



US SARS-CoV-2 Serology Standard: Background and How to use it

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A US SARS-CoV-2 Serology Standard



- **What is it and why do we use it?**
- **How we make it and what does it contain?**
- **How do we assign unitage and evaluate suitability?**
- **Calibration to the WHO International Standard (20/136)**

A US SARS-CoV-2 Serology Standard: What is it and why do we use it?



- The serology standard is to be used as **an assay calibrator** by laboratories conducting SARS-CoV-2 serology testing
 - to measure antibodies after infection or after vaccination
- The main goal of a serology standard is **to harmonize assays** that measure anti-SARS-CoV-2 antibodies **to increase comparability** of results from different studies, including different candidate vaccines

How was the standard generated?

Screening Study to Select Candidates



Four Laboratories screened 9 convalescent ACD plasma samples (high volume) donated by BARDA to select suitable samples to use as a SARS-CoV-2 standard

Testing laboratories

- CDC
- NIAID-Integrated Research Facility (IRF)
- NIH Clinical Center
- FNLCR

Assays

- Spike IgG and IgM ELISA
- Neutralization Assay
- Roche Nucleocapsid Total Antibody
- Nucleocapsid IgG and IgM

Sample ID	Sample Type	Sample Volume (mL)	IRF Neutralization	HSL Spike IgM (AU/mL)	HSL Spike IgG (AU/mL)	HSL Nucleocapsid IgG (AU/mL)	OD- FNL Krammer SOP RBD IgG	Titer- FNL CDC SOP IgM	Titer- FNL CDC SOP IgG	Titer- CDC Spike PanIg	Titer- CDC Spike IgG	Titer- CDC Spike IgM
NR-53584	Plasma	124	1:34	116.1	814.2	4146.9	2.1	100	400	400	400	NEG
NR-52709	Plasma	500	1:33	NEG	295.8	647.1	1.6	NEG	400	100	400	NEG
NR-52708	Plasma	500	1:80	610.8	12281.5	10813.7	2.8	1600	6400	6400	6400	100
NR-52707	Plasma	470	NEG	NEG	NEG	NEG	0.1	NEG	NEG	NEG	NEG	NEG
NR-52706	Plasma	400	1:99	201.6	4971.0	15838.9	2.8	100	6400	1600	1600	NEG
NR-53573	Plasma	110	1:843	124.7	31893.3	20297.7	2.8	400	6400	6400	25600	100
NR-52699	Plasma	400	1:40	230.2	923.3	6240.1	2.4	100	1600	400	400	NEG
NR-53569	Plasma	110	1:618	1195.0	13597.1	16618.8	3.3	1600	6400	6400	6400	400
NR-53582	Plasma	300	1:282	473.1	5319.1	12356.2	2.7	100	6400	1600	6400	NEG

How did we assign unitage and evaluated suitability across assay types?



COLLABORATIVE STUDY

Panel

16 samples (7 samples + US Standard) in duplicate, deidentified

Panel Testing

Three sets of the samples were sent to participating labs

Each set of 16 samples were tested on three separate days

Testing laboratories

- CDC
- NIAID-IRF
- Mount Sinai
- NIH Clinical Center
- Quest Diagnostics
- FNLCR
- NIST
- NIAID-VRC

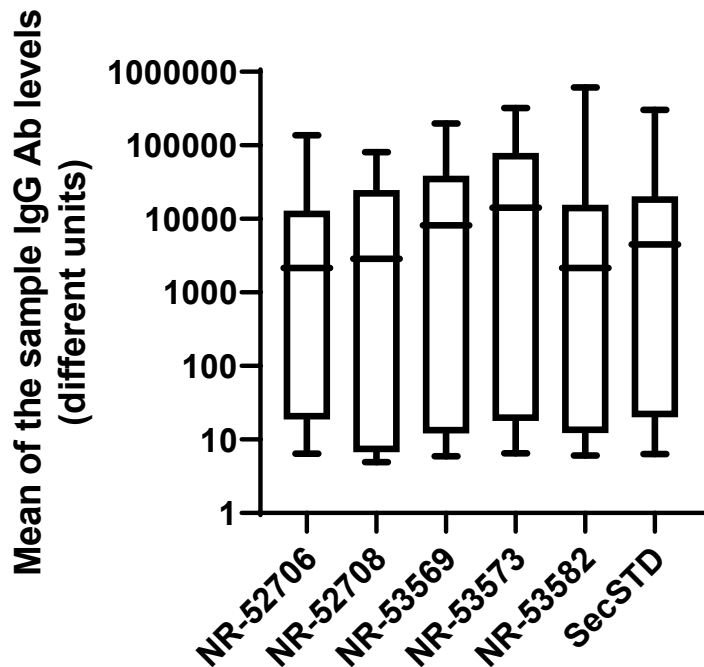
Assays

- Spike IgG and IgM ELISA
- Neutralization Assay
- Orthogonal (RBD IgG and Spike IgG)
- Abbott Nucleocapsid IgG
- Roche Nucleocapsid Total Antibody
- Euroimmun Spike IgG
- Nucleocapsid IgG and IgM
- Inhibition Assay (RBD-ACE2)
- MSD (RBD, Spike, Nucleocapsid) IgG

Collaboration Study Analyses of IgG Assays according to NIBSC guidance

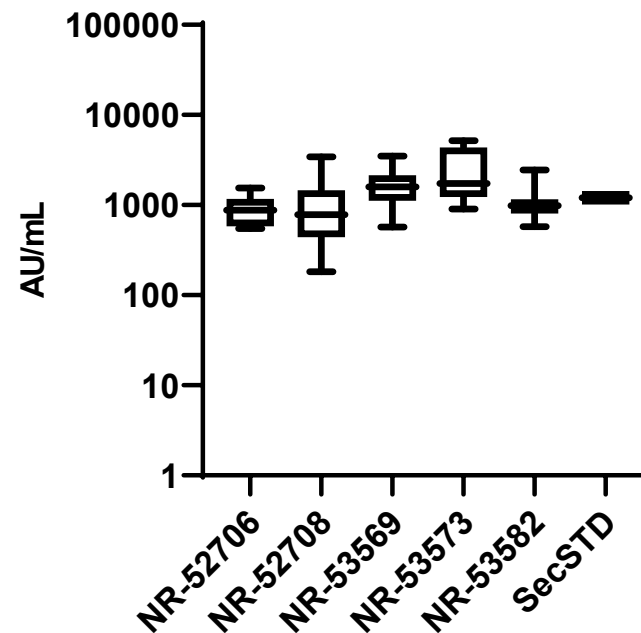


SARS-CoV-2 IgG Ab levels



ID	Interlab GM	Interlab SD	Interlab %GCV	Max GM	Min GM
NR-52706	844.8	1.5	3416.3%	137642.1	6.4
NR-52708	822.3	1.7	4393.8%	80929.4	4.9
NR-53569	1498.9	1.7	5223.8%	197620.6	5.9
NR-53573	2096.9	1.8	6160.8%	321956.8	6.5
NR-53582	1001.3	1.7	4703.3%	614402.9	6.1
SecSTD	1241.1	1.6	4334.8%	303158.3	6.4

Harmonization of SARS-CoV-2 IgG Ab levels (Standard set at 1200 AU/mL)

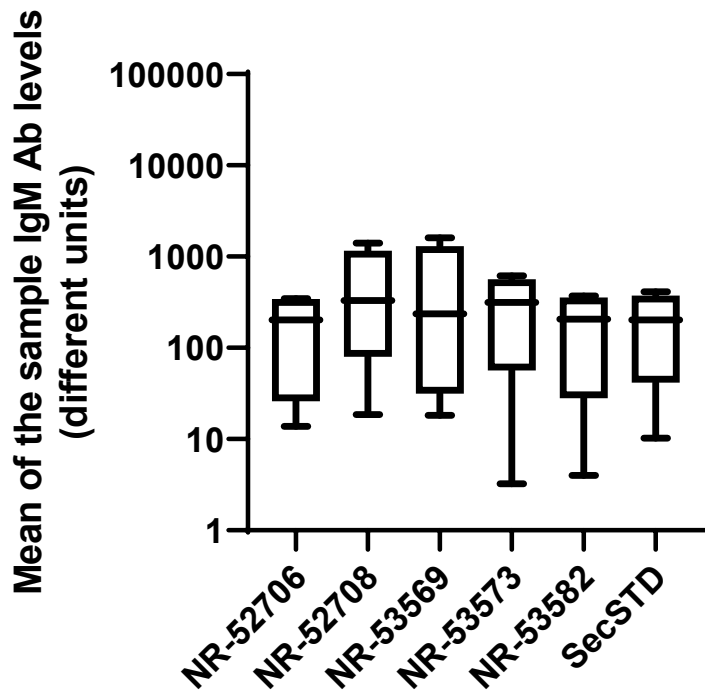


ID	Interlab GM	Interlab SD	Interlab %GCV	Max GM	Min GM
NR-52706	841.8	0.2	41.6%	1547.5	547.8
NR-52708	807.5	0.4	134.6%	3428.8	182.1
NR-53569	1490.5	0.2	60.6%	3508.8	571.7
NR-53573	2097.3	0.3	90.9%	5192.1	901.1
NR-53582	998.5	0.2	43.3%	2449.3	576.9
SecSTD	1200.0	0.0	0.0%	1200.0	1200.0

Collaboration Study Analyses of IgM Assays according to NIBSC guidance

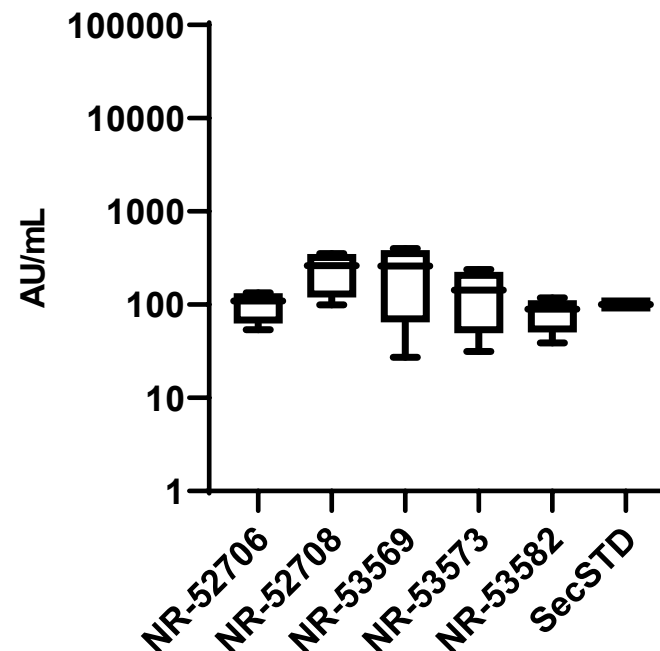


SARS-CoV-2 IgM Ab levels



ID	Interlab GM	Interlab SD	Interlab %GCV	Max GM	Min GM
NR-52706	100.5	0.7	370.6%	345.0	13.7
NR-52708	228.9	0.8	516.7%	1406.6	18.6
NR-53569	170.2	0.9	609.8%	1605.7	18.1
NR-53573	115.5	1.1	1026.7%	618.3	3.2
NR-53582	82.5	0.9	718.7%	371.4	4.0
SecSTD	111.4	0.7	423.0%	409.8	10.3

Harmonization of SARS-CoV-2 IgM Ab levels (Standard set at 100 AU/mL)



ID	Interlab GM	Interlab SD	Interlab %GCV	Max GM	Min GM
NR-52706	95.7	0.2	52.8%	133.9	53.9
NR-52708	215.7	0.3	82.3%	351.5	99.4
NR-53569	160.2	0.5	242.3%	398.7	27.3
NR-53573	109.4	0.4	145.7%	238.4	31.6
NR-53582	77.9	0.2	62.0%	118.2	38.9
SecSTD	100.0	0.0	0.0%	100.0	100.0

Collaboration Study Summary



- **All assays correctly identified the two negative samples**
- **Harmonization of assays with candidate US SARS-CoV-2 Standard significantly reduced Inter-laboratory coefficient of variation**

Calibration to WHO Serology SARS-CoV-2 International Standard (IS)



Study Design

FNLCR tested the WHO IS along with the US SARS-CoV-2 serology standard in Spike and Nucleocapsid assays.

Testing Plan

Day 1: Reconstitute a single vial of WHO IS and test the material in triplicate in the four FNLCR assays (Spike IgG and IgM; Nucleocapsid IgG and IgM). The US SARS-CoV-2 serology standard was also included in the same plate and tested in triplicate.

-Repeat Day 1 testing for a total of three separate testing days.

Day 1	1	2	3	4	5	6	7	8	9	10	11	12
Plate 1	C_STD	C_STD	NEG	PC1	STD-C1	STD-C2	STD-C3	STD-T1	STD-T2	STD-T3	C_STD	C_STD
A	50	50	50	50	200	200	200	200	200	200	50	50
B	100	100	150	150	400	400	400	400	400	400	100	100
C	200	200	450	450	800	800	800	800	800	800	200	200
D	400	400	1350	1350	1600	1600	1600	1600	1600	1600	400	400
			No Sample	PC2								
E	800	800	50	150	3200	3200	3200	3200	3200	3200	800	800
F	1600	1600	150	450	6400	6400	6400	6400	6400	6400	1600	1600
G	3200	3200	450	1350	12800	12800	12800	12800	12800	12800	3200	3200
H	6400	6400	1350	4050	25600	25600	25600	25600	25600	25600	6400	6400

STD-C: WHO IS 1000 (IU/mL)

STD-T: US Serology Standard

C_STD: Assay Daily Use Standard (Internal Reference Standard)

Calibration to WHO Serology SARS-CoV-2 International Standard

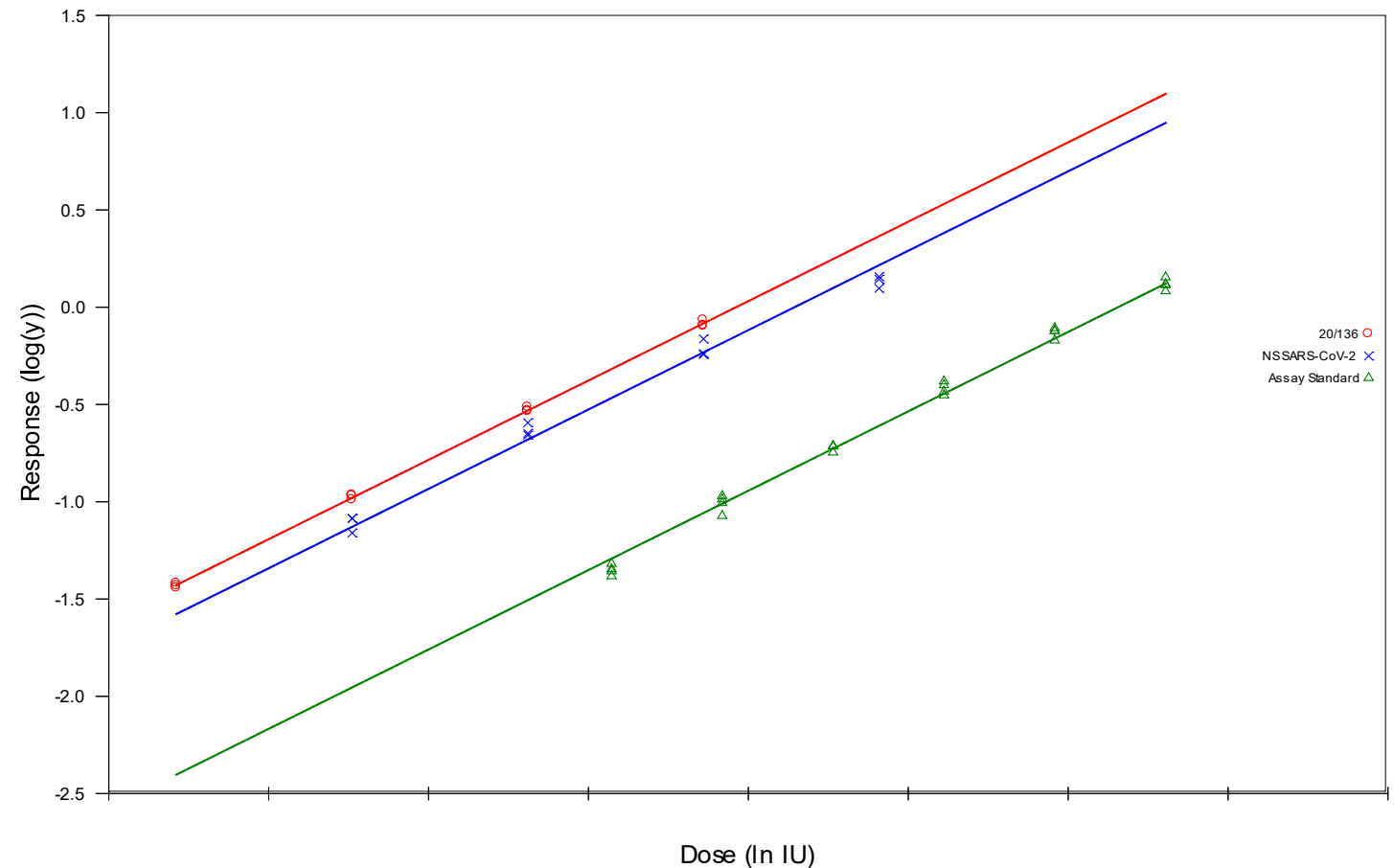


Data Analyses

Test for parallelism between the dose-response curve of the “Calibrator” and the dose-response curve of the “Test” reagent.

Molecular Devices (SoftMax Pro 6.5+), Combistats¹, and R are programs that can perform parallel line analysis.

Parallel Line Method: Combistats



¹<https://www.edqm.eu/en/combistats>

Calibration to WHO Serology SARS-CoV-2 International Standard



Data Analyses

Dashed lines- Response range of WHO IS (20/136)

Notes:

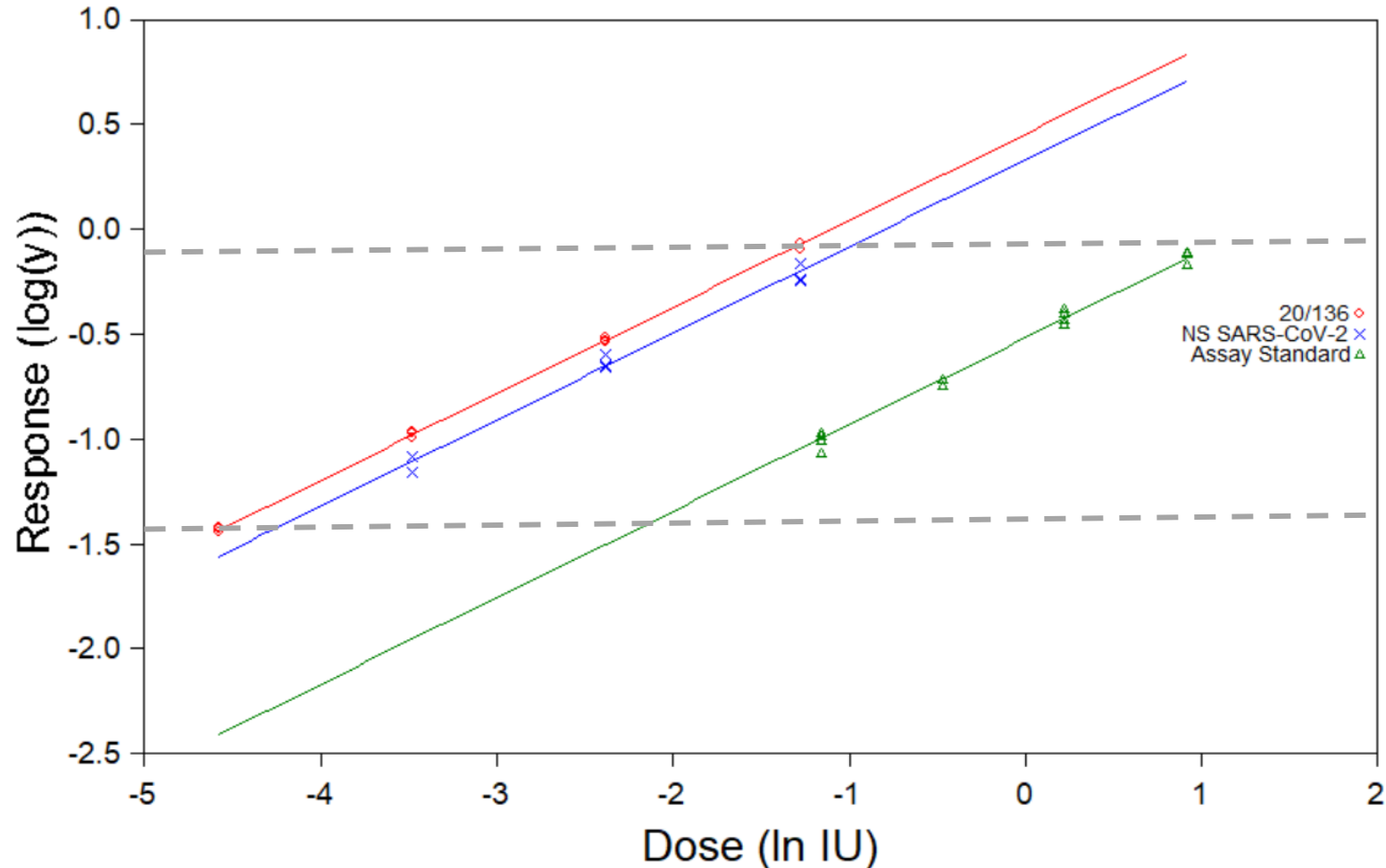
1. Response range of Samples needs to fall within the calibrator response range
2. Dose-response lines should be parallel and linear

Calibrator-WHO Serology SARS-CoV-2 International Standard (Red Line)

Test- US SARS-CoV-2 Serology Standard (Blue Line)

Assay Standard- Internal Reference Standard (Green Line)

Parallel Line Method: Combistats



Calibration to WHO Serology SARS-CoV-2 International Standard



IgG Data Analyses- with Parallel Line Analysis (Combistats)

SPIKE

SID	Dilution	Mean Day 1	Mean Day 2	Mean Day 3	Geometric Mean
STD-C	400	1000	1000	1000	1000
STD-T	400	740	783	770	764
C_STD	50	95	98	90	94

STD-C: WHO IS 1000 (BAU/mL)

STD-T: US Serology Standard

C_STD: Assay Daily Use Standard (Internal Reference Standard)

NUCLEOCAPSID

SID	Dilution	Mean Day 1	Mean Day 2	Mean Day 3	Geometric Mean
STD-C	400	1000	1000	1000	1000
STD-T	400	714	655	676	681
C_STD	100	74	76	73	74

Calculation:
Set STD-C value as 1000 BAU/mL, and software calculates STD-T value

Calibration to WHO Serology SARS-CoV-2 International Standard



IgM Data Analyses- with Parallel Line Analysis (Combistats)

SPIKE

SID	Dilution	Mean Day 1	Mean Day 2	Mean Day 3	Geometric Mean
STD-C	100	1000	1000	1000	1000
STD-T	100	208	264	271	246
C_STD	100	843	975	919	911

STD-C: WHO IS 1000 (BAU/mL)

STD-T: US Serology Standard

C_STD: Assay Daily Use Standard (Internal Reference Standard)

NUCLEOCAPSID

SID	Dilution	Mean Day 1	Mean Day 2	Mean Day 3	Geometric Mean
STD-C	50	1000	1000	1000	1000
STD-T	50	1132	950	1038	1037
C_STD	400	11454	10449	10256	10707

Calculation:
Set STD-C value as 1000 BAU/mL, and software calculates STD-T value

Calibrated US SARS-CoV-2 Serology Standard



	Spike IgG (BAU/mL)	Nucleocapsid IgG (BAU/mL)	Spike IgM (BAU/mL)	Nucleocapsid IgM (BAU/mL)	Neutralization (IU/mL)
WHO International Standard (20/136)	1000	1000	1000	1000	1000
US Serology Standard	764	681	246	1037	*

***- To be analyzed in the coming weeks**

US SARS-CoV-2 Serology Standard Promotion and Implementation



Promotion

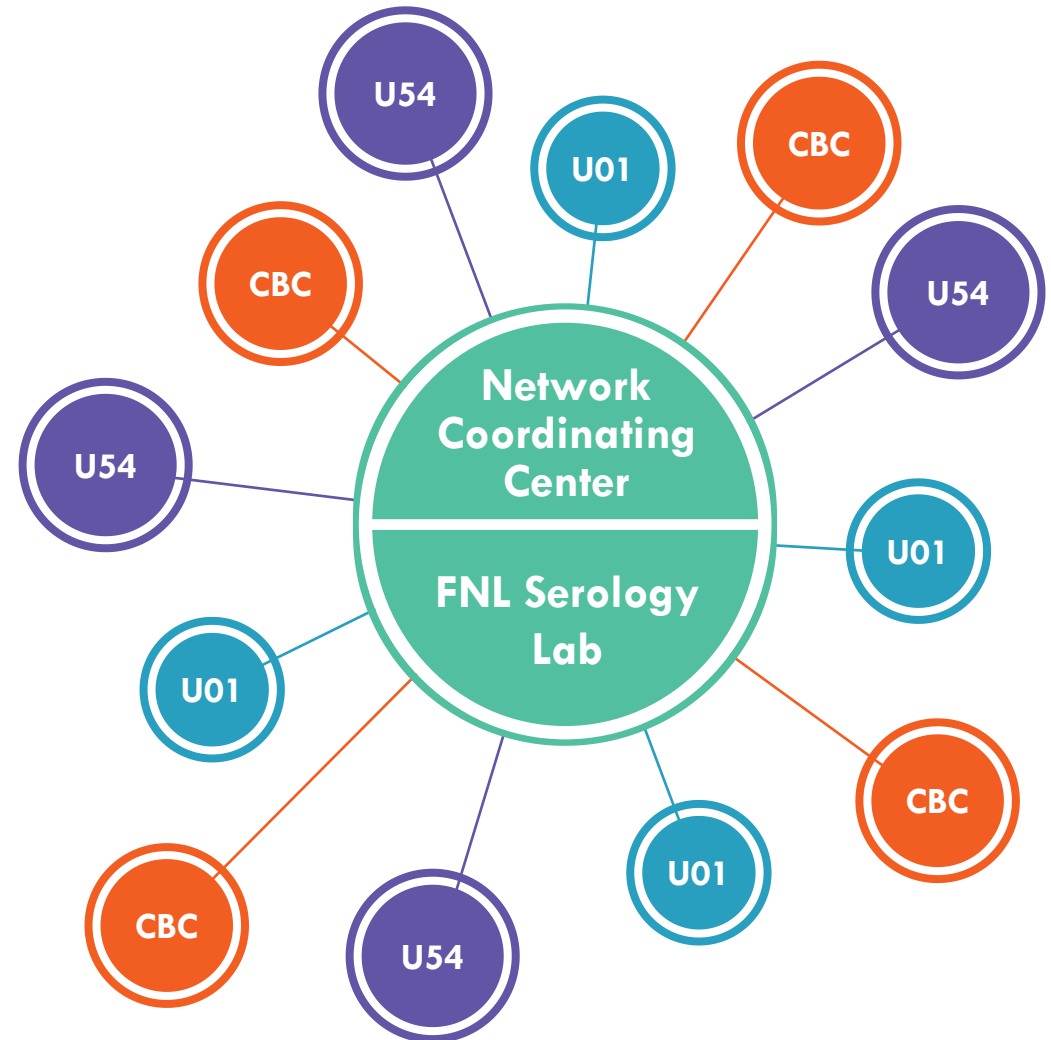
NCI and FNL have promoted the availability of the Serology Standard through various channels

FNL Serology site for request

<https://frederick.cancer.gov/seronet/serology-standard>

Serological Sciences Network (SeroNet)

Widespread emails



U.S. SARS-CoV-2 Serology Standard: Shipments



Entity	US Serology Standard Requests	Evaluation Panel Requests
Pharma/Biotech	18	4
US Government	5	2
SeroNet	13	9
Academic	10	1
Total	46	16

Requests at <https://frederick.cancer.gov/seronet/serology-standard>

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