The next steps in typhoid diagnostics

(from a personal standpoint)

Stephen Baker 01/02/13

wellcome trust

UNIVERSITY OF OXFORD

Baker et al. BMC Infectious Diseases 2010, 10:45
http://www.biomedcentral.com/1471-2334/10/45

BMC
Infectious Diseases

COMMENTARY Open Access

Searching for the elusive typhoid diagnostic

Stephen Baker[1,2*], Michael Favorov[3], Gordon Dougan[4]

Abstract

Typhoid (enteric) fever is still a common disease in many developing countries but current diagnostic tests are inadequate. Studies on pathogenesis and genomics have provided new insight into the organisms that cause enteric fever. Better understanding of the
are limited in their diagnostic information
Here we discuss the current position of ty
and suggest potential ways of advancing

EXPERT REVIEWS

The utility of diagnostic tests for enteric fever in endemic locations

Expert Rev. Anti Infect. Ther. 9(6), 711–725 (2011)

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Lalith Wijedoru[2],
Amit Arjyal[3] and
Stephen Baker[†4]

Enteric fever, an infection caused by *Salmonella enterica* serovar Typhi and serovar *Paratyphi* A, is common and endemic in many areas of the Asian and African continents. In endemic areas, diagnostic tests are needed to diagnose acute cases for clinical management, to detect convalescent and chronic fecal carriage and for contact tracing. A suitable test may also allow an assessment of disease burden in a community to determine the need for vaccination programs.

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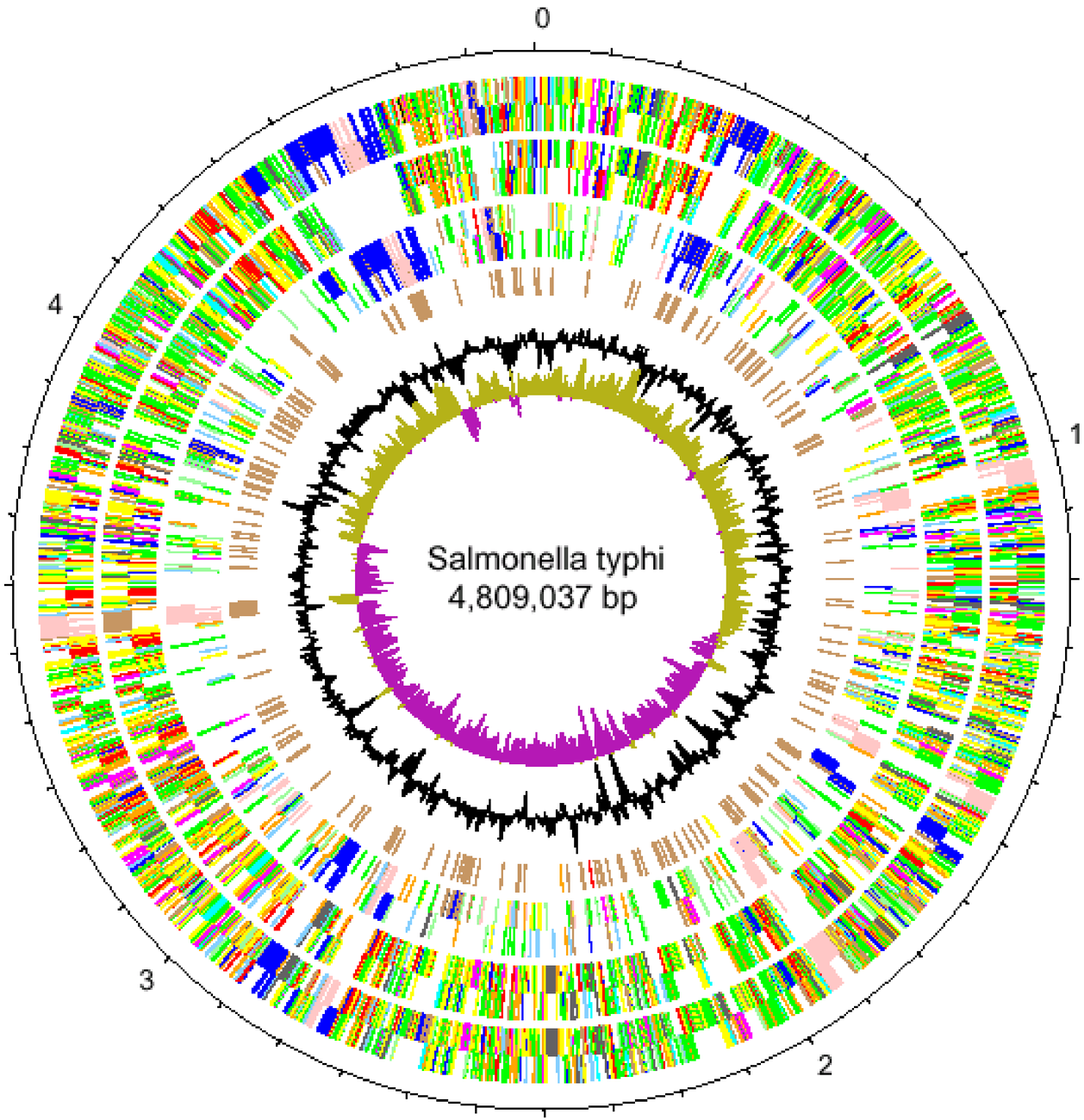
The Salmonella Typhi genome project



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Salmonella Typhi Genome sequencing project

- **Initiated in 1998 by the Sanger Centre**
  - 18 months to sequence and assemble
  - 10 months to analyze
  - Estimated cost of 600,000 GBP
  - Published in October 2001
  - Improvements in sequencing technology

- **Heralded as a landmark in studying typhoid fever**
  - Opportunity for new insight
  - Vaccines
  - Treatment
  - Diagnostics

- **These are yet to materialise – but are coming slowly**

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Typhoid is a disease that can be eliminated

- This is tangible target
- Should we invest in and improve
  - Diagnostics?
  - Treatment?
  - Vaccination?
  - Education?
  - Sanitation?
- How do we push research findings into policy?
- What are the long term perspectives for typhoid?
- Are there other infectious diseases that we benefit from particular approaches?
- Can we use current knowledge to reach local elimination?
- Can we apply genomics to tackle elimination?

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Why do we need new Diagnostics ?

- Current diagnostic tests are poor
- Since the genome sequence new diagnostics have entered the market place
- Yet the technology is pre-genome and rely on non-specific markers

- Important for patient and clinician
- Important for assessing burden and effect of intervention
- If elimination is a realistic target we have to calculate the nature of the problem

- Clinical, microbiological, serological, molecular
- Different advantages / disadvantages
- Practicality, sensitivity, specificity, time, cost
- No agreement on standard
- Confusion on interpretation of data and methods

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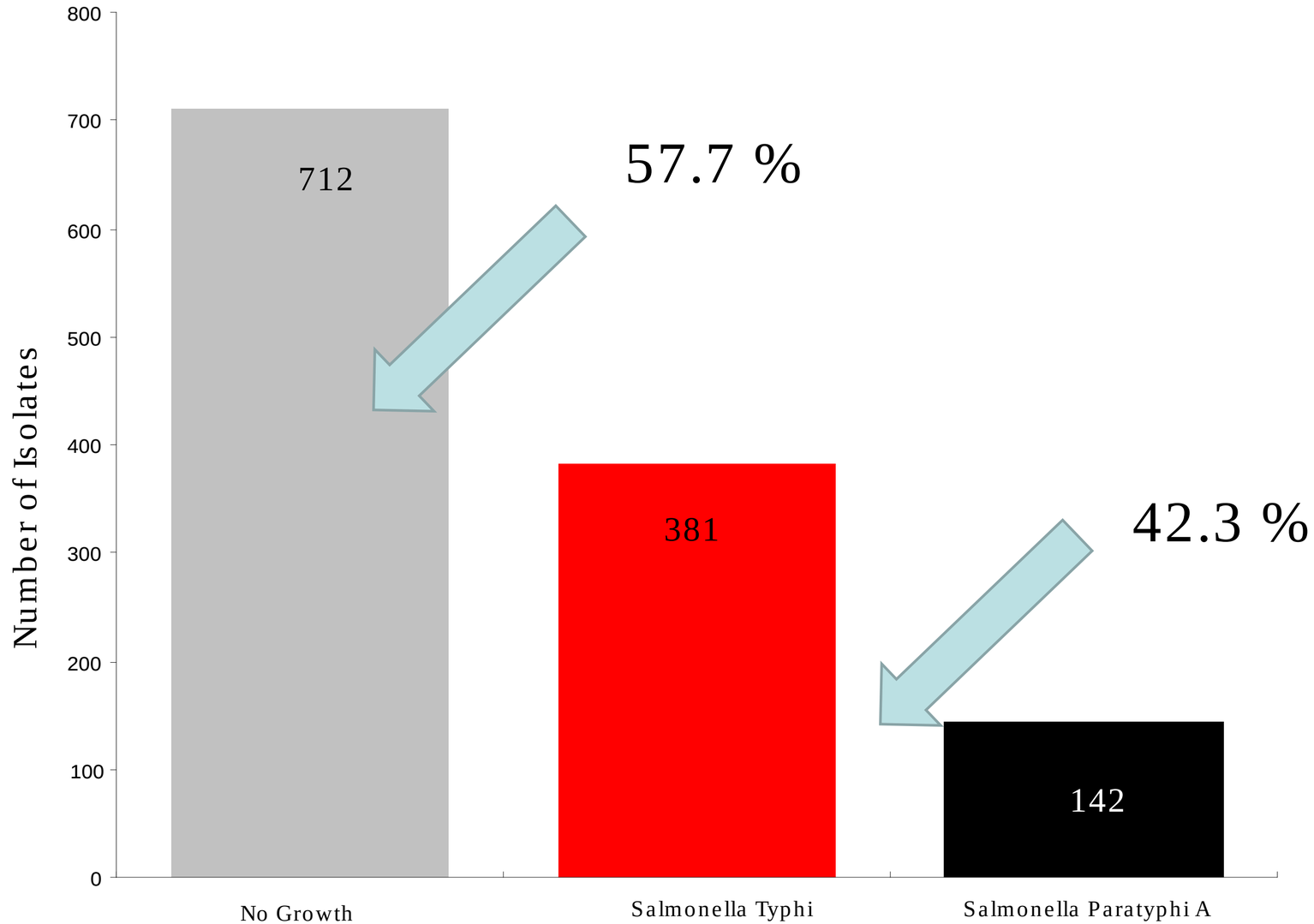
Can we do better than this?

Blood culture is a gold standard……



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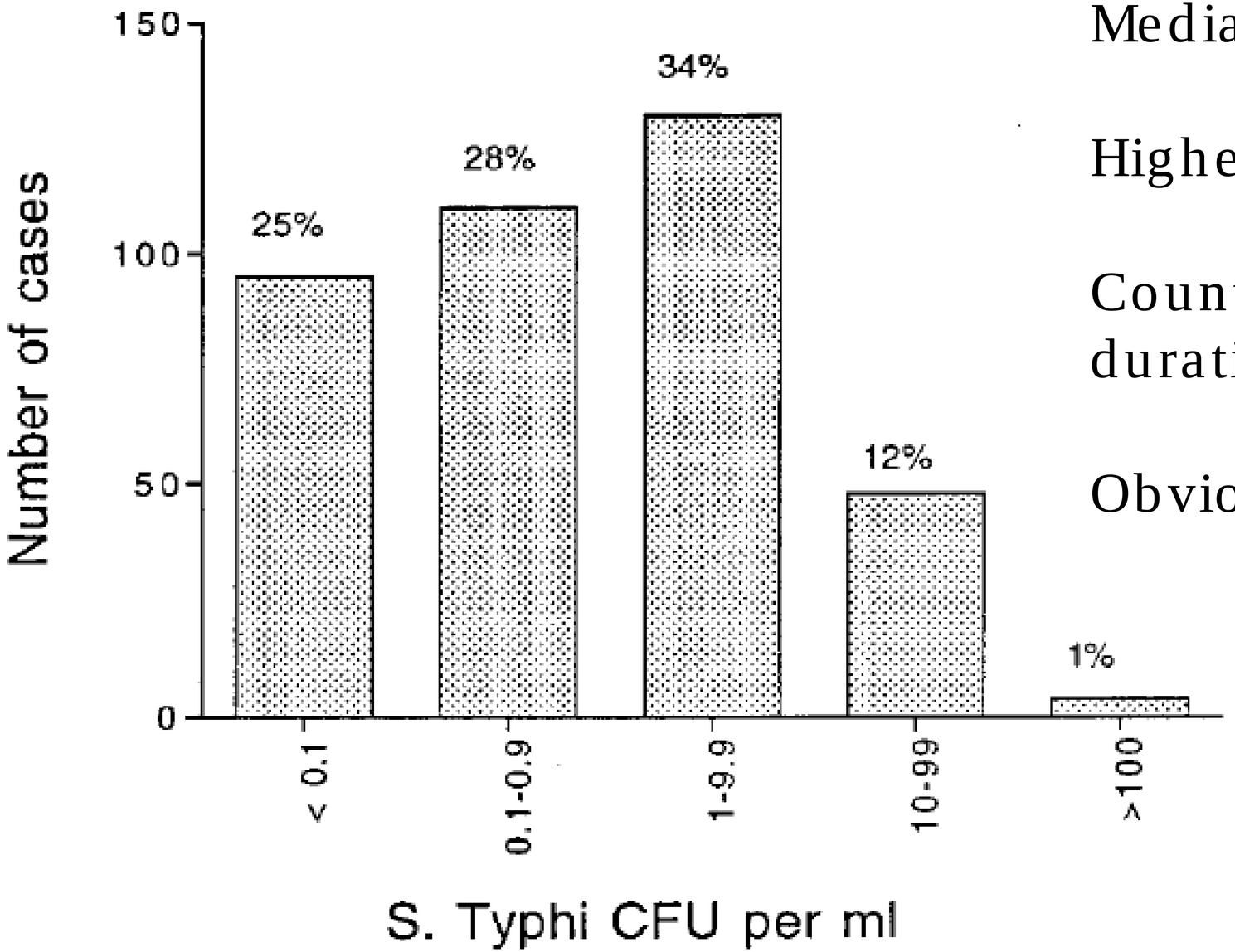

FIG. 1. Distribution of blood bacterial counts in acute typhoid fever.

Median 1 CFU/mL

Higher counts in children

Counts decline with illness duration

Obvious effects of antimicrobials

Wain J et al. J Clin Micro 1998

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George Fernand Widal 1862-1929

The Widal

- **However……**
  - **Unspecific**
    - Due to the nature of the bacterial antigens
    - Difficult to know if the patient is in the acute phase
    - Patients rarely demonstrate a 4 fold increase
    - Often no increase or only 2 fold increase
    - Not rapid (takes 14 days)
    - Not standardized
    - Negative in 30% of cultured confirmed cases
    - Often done in a single tube
    - Lack of seroprevalence data
- **Still commonly performed**
    - Fraught with issues
    - Lacks sensitivity and specificity

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Rapid diagnostic kits

- A number of rapid serological tests are available
- Range in cost and specificity
- Different performance

The sensitivity of real-time PCR amplification targeting invasive Salmonella serovars in blood (BMC infect dis 2010, 10:125)

- 100 blood samples culture positive for S. Typhi (54) or S. Paratyphi A (46)
- 23/54 Typhi positive samples also positive by RT-PCR
- 18/46 Paratyphi A samples also positive by RT-PCR
- Sensitivity 42 % and 39 %
- Specificity 100 %
- 50 blood samples from patients with ‘clinical’ enteric fever – all negative
- 28 bone marrow samples positive for S. Typhi – 28 (100 %) positive
- Final sample volume in PCR 75 µL
- For 100 % need to extract DNA from 5 – 10 mls

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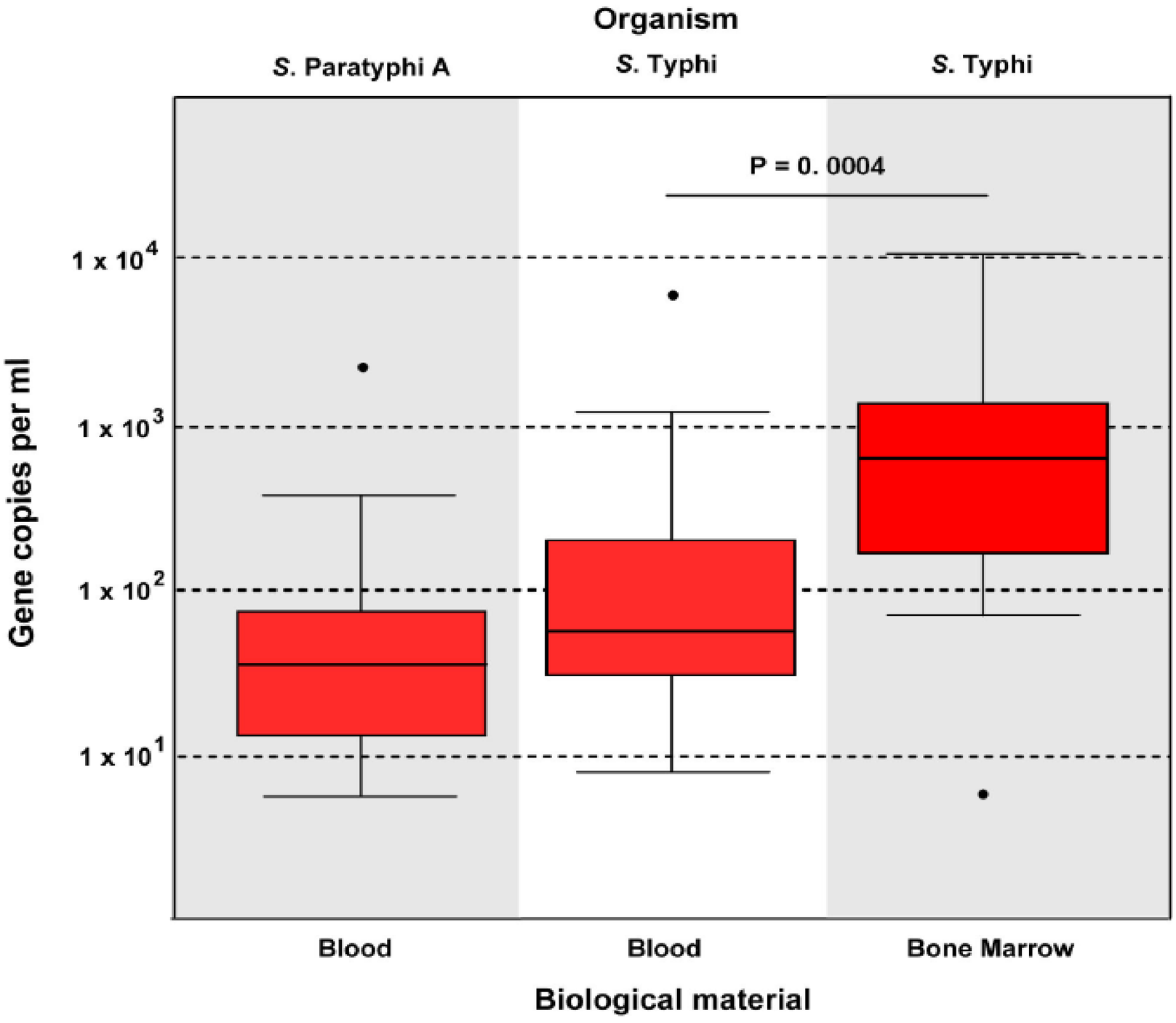
Organism
S. Paratyphi A
S. Typhi
S. Typhi
P = 0. 0004
1 x 10^4
1 x 10^3
1 x 10^2
1 x 10^1
Gene copies per ml
Blood
Blood
Bone Marrow
Biological material

Summary of current methods

- **Clinical**
  - Cheap, essential for treatment, further testing, rapid
  - Un-reliable without confirmation
  - Limited use for reporting
- **Serological**
  - Varying cost, useful when combined with other tests, rapid
  - High % of false negatives in endemic areas
  - Some use in reporting, lack of standardization
  - Rapid tests ….. Same issues
- **Molecular**
  - Can be cheap, not very useful in resource poor setting
  - Results appear good, but un-physiological bacterial loads
  - No use in reporting
- **Microbiological**
  - Blood or Bone marrow +/- Stool sample
  - Essential for reporting and burden assessment

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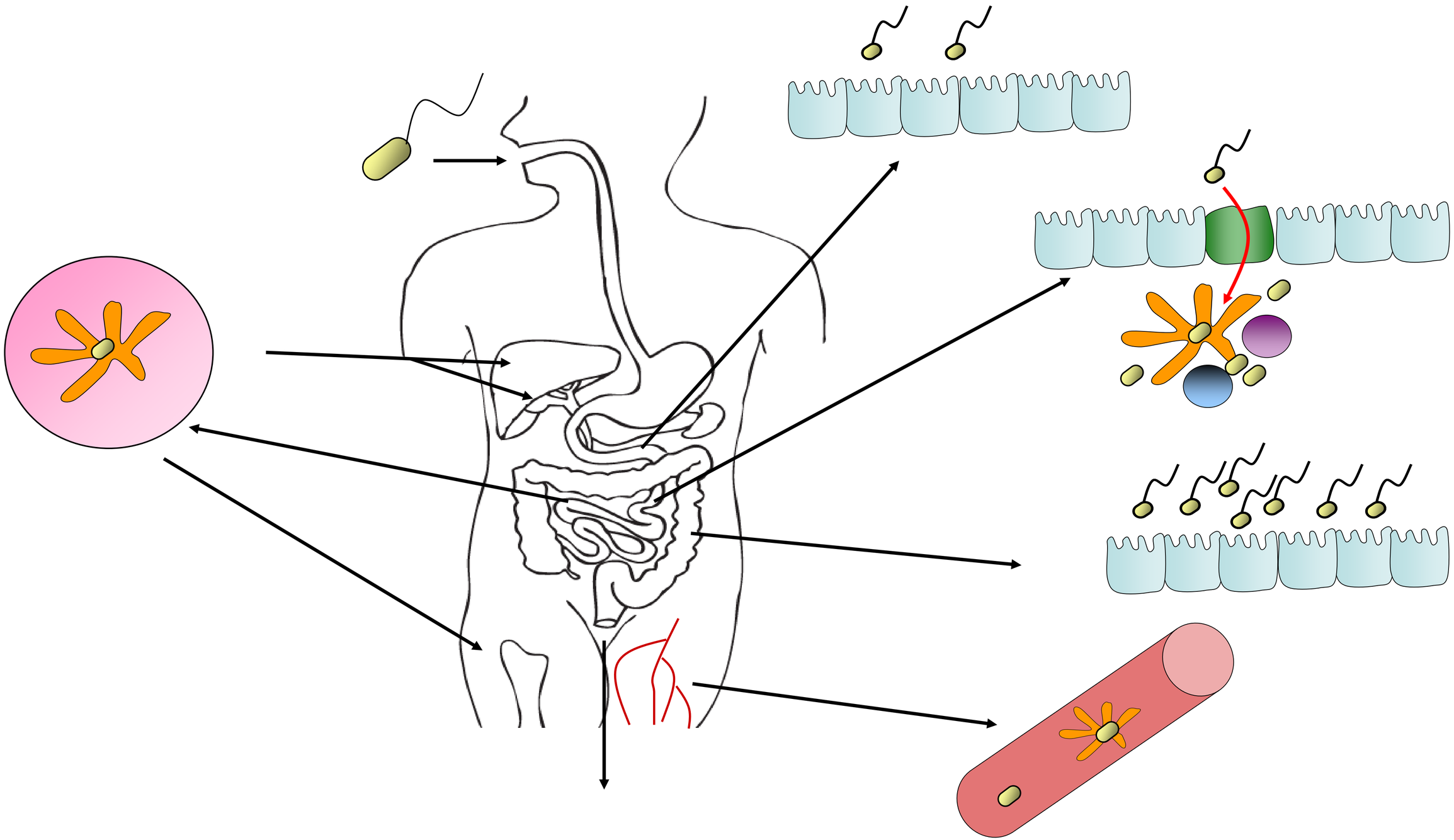

Transcriptional response in the peripheral blood of patients infected with *Salmonella enterica* serovar Typhi

Lucinda J. Thompson[a], Sarah J. Dunstan[b], Christiane Dolecek[b], Tim Perkins[c], Deborah House[c], Gordon Dougan[c], Nguyen Thi Hue[b], Tran Thi Phi La[d], Doan Cong Du[d], Le Thi Phuong[e], Nguyen Thi Dung[b], Tran Tinh Hien[b], Jeremy J. Farrar[b], Denise Monack[a], David J. Lynn[f,g], Stephen J. Popper[a], and Stanley Falkow[a,1]

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We used microarrays and transcriptional profiling of peripheral blood to investigate the host response of 29 individuals who contracted typhoid fever in the Mekong Delta region of Vietnam. Samples were taken over a nine month period encompassing acute disease, convalescence, and recovery. We found that typhoid fever induced a distinct and highly reproducible signature in the peripheral blood that changed during treatment and convalescence, returning in the majority of cases to the "normal" profile as measured in healthy uninfected controls. Unexpectedly, there was a strong, distinct signature of convalescence present at day 9 after infection that remained virtually unchanged one month after acute infection and in some cases persisted as long as nine months despite a complete clinical recovery in all patients. Patients who retain the convalescent signature may be genetically or temporarily incapable of developing an effective immune response and may be more susceptible to reinfection, relapse, or the establishment of a carrier state.

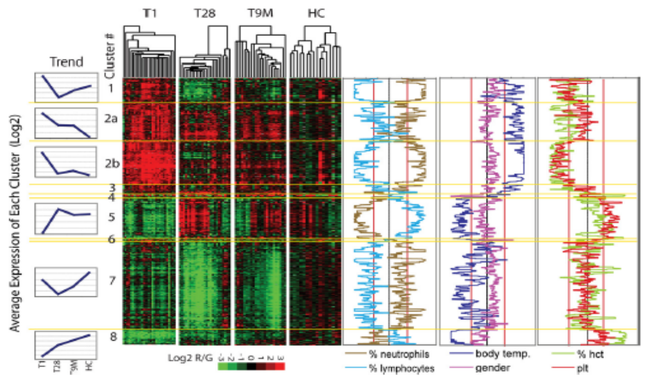

Fig. 2. Temporal changes in gene expression in typhoid patients. Transcripts (1,082) determined by SAM analysis to vary significantly in abundance from the HC samples at T1, T28, or T9M in the typhoid samples were hierarchically clustered; arrays were clustered by time point. Red indicates high expression, and green indicates low expression as shown in the legend; gray indicates missing data. Pearson correlation coefficients and *P* values were calculated for the expression of every gene and each CP [percent neutrophils, percent lymphocytes, body temperature, gender, percent hematocrit (HCT), and platelets (PLT)] across a selected set of 85 samples that had full clinical data. The plots to the right of the clusters show the negative $\log_{10}$ of the *P* value signed according to the sign of the calculated correlation coefficient. The *P* values are plotted as moving averages of three genes (along the vertical axis). The red vertical lines on each plot indicate a *P* value of 0.05. Gene clusters (1–8) referred to in the text are demarcated by horizontal yellow lines.

Proc Natl Acad Sci USA. 2009 Dec 29;106(52):22433-8.

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The challenges

- The gap between academia and endemic locations
- Raising the Typhoid profile
- The difficulty of detecting the organism
- Diagnosing other febrile diseases
- Persuading groups to be involved
- Ensuring accuracy
- Validating tests
- Implementation
- Finances
- The people in this room working together!!

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The technology

- Kit/assay development
- Rapid diagnosis with a simple yes/no test

- Serological
- Proteomics
- Biochemistry
- Metabolomics
- Mass spectrometry
- Molecular biology

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Ongoing research….. And a way forward

- Typhoid diagnostics consortium
- A universal biobank
- Biomarkers
  - IVIAT
  - ALS
  - Metabolites
  - Enrichment PCR
  - LAMP-PCR
  - Immunochip screening

- We are at the point when some of these methods should have more in depth field testing
- Get a second opinion!

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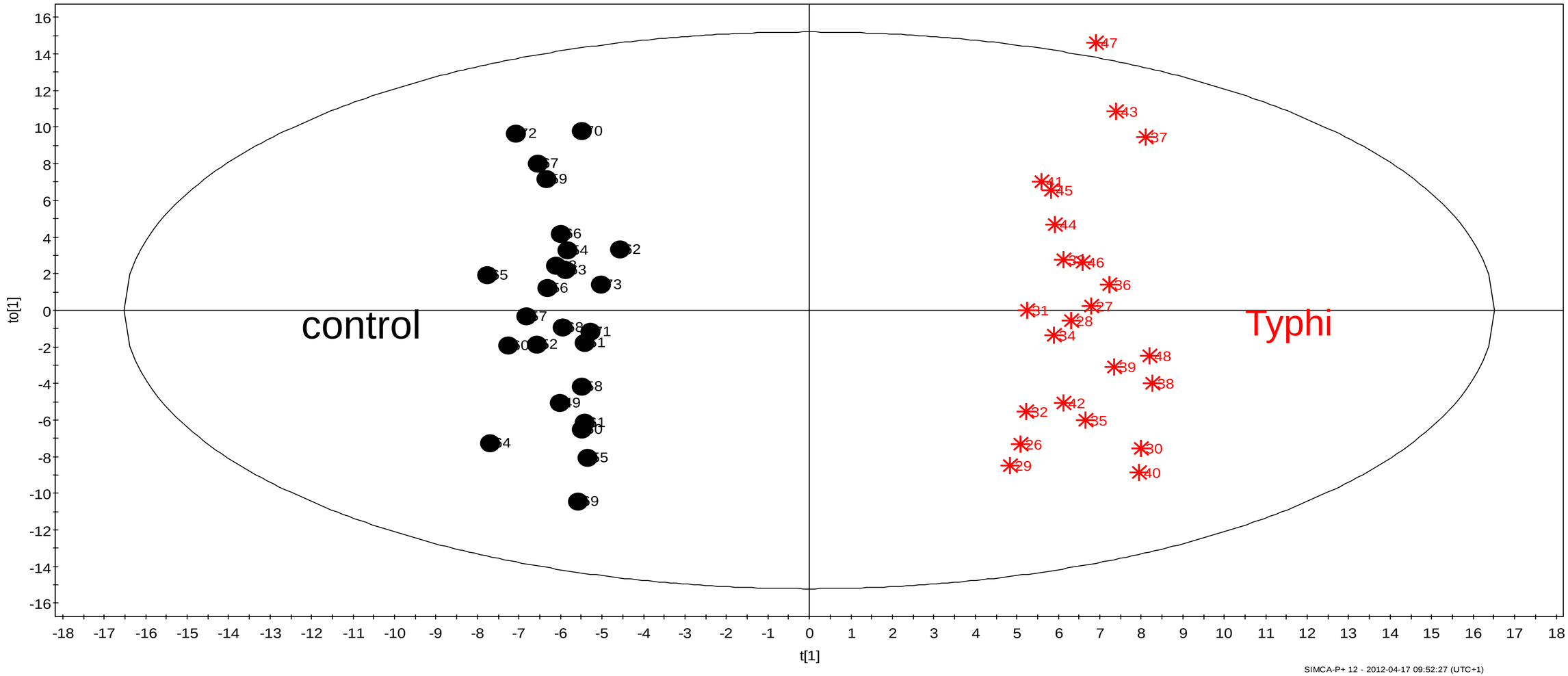
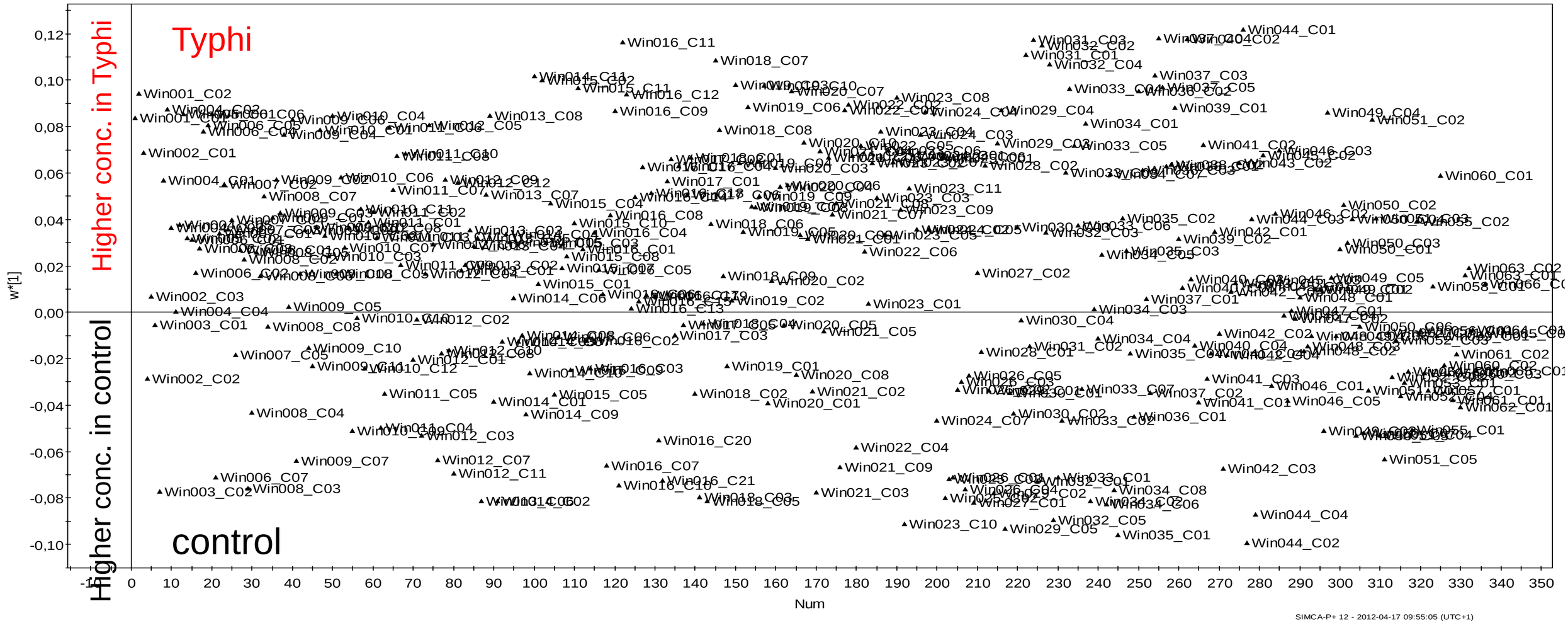
GC-TOFMS analysis – Classification model; control vs Typhi

- **Clear difference between control and Typhi.**
- **$p = 9.8*10^{-12}$**

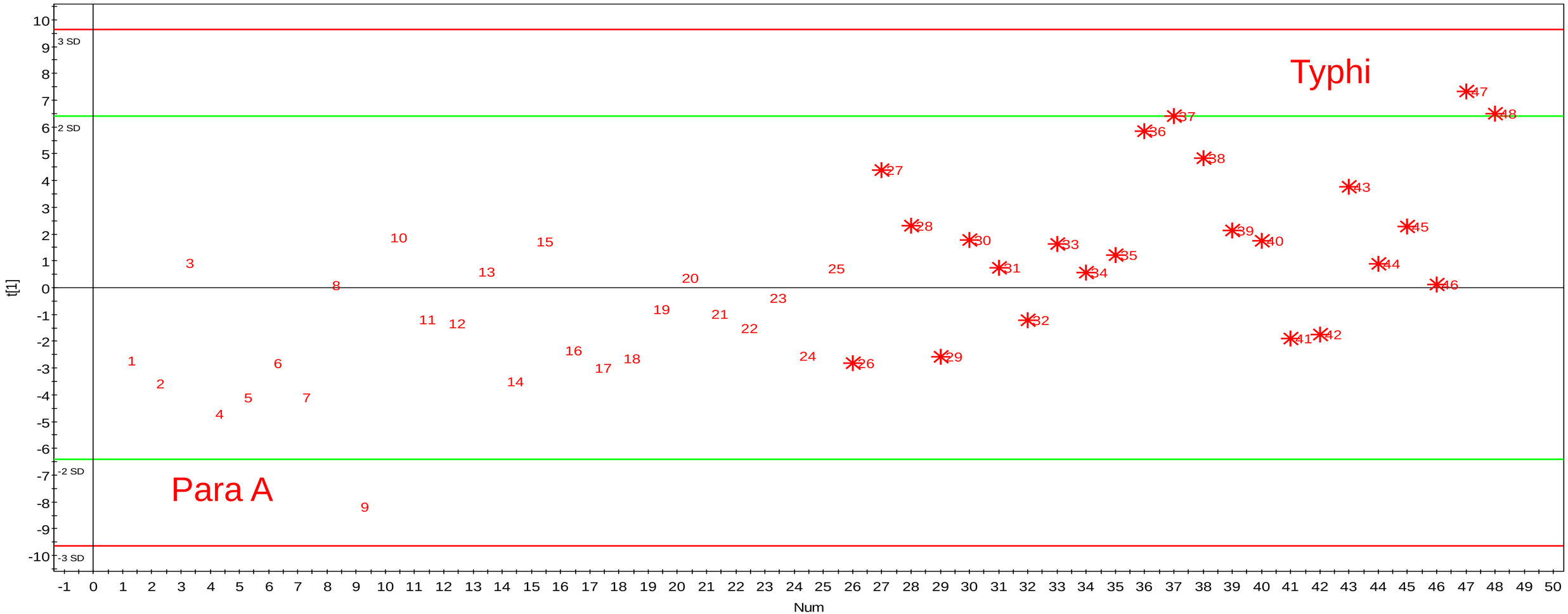


GC-TOFMS analysis – Classification model; control vs Typhi



GC-TOFMS analysis – Classification model; Typhi vs Para A

- **Difference can be found between Typhi and Para A.**
- **p = 0.048**



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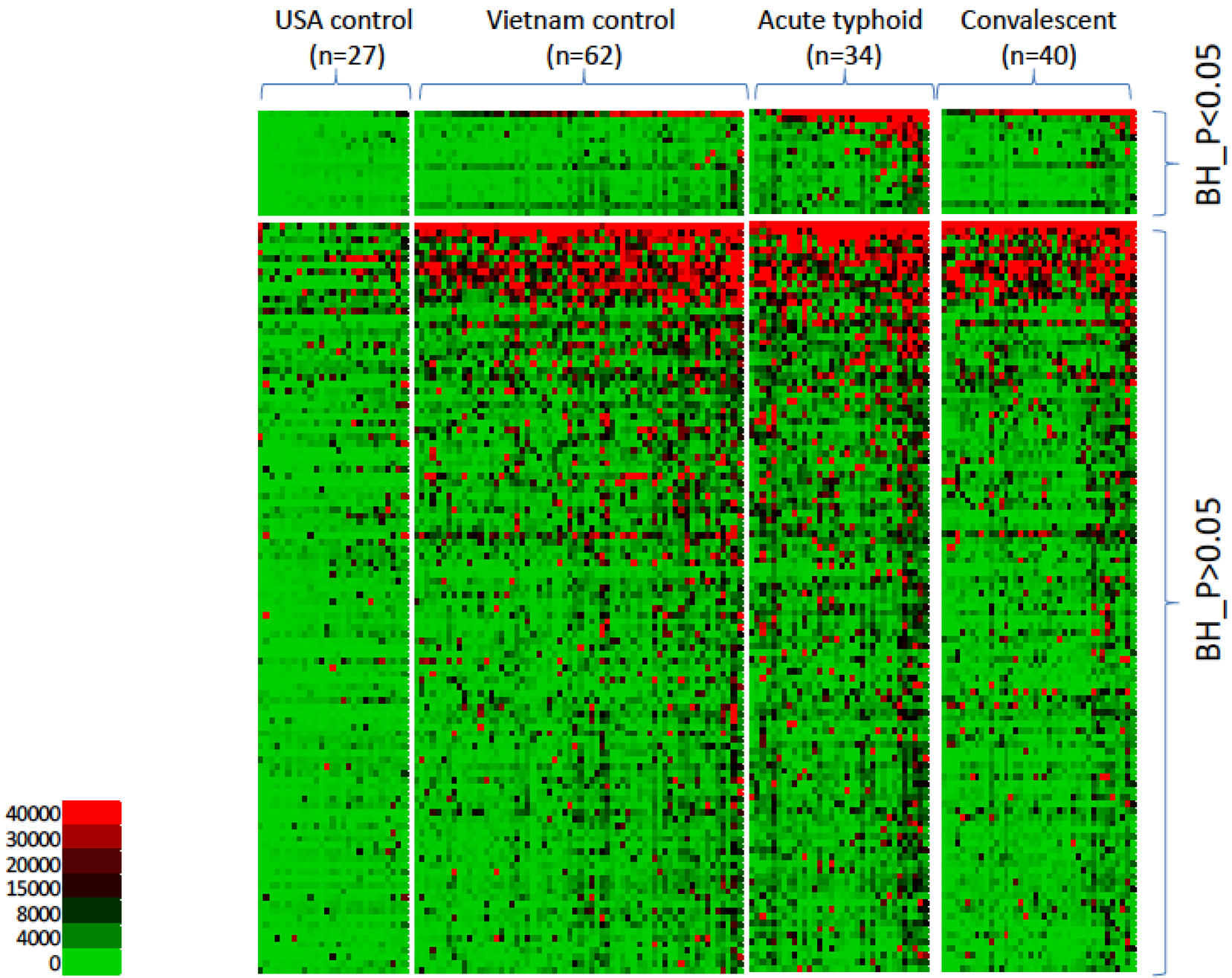
USA control
(n=27)
Vietnam control
(n=62)
Acute typhoid
(n=34)
Convalescent
(n=40)
BH_P<0.05
BH_P>0.05
40000
30000
20000
15000
8000
4000
0

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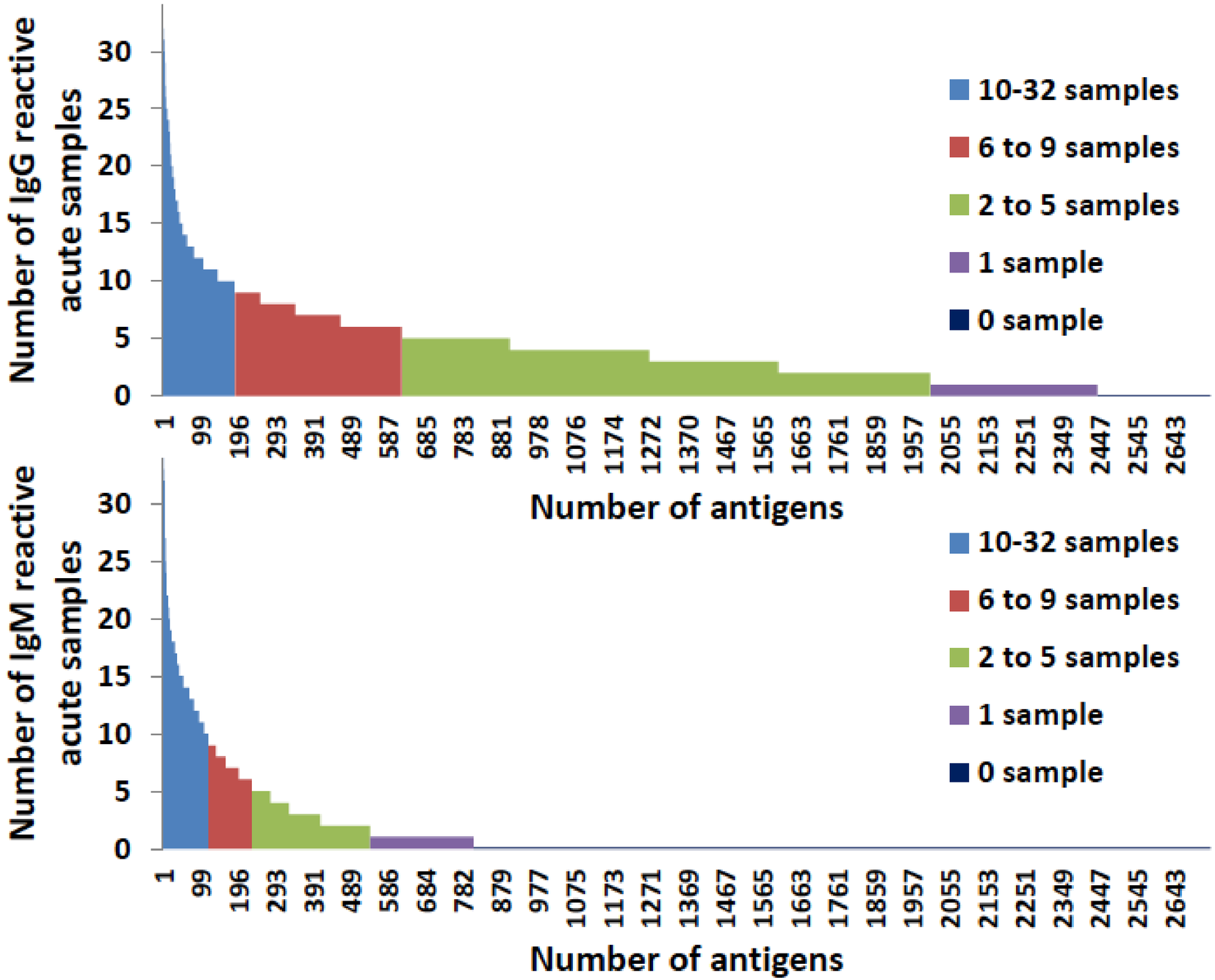
Number of IgG reactive acute samples
30
25
20
15
10
5
0
1
99
196
293
391
489
587
685
783
881
978
1076
1174
1272
1370
1467
1565
1663
1761
1859
1957
2055
2153
2251
2349
2447
2545
2643
Number of antigens
10-32 samples
6 to 9 samples
2 to 5 samples
1 sample
0 sample
Number of IgM reactive acute samples
30
25
20
15
10
5
0
1
99
196
293
391
489
586
684
782
879
977
1075
1173
1271
1369
1467
1565
1663
1761
1859
1957
2055
2153
2251
2349
2447
2545
2643
Number of antigens
10-32 samples
6 to 9 samples
2 to 5 samples
1 sample
0 sample

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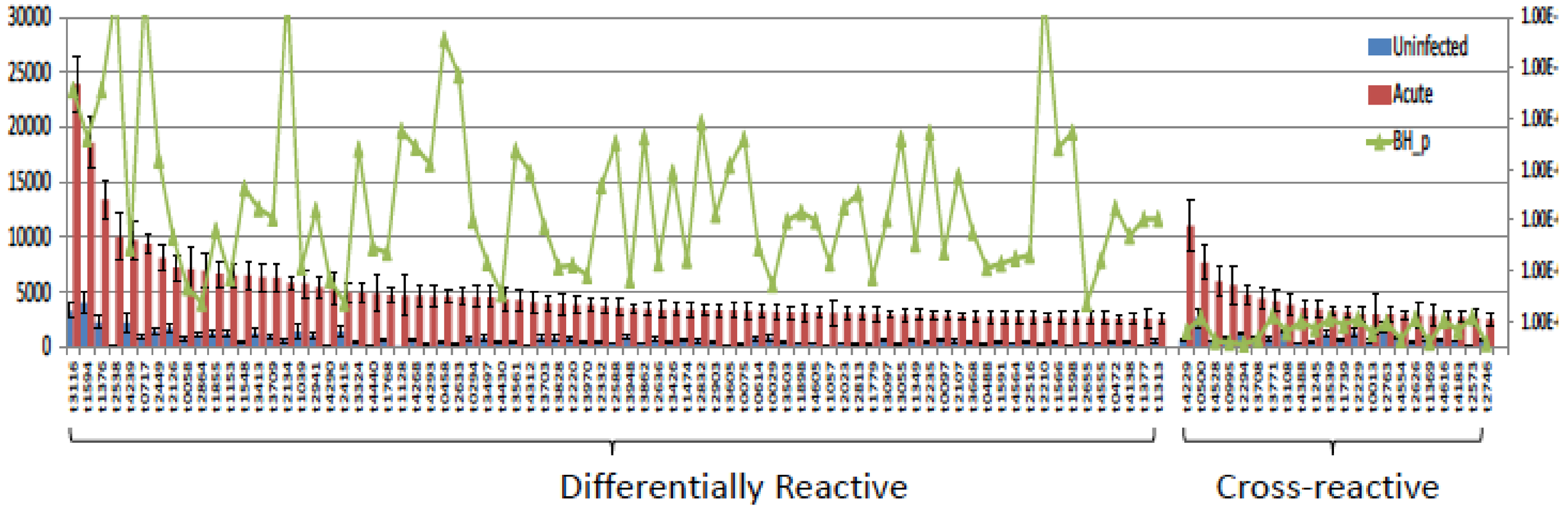
Serodominant IgM Antigens: uninfected vs acutely infected typhoid patients in Vietnam



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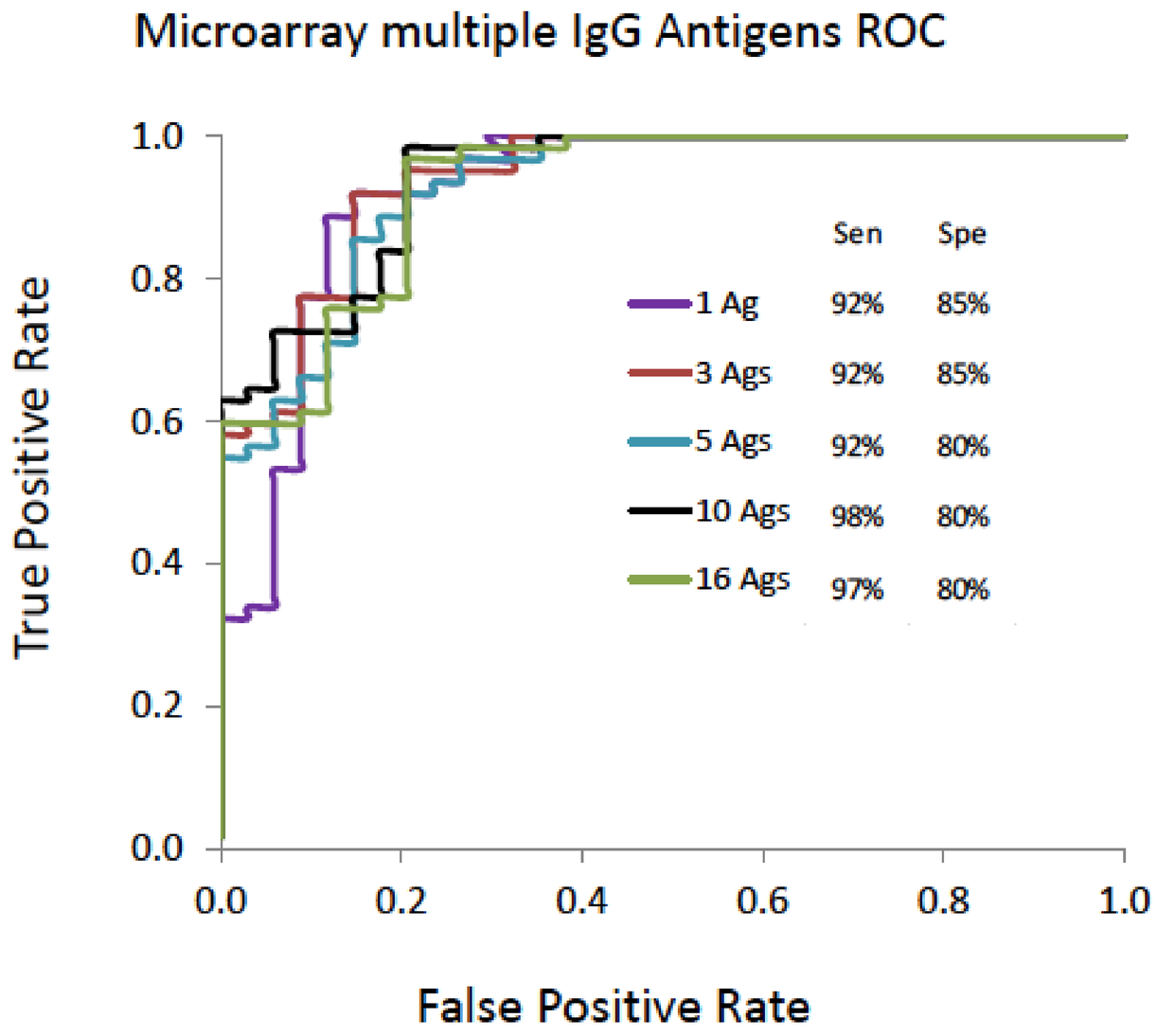
Microarray ROC on normalized data for IgG antigens:
Uninfected vs infected typhoid patients in Vietnam



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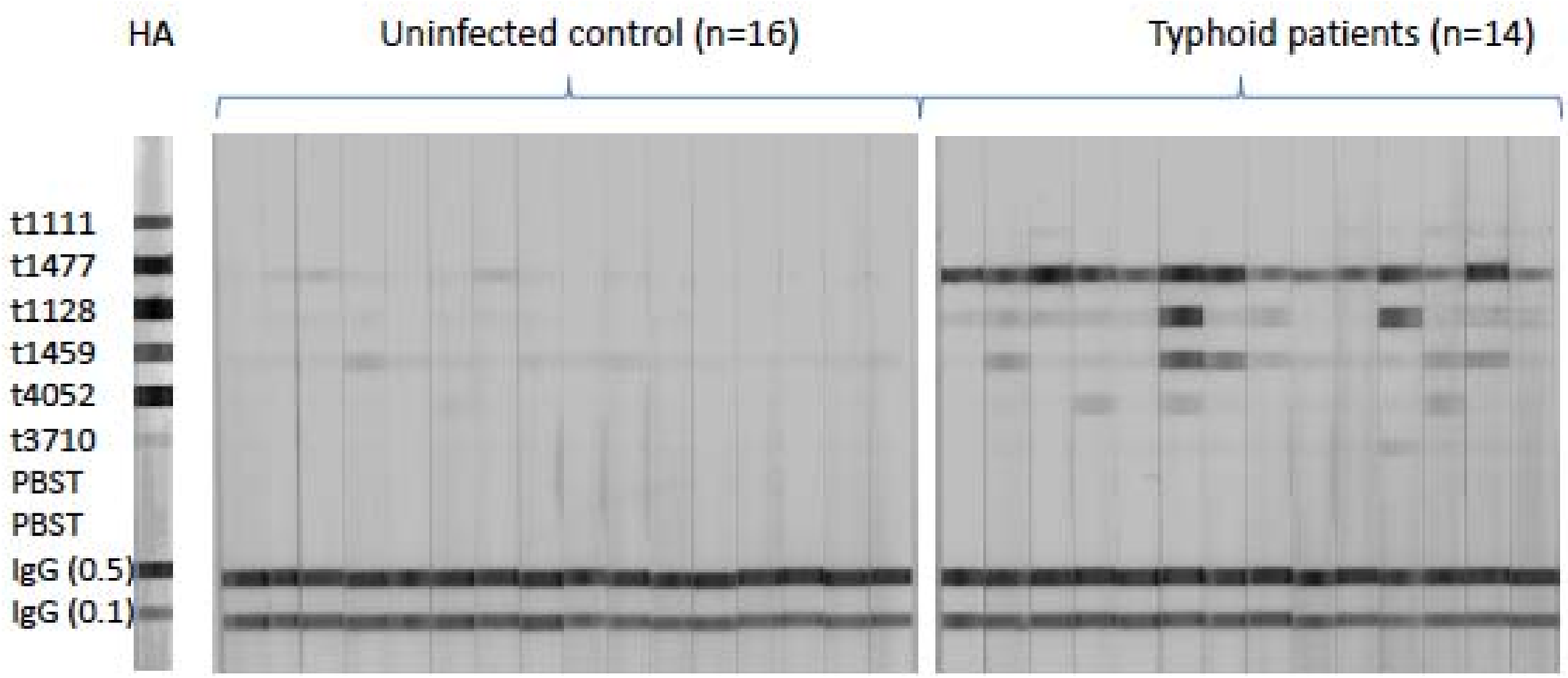
HA
Uninfected control (n=16)
Typhoid patients (n=14)
t1111
t1477
t1128
t1459
t4052
t3710
PBST
PBST
IgG (0.5)
IgG (0.1)

Acknowledgements

Abhilasha Karkey
Buddha Basnyat
Amit Arjyal
Patan Hospital Typhoid group
Kathryn Holt
Christiane Dolecek
Gordon Dougan
Jeremy Farrar
Ed Ryan
Phil Felgner
Li Liang
Henrik Antti
Kumar Rajakumar

Sabina Dongol
Jim Campbell
Tran Vu Thieu Nga
Duy Pham Thanh