SARS-CoV-2 Testing Update from the Pathogen Detection Working Group (PDWG) March 2021

Surveillance: As of January 2021, the seven National Primate Research Centers (NPRCs) have tested more than 1305 animals for virus using RT-PCR and 8207 animals for antibody using various immunoassays. The survey population includes rhesus macaques, pigtailed macaques, cynomologus macaques, Japanese macaques, baboons, mangabeys, squirrel monkeys, African green monkeys, and chimpanzees, from 8 different geographic locations throughout the NPRC system. **No infections have been confirmed.**

RT-PCR: The laboratories are all using nasopharyngeal swab samples on variations and combinations of the CDC recommended nucleocapsid (N1, N2, N3) primers and probes. Modifications to streamline testing have been implemented at the different centers. No particular problems have been observed. Although much improved, some supply chain issues for both specific and general materials still exist.

Antibody / Cross Center Survey: Six PDWG testing laboratories shared and tested samples using various commercial and laboratory developed assays. Eight different assays were used. Due to logistical constraints not all samples could be tested on all assays. The overall results demonstrated acceptable (>95%) sensitivity and specificity for most assays. The PDWG also gratefully acknowledges the contribution of Dr. Diogo Magnani and the Nonhuman Primate Reagent Resource for providing a monoclonal antibody standard to SARS-CoV-2 Spike for use as an assay control. Although the purpose of this study was to compare assays (not laboratories) several of the assays were run on the same samples in multiple laboratories and no discrepancies were noted.

Interpretation:

- No reactivity to any spike (S) or nucleocapsid (NC) antigen was interpreted as **negative** for all assays.
- Reactivity to both S and NC antigens were required to interpret multiple antigen assays as **positive**. Single antigen assays were positive or negative based on reactivity to the single antigen.
- Other patterns of reactivity are categorized as either **indeterminate** or **negative** according to the criteria established for the different assays. Some assays include an indeterminate category for reactivity to one but not all antigens while others include incomplete patterns of reactivity in the negative category (see Specificity Panel notes for details). Regardless, indeterminate reactivity should NOT be interpreted as infection; but rather suggests a possible need for further analysis.

Antigen targets for each assay.

Assay	S-CoV-2 Antigens	Other Antigens
Xpress Spike ELISA	S1,S2	
Xpress Nucleocapsid ELISA	NC	
Intuitive Panel	S1, S2, NC	
MesoScale Dx Panel	S, NC, RBD, NTD	HKU1 S, OC43 S, NL63S, 229E S, SARS-CoV-1 S
Charles River Lab MMIA	S, NP	HKU1 S, OC43 S, NL63S, 229E S
Xmap MMIA	S1, NC, RBD	
California NPRC MMIA	S Trimer, NC, RBD, Viral Lysate	
Washington NPRC MMIA	S1 (includes RBD), NC	HKU1 S1, OC43 S1, NL63S1, 229E S1, OC43 NP, NL63 NP 229E NP
Yerkes NPRC MMIA	S	

Although not used on all panel samples, Washington NPRC also has an immunoblot using S1 and NC.

Specificity Panel #1: Samples from colony animals with no known exposure to SARS-CoV-2 that were initially screened as negative or indeterminate by one or more assays before being included.

		Interpretation		
	# Tested	Positive (Reactive to S **and** NC)	Indeterminate (Reactive to S **or** NC)	Negative (No reactivity to S and NC)
Xpress Spike ELISA	62	4*		58
Intuitive Panel	62	0	1	61
MesoScale Dx Panel	62	0	62**	
Charles River Lab MMIA	62	0	62**	
Xmap MMIA	50***	0	2	48
California NPRC MMIA	62	0	6	56
Washington NPRC MMIA	62	0	62**	
Yerkes NPRC MMIA	62	2*		60

*Since the Xpress ELISAs and Yerkes NPRC assays in this study include S without NC, there is no difference between indeterminate and positive interpretations.

** These assays do not use the indeterminate category and interpret any S without NC or NC without S as negative.

***The Xmap Multiplex Microbead ImmunoAssay (MMIA) was designed for human samples and was modified for use with nonhuman primate samples. Twelve (12) samples were excluded from the analysis because high background reactivity made them uninterpretable.

There are no false positive SARS-CoV-2 final interpretations using any of the multiple antigen assays.

Seasonal Coronavirus (human) assays were also run on this negative/indeterminate panel with the following results:

		# Reactive						
	# Tested	HCoV-HKU1 S1	HCoV-OC43 S1	HCoV-OC43 NP	HCov-NL63 S1	HCov-NL63 NP	HCoV-229E S1	HCoV-229E NP
CRL MMIA	62	19	8	NT	21	NT	20	NT
MSD Panel*	62	3	0	NT	1	NT	13	NT
WANPRC MMIA*^	62	0	0	2	0	1	9	2

NT= not tested

11/11 positive human controls were reactive on the WANPRC and MSD panel antigens.

^ Testing using these reagents was replicated at a second laboratory

Sensitivity Panel #2a: Samples from animals experimentally infected at least 14 days prior to sampling.

			Interpretation		
Assay	Species	# Tested	Positive	Indeterminate	Negative
Xpress Spike ELISA	MMU	8	8*		0
Xpress NC ELISA	MMU	4	4*		0
MesoScale Dx Panel	MMU	4	4	0**	
Charles River Labs MMIA	MMU	8	7	1**	
California NPRC MMIA	MMU	8	8	0	0
Washington NPRC MMIA	MMU	8	8	0**	
Yerkes NPRC MIA	MMU	8	8*		0
Xpress Spike ELISA	AGM	3	3*		0
MesoScale Dx Panel	AGM	2	2	0**	
Charles River Labs MMIA	AGM	3	3	0**	
California NPRC MMIA	AGM	3	3	0	0
Washington NPRC MMIA	AGM	3	3	0**	
Yerkes NPRC MIA	AGM	3	3		0
Charles River Labs MMIA	PAPIO	12	6	6**^	
XMap MMIA	PAPIO	12	10	0	2^

*Since the Xpress ELISAs and Yerkes NPRC assays in this study include S without NC, there is no difference between indeterminate and positive interpretations.

** These assays do not use the indeterminate category and interprets any S without NC or NC without S as negative

^ Although experimental infection was attempted no evidence of infection was found in 2 baboons.

Single antigen (NC or S) reactivity was detected in some assays without an indeterminate category resulted in a negative interpretation. However, if reactivity to the single antigens are included along with the positives, all the infections were detected by all assays except for 3 experimentally infected baboons, and in 2 of them other evidence indicates that the attempted infection failed.

Sensitivity Panel #2b: Samples from animals experimentally infected less than 14 days prior to sample collection.

			Interpretation		
Assay	Species	# Tested	Positive	Indeterminate	Negative
Xpress Spike ELISA	MMU	6	2*		4
Xpress NC ELISA	MMU	6	4*		2
Charles River Labs MMIA	MMU	6	1	3**	
California NPRC MMIA	MMU	6	6	0	0
Washington NPRC MMIA	MMU	6	4	2**	
Yerkes NPRC MIA	MMU	6	6*		0
Xpress Spike ELISA	AGM	1	0*		1
Charles River Labs MMIA	AGM	1	0	1**	
California NPRC MMIA	AGM	1	0	0	1
Washington NPRC MMIA	AGM	1	0	1**	
Yerkes NPRC MIA	AGM	1	0*		1

As expected, in early (prior to day 14) infection, antibody could not always be detected. However, some of the assays were able to detect signal to S and/or NC antigens in some early samples. No attempt was made to distinguish IgM from IgG or specific antigen sequences, which could be contributing factors to these differences.

Sensitivity Panel #3a: Individual antigen reactivity (not interpretation) for experimental Spike Vaccine Recipients.

			# Reactive	
Assay	Species	*# Tested*	*Nucleocapsid*	*Spike and/or RBD*
Xpress Spike ELISA	MMU	6	n/a	5
Xpress NC ELISA	MMU	6	0	n/a
MesoScale Dx Panel	MMU	4	0	4
Charles River Labs MMIA	MMU	4	0	3
California NPRC MMIA	MMU	12	6	12
Washington NPRC MMIA	MMU	12	0	10
Yerkes NPRC MIA	MMU	12	n/a	12
MSD Panel	MNE	3	0	3
California NPRC MMIA	MNE	5	0	5
Washington NPRC MMIA	MNE	5	0	5
Yerkes NPRC MIA	MNE	5	n/a	5

n/a= not applicable

Sensitivity Panel #3b: Individual antigen reactivity (not interpretation) for experimental Nucleocapsid Vaccine Recipients.

			# Reactive	
Assay	Species	*# Tested*	*Nucleocapsid*	*Spike and/or RBD*
Xpress Spike ELISA	MMU	4	n/a	0
Xpress NC ELISA	MMU	4	4	n/a
Charles River Labs MMIA	MMU	4	2	0
California NPRC MMIA	MMU	4	4	0
Washington NPRC MMIA	MMU	4	4	0
Yerkes NPRC MIA	MMU	4	n/a	0

n/a= not applicable