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Laos Oxford
LOMWRU



Lao - Oxford - Mahosot Hospital - Wellcome Trust Research Unit

Loop- mediated isothermal amplification for
murine typhus (*Rickettsia typhi*) –
problems with diagnosis at the limit of detection

Sabine Dittrich, PhD

Outline

Background

- Rickettsia typhi – murine typhus
- Rational for the study

Methodology

Results

- Analytical / diagnostic sensitivity data
- Bacterial load investigations

Conclusions

- LAMP assay for murine typhus in Lao
- Diagnosis of murine typhus in general

Rickettsia

Antigenic groups and species

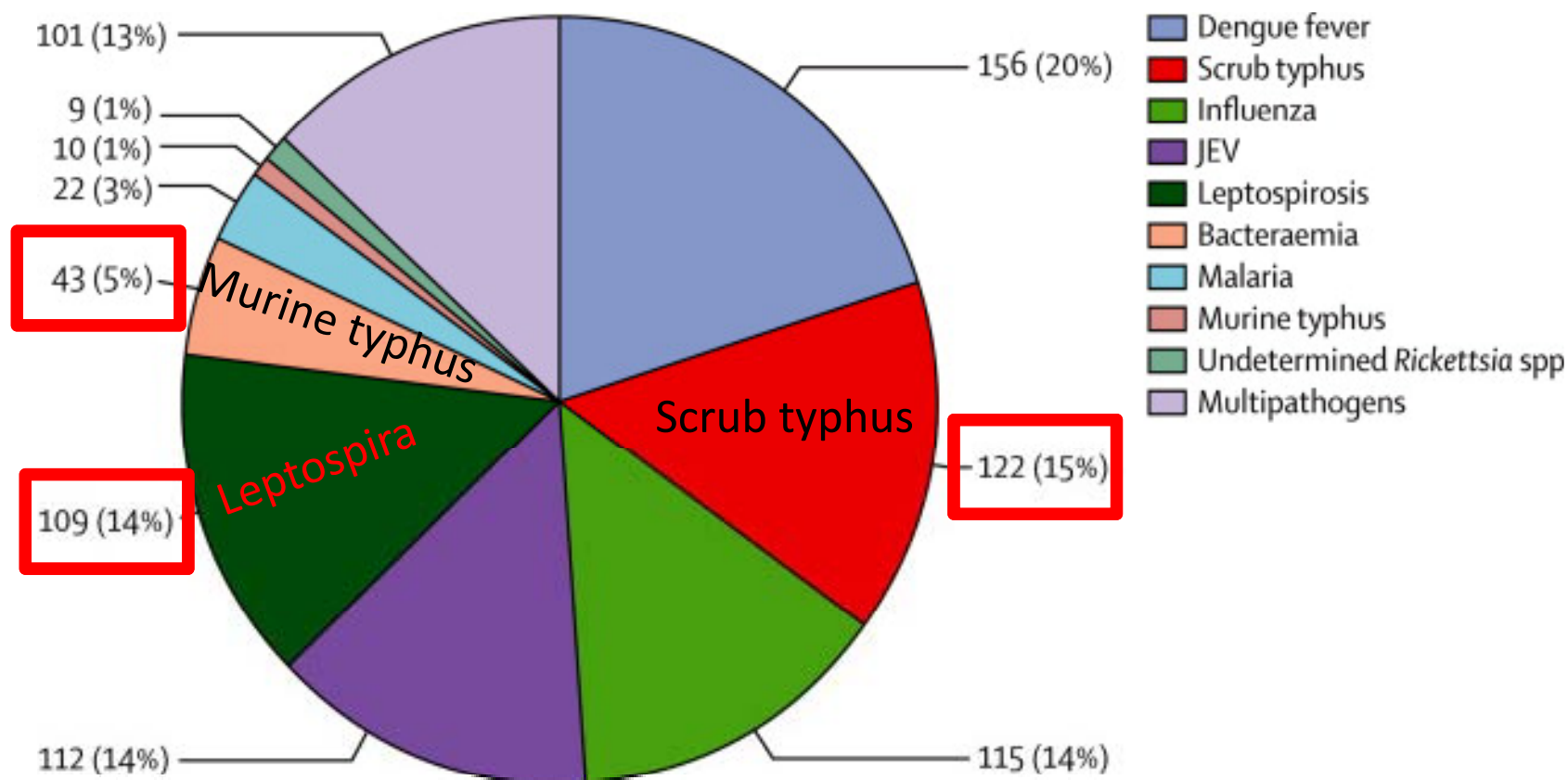
- Typhus Group (distribution: world wide)
 - *Rickettsia prowazekii* (Epidemic typhus)
 - *Rickettsia typhi* (Murine typhus)
- Scrub Typhus Group (distribution: Asia/Pacific)
 - *Orientia tsutsugamushi* (Scrub typhus, Japanese flood fever)
- Spotted Fever Group (distribution: world wide)
 - *Rickettsia rickettsii* (Rocky Mountain spotted fever)
 - *Rickettsia japonica* (Oriental spotted fever)
 - *Rickettsia australis* (Queensland tick typhus)
 - *Rickettsia conorii* (Mediterranean spotted fever)
 - *Rickettsia honei* (Flinders Island spotted fever)
 - *Rickettsia akari* (Rickettsial pox)
 - *Rickettsia siberica* (Siberian tick typhus)
 - *Rickettsia felis* (Cat flea fever)
 - *Rickettsia helvetica*



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Causes of non-malarial fever in Laos: a prospective study

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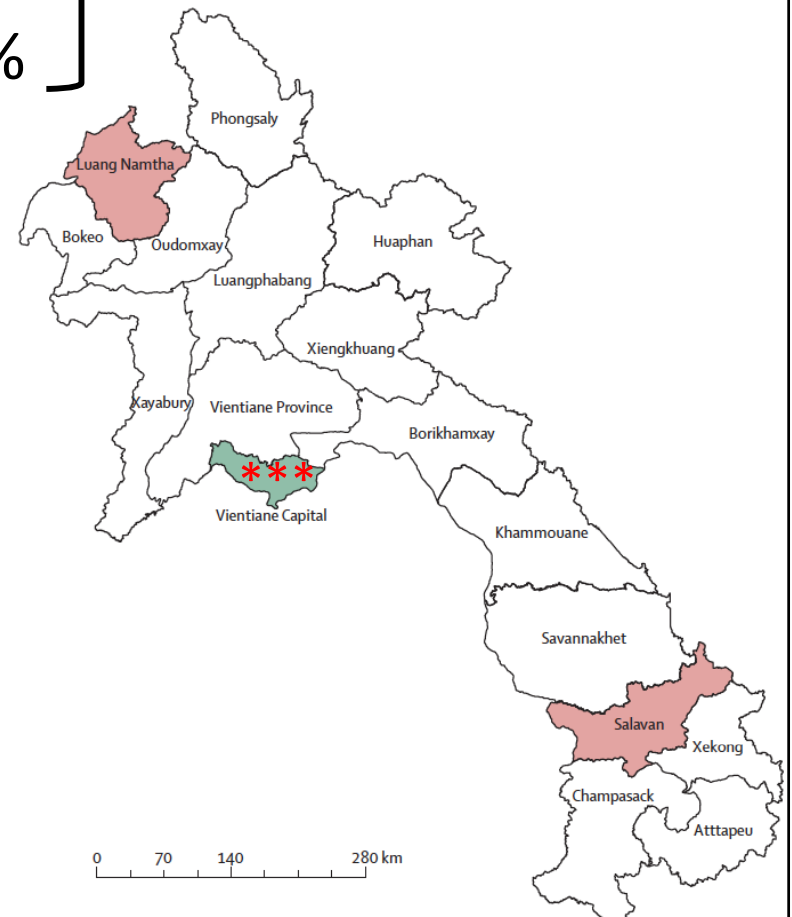


Current diagnostic of *R. typhi* in Lao

Antibody detection

- RDT rapid test (ImmunoDOT™)
 - Sensitivity: 61% / Specificity: 87%
- Weil Felix test
 - Sensitivity ~ 33% / Specificity: ~ 50%
- IFA
 - 4-fold titer rise : Gold Standard

Do-able in “low-tech” facilities



Direct detection

- qPCR ***
- Culture ***

Loop-mediated isothermal amplification (LAMP) ASSURED?

A affordable

S sensitive

S specific

U user friendly

R reliable and robust

E equipment free

D delivered to those who need it

Can we develop a LAMP assay to accurately detect murine typhus in Lao patients?

Material & Methods

Primer development

- *Rickettsia* spp. genomes (GenBank; n=5)
- Primer Explorer software (Eiken Homepage)

Developmental phase (n=142)

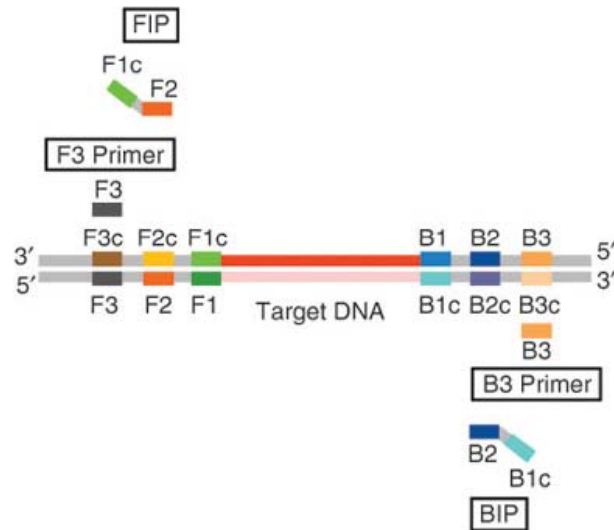
- *R. typhi* culture isolates (n=12)
- *R. typhi* qPCR (*ompB*) positive patients (n=42)
- Specificity panel
 - Patient sample: n=47
 - Bacterial isolates: n=41

Prospective study population

- May – December 2012, Mahosot hospital
- Consecutive, suspected typhus patients
- Age > 15 years



Selection of primers (234bp amplicon) cell surface associated protein 1-gene (*sca1*-gene)



14699 TCAAGATTTTTAAAATATTCTTTGCTCGCCTCTATTT**CAGTAGGAGCGGTAATGGCAATG**
F3

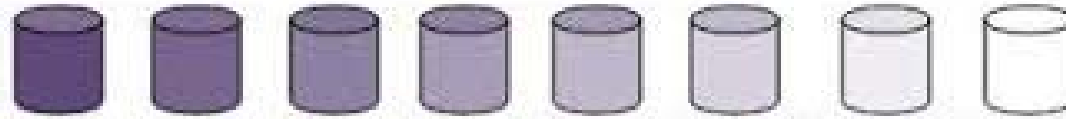
14759 **CCTGTTGAAGGAATTGCTATGGGCATAGATAAAAAGGCATTTTGTACCGAATTAAATACG**
F2 LF

14819 AAATTATTGCTCAAATTTTCACAATCAAGCGTGAATAAAGATACAAT**CAGTACAACACAG**
F1c B1c

14879 **GAACTAACAATAACTTATCTAACAATGTGCAAAGCAATAAAAGTGACATGACAGAAGAG**
LB B2

14939 **GTAGCTAATGTGACTACCGAATCGTTGTGCAAAGTTAGCAAGATGCAAGCACCAGAATTC**
B3

Developmental phase



Analytical sensitivity*: < 10 DNA copies / LAMP reaction

Limit of detection (LoD)**: ~ 40 DNA copies / LAMP reaction

(*minimum number of copies in a sample that can be measured accurately

**95% of positive samples are detected)

Diagnostic sensitivity: 48% (95%CI: 32.5 – 62.7)

Diagnostic specificity: 100%

		qPCR (<i>ompB</i>)		
		Pos +	Neg -	
LAMP	Pos +	20	0	20
	Neg -	22	142	164
		42	142	184

Prospective evaluation study

May – December 2012 : n=266

- qPCR positive for *R. typhi*:
15 / 266 (5.6%) Jiang *et. al.* 2004



- LAMP positive for *R. typhi*:
5 / 266 (1.9%)

- Diagnostic sensitivity / specificity (compared to qPCR)

Sensitivity: 33 % (CI 95%: 9.2 - 56.8)

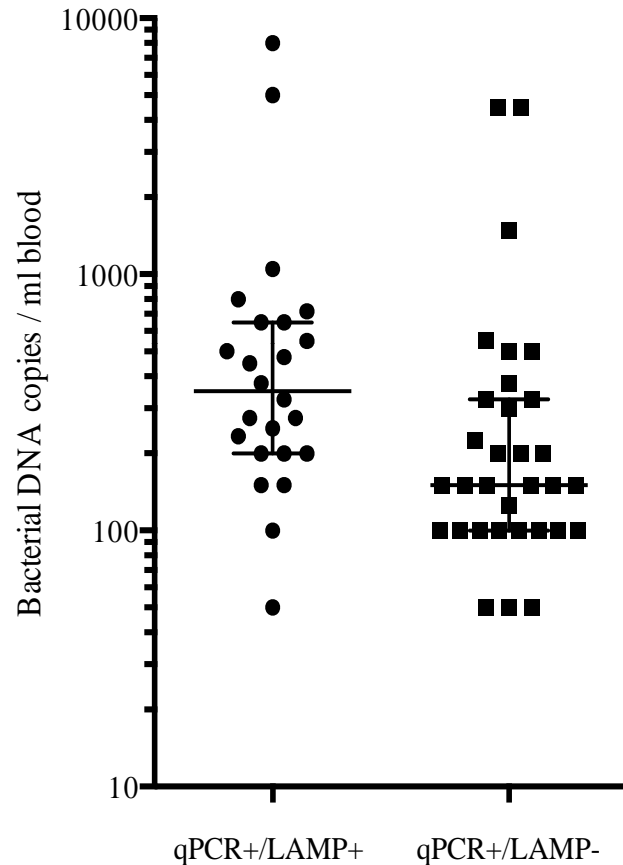


Specificity: 98.5% (CI95%: 97.0% - 100%)

What is the problem?

Why does the low limit of detection not translate into a acceptable diagnostic sensitivity?

Bacterial load per mL of blood the problem?



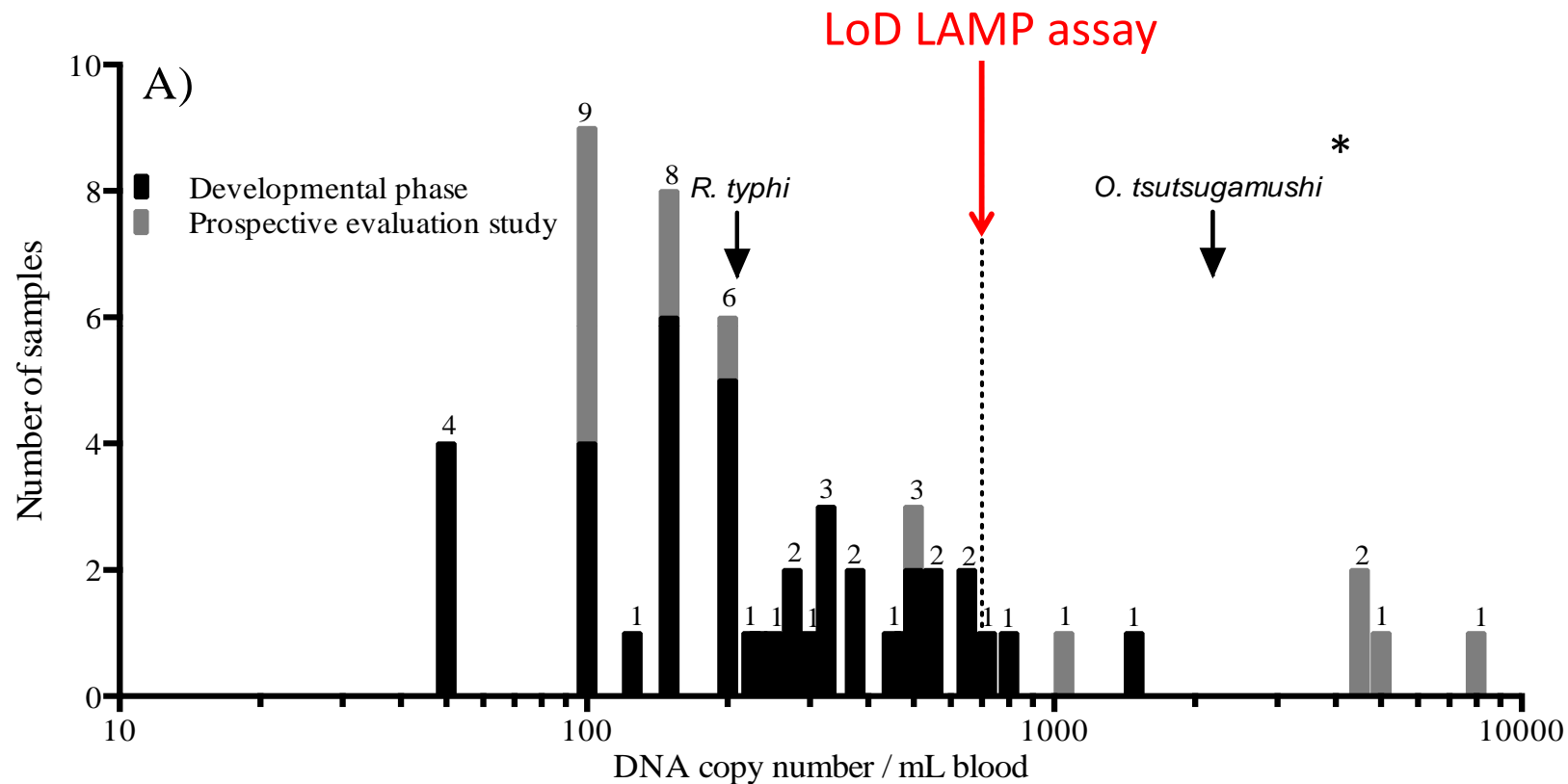
~1/4 of samples
<10 DNA copies / qPCR reaction -> “grey zone”

Bustin *et. al.* 2009 (MIQE guidelines)

qPCR+/LAMP- : 150 copies/mL blood; IQR: 100 – 325

qPCR+/LAMP+ : 350copies/mL blood; IQR: 200 – 650, $p=0.001$

Bacterial loads in acute murine typhus patients n=57



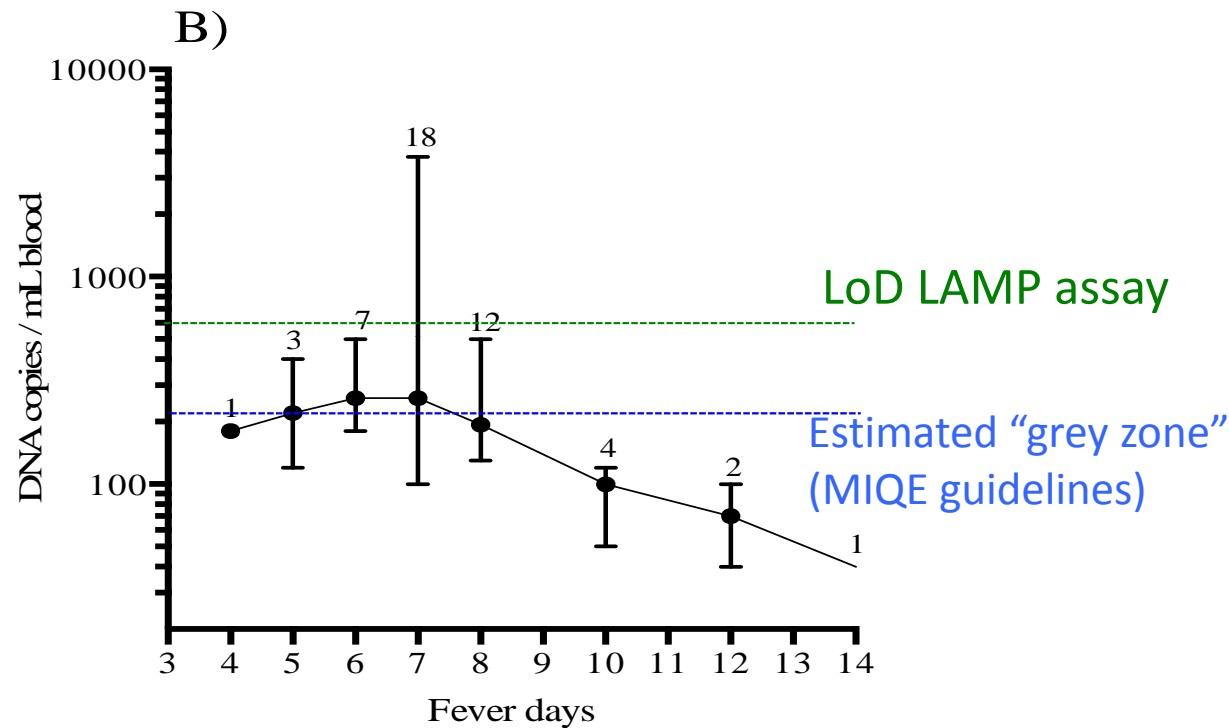
Median: 210 copies/mL of blood (IQR: 130 – 500) -> 12 copies/LAMP reaction

In comparison:

Scrub typhus (n=81, Mahosot Hospital, Lao): 490 copies/mL blood (IQR: 200 – 1750)

* Paris et. al. 2008; Paris et. al. 2009; Singilarak et. al. 2005; Sonthayanon et. al. 2009

R. typhi load at days of fever



Murine typhus: 14 days bacteriemia, 7 days of fever, IQR: 4 – 7

In comparison:

Scrub typhus: 10 days of bacteremia; 6 days of fever IQR: 4 – 7

Conclusion

- Unacceptable sensitivity for patient care!
- Antibody based test remains only available POC assay
- Surprisingly low overall bacterial loads in (Lao) patients
- Low bacterial loads present problem for all direct tests (even qPCR)
- Low bacterial loads raise question about “missed cases”
- Improved DNA preparation/concentration tools needed
- Research dedicated to development of innovative diagnostic tools needed

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Dr David Dance

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Dr Rattanaphone Phetsouvanh



MORU & other collaborators

Dr Daniel Paris

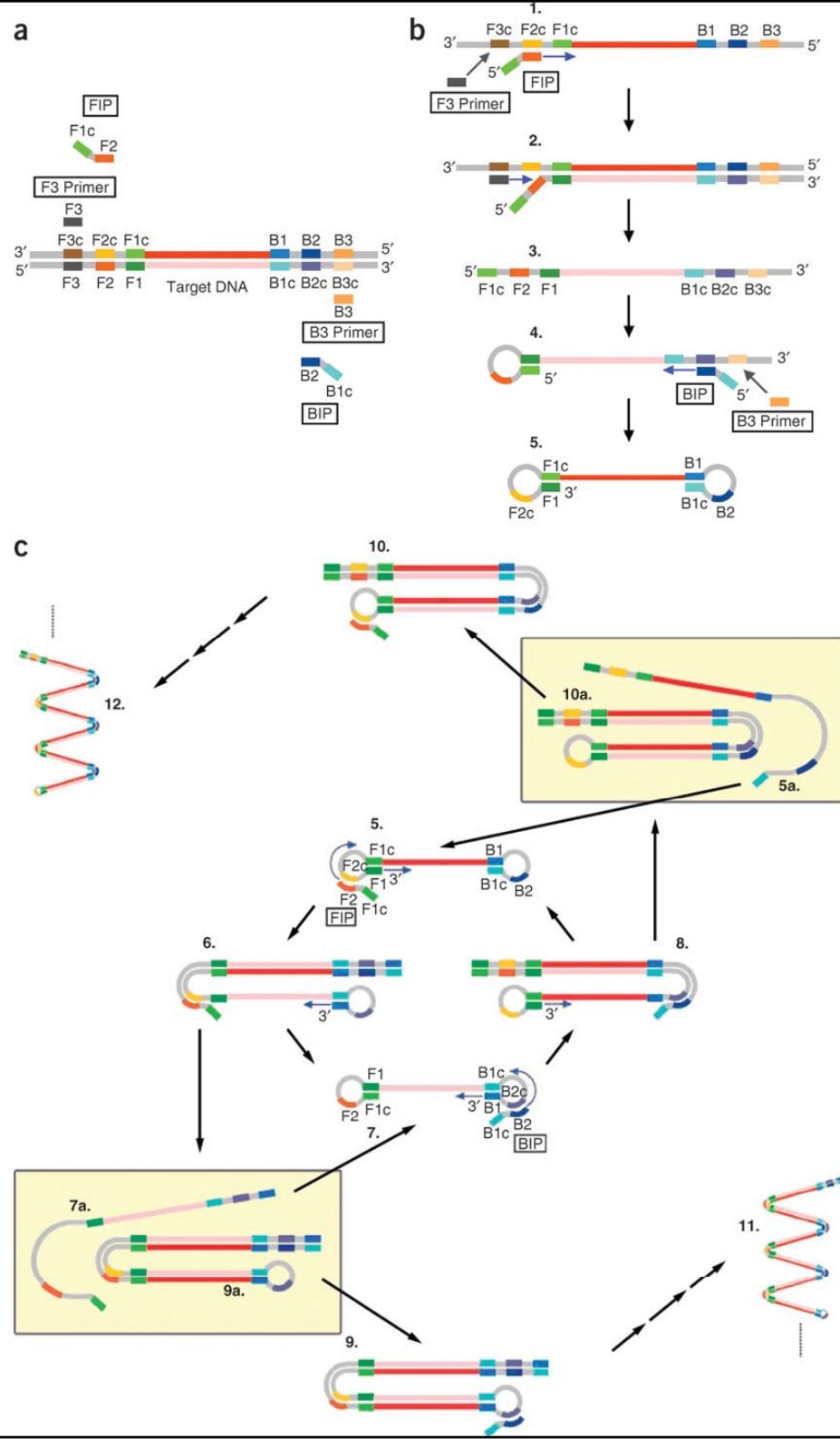
Dr John Stenos

Dr Al Richards



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Backup slides



Variable	All (n=266)§	<i>R. typhi</i> PCR positive		p-value
		LAMP positive (n=5)§	LAMP negative (n=10)§	
Age (median, IQR)	32.3 (23 – 48) (n=218)	50 (37 - 50)	29 (26 - 43)	0.62
Gender (% male)	102 / 221 (46.2)	1 / 5 (20)	3 / 10 (30)	1
No. days fever (median, IQR)	7 (5 - 10) (n=217)	7 (7 - 8)	7 (6 - 7) (n=9)	0.10
Headache (%)	189 / 220 (85.9)	3 / 5 (60)	8 / 10 (80)	0.56
Vomiting (%)	77 / 218 (35.3)	2 / 5 (40)	4 / 9 (44.4)	1
Diarrhea (%)	37 / 217 (17.1)	0 / 5 (0)	1 / 9 (11.1)	1
Rash (%)	18 / 211 (8.5)	1 / 5 (20)	2 / 9 (22.2)	1
Convulsions (%)	19 / 216 (8.8)	0 / 5 (0)	0 / 9 (0)	1
Jaundice (%)	24 / 216 (11.1)	1 / 5 (20)	1 / 9 (11.1)	1
Bleeding (%)	4 / 76 (5.3)	0 / 3 (0)	0 / 5 (0)	1
Myalgia (%)	165 / 220 (75)	3 / 5 (60)	8 / 10 (80)	0.56
Lymphadenopathy (%)	25 / 213 (11.7)	0 / 4 (0)	1 / 9 (11.1)	1
Meningism (%)	24 / 216 (11.1)	1 / 5 (20)	1 / 9 (11.1)	1
Temperature (°C; median, IQR)	38.0 (37.5 – 38.8) (n=208)	38.4 (37.3 – 39.3) (n=4)	38.0 (37.6 – 38.5) (n=9)	0.67
Glasgow Coma Scale (median, range)	15 (4 – 15) (n=186)	15 (15 – 15)	15 (15 – 15) (n=7)	1
Pulse / min (median, IQR)	94 (82 – 100) (n=207)	100 (97 – 104)	96 (91 – 102) (n=8)	0.61
Died (%)	5 / 90 (5.6)	0 / 3 (0)	0 / 6 (0)	1
Antibiotic within the last week (%)	48 / 116 (41.4)	3 / 4 (75)	0 / 4 (0)	0.14
Estimated bacterial load (/mL blood; median, IQR)	n.a.	~3030 (410 – 720) (n=4)	~130 (100 – 500)	0.03*