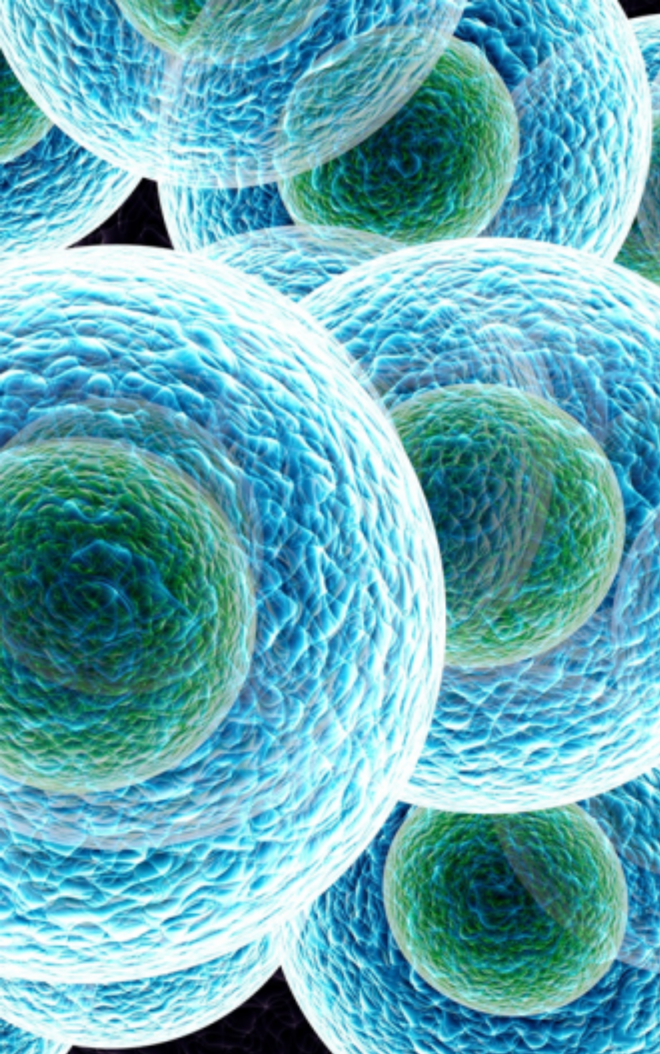

Development of SARS-CoV-2 ECL Serology Assays

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HELPING DELIVER LIFE CHANGING THERAPIES

PPD®

SARS-CoV-2 IgG/IgM/IgA Serology Assays
Assay Format

+ Assays aimed to quantitate the levels of anti-RBD antibodies, specific for SARS-CoV2 infection

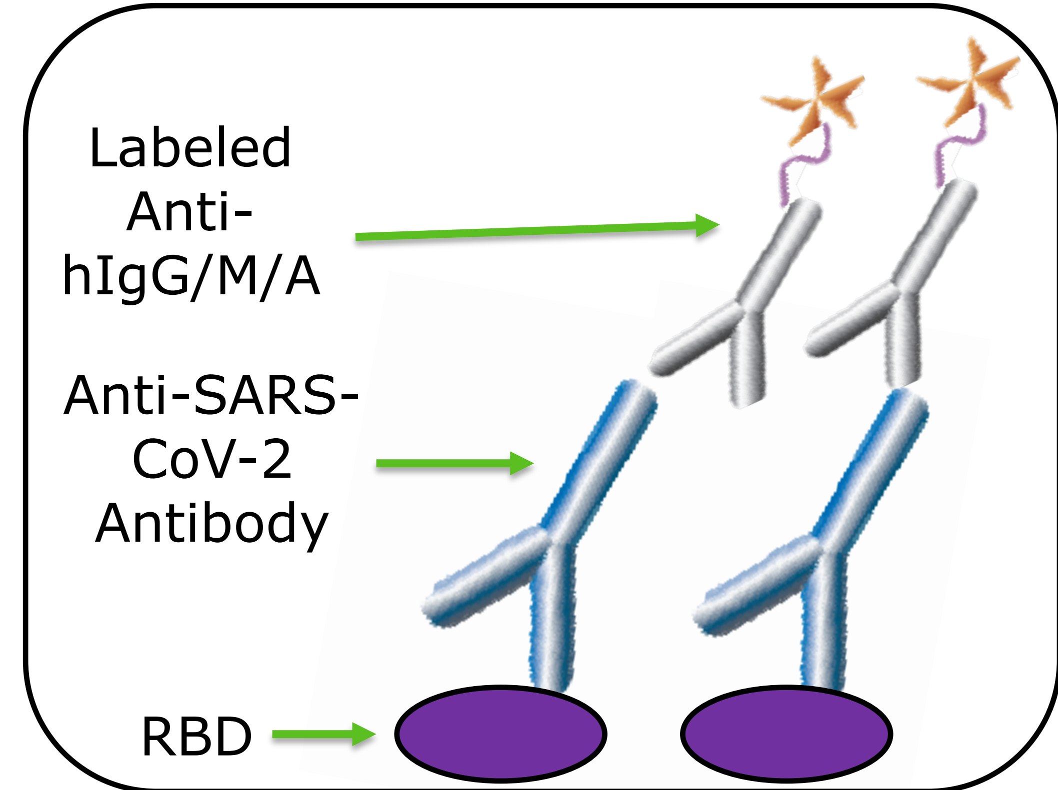
+ Utilized commercial RBD reagents and in-house prepared labeled reagents (SulfoTag detection Ab)

+ Unique elements to the approach:

  --Use of MSD/ECL platform

  --Aim to eliminate need for titering

  --Use mix of ADA and biomarker approaches

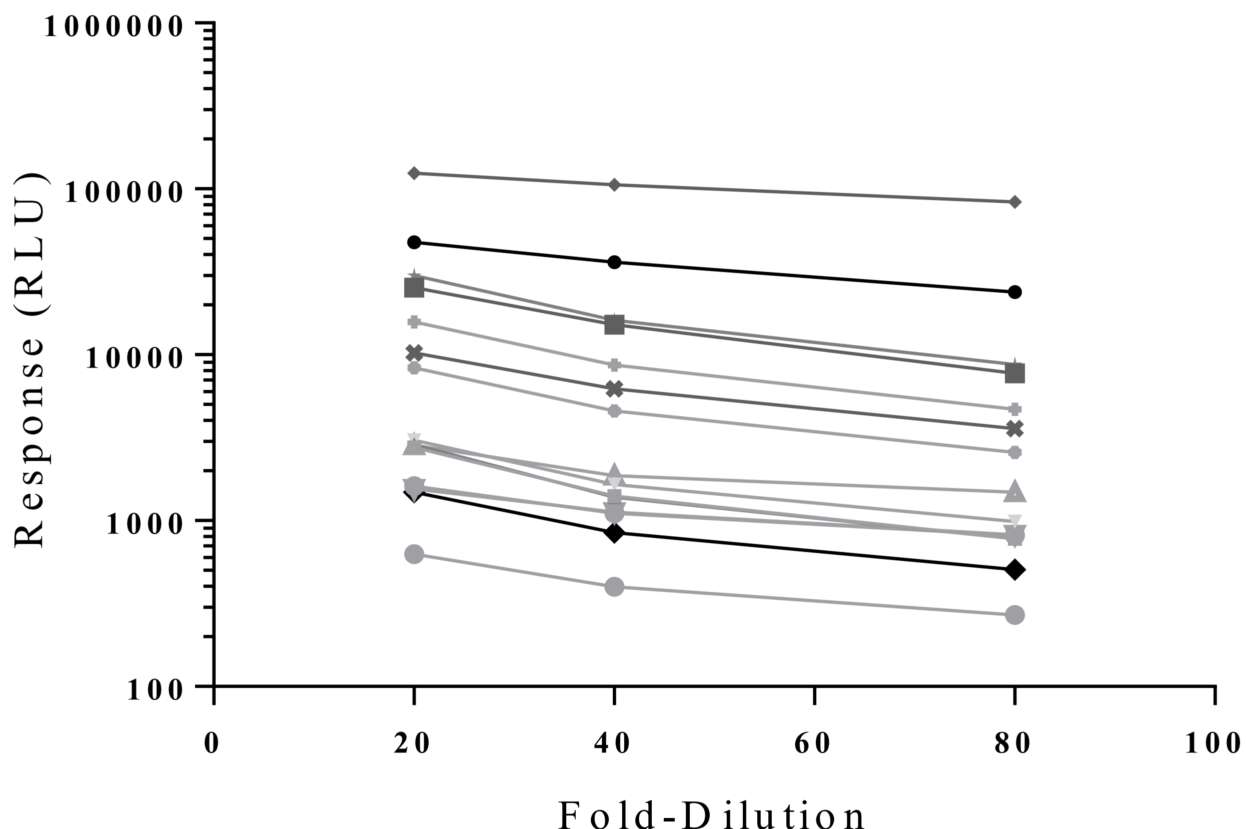


Optimization of Assay Format: Need for Custom Normalization

+ Initial assessment revealed that assay potential varied with the subset of COVID-negative serum samples used

+ For example, normal serum samples typically had signals in the thousands, but certain individuals showed very elevated signals (false positive)

+ Optimized buffer composition (base buffer formulation utilizes Blocker Casein), which improved background, but issues persisted



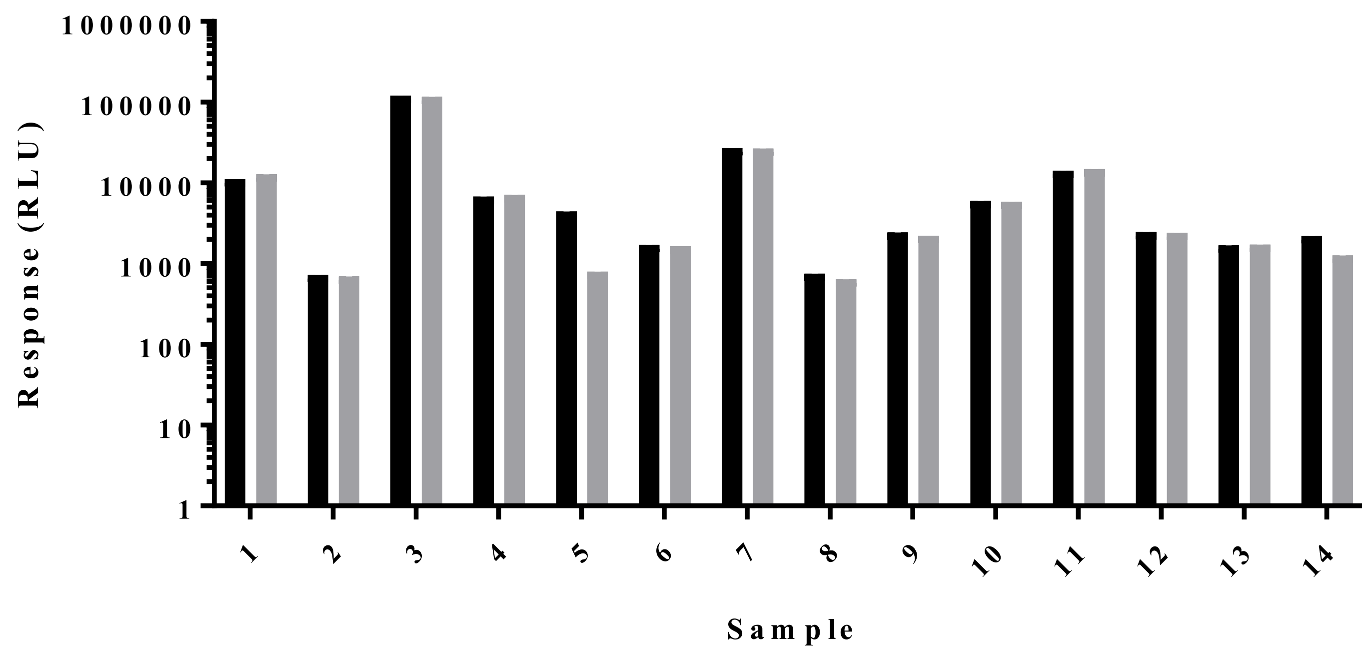
Optimization of Assay Format: Need for Custom Normalization

- The background was not specific to coated wells!
- Similar background profiles observed for coated wells vs. wells that were blocked only.
- Therefore, not due to the issue of cross-reactivity from other anti-coronavirus antibodies.
- Allows for a custom normalization strategy: subtract the signal from blank wells from the coated wells.

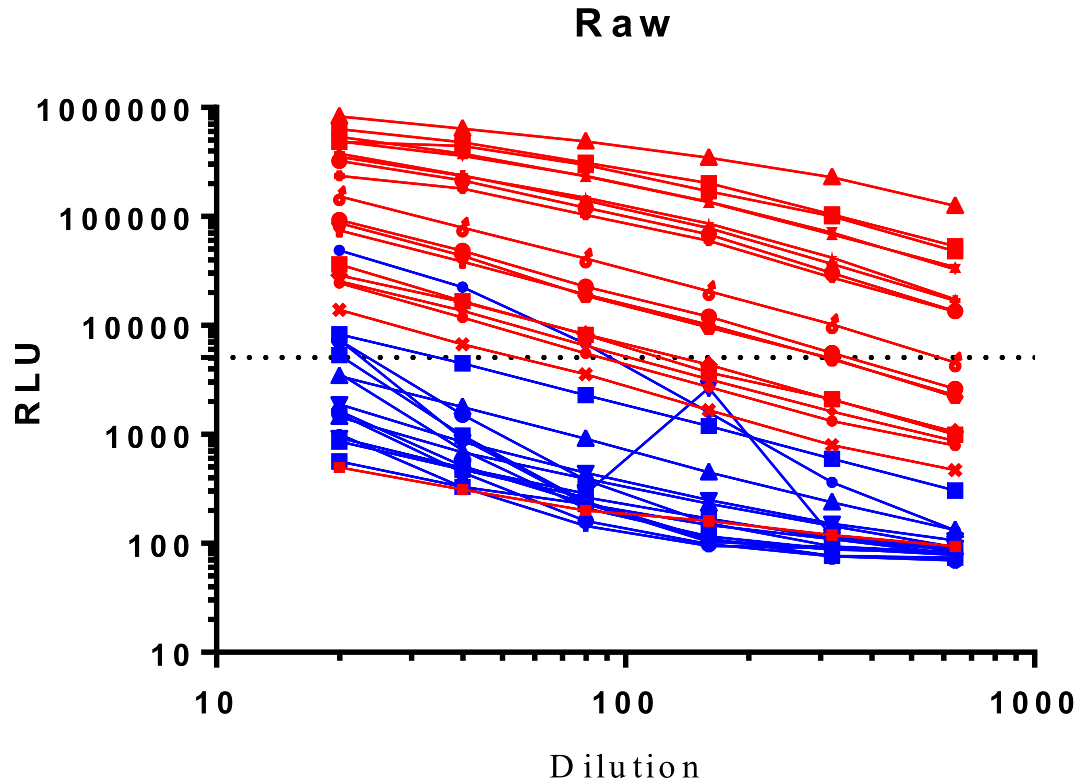
ECL Signal: Negative serum

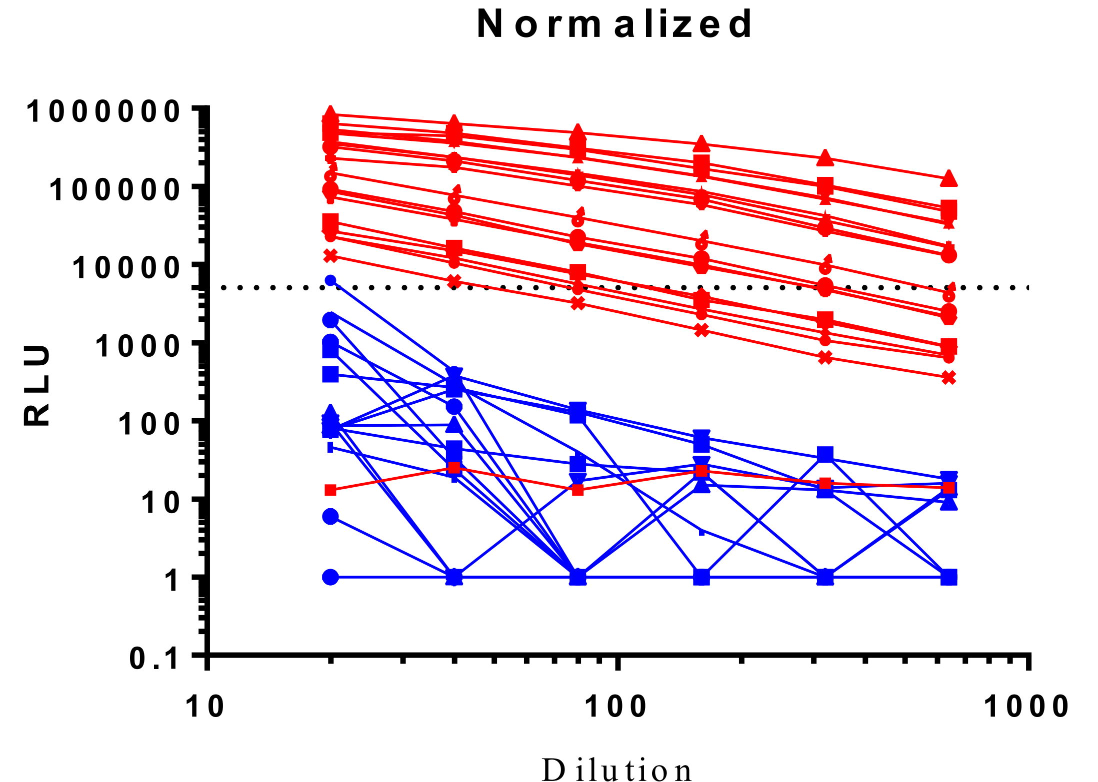
Black bars: Coated & blocked wells

Gray bars: Blocked wells



Advantages of normalization apparent in patient sample comparison



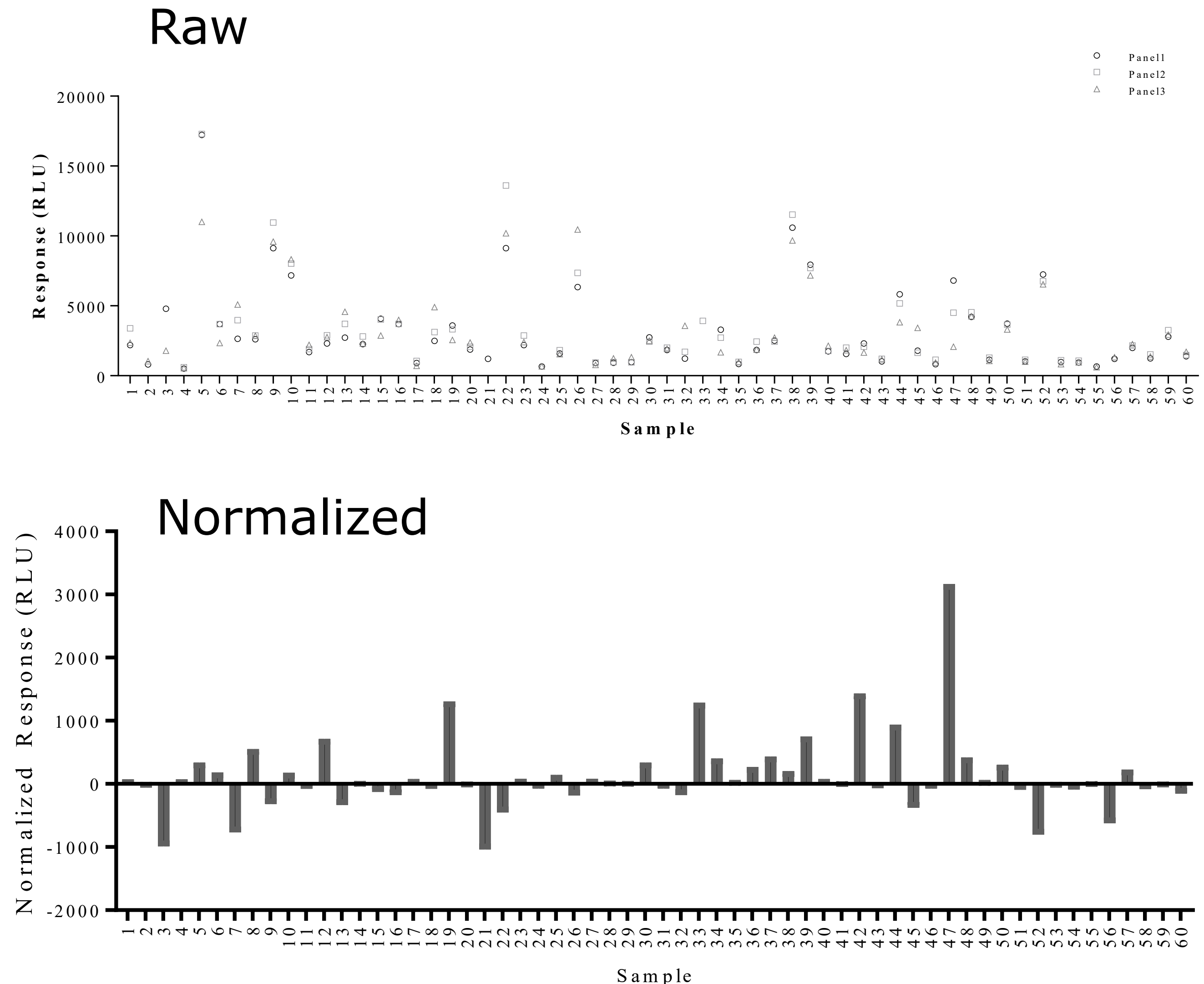


Red lines = Positive Samples

Blue = Negative Samples

Cut point approach demonstrates potential of the method

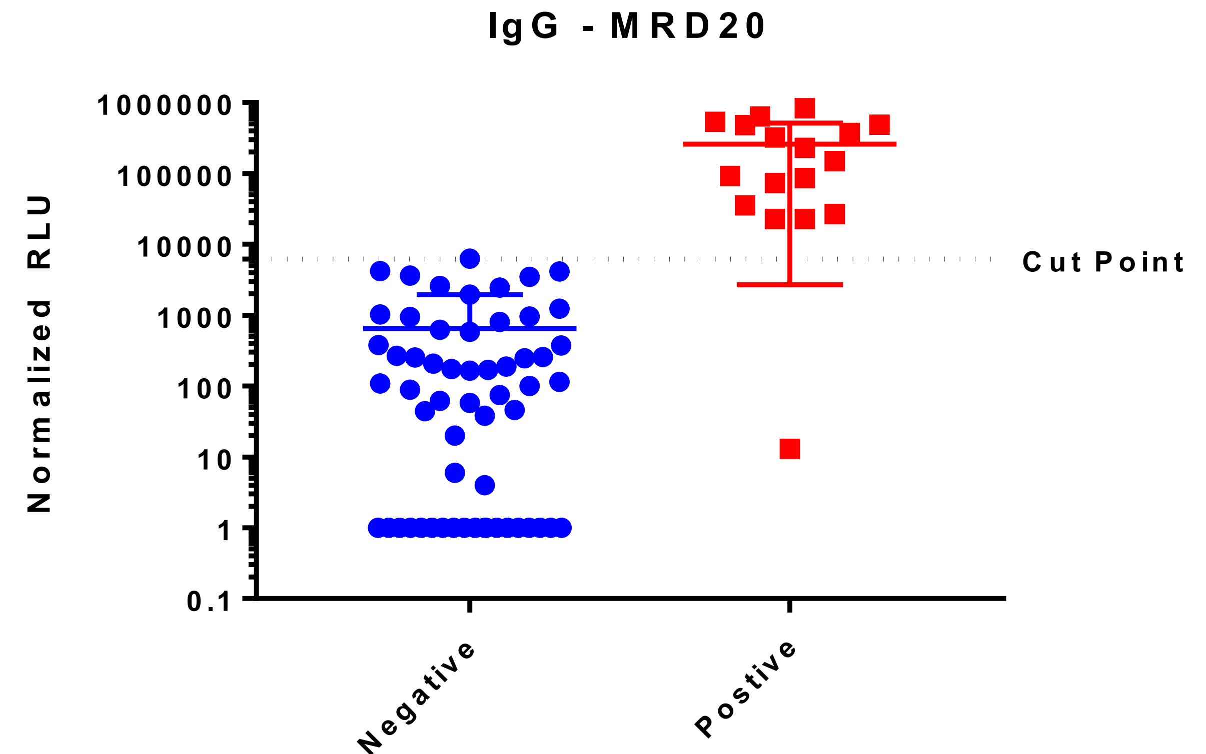
+ Assessed the performance of the method with background correction across a set of 60 samples, run across 3 days (commercial serum)
+ All samples were COVID-negative: collected in U.S., before Jan. 2020
+ Upper panel: Demonstrates reproducibility of raw (prior to correction) signals
+ Lower panel: Demonstrates utility of the background correction approach.



Anti-SARS-CoV-2 IgG Serology Assay
Sample results at 1:20 dilution

+ A panel of negative samples (n = 59) was used to estimate a statistical cut point to discriminate between positive and negative samples
+ This allows an estimate of clinical sensitivity and specificity
+ Samples with PCR confirmed COVID-19 diagnoses (n=19) were used to verify cut point performance

	Negative Sample	Positive Sample
Positive Result	1/59 (1.7%)	18/19 (94.7%)
Negative Result	58/59 (98.3%)	1/19 (5.3%)
Diagnostic Sensitivity	94.7%	
Diagnostic Specificity	98.5%	



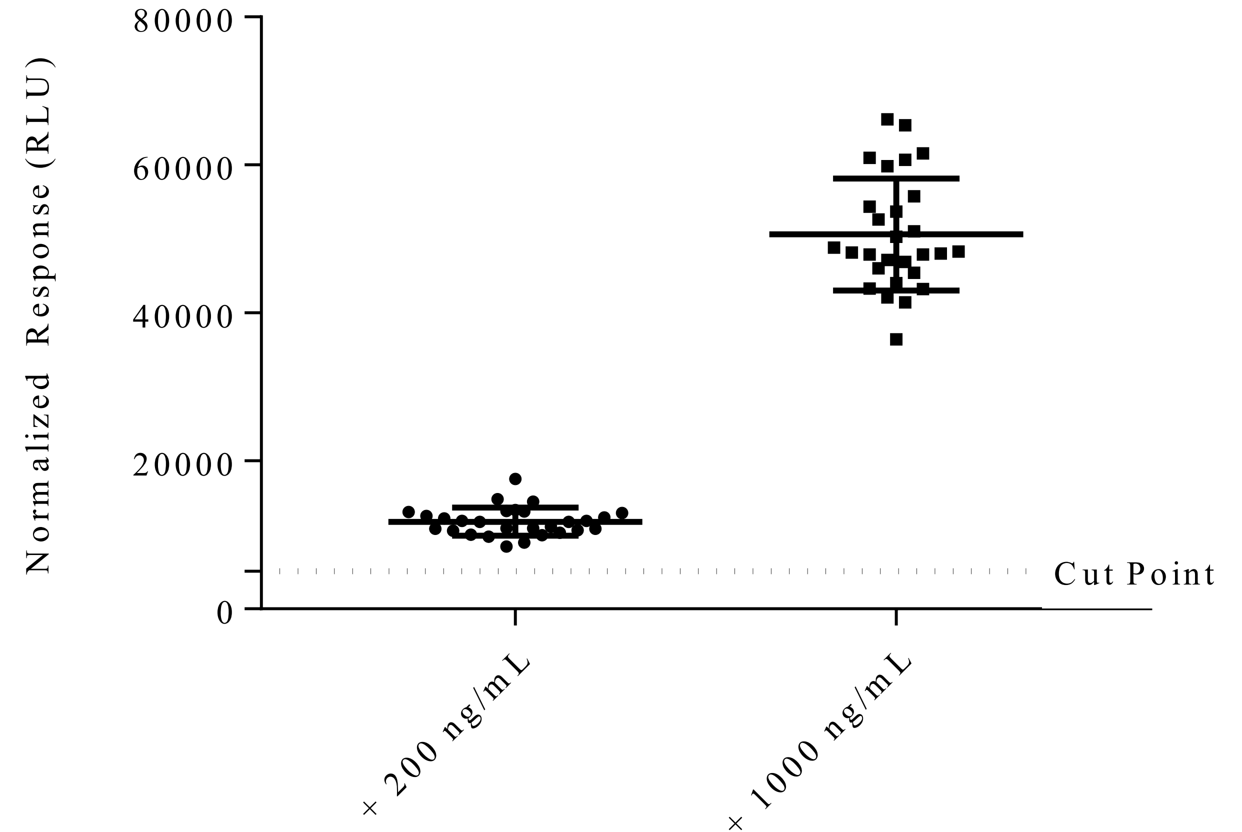
Spiked samples further demonstrate potential of the method

+ Artificially-spiked sample further demonstrate the utility of the method

+ Used an anti-RBD IgG PC spiked into COVID-negative serum samples

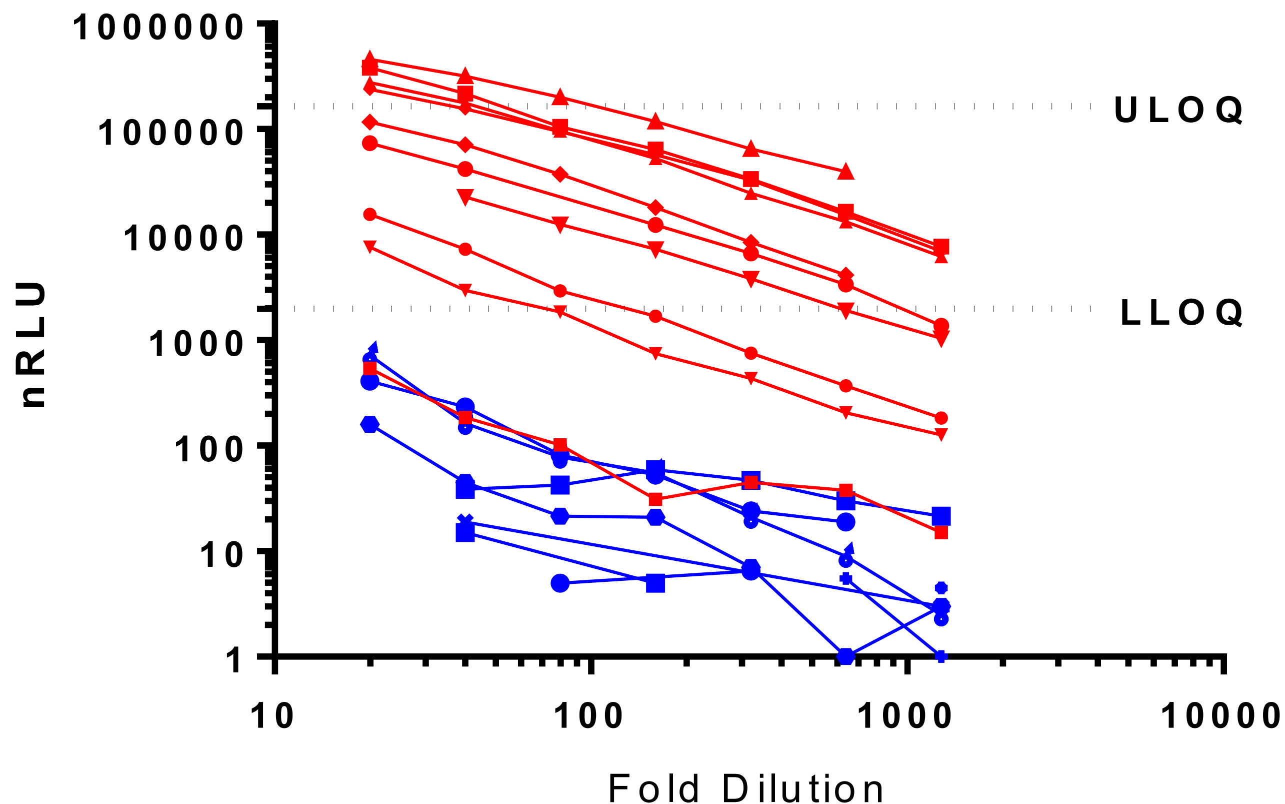
+ Even at a low dilution (MRD20), results clustered across numerous individuals

+ Readily able to detect only 200 ng/ml PC antibody vs. negative samples



Selectivity Validation

- Positive and negative serum samples were analyzed
- Titered using a 2-fold dilution scheme
- 20- to 1280-fold dilutions were tested
- The calibration curve LLOQ is provided here—can adjust based on desired specificity
- Samples with results in the quantitation range can be reported as relative concentrations



Red lines = Positive Samples

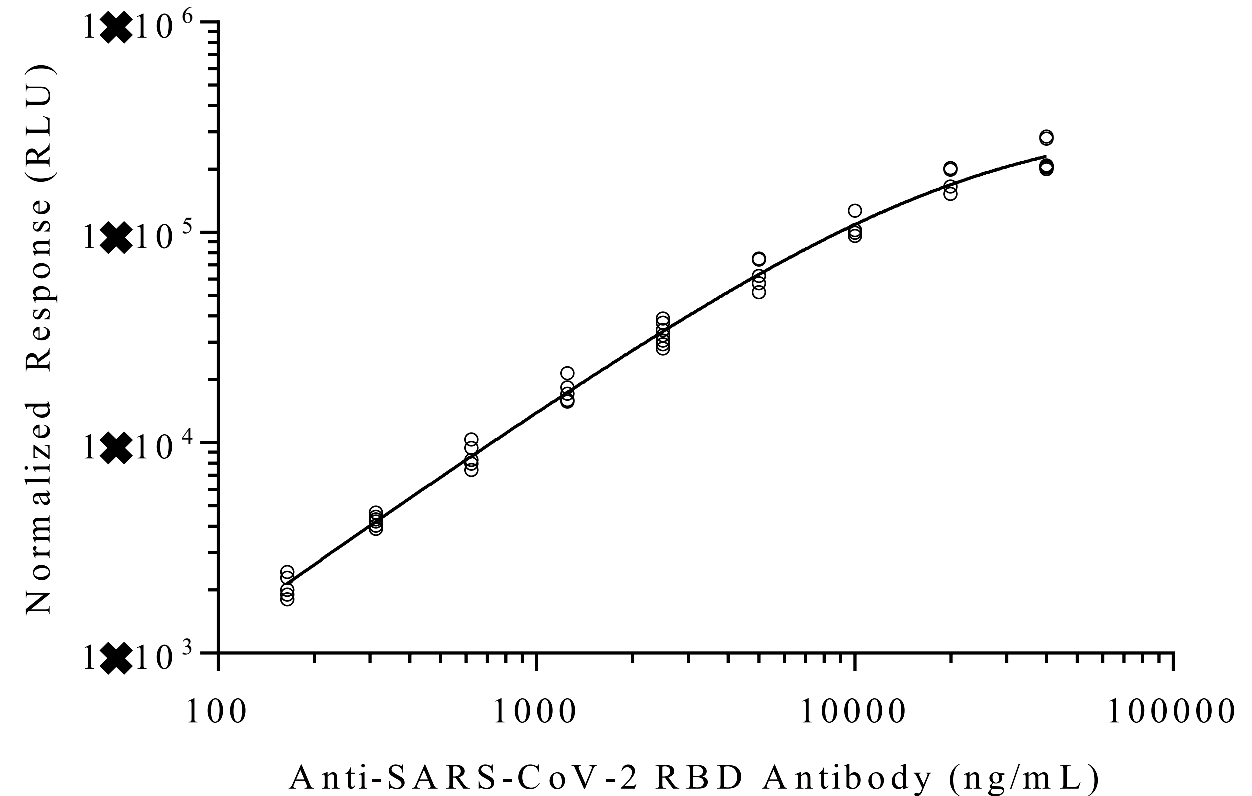
Blue = Negative Samples

Statistic	Value	95% CI
Sensitivity	90.0%	55.5% to 99.8%
Specificity	100%	69.2% to 100%
Positive Likelihood Ratio		
Negative Likelihood Ratio	0.10	0.02 to 0.64
Disease prevalence (*)	10.0%	
Positive Predictive Value (*)	100%	
Negative Predictive Value (*)	98.9%	93.3% to 99.8%
Accuracy (*)	99.0%	81.4% to 100%

(*) These values are dependent on disease prevalence.

Sample Quantitation: Use biomarker-like approach, reference curve

- While an ADA cut point approach could be used, observed some differences in signal across plates, but not necessarily tracking with “noise’
- Therefore, instead of targeting S/N semi-quantitation, used a reference calibration curve on plates
- Reference curve: screen matrix to remove outliers, spike commercial PC (anti-RBD PC) into matrix
- Quantitate normalized sample signals from the curve



Formal validation supports the robustness of the method

+ Inter-assay precision of calibrators (back-calculated values): 2-7%
+ %CV of calibrator raw responses across 17 runs (4 analysts) = 12-18%
+ Run acceptance based on suitability of calibrators (%CV) and adherence to reference ranges as established in validation (similar to ADA HPC, LPC), as well as blank assessment (uses blank matrix pool)
+ No interference from sample lipemia or hemolysis

Parallelism analysis: increased granularity of quantitation

- Titer assessment reports within 2-fold range on each side of mean
- Quantitative approach allows for further granularity of reporting
- Assessed parallelism of set of samples—dilute into sample diluent
- Quantitated as diluted into buffer; established against the matrix pool curve (which is spiked into buffer to create the MRD40)

Sample	Dil 20	Dil 40	Dil 80	Dil 160	Dil 320	Dil 640	Dil 1280
1	0	-9.2	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
2	> ULOQ	> ULOQ	0	-3.7	-13.4	-6.4	-12.8
3	0	-29.4	< LLOQ	< LLOQ	< LLOQ	< LLOQ	< LLOQ
4	> ULOQ	> ULOQ	rcv	0	5.7	-6.7	-15.5
5	0	0.30	rcv	9.3	18.0	26.1	< LLOQ
6	> ULOQ	> ULOQ	0	5.3	2.4	-0.96	-6.7
7	> ULOQ	> ULOQ	> ULOQ	0	-9.2	3.5	< LLOQ
8	rcv	0	8.8	27.7	40.3	< LLOQ	< LLOQ
9	> ULOQ	0	-0.85	-7.6	-15.3	-20.8	-19.2

Overall method format is easily adapted to IgM and IgA

- Level of background was less for IgA and IgM variations
- However, background correction still appeared to be valuable
- Commercially available PCs; however, need to confirm parallelism of this material against true positive samples

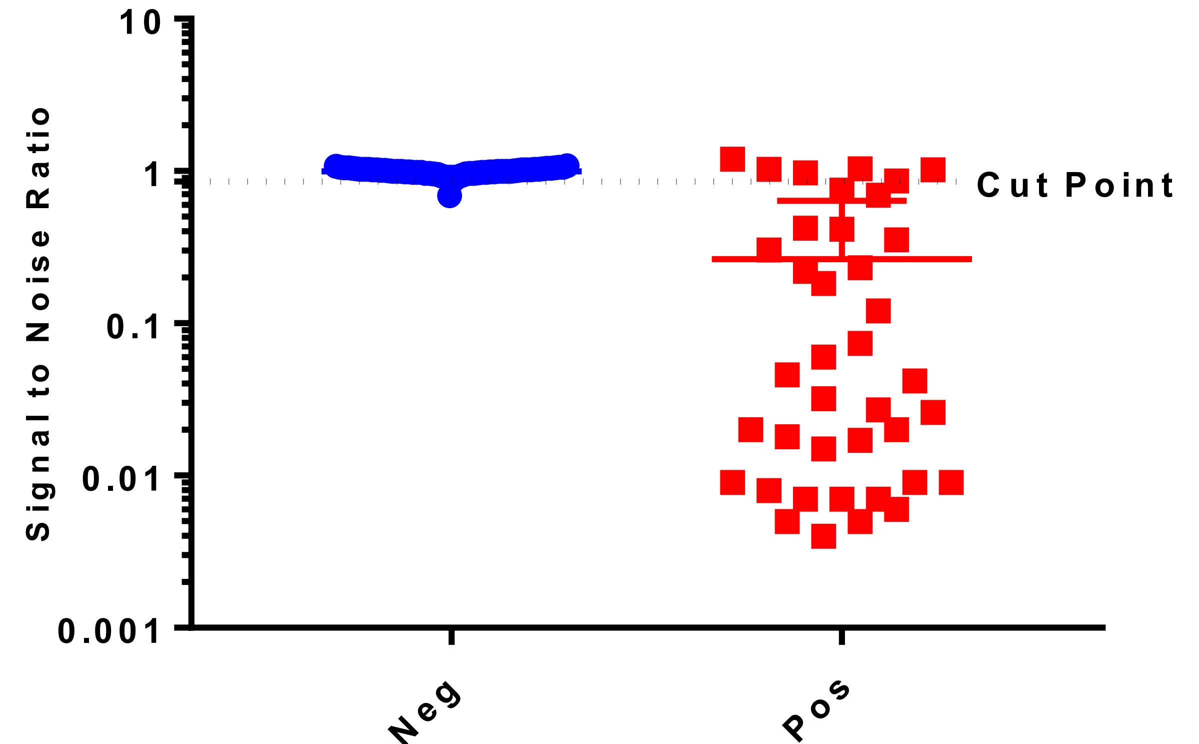
--Noted loss of parallelism below certain curve range with some PCs

--Can set range of quantitation

Assay components have also been adapted to plate-based Nab

Nab Format:

- Standard cell-based assay examines ability of anti-RBD antibodies in serum to block (pseudo)viral uptake to cells—by preventing ACE2 binding
- Plate-based format assesses ability of serum Nabs to block ACE2-RBD binding interaction
- Used cut point approach to assess presence of neutralizing antibodies in the serum



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