Evaluation of a new Real-Time PCR assay to identify S. Typhi, S. Paratyphi A and s. spp from patients with fever in Bangladesh

des racines pour la vie

roots for life

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Objectives:

- Develop a PCR test for identification *S.* Typhi and *S.* Paratyphi A (improve performance of detection)

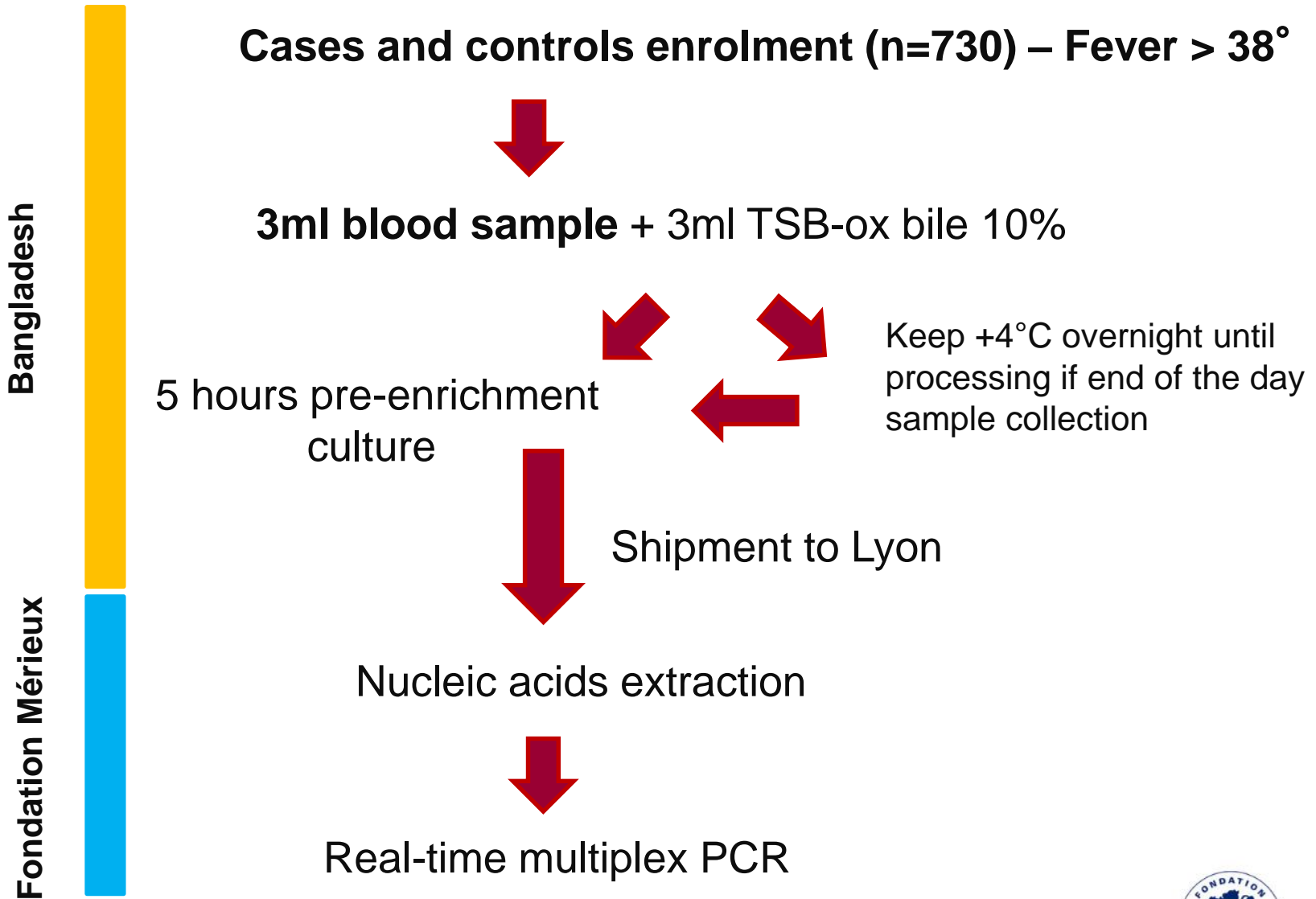- Validation of the assay in clinical settings where typhoid is endemic

- 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 3 ml

 ➢ Use of a pre-enrichment media (5% ox bile final)

Study design

Bangladesh

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Cases and controls enrolment (n=730) – Fever > 38˚

3ml blood sample + 3ml TSB-ox bile 10%

Keep +4°C overnight until processing if end of the day sample collection

5 hours pre-enrichment culture

Shipment to Lyon

Nucleic acids extraction

Real-time multiplex PCR

Results: Blood culture

Characteristics	Blood culture positive (N=98)	Blood culture negative (N=527)	*P*
Categorical variables, N			
vomiting	30/53	90/323	.0053
Diarrhoea	5/56	47/404	.59
Continuous variables, median (IQR)			
Volume PCR, ml	3.4 (3.13-3.7)	3.41 (3.19-3.7)	.87
Volume Blood culture, ml	2.6 (1.98-3.4)	2.51 (1.97-3.4)	.98
Delay sample-blood culture, min.	247.94 (53.52-348.43)	229.38 (85.2-321.1)	.84
Delay sample-PCR, min.	74.27 (22.94-254.5)	76.46 (24.03-214.08)	.7
Time to positive Blood Culture, h.	18,3		

PCR results on Blood Culture positive

Blood culture positive (n=98)
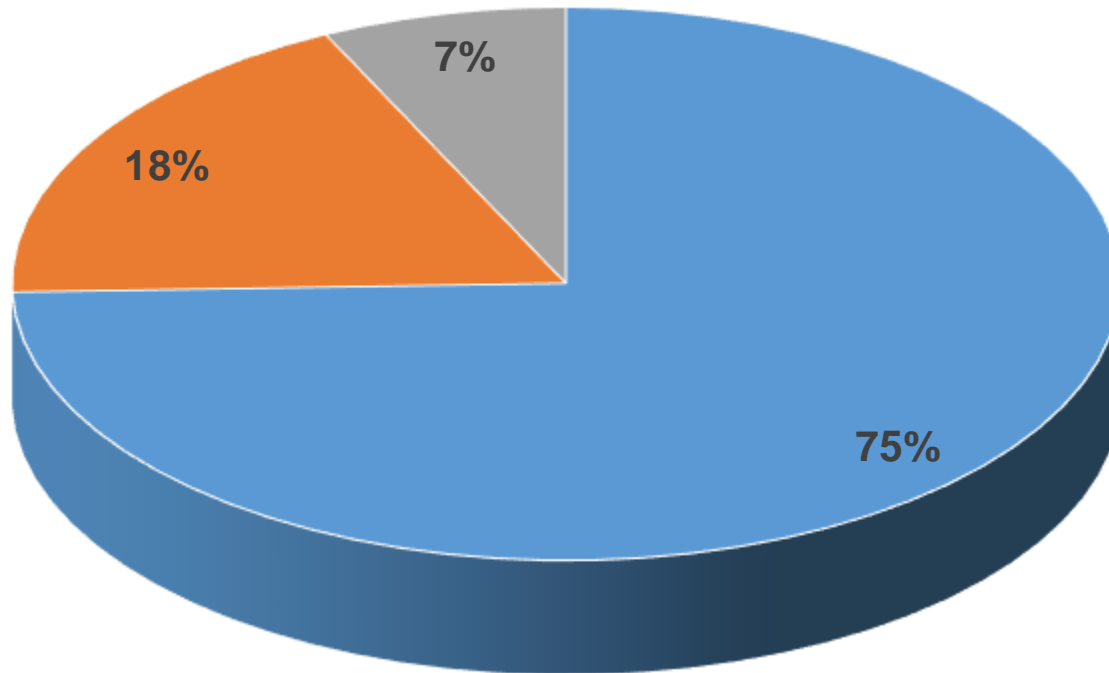
73/98 *S.* Typhi
18/98 *S.* Paratyphi A
7/98 negative

93% positive detection

- S. Typhi
- S. Paratyphi A
- Negative

PCR results on Blood Culture negative

Blood culture negative (n=587)

11%

1%

88%
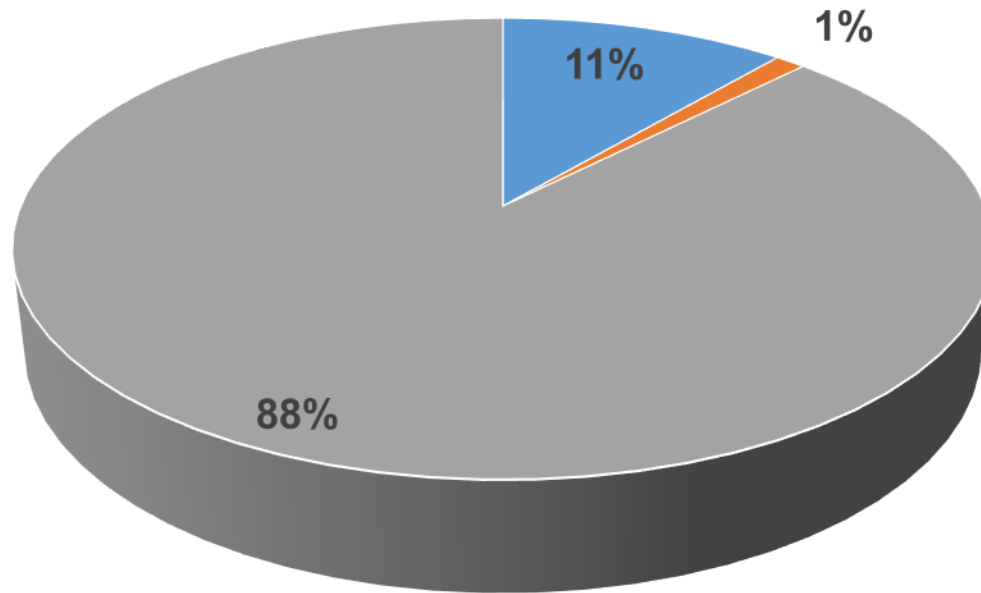
- S. Typhi
- S. Paratyphi A
- Negative

66/587 *S.* Typhi
7/587 *S.* Paratyphi A
514/587 negative

12% positive detection

73 additional samples identified by PCR

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Results: PCR assay performance

Characteristics	PCR positive (N=164)	PCR negative (N=521)	*p*
Categorical variables, N (%)			
Blood Culture positive	91	7	-
Blood culture negative	73	514	-
Continuous variables, median (IQR)			
Volume PCR, ml	3.43 (3.19-3.7)	3.40 (3.19-3.7)	0.4
Delay sample-PCR, min.	**107.04 (26.21-251.22)**	72.09 (21.84-209.71)	.0278
Time to positive Blood Culture, h.	17.8 (15.1-23.4)	**28.5 (24.3-36.3)**	.0025

Characteristics	Gold standard : blood culture Tested: Molecular assay	
	Result	(95% CI)
Overall (n=685)		
Sensitivity	92.9%	(85.3-96.8%)
Specificity	87.6%	(84.6-90.1%)
Positive likelihood ratio	7.5	(6.0-9.4)
Negative likelihood ratio	0.08	(0.04-0.17)

Conclusion

- **93%** of the typhoid positive blood samples are correctly identified

- **100%** match with the specie identified in blood culture

- **100%** of the controls samples confirmed negative

- Increase of **74%** in identified patients

- Salmonella detection as low as **1cfu/ml** in blood samples

Conclusion: strengths and limitations

- **Strengths:**

    - ✓ **Relevant number of clinical samples tested (n=730)**
    - ✓ **Robustness of the multiplex molecular assay**
    - ✓ **Fine tune disease burden results (+74%)**

- **Limitations:**

    - ✓ **7 samples not identified**
    - ✓ **No method for comparison et performance evaluation**
    - ✓ **ATB not assessed**
    - ✓ **Invasive and large (>1ml) sampling**
    - ✓ **Need of infrastructure and skilled staff**
    - ✓ **Not diagnostic test for patient care management**

Perspectives

- **Study implemented in Africa:**

    - ✓ **3 countries: Ghana, Malawi, Burkina Faso**

    - ✓ **1000 suspected cases and 200 controls**
        - ✓ **3 consecutive days with fever > 38°C**
        - ✓ **Children and adults**
        - ✓ **2-3ml of blood**

    - ✓ **Preliminary results:**

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