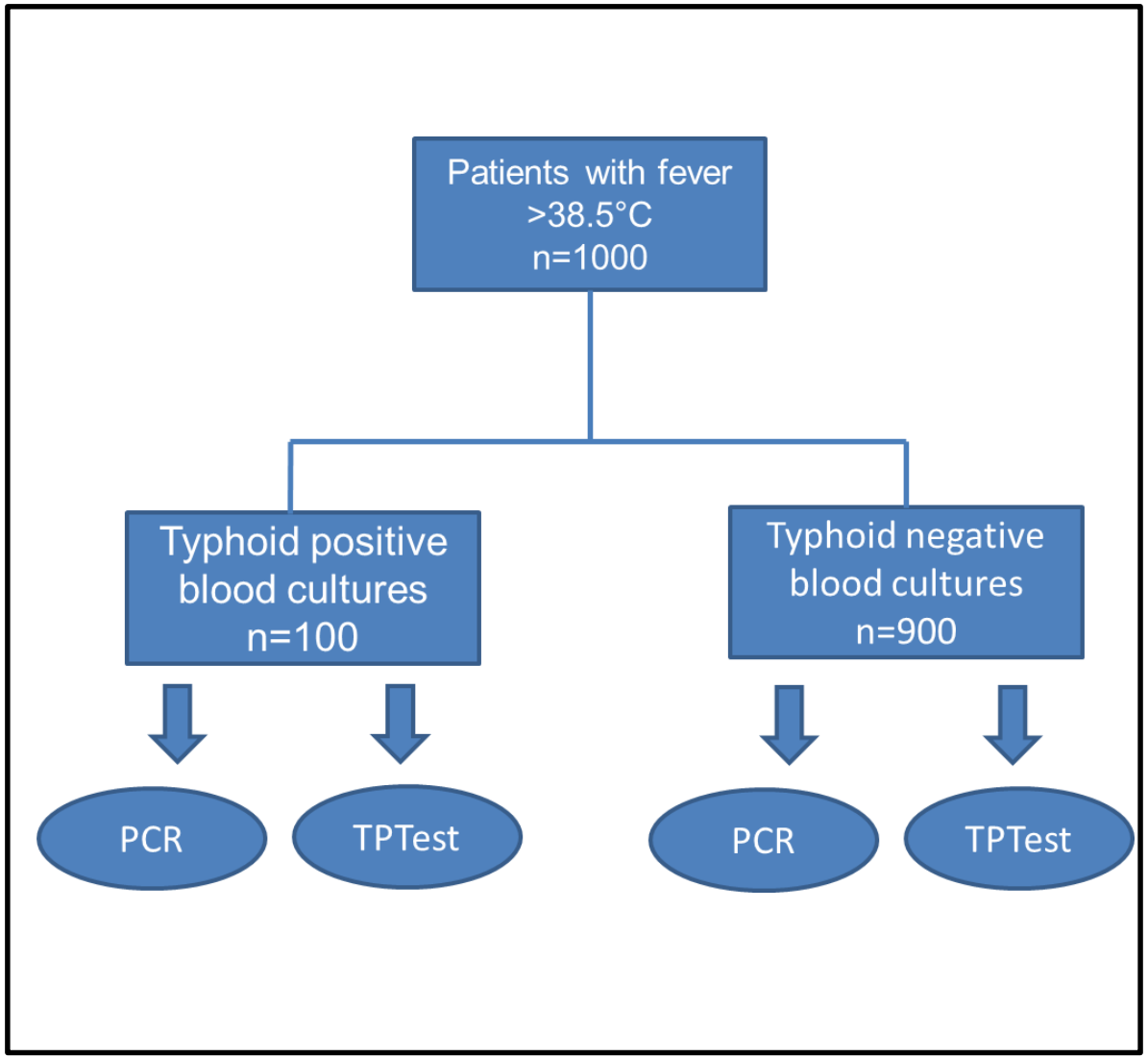
Study protocol



First patient included: 09/01/2015

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

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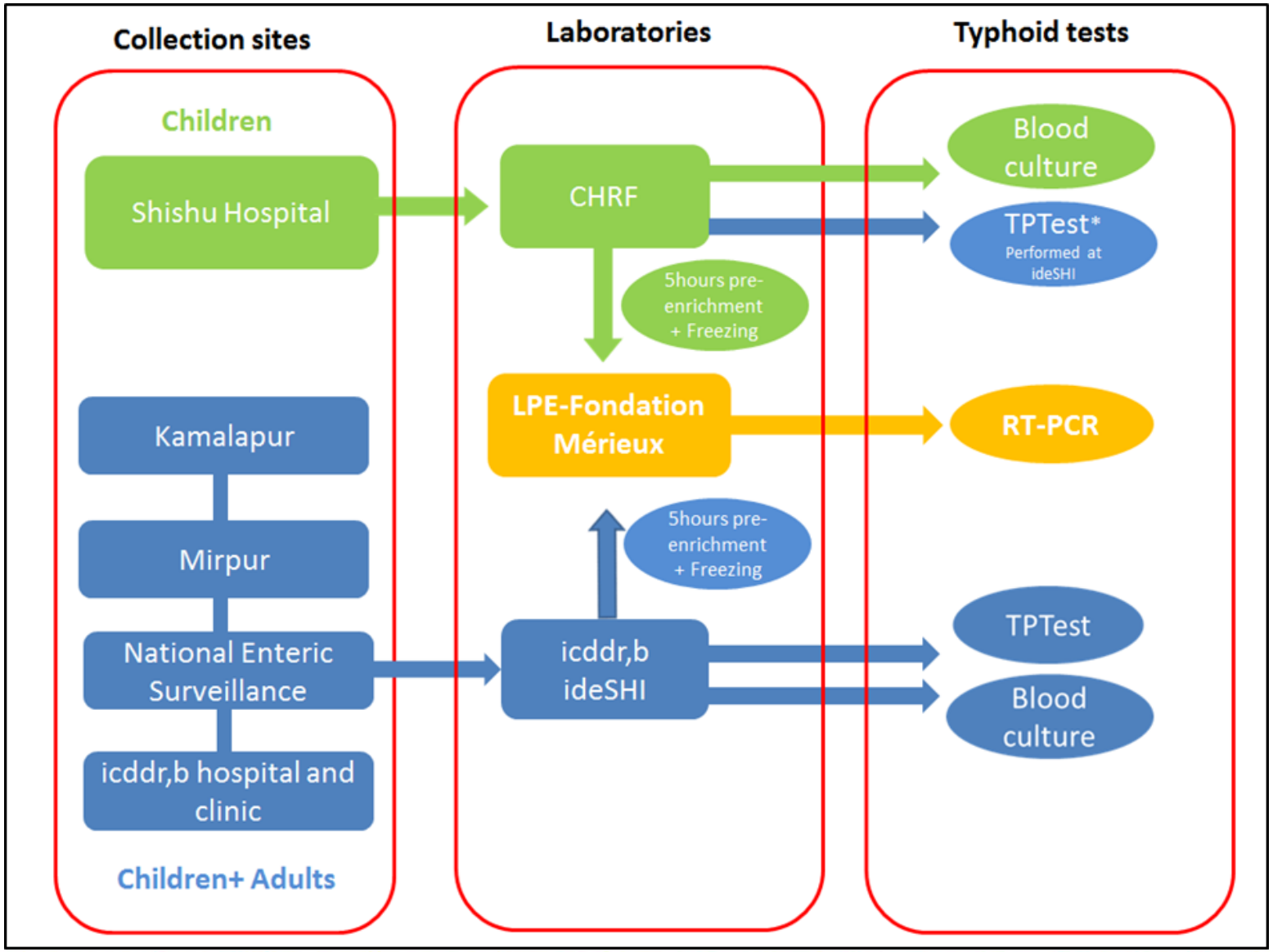
Study protocol



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Blood volume & age

Age (years)	Blood culture	PCR	TPTest	Total
18+	5 ml	3 ml	1 ml	9 ml
5-17	3-5 ml	3 ml	1 ml	7-9 ml
2-4	3 ml	3 ml	-	6 ml
0-2	Excluded	Excluded	Excluded	Excluded

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Preliminary results (n=350)

(n=350)	Blood culture +	Blood culture -	Total
PCR +	41	49	**90**
PCR -	3	257	260
Total	**44**	306	350

- Definition true positive: blood culture and/or PCR positive
- Sensitivity blood culture: 44/93 = **47,3%**
- Sensitivity PCR: 90/93 = **96,8%**
- 100% match identification (*S*. Typhi and *S*. ParatyphiA)

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Study extension and evaluation in Africa

Funder: BMGF

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Objectives & Study sites

- 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

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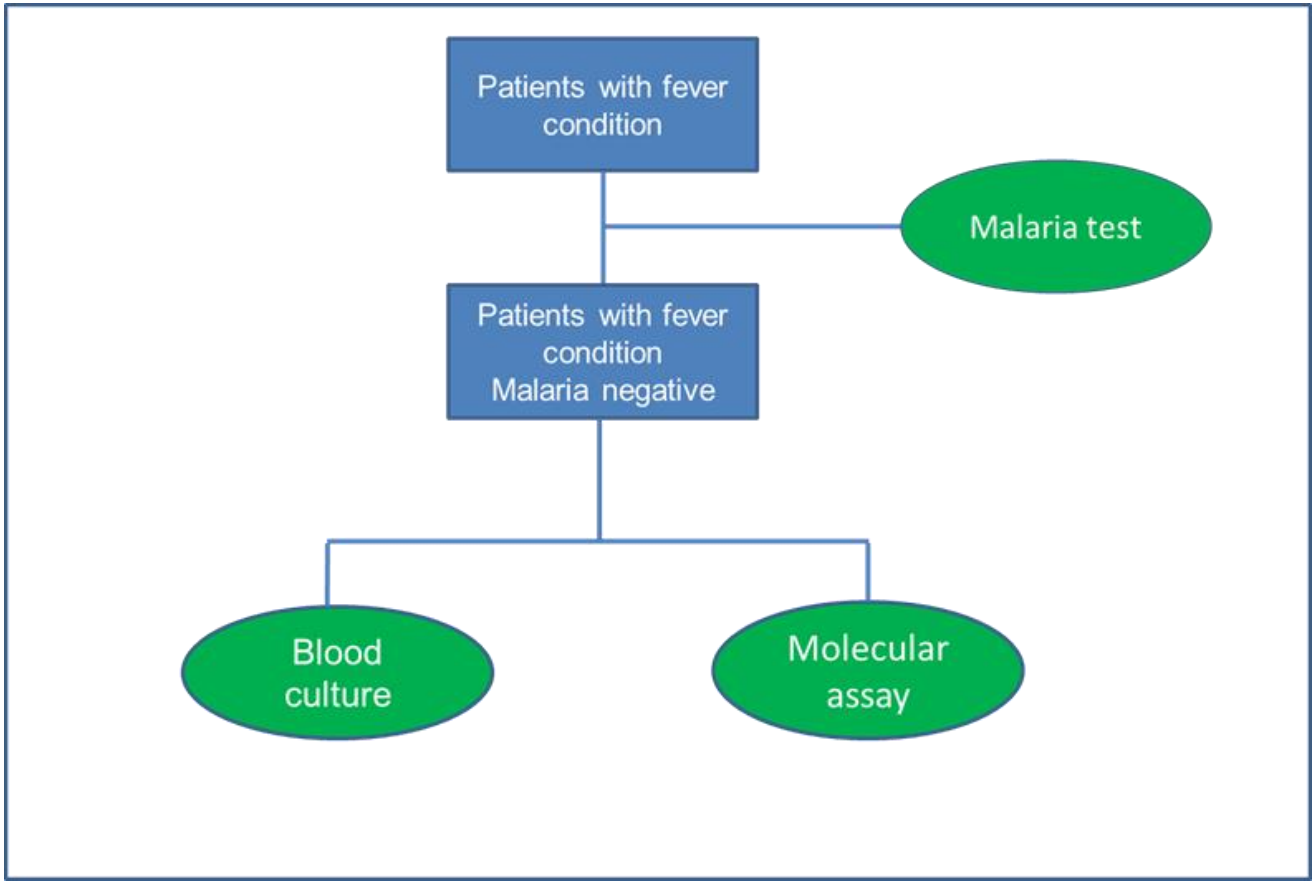
Objectives & Study sites



Objectives & Study sites

- 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)

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Objectives & Study sites

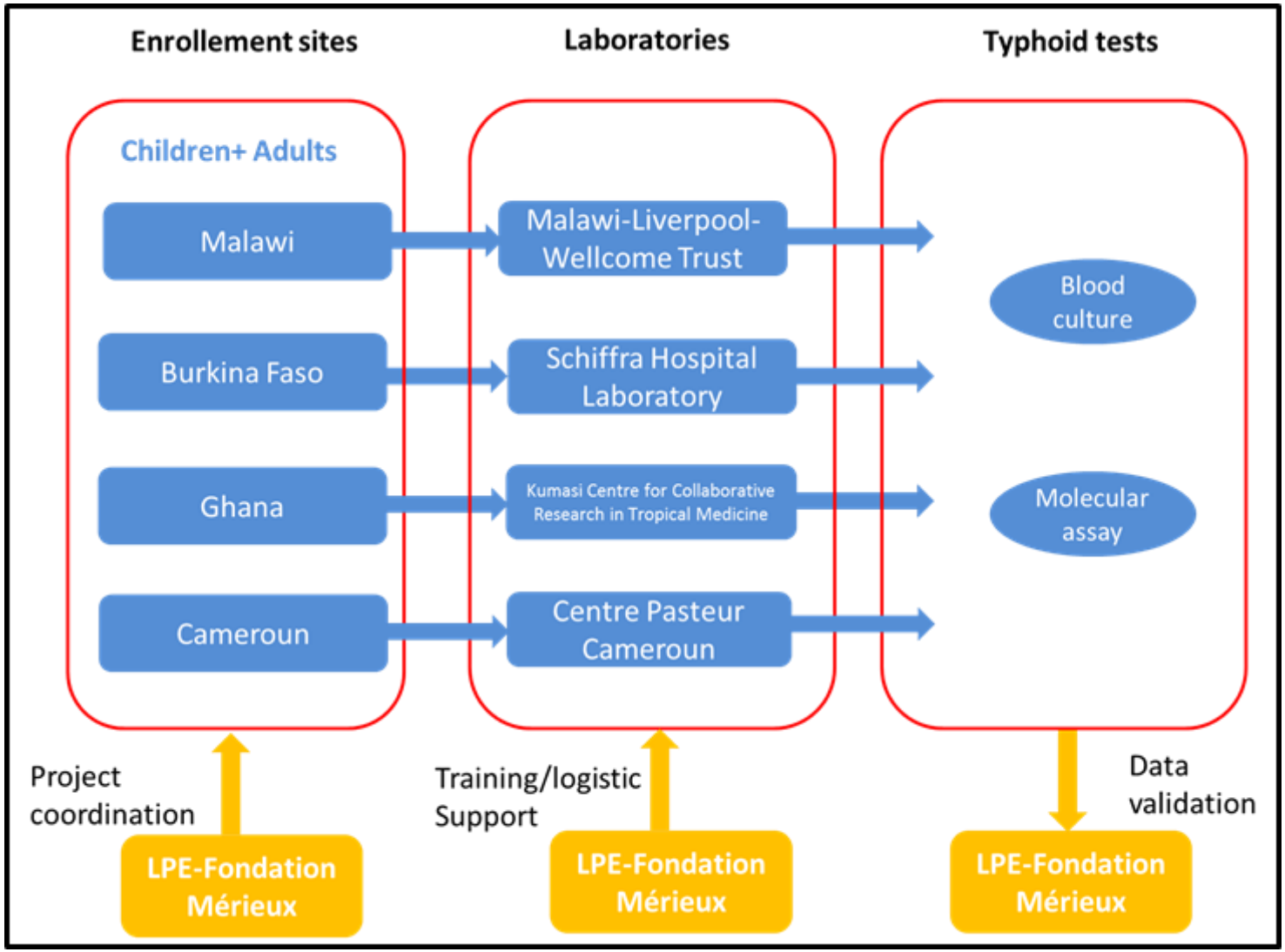
- Exclusion criteria

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

Objectives & Study sites

Age (years)	Blood culture	PCR	Total
18+	5 ml	3 ml	8 ml
5-17	3-5 ml	3 ml	6-8 ml
2-4	2-3 ml	2-3 ml	4-6 ml
3mths-2yo	1-2ml	1-2 ml	2-4 ml

Objectives & Study sites



Asian typhoid surveillance

Funder: BMGF

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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

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