vitalant℠
Research Institute

Criteria and Selection of SARS-CoV-2 Antibody Assays for Serosurveillance and other Applications, including Consideration of Impact of Vaccines

CDC Multistate Assessment of SARS-CoV-2 Seroprevalence in Blood Donors (MASS-BD)

Michael P. Busch*, Mars Stone*,Eduard Grebe*†

** Vitalant Research Institute*
** Department of Laboratory Medicine, University of California San Francisco*
† SACEMA, Stellenbosch University

Applications and use cases for SARS-CoV-2 serology assays

- Adjunct to swab (molecular or Ag) testing for diagnosis of recent infections in symptomatic COVID-19 cases
- Serosurveillance to estimate cumulative SARS-CoV-2 infection rates (relative to case diagnoses & deaths)
  - *Discrimination from vaccine induced seropositivity (VISP)*
- Assessment of therapeutic and prophylactic utility of CCP & mAbs
- Persistence (breadth/potency) of humoral immunity after infection and vaccination
- Detection of reinfections (anamnestic boosting of Abs)
- Detection of vaccine breakthrough infections (Abs to NC or other non-S1 antigens)
- Impact of vaccination of previously infected persons
  - *Boosted immunity to reduce risk of reinfection*
  - *Boosted potency of CCP and hyperimmune IgG therapeutics and prophylactics*
- Serotyping for variants of interest/concern (VOI/VOC)
  - *Quantify bAbs and nAbs to VOI/VOC as correlates of immunity and to characterize reinfections and vaccine breakthrough infections*

Resources on serological testing and use cases

- Serosurveillance for SARS-CoV-2 Incidence using Global Blood Donor Populations
  Busch & Stone; J Clin Micro 2020
- Urdea M, Halteres Associates – SARS-CoV-2 Use Cases White Paper
- Halteres Associates – Use Cases for SARS-CoV-2 Assays
- US CDC – Interim Guidelines for COVID-19 Antibody Testing in Clinical and Public Health Settings
- US FDA – EUA Authorized Serology Test Performance
- FIND – The impact of novel variants of SARS-CoV-2 on diagnostic testing

Variant designation	B.1.1.7 (VOC-202012/01, 501y.v1)	B.1.351 501Y.V2	P.1 (501Y.V3) and P.2
Location first identified	United Kingdom[1]	South Africa[2]	Brazil[3]
Impact on performance of serological antibody tests	**No data**	**No data**	**No data**
	There is potential for the performance of assays detecting antibodies to viral spike protein or nucleocapsid to be affected, but to date no evaluations have been performed		

vitalant Research Institute

Key performance characteristics for different applications

Application	Sensitivity	Specificity	Stable Ab detection	Declining Ab reactivity	Correlation with neut titers	Quant repro-ducibility	Qual repro-ducibility	Broad dynamic range*	Linear dilutional response
Serosurveillance (assessing seroprevalence/cumulative incidence)	X	X	X				X		
Adjunct to molecular diagnostics	X	X					X		
Assessment of Ab-based treatment utility (CCP, mAb)		X		X	X	X		X	
Characterizing persistence & potency of post-infection humoral immunity	X	X		X	X	X		X	
Characterizing persistence/potency of post-vaccination humoral immunity		X		X	X	X		X	
Detection of reinfections (anamnestic boosting)		X		X		X		X	X
Detection of vaccine breakthrough infections		X		X		X		X	X
Characterizing anamnestic boosting by vaccination in previously infected		X		X	X	X		X	

* Dynamic range can be extended with on-board dilutions

vitalant℠
Research Institute

Study overview

vitalant℠
Research Institute

Study aims

Aim 1: Distribute 1,000 sample panels to evaluate:

- 21 high-throughput antibody assays and develop screening and confirmatory algorithms
- Comparison of binding Ab S/CO to neutralization Ab titers by multiple assays (Broad PRNT, VRI & Monogram pseudovirus RVPNT & OB kinetic RBD ELISA)
- Multiplexed (MSD, Genscript, Luminex) & highly multiplexed (COVAM) Ab assays

Aim 2: Apply optimally performing and logistically most-feasible assay/algorithm to test 2,000-6,000 samples from each of ~60 blood center regions (17 participating BCOs) throughout the US for 18 months

Aim 3: 18-month longitudinal follow-up of 150 CCP donors (50 mL blood at months 1, 6, 12 and 18) to characterize and understand the time course of the SARS-CoV-2 immune response; this will inform the waning of (herd) immunity and risk of reinfection

vitalant℠
Research Institute

Commercial high-throughput serological assays

Manufacturer	# of Assays	Assay	Antigen	Format	US Regulatory Status	Testing Lab	Testing Volume (µl)	Dead Volume (ul)	Total (ul)	Panel Volume (ul)
Ortho	2	Ortho Vitros®	S1	Total Ig antigen sandwich & IgG CLIAs	EUA	VRI	80	300	380	400
Euroimmun	4	Euroimmun AG Anti-SARS-CoV-2 qualitative & semi-quantitative	NC, S1	IgG S1 (qual), IgA S1 (semi-quant), IgG S1 (quant), IgG NC (semi-quant) antigen sandwich ELISAs	IgG NC qual - EUA; other RUO	Advent Health	10 each	200	250	300
Roche	2	Elecsys® Anti-SARS-CoV-2 qualitative & quantitative	NC (qual) & S1/S2 (quant)	Total Ig antigen sandwich ECLIAs on Roche cobas	Total Ig NC - EUA; Total Ig S1/S2 - RUO, EUA application submitted	UC Davis	20 each	250	290	300
DiaSorin	1	LIAISON® 28 SARS-CoV-2 S1/S2 IgG (quantitative)	S1/S2	IgG magnetic particle CLIA	EUA	BC CDC	10	150	310	400
Siemens	2	ADVIA Centaur SARS-CoV-2 Total (COV2T) and IgG	S1-RBC (qual) & S1-RBD (quant)	Total Ig & IgG Ag sandwich CLIA	EUA		50 each	100		
Abbott	4	SARS-CoV-2 IgG Architect & Alinity (qual and quant)	NC & S1	IgG NC (qual) & IgG S1 (quant) microparticle CMIA	EUA	Fred Hutchinson UW Architect/ Duke Alinity	25 each	50	100	400
Bio-Rad	2	Platelia NC microplate assay (Evolis) & Multiplex Assay (Bio-Plex)	Platelia NC Multi-antigen assay (S1/S2, RBD)	Platelia NC Total Ig (semi-quant) ELISA; Total Ig S1/S2 multiplexed microbeads	NC - EUA Multi-Antigen - EUA application submitted	BWNW	10 (Platelia) + 5 (Multiplex)	200	510	500
Quotient	1	MosaiQ™ COVID-19 Antibody Microarray	S1/S2	IgM & IgG Array (pre-printed on glass)	EUA		10	500		
Diazyme	1	DZ-Lite SARS CoV-2	NC & S1/S2	IgG microbead CLIA	EUA	UCI	10	200	210	400
Beckman Coulter	1	Access SARS-CoV-2 IgG	S1 RBD	IgG 2-step paramagnetic particles CLIA	EUA		100	200	300	
Wantai	1	SARS-CoV-2 Total Ig	S RBD	Total Ig sandwich ELISA	EUA	Sanquin	100	10	110	300

The 1000 sample blinded panel

Sample type	Description	N
Ab+ CCP	HLA+, HLA-, randomly chosen from CCP repository	200
Longitudinal CCP series	4-14 serial timepoints over ~4 months	200
Seroconversion panel	1 donor with 14 serial timepoints	14
Blinded replicates	6 replicates of 15 CCP collections, low medium and high titer/reactivity	90
Serial dilution series	Six-step serial dilutions of 5 samples	30
Apparent serosilent cases	Ab- CCP donation samples	25
Pre-COVID negative controls	Specificity panel	450

vitalant℠
Research Institute

Sensitivity and specificity

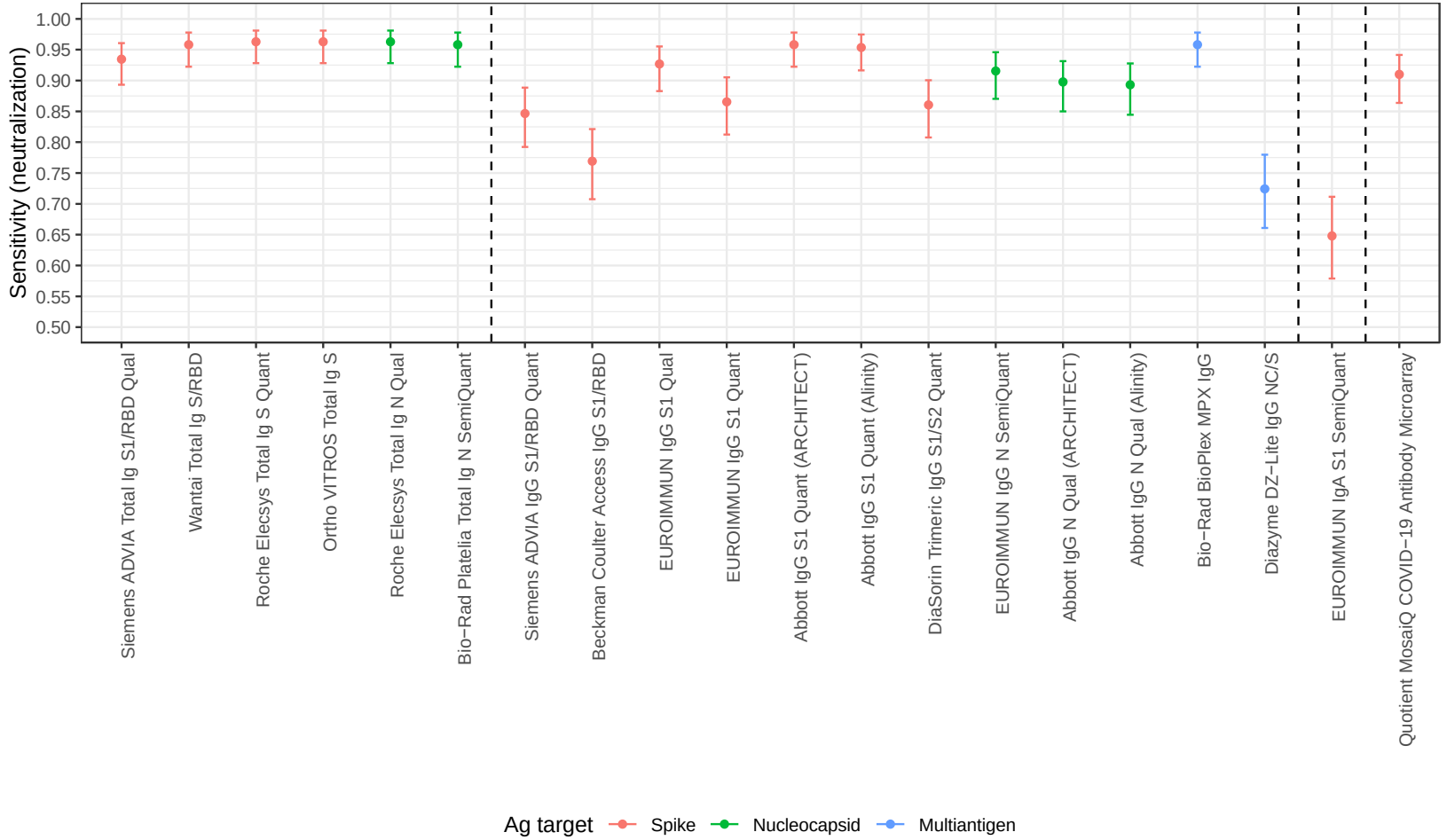
vitalant℠
Research Institute

Methods

- **Sensitivity**
  - Definitions of positivity or positive percent agreement (PPA)
    - Primary: qualification as CCP donors
    - Secondary: neutralizing activity as determined by Broad PRNT
    - Additional: at least 3 bAb assays reactive (at least 2 besides the one being evaluated)

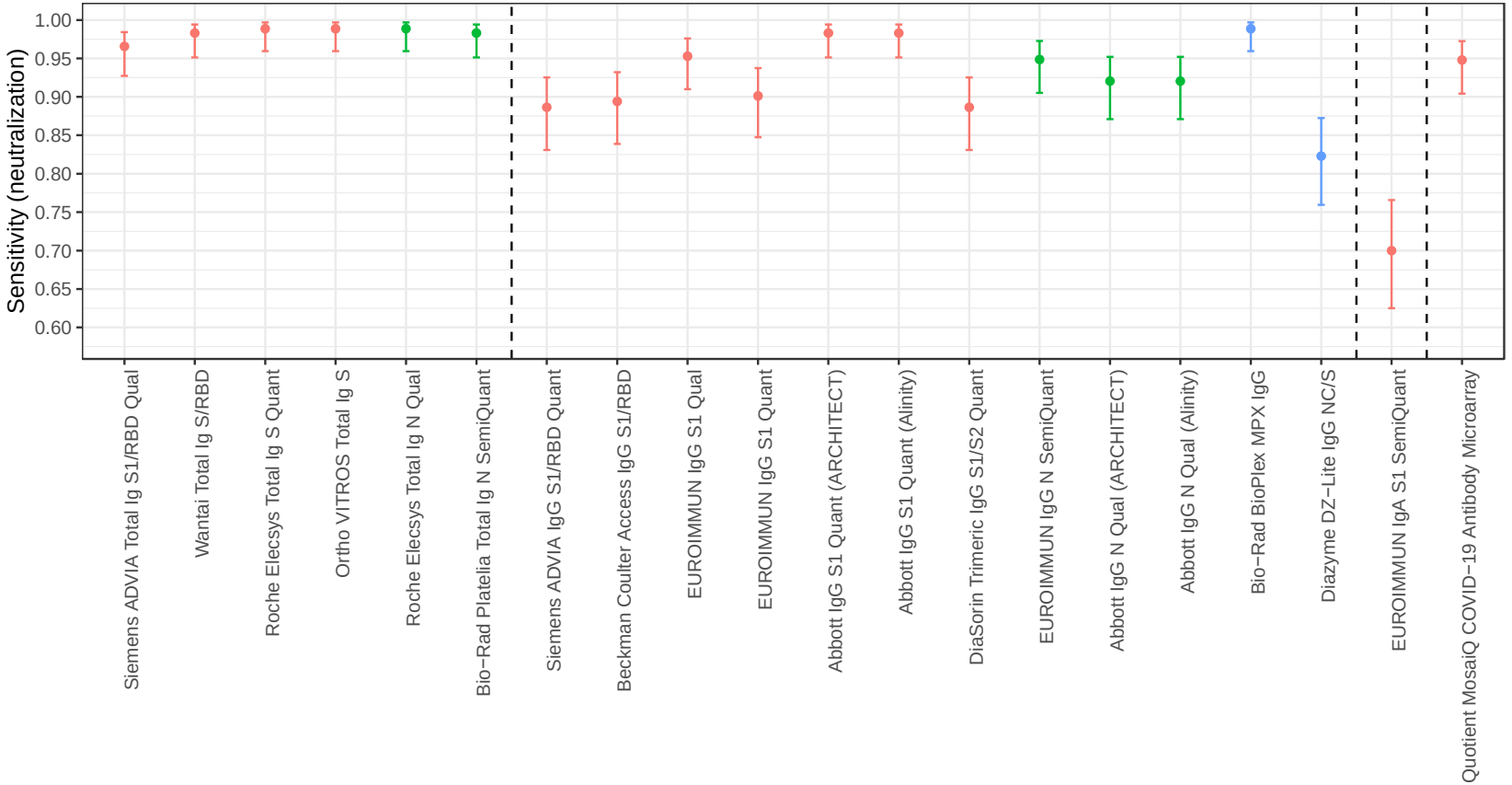
- **Specificity**
  - 2019 pre-COVID-19 controls presumed negative and negative by RVPNT

Sensitivity/PPA (qualified CCP donors)



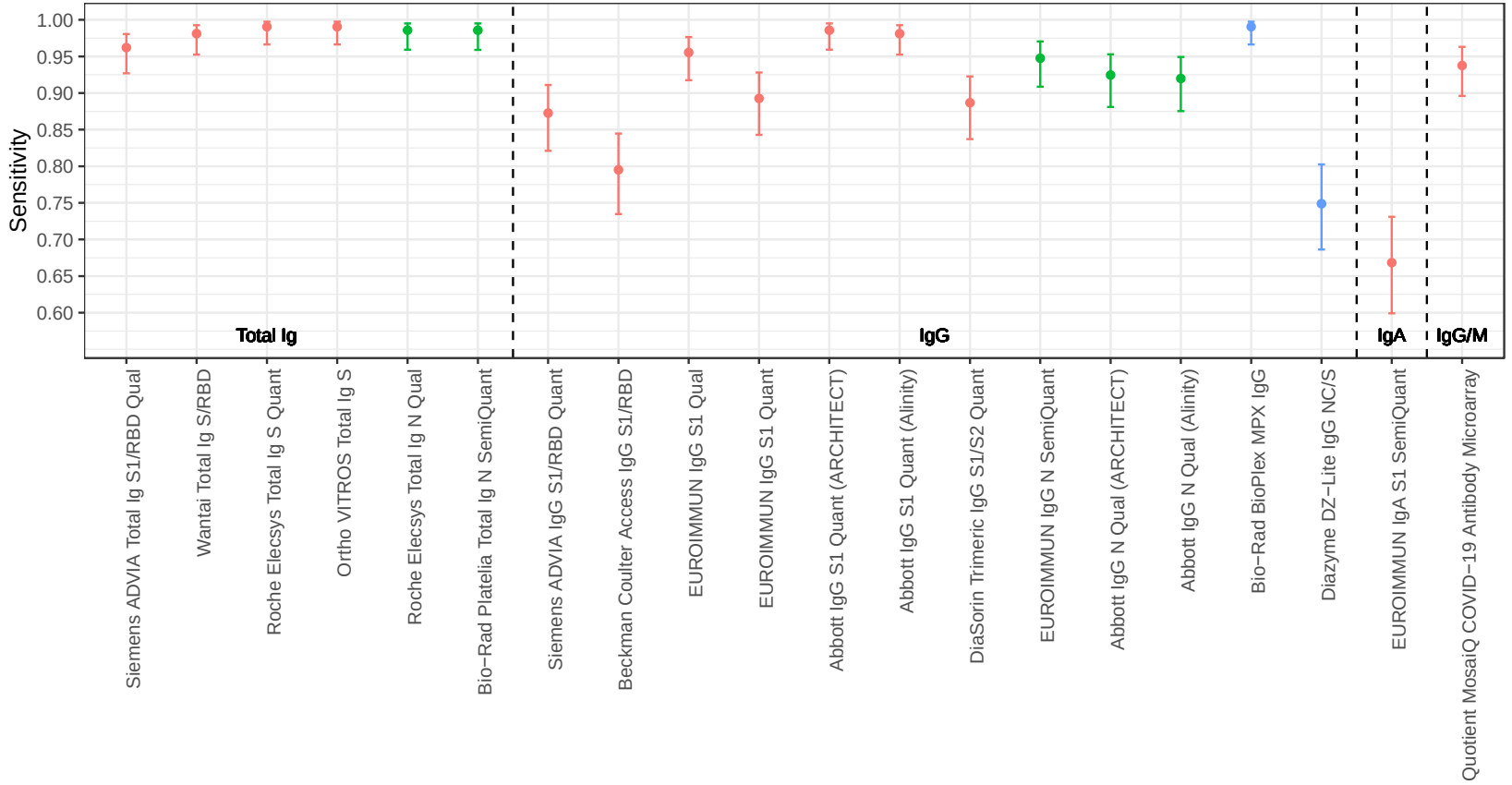
lant℠
Research Institute

Sensitivity/PPA (SARS-CoV-2 neutralization by Broad PRNT)



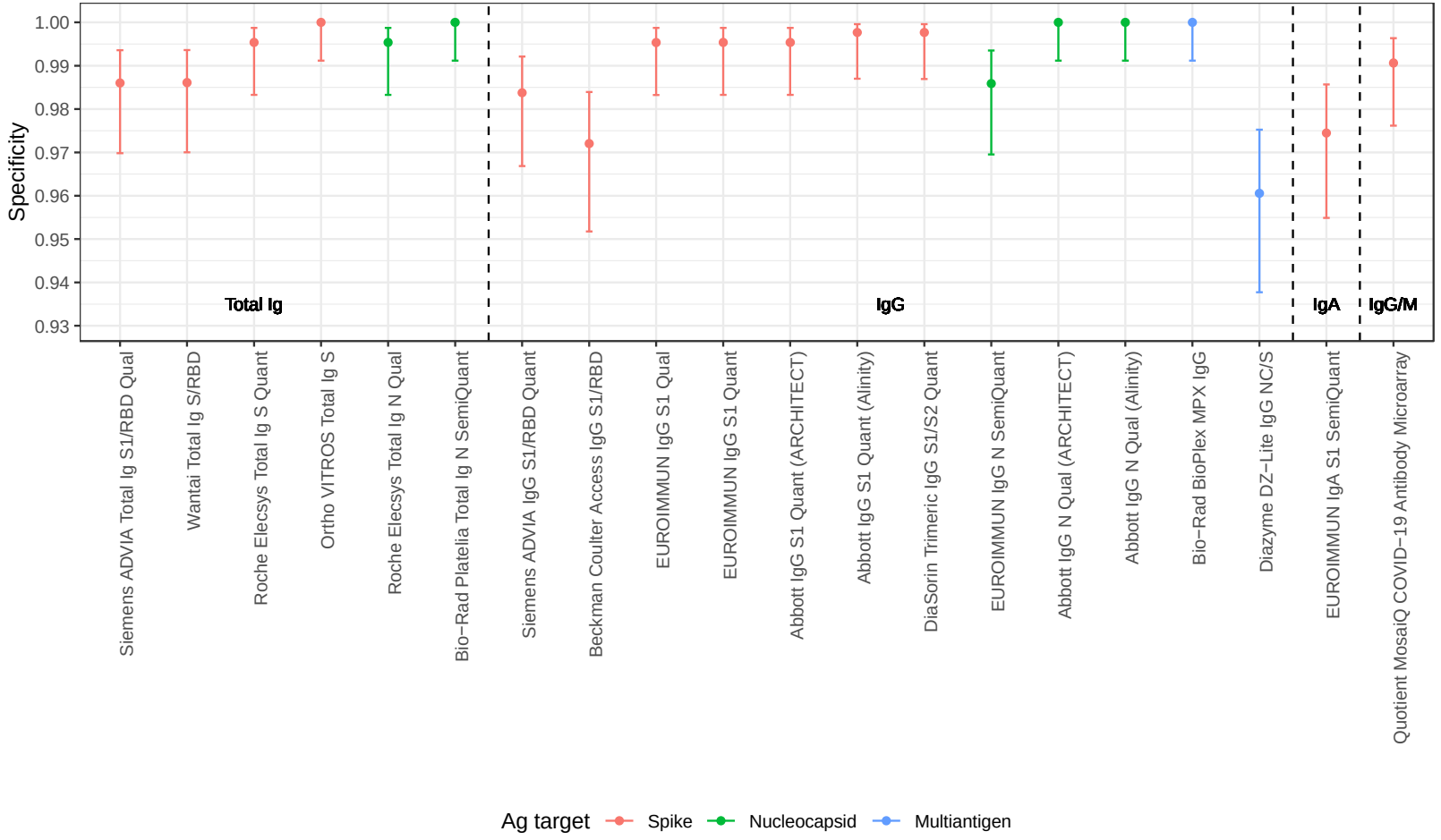
lant℠
Research Institute

Sensitivity/PPA (‘operational gold standard’: 3+ reactive assays)



lant℠
Research Institute

Specificity (pre-COVID-19 controls)



Research Institute

Repeatability and precision

vitalant
Research Institute

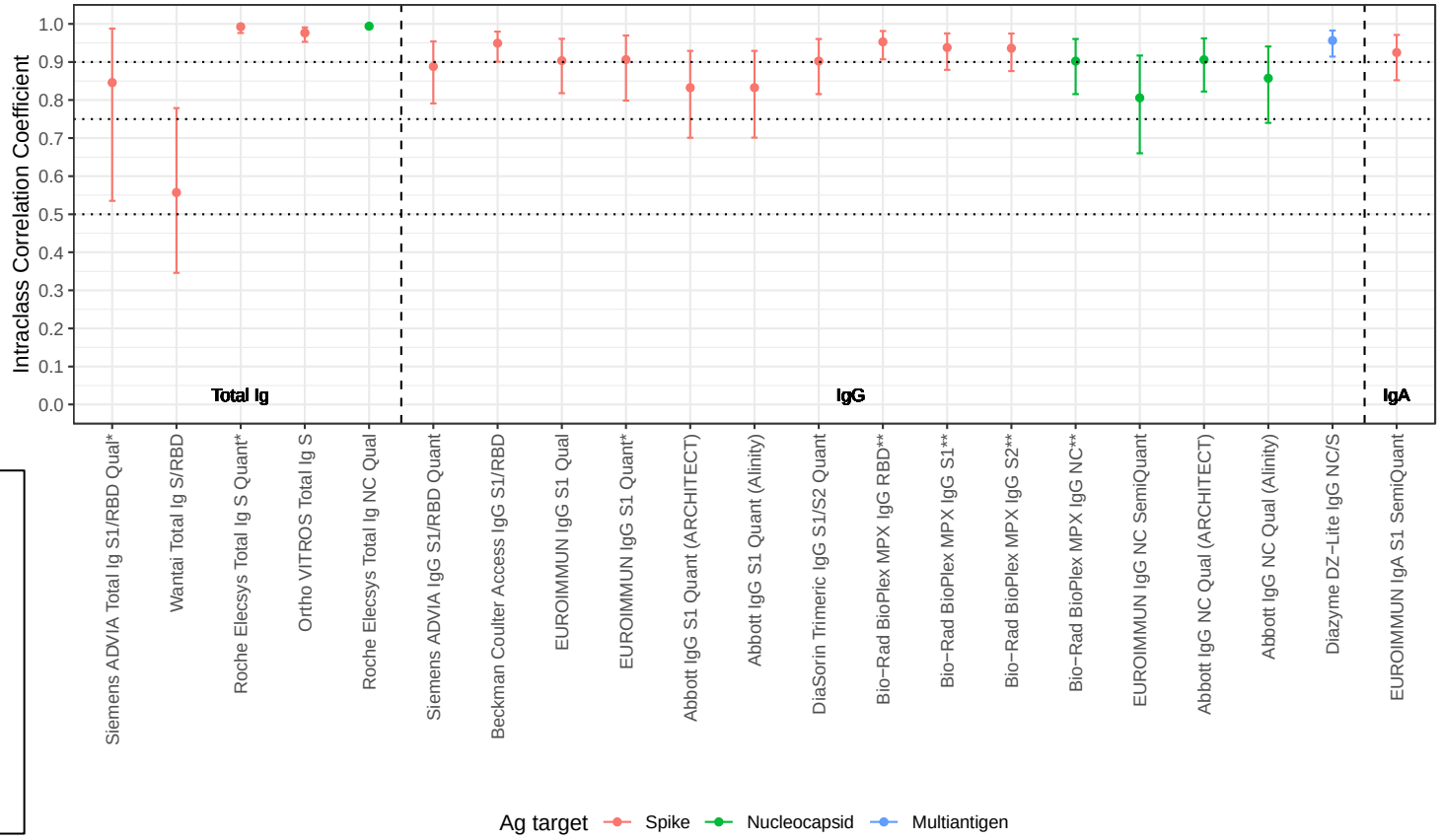
Coefficients of variation from blinded replicates

Blinded replicate sample	EUROIMMUN IgG S1 Qual	EUROIMMUN IgG S1 Quant	EUROIMMUN IgG N SemiQuant	EUROIMMUN IgA S1 SemiQuant	Roche Elecsys Total Ig N Qual	Roche Elecsys Total Ig S Quant	DiaSorin Trimeric IgG S1/S2 Quant	Siemens ADVIA Total Ig S1/RBD Qual	Siemens ADVIA IgG S1/RBD Quant	Abbott IgG N Qual (Alinity)	Abbott IgG S1 Quant (Alinity)	Abbott IgG N Qual (ARCHITECT)	Abbott IgG S1 Quant (ARCHITECT)	Bio-Rad BioPlex MPX IgG RBD	Bio-Rad BioPlex MPX IgG S1	Bio-Rad BioPlex MPX IgG S2	Bio-Rad BioPlex MPX IgG N	Bio-Rad Platelia Total Ig N SemiQuant	Beckman Coulter Access IgG S1/RBD	Diazyme DZ-Lite IgG NC/S	Wantai Total Ig S/RBD	Ortho VITROS Total Ig S
Sample 1	29.8	39.7	33.1	19.3	84.0	60.6	21.1	12.3	24.8	70.7	62.5	66.5	55.4	21.4	24.0	21.1	25.7	24.4	9.5	18.6	23.5	11.0
Sample 2	6.0	8.8	12.4	23.6	4.7	4.4	37.9	25.8	39.8	17.7	19.1	15.5	21.8	29.3	34.9	22.3	30.9	11.3	23.3	22.4	8.2	11.3
Sample 3	90.2	140.7	24.8	53.6	2.8	14.4	18.3	21.4	20.8	6.6	28.4	6.1	22.6	19.0	20.9	**	22.0	6.6	11.3	22.6	24.7	7.3
Sample 4	7.2	15.5	13.6	12.6	4.1	4.7	15.4	0.0	17.1	9.1	19.0	11.6	15.7	7.2	6.3	5.0	5.1	0.0	19.2	22.3	13.2	7.3
Sample 5	6.9	12.4	13.6	9.1	1.9	7.9	21.6	0.0	21.5	6.4	13.0	9.5	20.2	11.8	10.4	12.9	12.7	0.0	25.0	24.3	13.0	4.4
Sample 6	2.1	6.3	17.1	6.5	1.7	0.0	18.9	0.0	20.6	8.9	19.7	5.4	12.2	6.5	7.3	11.9	8.6	0.0	17.2	30.8	11.7	7.2
Sample 7	15.8	24.3	17.5	26.9	4.4	5.6	37.2	1.9	39.7	19.7	40.1	18.9	43.0	18.6	18.4	19.0	20.1	0.0	16.9	27.1	11.8	14.6
Sample 8	14.3	26.5	26.2	18.0	2.6	0.0	33.6	0.0	35.4	31.1	54.7	33.3	53.4	16.7	20.7	14.8	21.3	0.0	44.7	76.5	10.8	3.3
Sample 9	9.1	20.9	33.6	51.8	4.8	0.0	15.7	0.0	8.9	35.8	15.4	7.7	12.7	15.9	15.9	10.8	8.1	0.0	12.9	8.8	14.2	13.4
Sample 10	6.7	7.9	15.2	8.4	10.8	0.0	22.5	0.0	17.4	7.5	20.4	8.3	19.4	15.9	13.3	13.3	13.0	0.0	12.6	17.0	77.6	6.4
Sample 11	8.1	0.5	6.2	8.5	3.5	0.0	11.1	0.0	19.4	8.9	22.1	2.0	22.4	5.6	5.4	11.7	6.2	0.0	16.7	25.2	39.3	16.1
Sample 12	8.5	0.0	23.4	16.5	7.4	0.0	20.9	0.0	23.0	13.1	37.2	14.6	28.1	17.8	21.8	20.5	20.1	0.0	17.0	33.3	9.3	6.4
Sample 13	7.6	0.0	15.8	8.8	7.0	0.0	42.2	0.0	47.9	9.4	16.0	4.4	14.2	22.2	25.6	14.2	16.1	0.0	21.5	14.4	4.8	4.6
Sample 14	7.7	0.0	14.6	*	3.5	0.0	11.0	0.0	7.3	12.1	14.6	3.5	16.4	10.4	10.8	10.0	15.0	0.0	9.8	37.7	7.6	7.4
Sample 15	9.4	0.0	22.6	23.1	4.3	0.0	24.2	0.0	29.9	13.0	51.1	2.3	50.6	18.4	21.0	19.0	23.8	0.0	8.4	14.1	9.5	7.1

*** Not tested**
**** Negative**

vitalant℠
Research Institute

Intraclass Correlation Coefficients



ICC captures the proportion of total variation that is between-sample variation rather than within-sample variation (i.e., proportion of variation that is not from repeat measurements of the same sample).

*Measurements at maximum reactivity level excluded

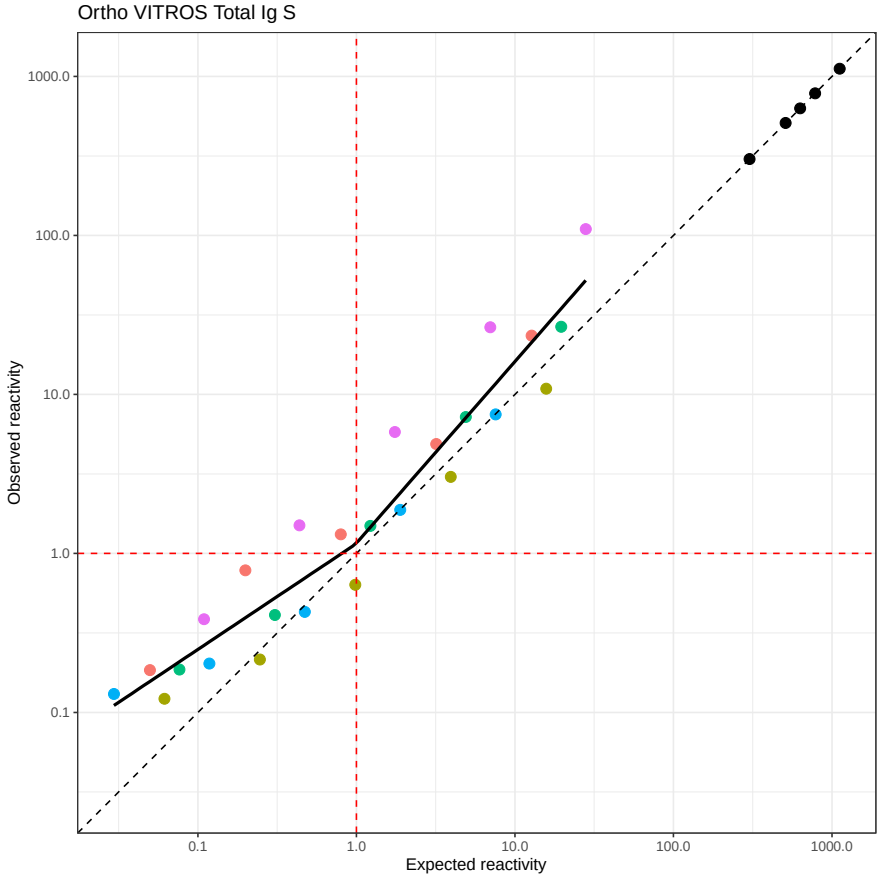
**On-board dilutions used to estimate U/mL in high reactivity samples

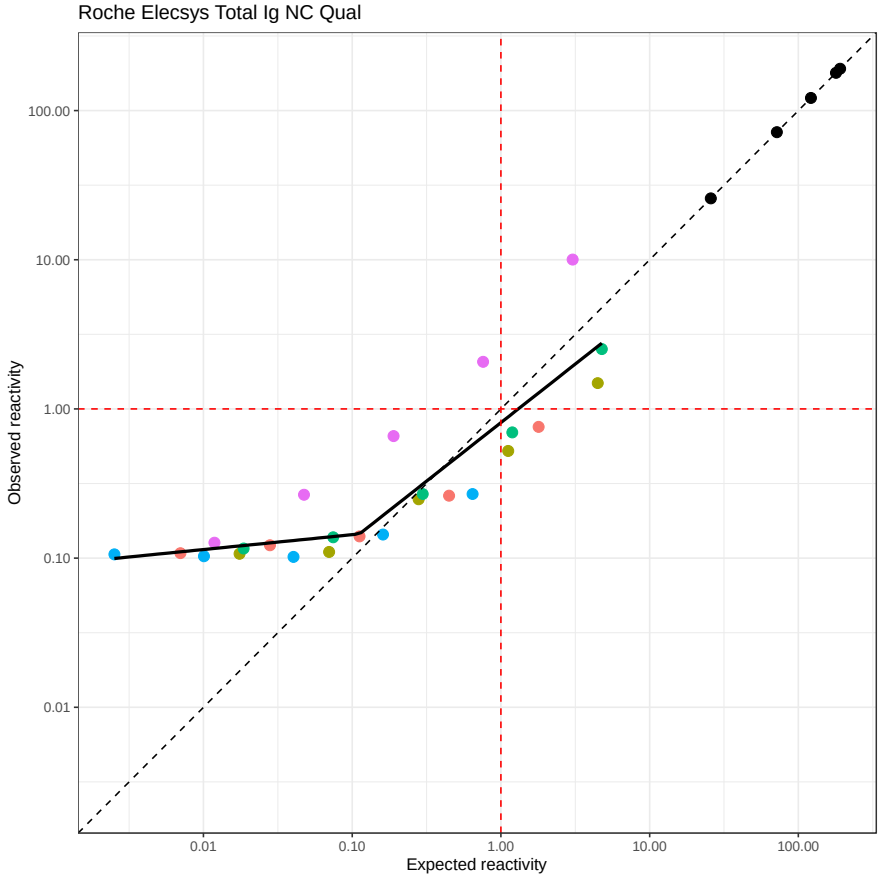
vitalant Research Institute

Dilutional performance

(examples)

vitalant℠
Research Institute





'Inflection point' is fitted. In general, the greater the dynamic range the more linear the response below the assay cutoff.

vitalant℠
Research Institute

Durability of bAb detection

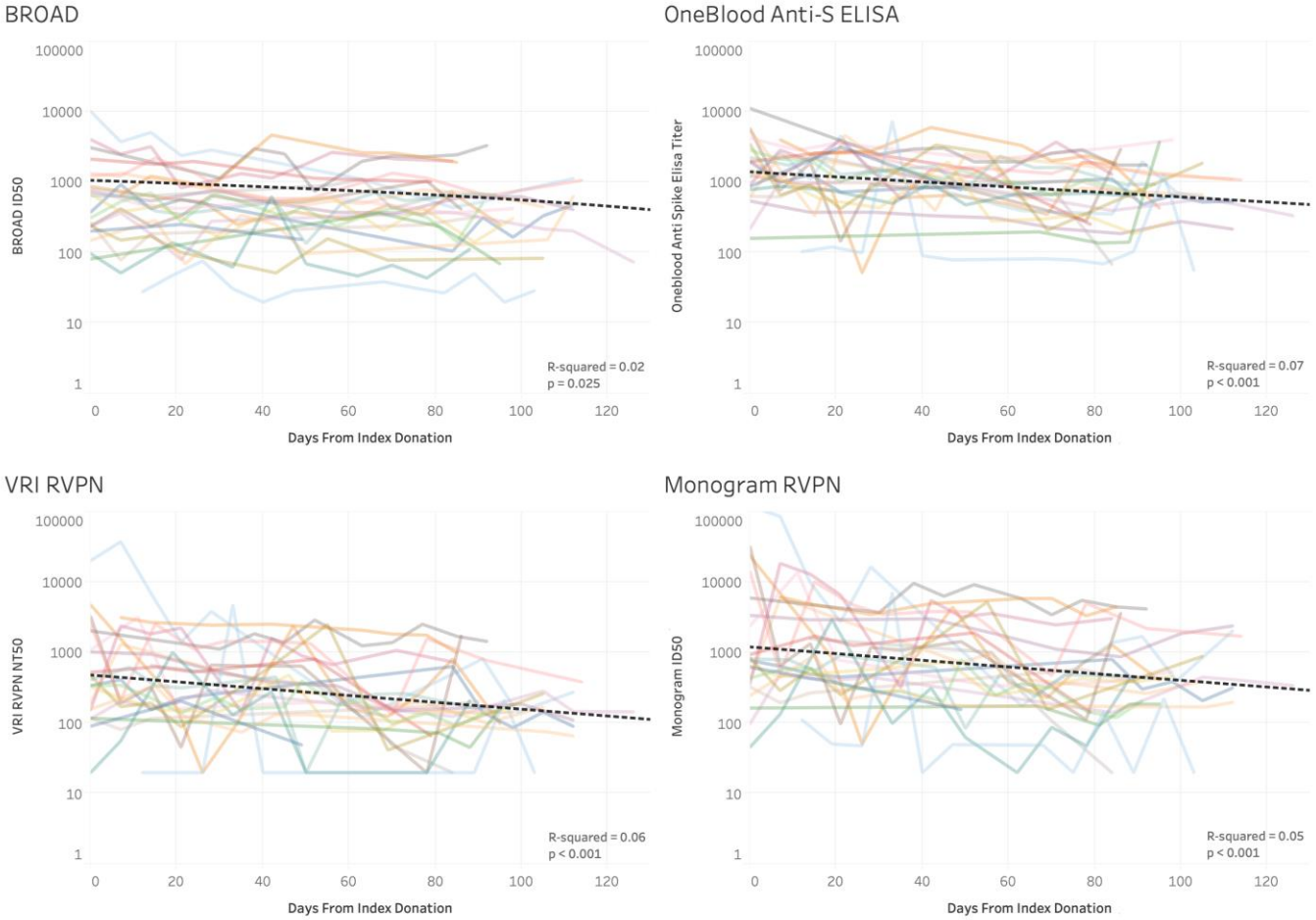
vitalant℠
Research Institute

Methods

- We fit linear mixed effects models for assay signal over time with donor random effects
  - *donor-specific slopes and intercepts*
  - log-transformed and rescaled assay signal for easier comparison of assays
  - if population average (‘fixed’) slope and its 95% confidence interval are < 0 this constitutes evidence of waning
- To estimate assay signal half-lives we fit models on the $\log_2$ scale and transform the slope into a half-live estimate

$$t_{1/2} = -\frac{1}{\beta}$$

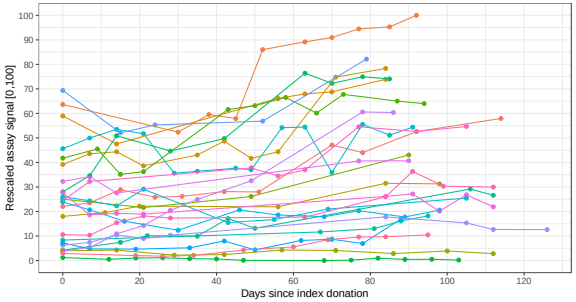
nAb titers over time



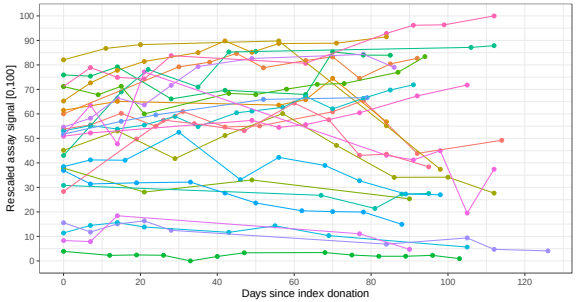
vitalant Research Institute

Assay signal over time (rescaled)

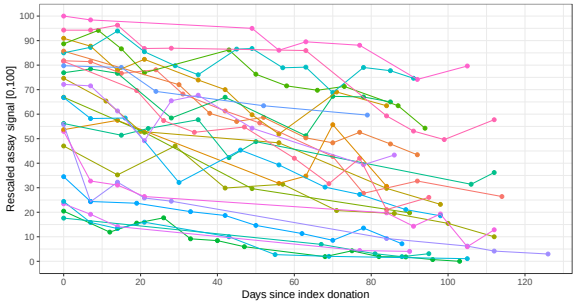
Ortho VITROS Total Ig S



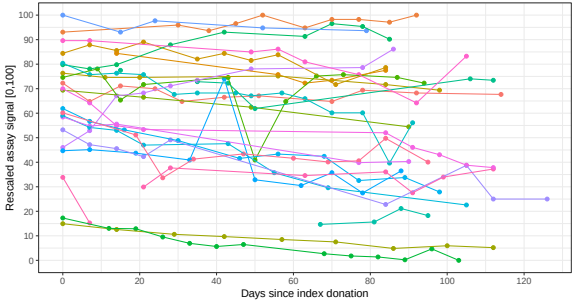
Roche Elecsys Total Ig N Qual



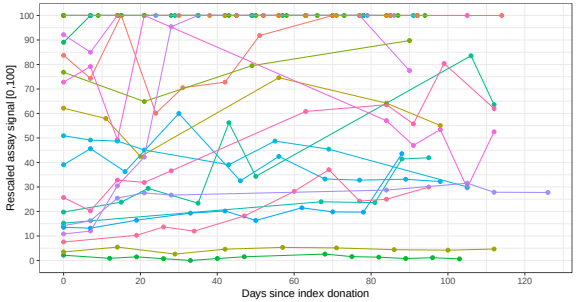
Abbott IgG N Qual (ARCHITECT)



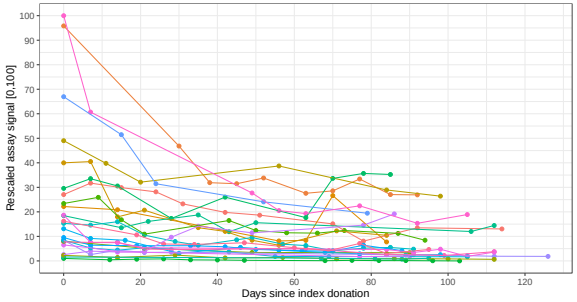
Ortho VITROS IgG S



Roche Elecsys Total Ig S Quant

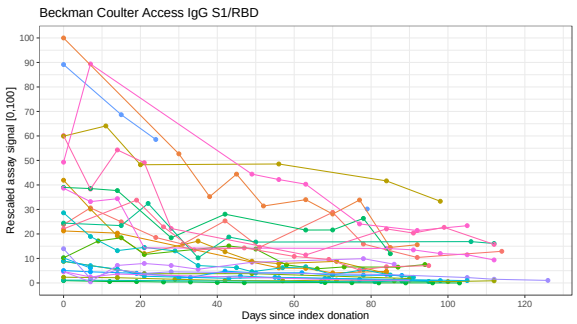


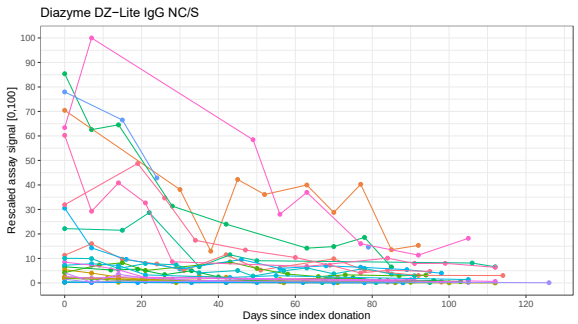
Abbott IgG S1 Quant (ARCHITECT)

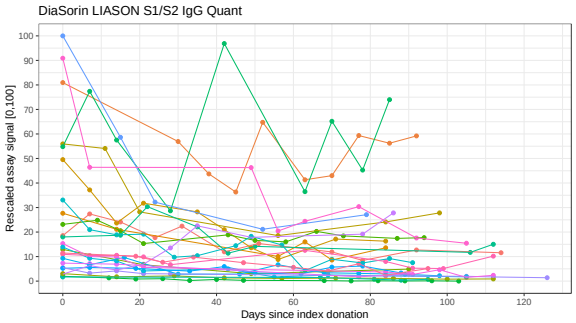


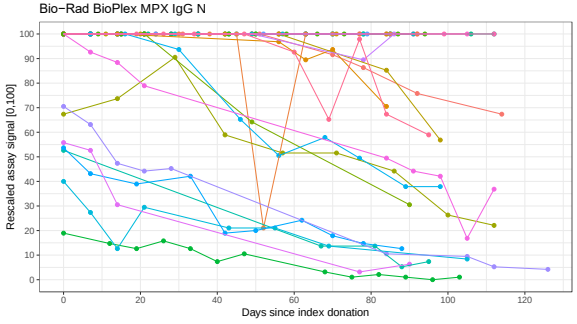
vitalant℠
Research Institute

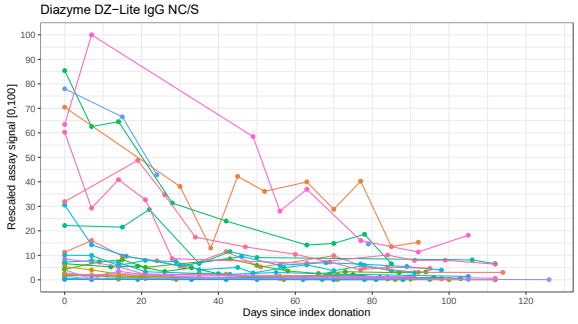
Assay signal over time (rescaled)

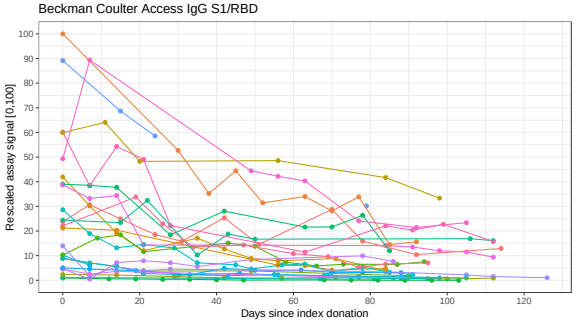






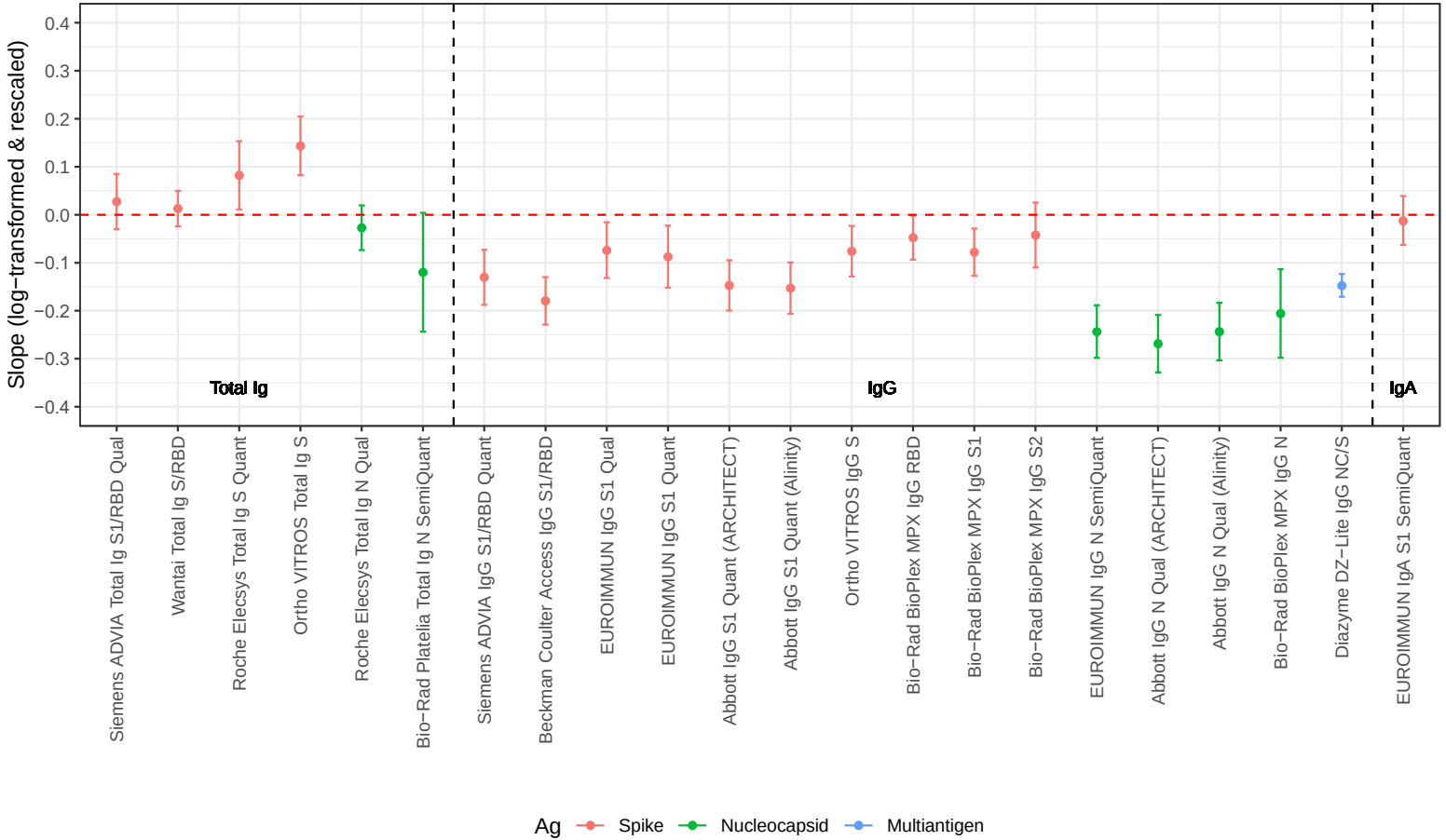






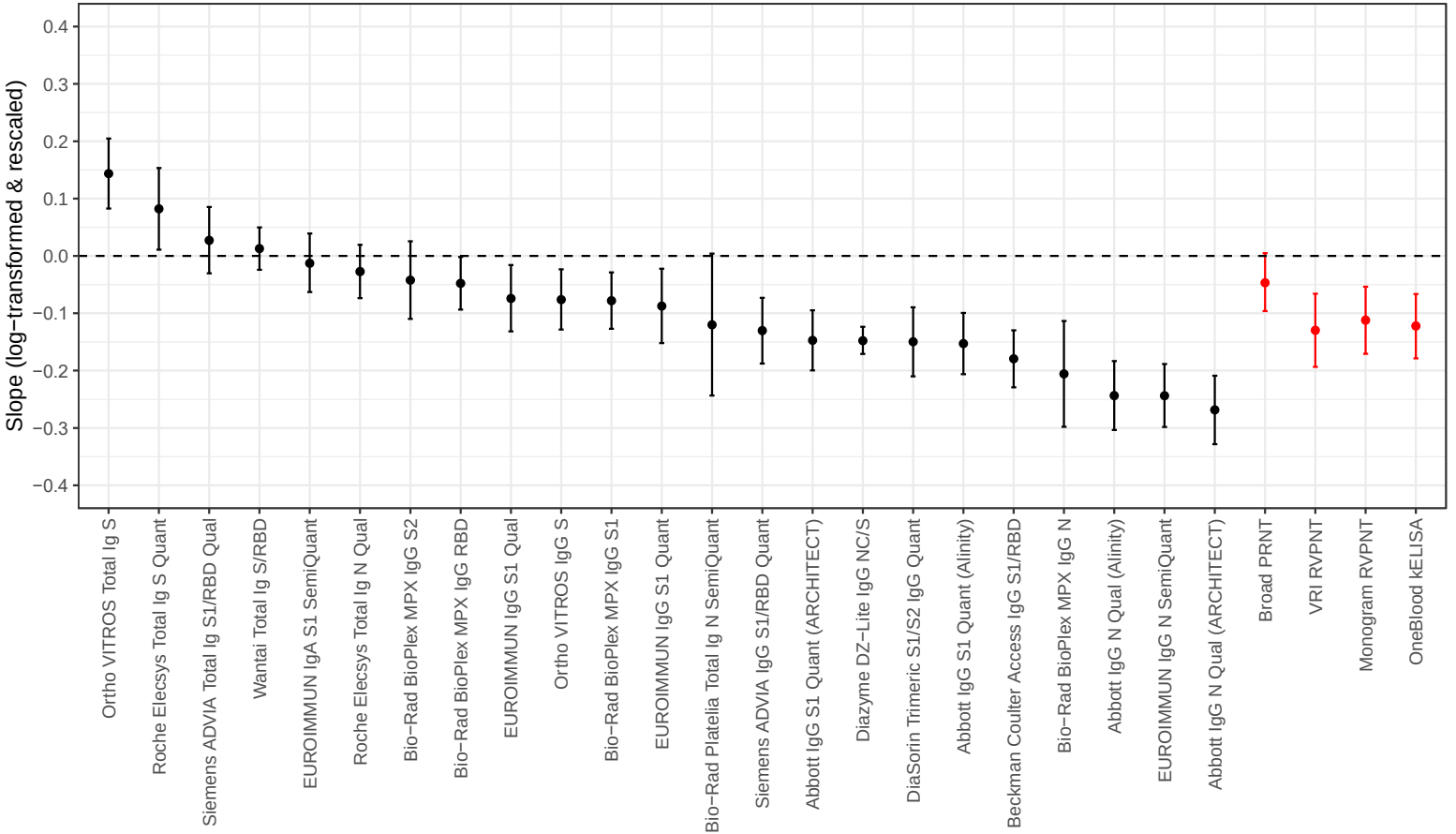
vitalant℠
Research Institute

Standardized slopes of bAb signal intensity from regression analysis



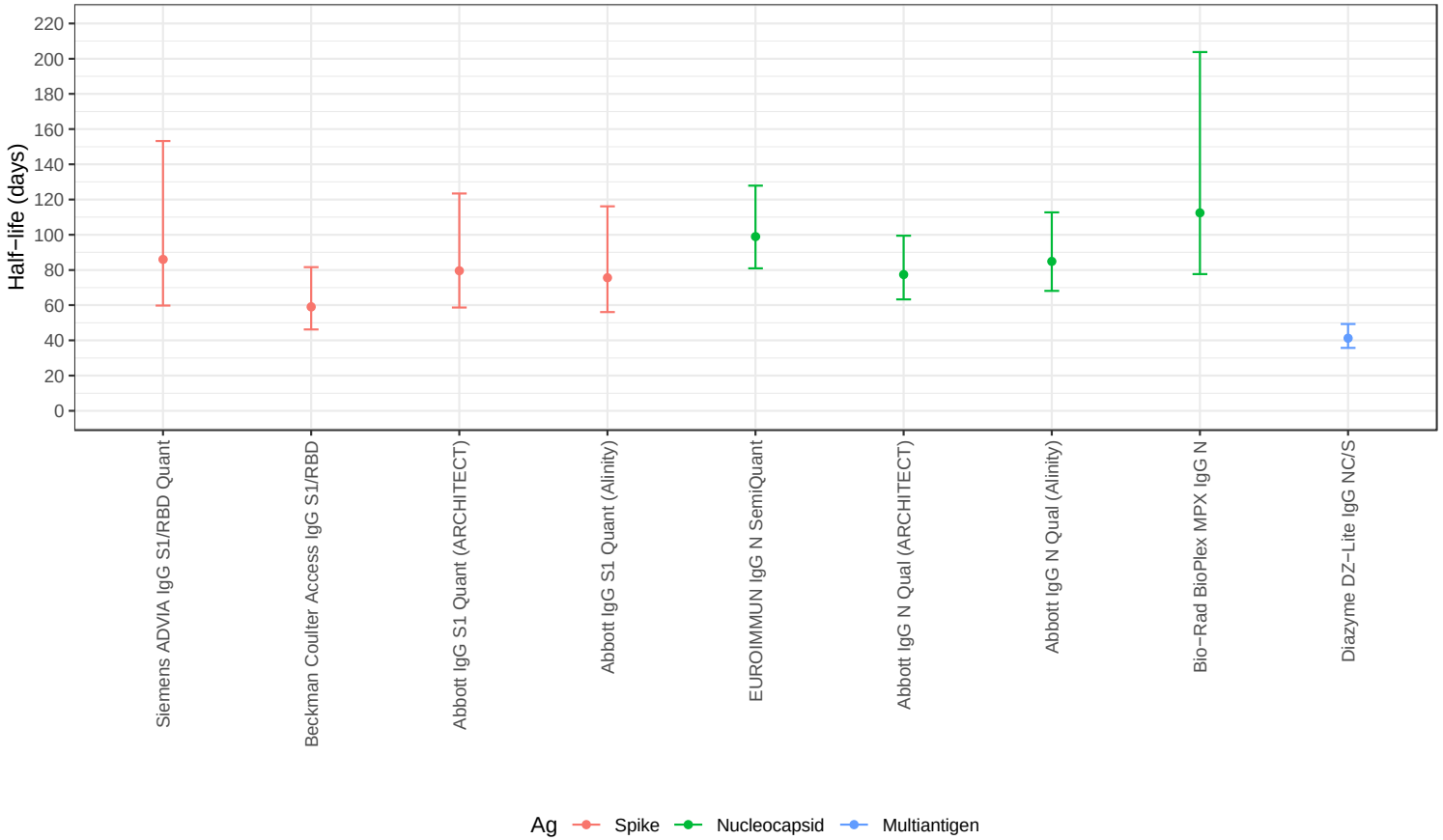
vitalant
Research Institute

Standardized slopes of bAb signal intensity and nAb titers



vitalant℠
Research Institute

Assay signal half-lives for assays showing rapid waning



Mean time to seroreversion depends on distribution of initial reactivity.

vitalant℠
Research Institute

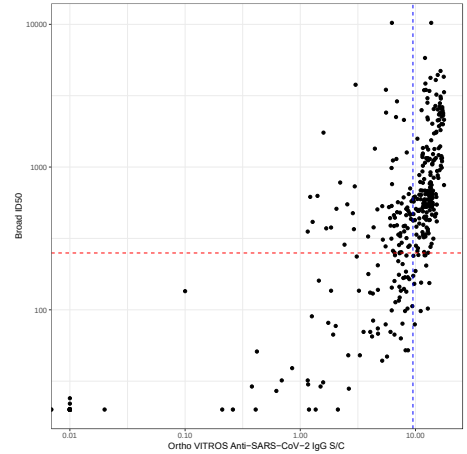
Assay characteristics associated with waning

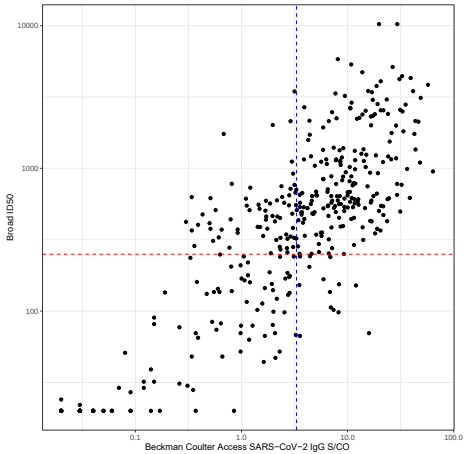
- Neutralizing Ab titers wane after recovery over several months
- Regression analysis suggests both assay format (‘direct’/’indirect’) and Ag target matter
  - similar impact on rate of waning
  - total Ig assays are stable or even show increasing reactivity over time
  - anti-N assays show more rapid waning, probably reflecting underlying Ab dynamics

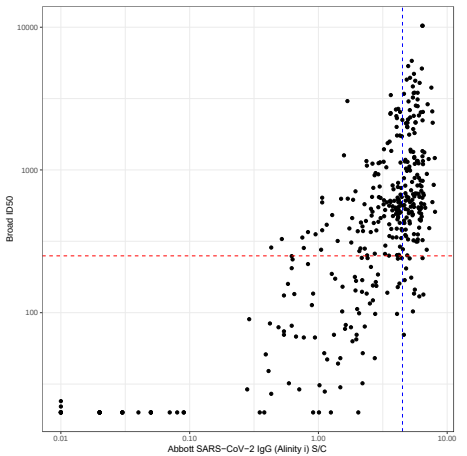
Correlation of bAb signal intensity with nAb titers

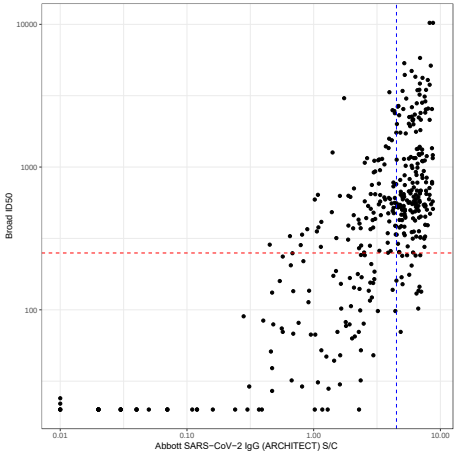
vitalant Research Institute

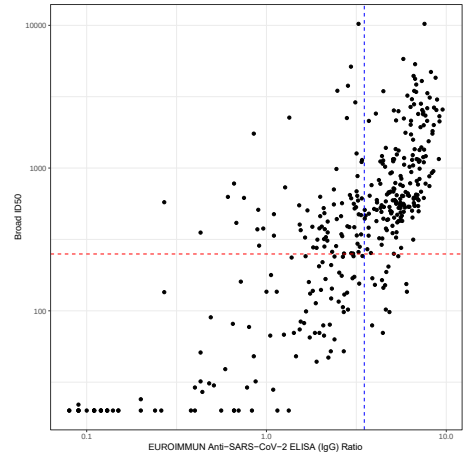
Correlation of bAb assays signal with nAb titer

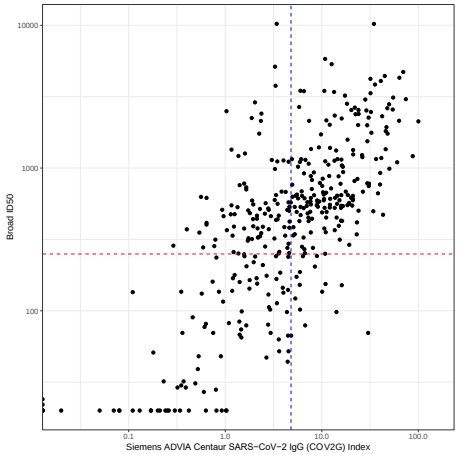


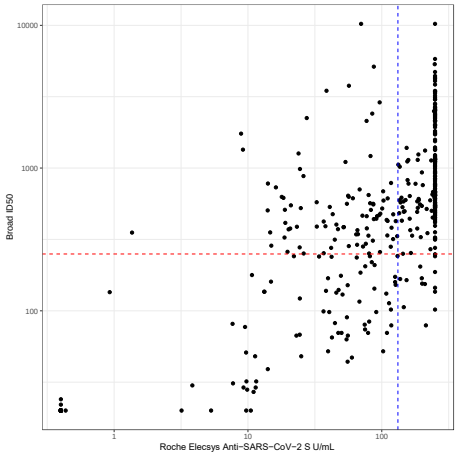


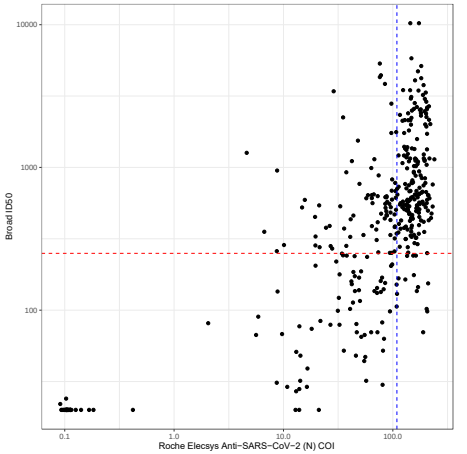




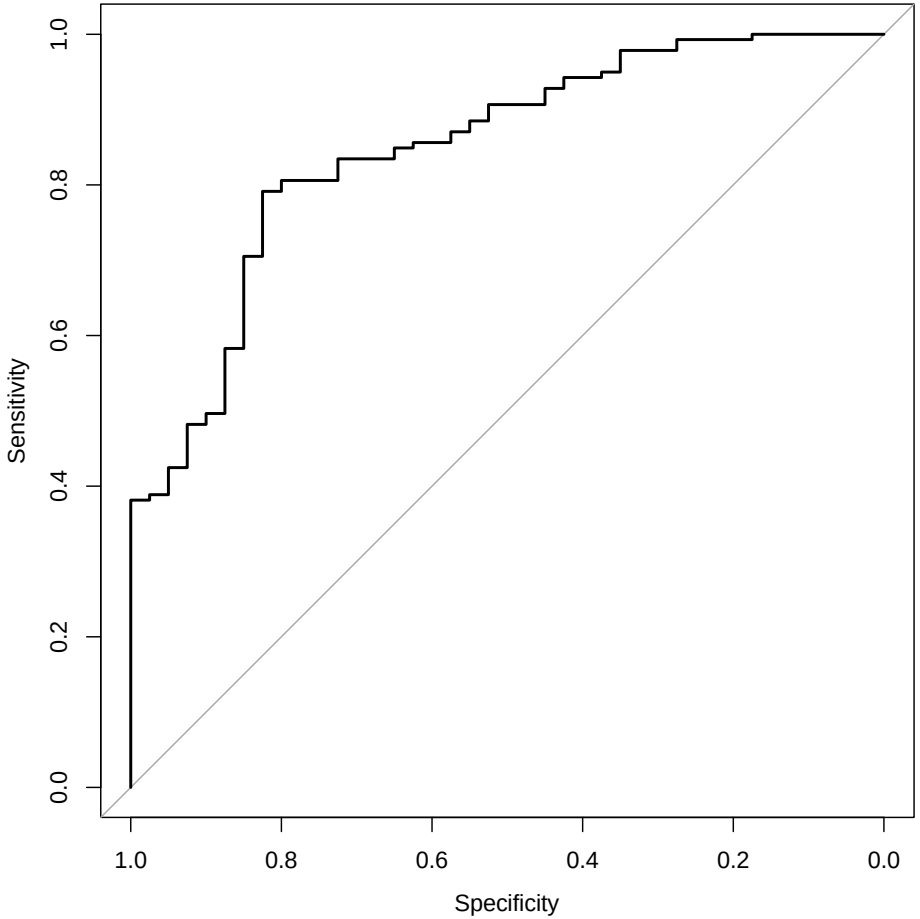




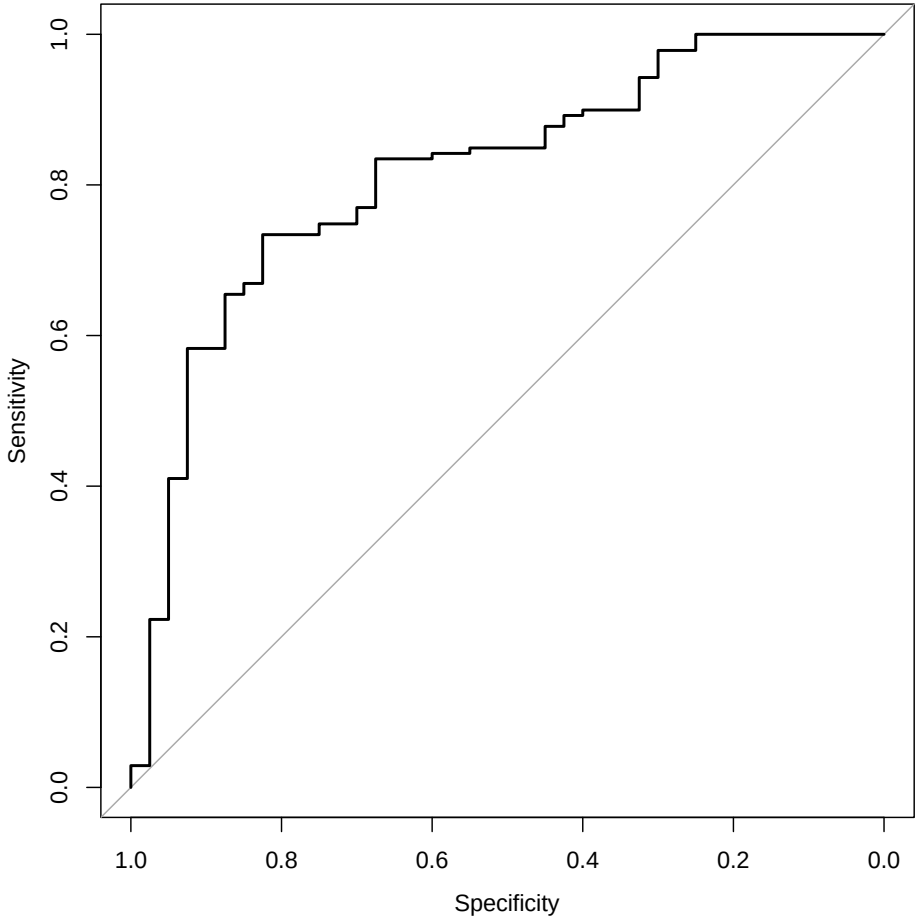




Ortho VITROS Total Ig S



Roche Elecsys Total Ig NC Qual



vitalant℠
Research Institute

ROC analysis for Broad ID50 ≥ 1:250

Assay	AUC	Optimal threshold (Youden J statistic)	Se	Sp	PPV	NPV
Siemens ADVIA Total Ig S1/RBD Qual	0.827	6.545	75.4%	85.0%	94.5%	50.0%
Wantai Total Ig S/RBD	0.808	15.295	83.5%	75.0%	92.1%	56.6%
Roche Elecsys Total Ig S Quant	0.877	69.065	79.1%	90.0%	96.5%	55.4%
Ortho VITROS Total Ig S	0.847	140.985	79.1%	82.5%	94.0%	53.2%
Roche Elecsys Total Ig NC Qual	0.814	82.625	73.4%	82.5%	93.6%	47.1%
Bio-Rad Platelia Total Ig NC SemiQuant	0.635	2.584	97.8%	32.5%	83.4%	81.3%
Siemens ADVIA IgG S1/RBD Quant	0.862	2.12	85.5%	72.5%	91.5%	59.2%
Beckman Coulter Access IgG S1/RBD	0.894	2.88	81.6%	82.5%	94.1%	56.9%
EUROIMMUN IgG S1 Qual	0.904	2.815	82.0%	85.0%	95.0%	57.6%
EUROIMMUN IgG S1 Quant	0.905	27.21	79.9%	87.5%	95.7%	55.6%
Ortho VITROS IgG S	0.896	8.62	78.2%	89.5%	96.3%	54.0%
Abbott IgG S1 Quant (ARCHITECT)	0.888	450.5	88.5%	75.0%	92.5%	65.2%
Abbott IgG S1 Quant (Alinity)	0.893	362.15	89.9%	75.0%	92.6%	68.2%
DiaSorin Trimeric IgG S1/S2 Quant	0.876	44.35	74.1%	87.5%	95.4%	49.3%
EUROIMMUN IgG NC SemiQuant	0.852	2.25	84.9%	77.5%	92.9%	59.6%
Bio-Rad BioPlex MPX IgG RBD	0.886	65.5	87.1%	72.5%	91.7%	61.7%
Bio-Rad BioPlex MPX IgG S1	0.907	83	69.8%	95.0%	98.0%	47.5%
Bio-Rad BioPlex MPX IgG S2	0.866	5.5	72.7%	85.0%	94.4%	47.2%
Abbott IgG NC Qual (ARCHITECT)	0.833	3.24	88.5%	72.5%	91.8%	64.4%
Abbott IgG NC Qual (Alinity)	0.822	2.77	88.5%	72.5%	91.8%	64.4%
Bio-Rad BioPlex MPX IgG NC	0.853	75.5	76.3%	85.0%	94.6%	50.7%
Diazyme DZ-Lite IgG NC/S	0.790	1.68	82.7%	70.0%	90.6%	53.8%
EUROIMMUN IgA S1 SemiQuant	0.828	1.315	66.9%	82.5%	92.7%	42.9%

vitalant℠
Research Institute

Suitability of assays for serosurveillance

vitalant
Research Institute

Assay	Sensitivity >95%	Specificity >99%	Stable bAb detection	Reproducibility *(preliminary)**	Optimal for serosurveillance?
Siemens ADVIA Total Ig S1/RBD Qual	✓	✗	✓	✓	✗
Wantai Total Ig S/RBD	✓	✗	✓	✗	✗
Roche Elecsys Total Ig S Quant	✓	✓	✓	✓	✓
Ortho VITROS Total Ig S	✓	✓	✓	✓	✓
Roche Elecsys Total Ig NC Qual	✓	✓	✓	✓	✓
Bio-Rad Platelia Total Ig NC SemiQuant	✓	✓	✓	✓	✓
Siemens ADVIA IgG S1/RBD Quant	✗	✗	✗	✓	✗
Beckman Coulter Access IgG S1/RBD	✗	✗	✗	✓	✗
EUROIMMUN IgG S1 Qual	✓	✓	✗	✓	✗
EUROIMMUN IgG S1 Quant	✗	✓	✗	✓	✗
Abbott IgG S1 Quant (ARCHITECT)	✓	✓	✗	✓	✗
Abbott IgG S1 Quant (Alinity)	✓	✓	✗	✓	✗
DiaSorin Trimeric IgG S1/S2 Quant	✗	✓	✗	✓	✗
EUROIMMUN IgG NC SemiQuant	✗	✗	✗	✓	✗
Abbott IgG NC Qual (ARCHITECT)	✗	✓	✗	✓	✗
Abbott IgG NC Qual (Alinity)	✗	✓	✗	✓	✗
Bio-Rad BioPlex MPX	✓	✓	✓**	✓	✓
Diazyme DZ-Lite IgG NC/S	✗	✗	✗	✓	✗
EUROIMMUN IgA S1 SemiQuant	✗	✗	✓	✓	✗
Quotient Mosaiq Microarray	✗	✓	?	?	✗

* Based on quantitative repeatability assessment. ** One Ag target (S2) is stable.

vitalant℠ Research Institute

Surveillance of Blood Donors to Document Vaccine Penetrance and Potential Vaccine Breakthrough Infections

- Most current SARS-CoV-2 US vaccines will induce reactivity (VISP) to SARS-CoV-2 Spike S1 and ACE-2 receptor binding domains [RBD] but not nucleocapsid (NC) antigens

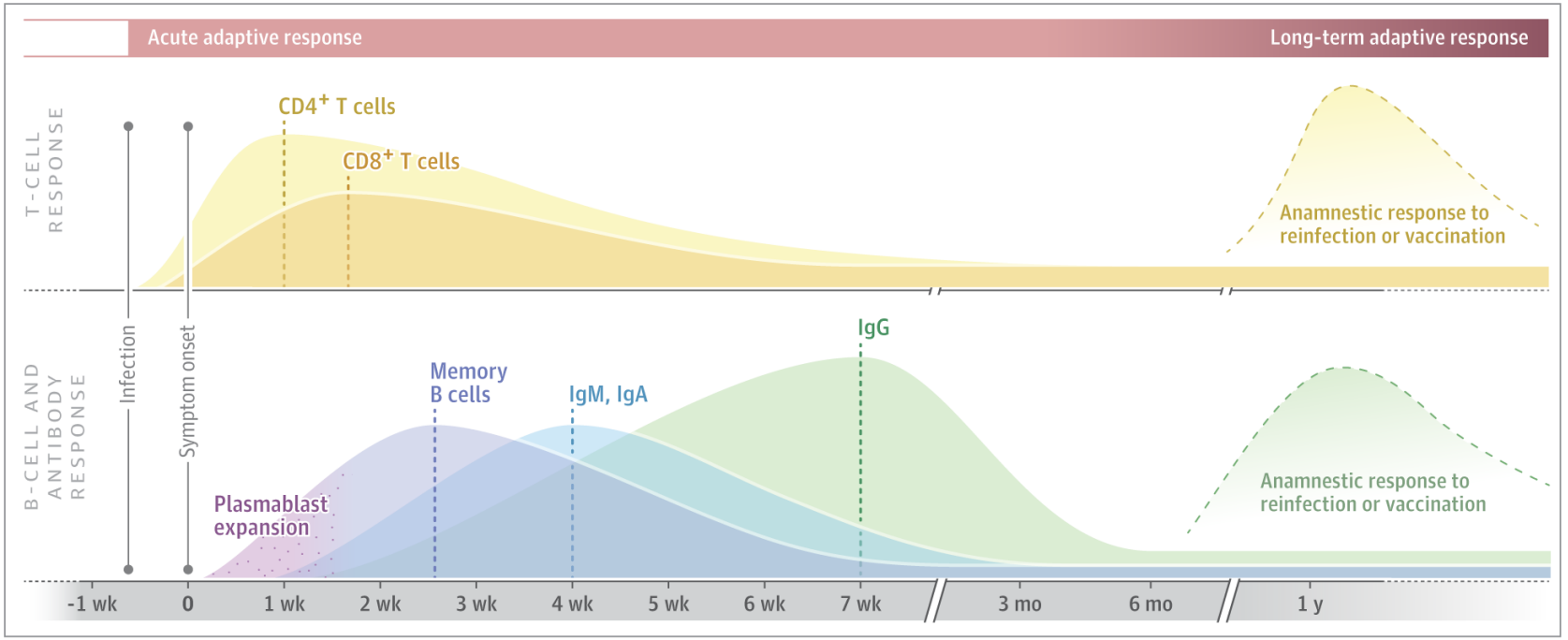
- Vaccinated donors screened reactive through MASS-D or universal testing would test discordant on S1 and NC Total Ig assays

- We are evaluating alternative multiplexed S, RBD, NC assays, such as the Bio-Rad BioPlex Luminex Assay, Meso-Scale Diagnostics (MSD). ThermoFisher/One-Lambda Luminex and UC Irvine COVAM Arrays, allowing for discrimination and quantitation of these differential immune responses as well as VOI and VOC antibodies

vitalant℠
Research Institute

COVID-19 and the Path to Immunity

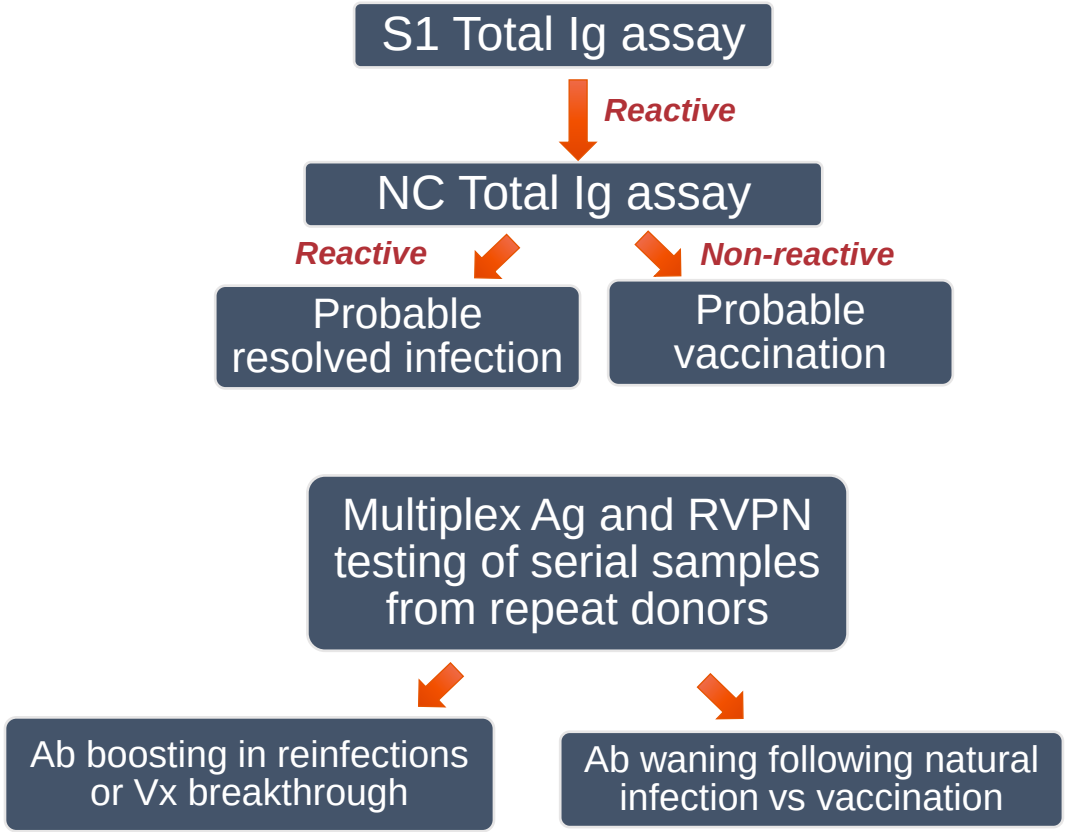
David S. Stephens, MD & M. Juliana McElrath, MD, PhD



JAMA. 2020;324(13):1279-1281

vitalant Research Institute

Algorithm for Serosurveillance following Vaccine Rollout



vitalant℠ Research Institute

Acknowledgements

VRI San Francisco

Mars Stone
Eduard Grebe
Graham Simmons
Clara di Germanio
Brian Custer
Hasan Sulaeman
Honey Dave
Lily McCann
Renata Buccheri

Research Operations Core
Immunology Core
Simmons lab

VRI Denver

Kathleen Kelly
Ray Moss
Larry J. Dumont

University of British Columbia

Steven H. Kleinman

University of São Paulo

Ester Sabino
Márcio K. Oikawa
Lewis Buss

US Centers for Disease Control and Prevention

Jefferson Jones
Brad Biggerstaff
Natalie Thornburg

British Columbia Centre for Disease Control

Mel Krajden

World Health Organisation

Polina Brangel

vitalant℠
Research Institute

Thank you

vitalant℠
Research Institute