

MALAWI LIVERPOOL  
WELCOMME PROGRAMME  
**Virology  
Group**



# Development of a rotavirus VP6-specific IgA and IgG binding antibody assay

Jonathan Mandolo

PhD student, Liverpool School of Tropical Medicine (LSTM)

15th International Rotavirus Symposium, Cape town South Africa

30<sup>th</sup> September 2025



# Importance of screening for rotavirus antibodies

- Rotavirus specific Immunoglobulin G (IgG) as indicator of maternal delivered antibodies, level of exposure to infection or vaccine
- Rotavirus specific Immunoglobulin A (IgA) as indicator of vaccine seroconversion, recent exposure

# Why a new assay?

- Supply chain break of rotavirus antibody (rabbit polyclonal)
  - ❖ Antibodies online, USA stopped production
  - ❖ Christian Medical College, Vellore, India stopped producing
- Cost effectiveness

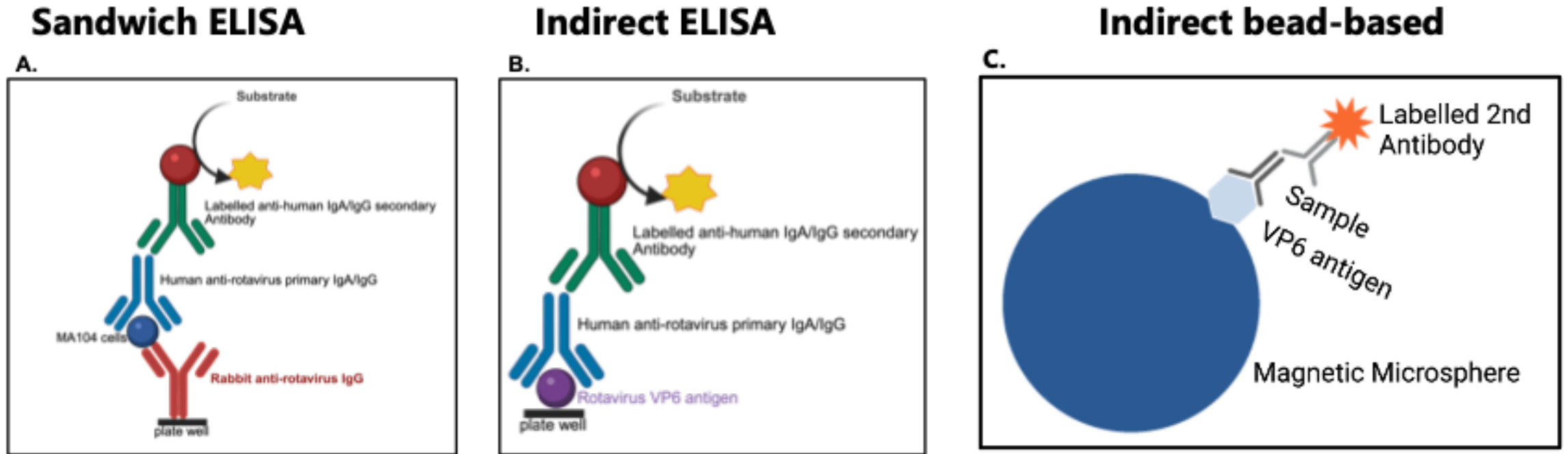
## Main aim

To establish and evaluate the sensitivity and specificity of a novel in-house recombinant VP6 antigen-based Immunoassay against the validated gold standard rabbit anti-rotavirus IgG based ELISA

# Study objectives

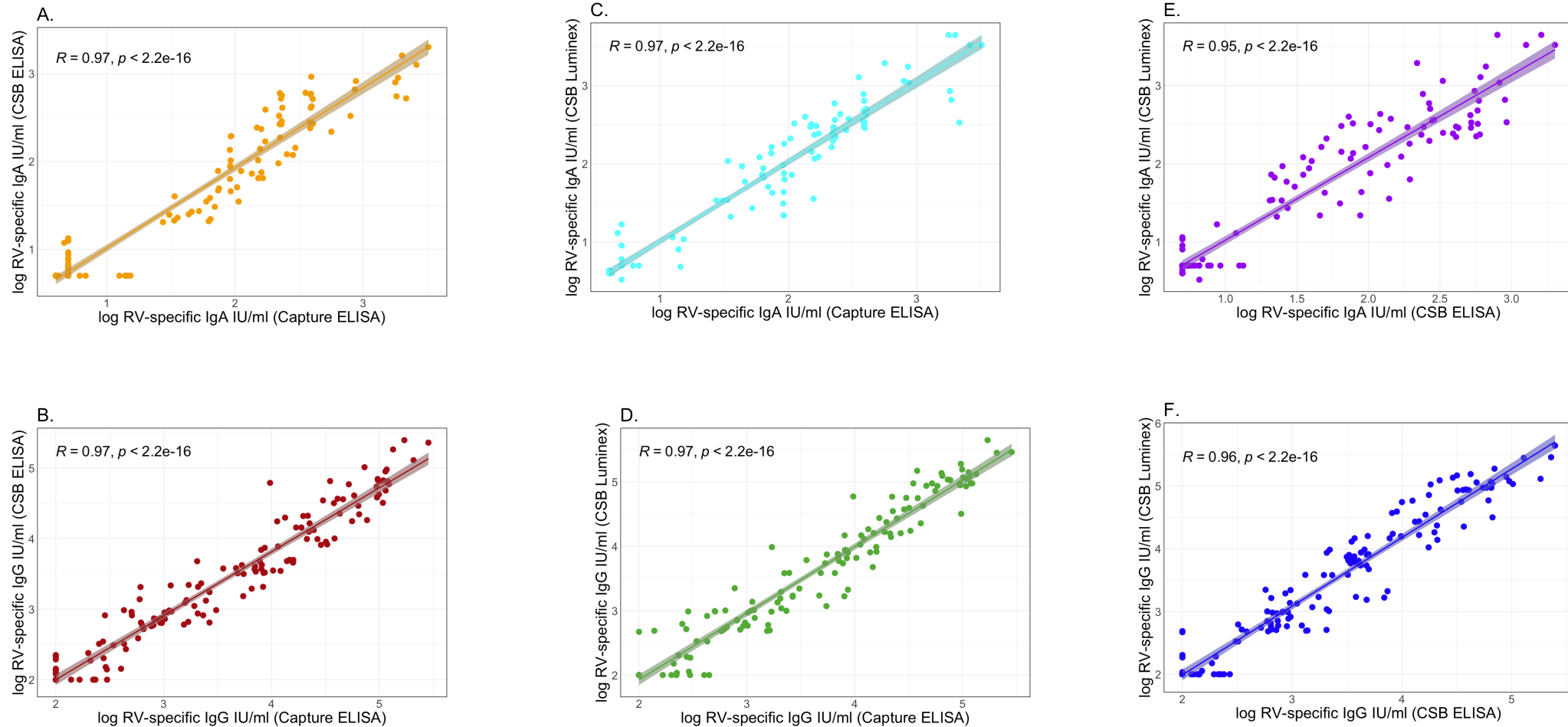
1. To develop the binding ELISA and Luminex bead-based assays using recombinant rotavirus VP6 antigen for the detection of rotavirus-specific IgA and IgG antibodies
2. To evaluate the sensitivity and specificity of the VP6-based assays compared to the validated gold standard rabbit anti-rotavirus IgG based ELISA
3. To assess the utility of the VP6-based assay in evaluating natural- and vaccine-induced rotavirus specific antibodies
4. To assess the utility of DBS samples in detecting rotavirus-specific antibodies using the recombinant VP6 antigen-based Immunoassay

# Schematic comparison of existing and developed assay formats



**Fig 1: Comparison of Immunoassay formats for detecting rotavirus-specific IgA/IgG antibodies. A)** Capture antibody ELISA. **B)** VP6 antigen-based ELISA. **C)** VP6 antigen-based Lumix assay. Fig created in BioRender. Mandolo, J. (2025) <https://BioRender.com>.

# Strong positive correlation in rotavirus-specific antibody titres across the platforms (n=143)



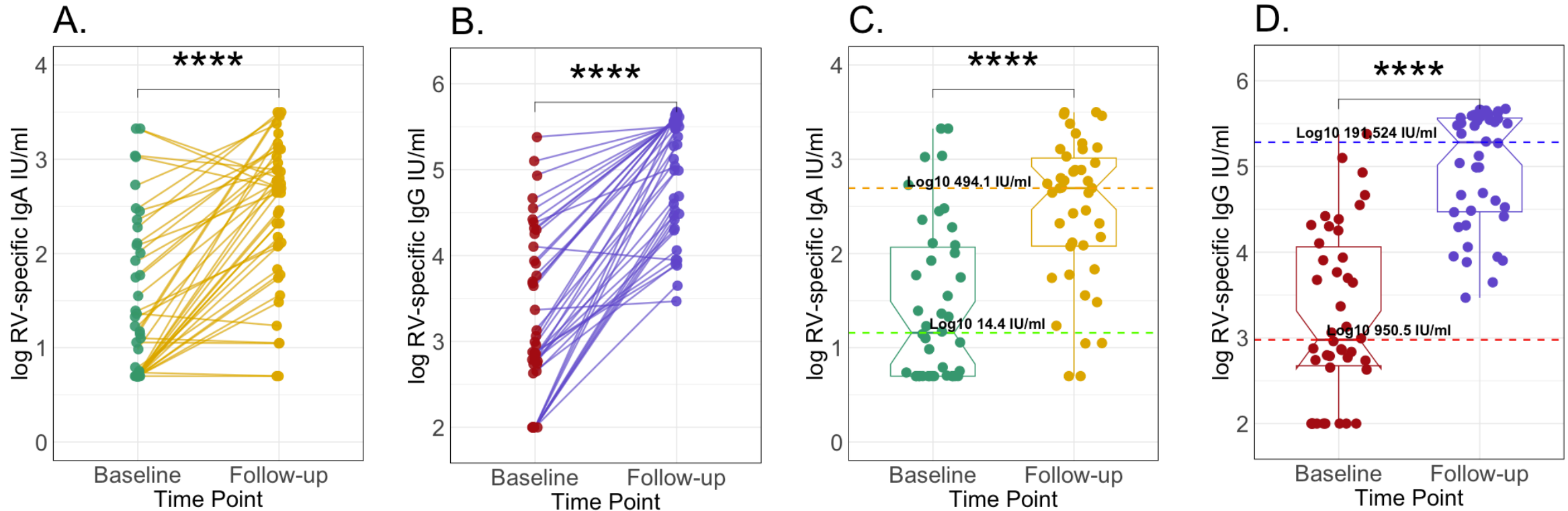
**Fig2. Correlation of rotavirus-specific antibody titres across immunoassay platforms.** Pearson correlation coefficients ( $r$ ) and associated  $p$ -values ( $p$ ) are reported for each comparison. Capture ELISA= rabbit anti-rotavirus IgG based ELISA . CSB ELISA = VP6 antigen-based ELISA. CSB Luminex = VP6 antigen Luminex bead-based assay

# VP6 antigen-based assays demonstrated high sensitivity and specificity compared to Capture ELISA, the gold standard

Assay		Capture ELISA		Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Overall concordance % (95% CI)
		Neg	Pos					
CSB ELISA	Neg	70	1	98.6 (92.6 – 100.0)	100.0 (94.9 – 100.0)	100.0 (95.0 – 100.0)	98.6 (92.4 – 100.0)	99.3 (96.2 – 100.0)
	Pos	0	72					
CSB Luminex	Neg	70	0	100.0 (95.1 – 100.0)	100.0 (94.9 – 100.0)	100.0 (95.1 – 100.0)	100.0 (94.9 – 100.0)	100.0 (97.5 – 100.0)
	Pos	0	73					

Values represent counts unless otherwise indicated. Pos = Positive; Neg = Negative. Capture ELISA= Anti-rotavirus rabbit polyclonal-based ELISA. CSB ELISA = VP6 antigen-based ELISA. CSB Luminex = VP6 antigen Luminex bead-based assay

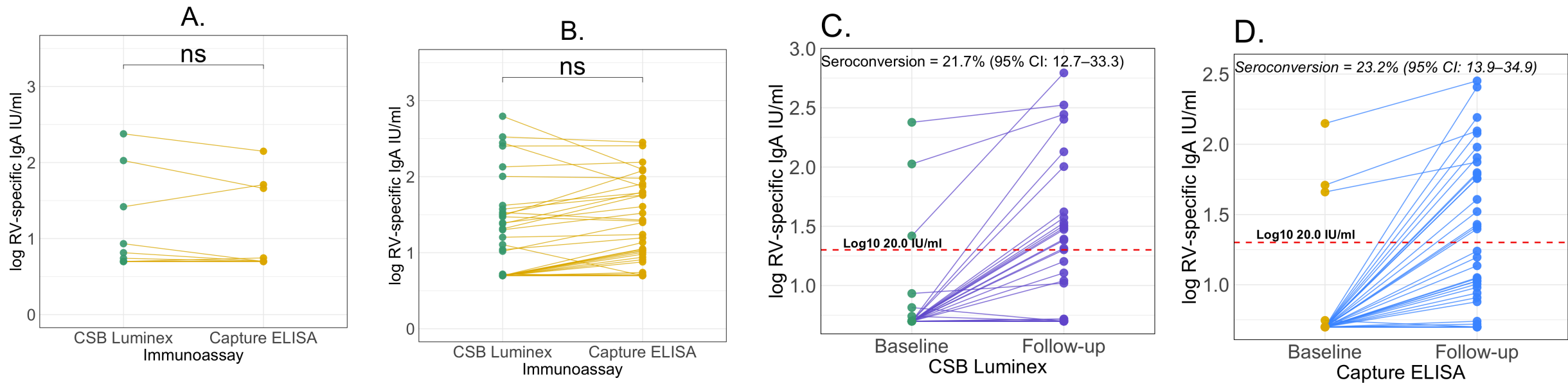
# The assay can detect humoral response following natural rotavirus exposure (n=42)



**Fig 3. Changes in RV-specific IgA and IgG antibody levels following natural exposure.** Each line connecting the baseline and follow-up dots represents an individual participant at the two timepoints. Horizontal dotted green line in C = median rotavirus-specific IgA titre at baseline. Horizontal dotted orange line in C = median rotavirus-specific IgA titre at follow-up. Horizontal dotted red line in D = median rotavirus-specific IgG titre at baseline. Horizontal dotted red line in D = median rotavirus-specific IgG titre at follow-up. Baseline = time when a child presented with severe rotavirus-related gastroenteritis. Follow-up = four weeks following presenting with severe rotavirus-related gastroenteritis

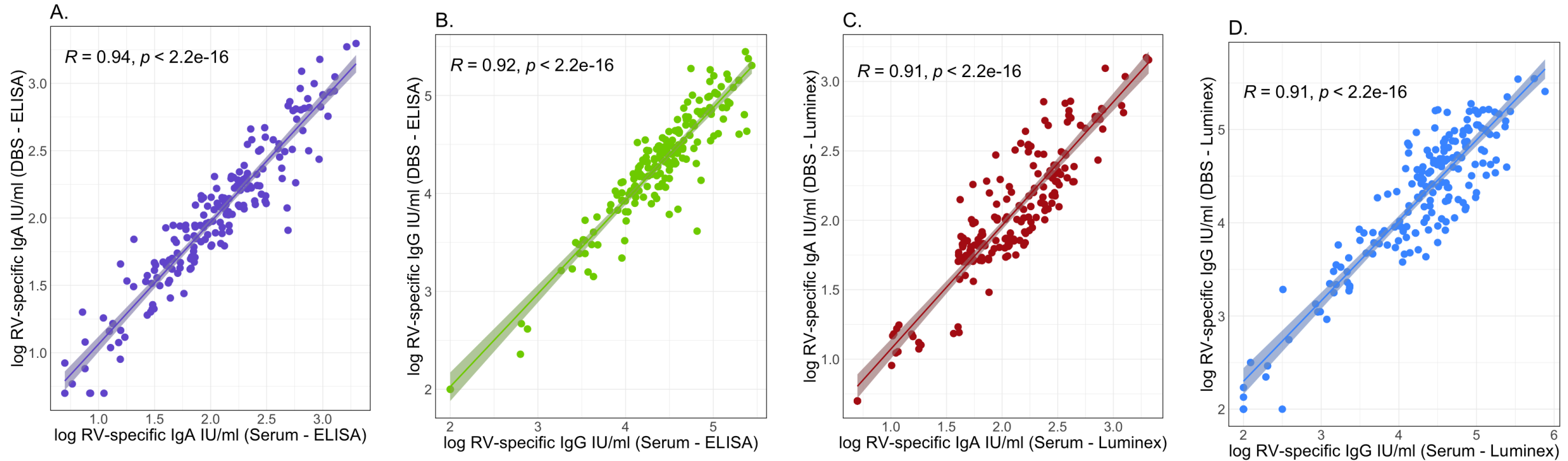
Gladstone et al. NEJM, 2011, Bernstein et al. JID, 1991, Velázquez et al. JID, 2000

# The assay can detect humoral response following Rotarix Vaccination (n=69)



**Fig 4. Comparison of VP6 antigen Luminex bead-based and Capture ELISA assays for detecting immune responses to Rotarix vaccination. A) Baseline rotavirus-specific IgA titres at 6 weeks. B) Follow-up rotavirus-specific IgA titres at 14 weeks. C) Comparison of baseline and follow-up IgA titres on Luminex platform. D) Comparison of baseline and follow-up IgA titres on Rabbit polyclonal rotavirus IgG**

# Strong positive correlation in rotavirus antibody titres between DBS and Serum samples across the platforms (n= 184)



**Fig 5. Correlation of rotavirus-specific antibody titres between serum and DBS samples on different immunoassay platforms (Pearson correlation).** ELISA = VP6 antigen-based ELISA. Luminex = VP6 antigen Luminex bead-based assay

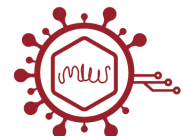
# DBS samples demonstrated high sensitivity and specificity compared to serum samples

Assay	DBS	Serum		Sensitivity %	Specificity %	PPV %	NPV %	Overall concordance %
		Neg	Pos					
CSB ELISA		Neg	Pos	98.8 (95.7 – 99.9)	94.1 (71.3 – 99.9)	99.4 (96.7 – 100.0)	88.9 (56.3 -98.6)	98.4 (95.3 – 99.7)
	Neg	16	2					
	Pos	1	165					
CSB Luminex		Neg	Pos	98.2 (94.8 – 99.6)	100.0 (80.5 – 100.0)	100.0 (97.8 – 100.0)	85.0 (62.1 – 96.8)	98.4 (95.3 – 99.7)
	Neg	17	3					
	Pos	0	164					

Values represent counts unless otherwise indicated. Pos = Positive; Neg = Negative

# Advantages of the new assays

<b>Assay</b>	<b>Over night incubation</b>	<b>Samples per plate</b>	<b>Availability</b>	<b>Cost</b>
Capture ELISA	Yes	9	In-house	?
Antigen ELISA	yes	21	commercial	?
Luminex	no	40	commercial	?



# Conclusions

- VP6 antigen could be a viable replacement for the rabbit polyclonal rabbit anti-rotavirus IgG , ensuring continuity in rotavirus serosurveillance and immunological studies
- DBS samples could be reliable for rotavirus antibody detection, highlighting their potential for large-scale epidemiological studies, especially in resource-limited settings

# Acknowledgements

## Supervisors

- Prof. Khuzwayo C. Jere
- Prof. Kondwani C. Jambo

## Advisors

- Prof. Miren Iturriza-Gómara
- Prof. Nigel Cunliffe

## Malawi Liverpool Wellcome Programme

- Dr Kayla Barnes
- Leah Mulira
- Martha Moyo
- Memory Mvula
- Fatima Mtonga
- Daniel Mumba
- Infection and Immunity Group
- Virology Research Group
- ROTAHOST study team
- ROTAHOST study Participants
- SEROSURV study participants
- SEROSURV study team
- DIARSURV study participants
- DIARSURV study team
- ROTARITE study team
- ROTARITE study Participants



National Institutes  
of Health



With funding support from  
**Gates Foundation**

