

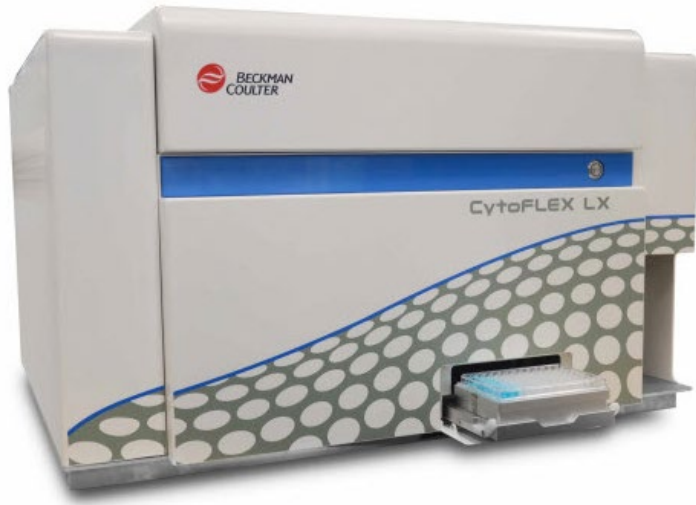
Development and performance evaluation of a
low-cost, high throughput, multiplex
immunoassay of 13 fever severity and aetiology
markers

Tegwen Marlais PhD

LONDON
SCHOOL of
HYGIENE
& TROPICAL
MEDICINE



Markers of fever severity and aetiology: Existing commercial kit examples



Flow cytometry

BioLegend LEGENDplex
Anti-Virus Response Panel
13-plex for \$150 per sample

Th Cytokine Panel
12-plex for \$140 per sample



Luminex

Bio-techne (customizable)
12-plex for \$50 per sample

Millipore MILLIPLEX
20-plex for \$115 per sample

Our aim & outcome: Develop a bead-based multiplex capture assay

It will use commercially-available reagents

- 13 capture and detection antibodies, 13 recombinant protein antigens
- Will be published as a fully open protocol for use and adaptation

Samples

- Dried blood spots in use- 2 per 100 ul elution buffer
- Serum and plasma being tested
- 80 samples per plate; throughput e.g. 4 plates (320 samples) per day, per operator

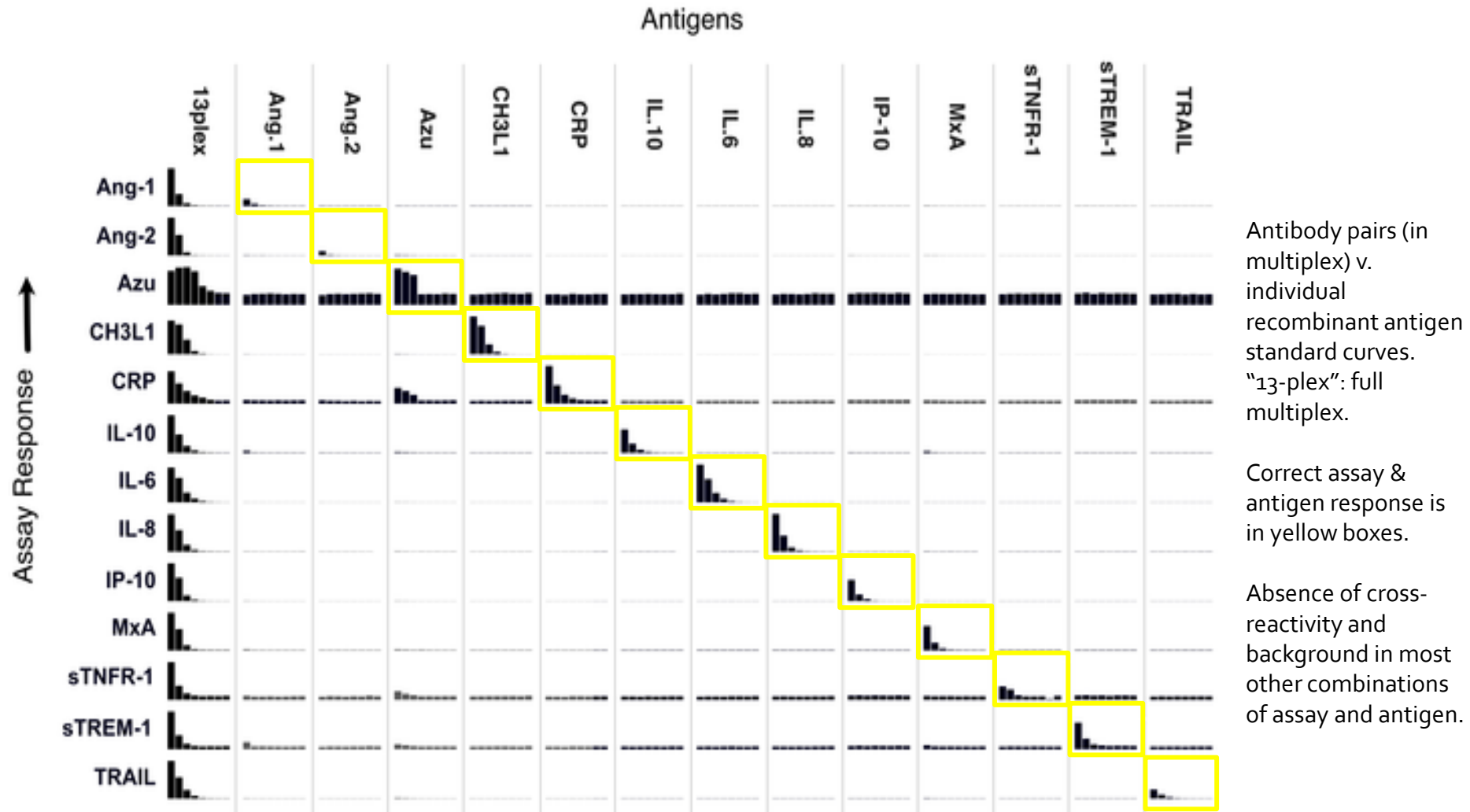
Cost for 13-plex assay after our optimisation

- \$320 per plate
- \$4 per sample

The 13 immune markers detected

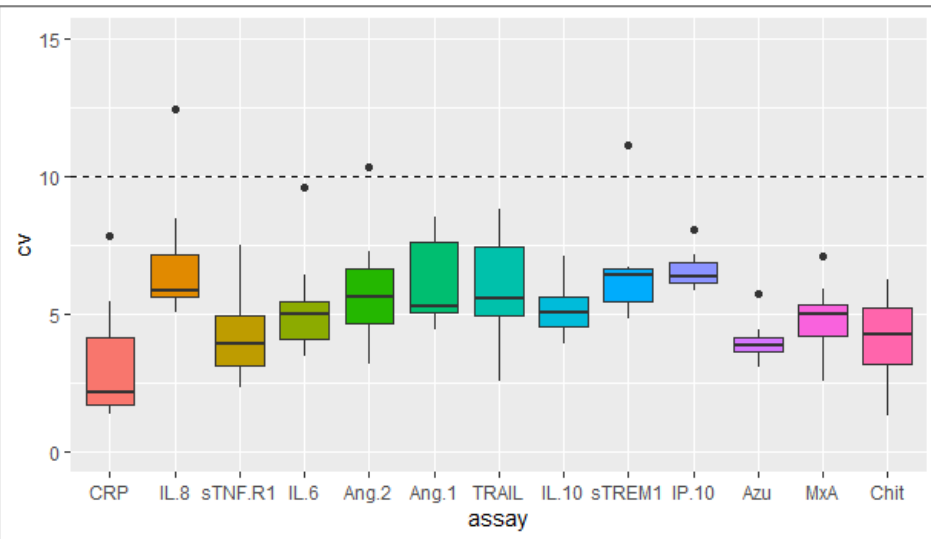
Associated with	Marker	Name
Bacterial infection	CRP	C-reactive protein
	sTREM-1	soluble Triggering receptor expressed on myeloid cells-1
Viral infection	TRAIL	TNF-related apoptosis-inducing ligand
	IP-10	Interferon gamma-induced protein 10
	MxA	Myxovirus resistance protein A
Severity of disease	CHI3L1	Chitinase 3-like 1
	Ang-1	Angiopoietin-1
	Ang-2	Angiopoietin-2
	Azu/HBP	Azurocidin 1/Heparin binding protein
	sTNF-R1	soluble Tumour necrosis factor receptor 1
	IL-6	Interleukin-6
	IL-8	Interleukin-8
IL-10	Interleukin-10	

Assay performance assessment: cross-reactivity

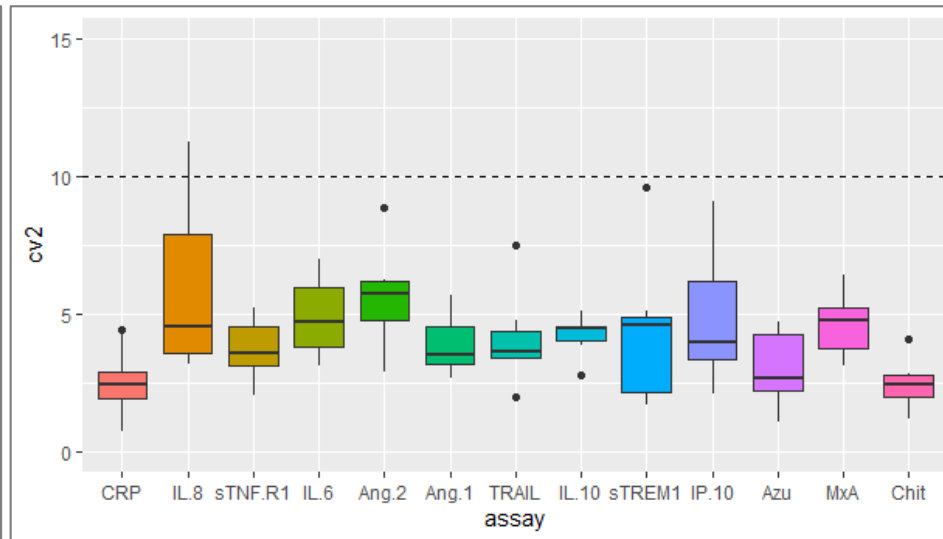


Assay performance assessment: coefficient of variation (repeatability)

Within-plate CVs



Between-plate CVs



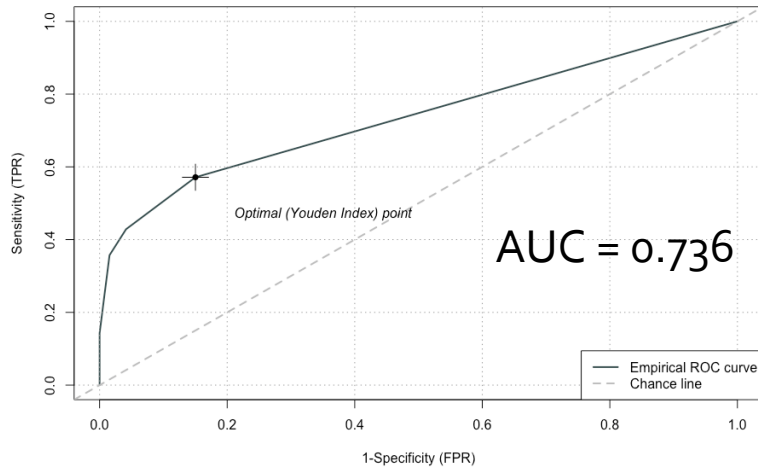
CV of 10 is considered the acceptable limit (dashed line).

CVs were calculated using DBS clinical samples and synthesized reference specimens covering all antigen concentrations.

Based on 12 replicates on one plate, plus 6 across 6 additional plates.

Preliminary results: Immune markers and fever severity scores

UVA for predicting death



UVA:
Universal Vital
Assessment

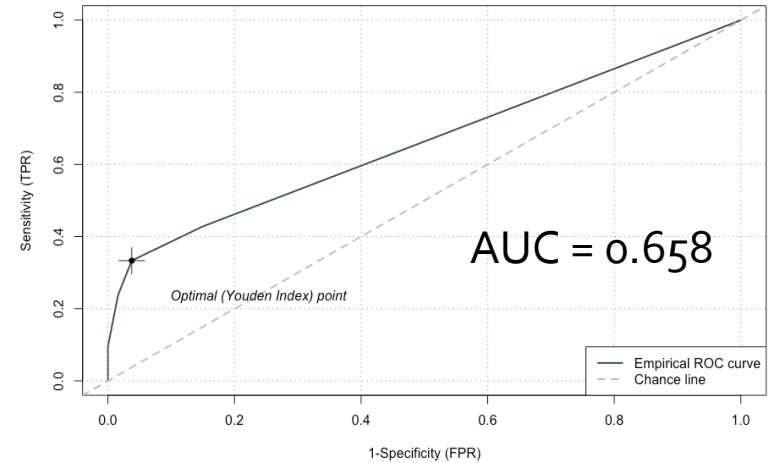
“bad outcome”:
dead, worse or
same at Day 28 as
at Day 0 in fever
cases.

CH₃L₁: chitinase
3-like 1

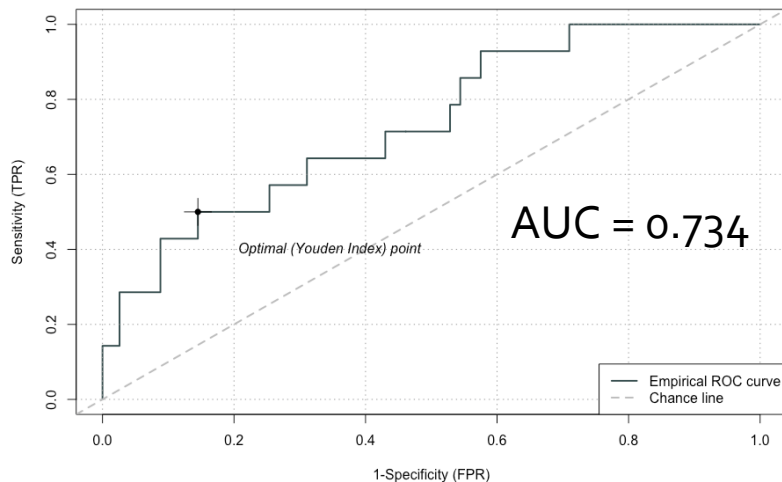
AUC: area under
the curve
(measure of
diagnostic
accuracy where 1
= 100%)

Samples from
Laos FIEBRE study
adult participants.
Dataset including:
14 deaths, 193
survivors.

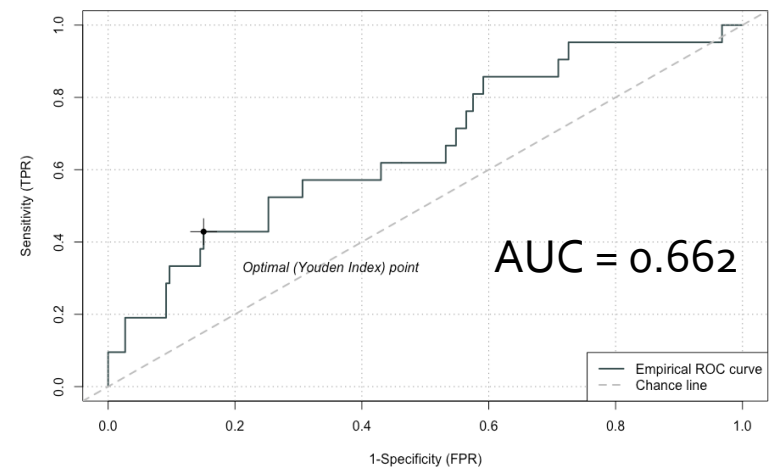
UVA for predicting any bad outcome



CH₃L₁ for predicting death



CH₃L₁ for predicting any bad outcome



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FIEBRE study partners and advisors

Dr Audrey Dubot-Pérèz, Unité des Virus
Emergent, Marseille, France
Dr Ben Amos
Prof Quique Bassat, ISGlobal
Prof John Crump, University of Otago
Prof David Laloo, LSTM, UK
Prof Paul Newton, University of Oxford
Prof Katharina Kranzer, BRTI
Dr Yoel Lubell, University of Oxford
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