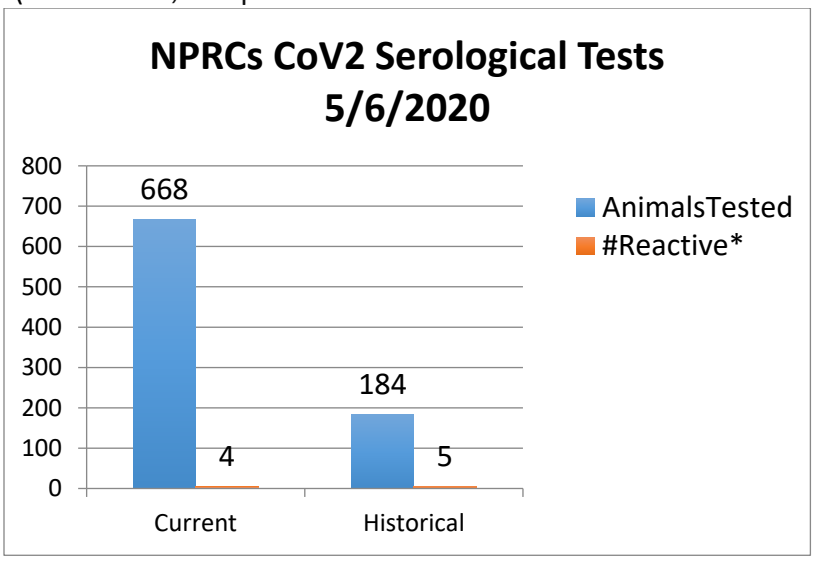
SARS-CoV-2 Testing reviewed by the National Primate Research Centers Pathogen Detection Working Group (PDWG) on 5/6/2020.

The National Primate Research Centers Pathogen Detection Working Group (PDWG) is developing and validating antibody and RNA PCR assays for SARS-CoV-2 testing. Recognizing the limits of our resources (for example, nonhuman primate positive controls) we are working collaboratively and synergistically to summarize our testing observations and data with various commercially available or laboratory developed assays. The NPRC Pathogen Detection Working Group is part of the NPRC Consortia and is a national resource for laboratory personnel, veterinarians and colony managers who conduct pathogen surveillance in nonhuman primate populations. As the availability of reagents, controls, and protocols and subsequent generation of data continues to evolve rapidly; we plan to continue to share our experience to inform updated, practical guidance for testing. For additional information and updates please contact JoAnn Yee (joyee@ucdavis.edu) and members of PDWG through the NPRC website (https://nprcresearch.org/primate/pathogen-detection/pathogens.php).

Since COVID 19 is a reportable Public Health disease, any testing program should be reviewed with your State Public Health Veterinarian or other authorities to ensure compliance with reporting and regulatory requirements.

- **1. Antibody testing:**
  Most centers are currently using the commercially available S1/S2 antigen coated plates from Xpress Bio. This antigen has successfully detected antibody in a limited number of sera from experimentally infected or immunized monkeys and human convalescent sera. ***We are actively seeking additional samples to use as positive controls.*** Reactivity in less than 2% of the samples has been attributable to non-specific cross reactivity with cell lines, purification tags, or seasonal corona viruses. No SARS-CoV-2 specific antibody was confirmed in current 2020 and past pre-2020 samples from four different colonies. The majority of samples were from rhesus macaques (*M. mulatta*), but smaller numbers of cynomologus macaques (*M. fascicularis*), pigtailed macaques (*M. nemestrina*), Japanese macaques (*M. fuscata*), sooty mangabeys (*C. atys*), chimpanzees (*P. troglodytes*) and African green monkeys (*C. sabaeus*) samples were included.



*None of the Reactive results were confirmed as positive by additional testing.

Additional Reagents being evaluated:

- Charles River Labs Multiplex
- Intuitive Biosciences Immunoassay
- In laboratory developed ELISAs or Multiplex Assays using Spike, Nucleocapsid, Receptor Binding Domain, or Seasonal Coronavirus antigens developed by the laboratory or its research collaborators or available from ATCC/BEI, AbClonal, Creative Diagnostics, Immune Tech, Mass Biologics, Ray Biotech, Sinobiologicals.

2 . Sample Collection and Extraction for RT-PCR

Sample type:

Nasopharyngeal or Oropharyngeal

Collection materials:

Users have had variable experience with different swab types.

Category	Style	Swab	Media	Vol	RNAse P or OSM Ct
Nylon	Flocked	BD UVT- H226-03	Universal Viral Transport Media	5ml	20.0-30.8
Nylon	Flocked	BD Diagnostics ESwab™		3 ml	20.0-30.8
Cellulose*	Compressed	Merocel- eye spear	Trizol	0.5 ml	30- 35
Merocel Polyvinylacetal (PVA)	Compressed	PVA Eye Spear		3 ml	24.15- 30.
Polyester	Spun	Polyester- Puritan #25-8061PD	Trizol	0.5 ml	20.1-27.8
Polyester	Spun	Polyester- Puritan #25-8061PD	Trizol	1 ml	27.9-28.3
Cotton	Spun	Medline Industries, Inc		3 ml	24.15 – 25.03
Rayon	Spun	BBL CultureSwab		3 ml	23.6-68.7
Rayon	Spun	Fisherfinest®		3 ml	26.9-110.8
Rayon	Spun	Starswab	Bartels	2 ml	24.5-29.6
Rayon	Spun	Starswab	Bartels	2 ml	24.5-29.6
Cotton			Qiagen RLT	0.5 ml	22.6-27.8

*There were some performance issues observed with this type

Transport medium

- Viral Transport Media may be difficult to acquire. Several companies are selling bulk quantities of VTM, which users then transfer into individual sample vials.
- The CDC Standard Operating Procedure for laboratories to create their own VTM is located at this link: https://www.cdc.gov/csels/dls/locs/2020/new_sop_for_creating_vtm.html
- Many labs are placing swabs directly into RNA preservatives such as guanidinium or Trizol solutions

Extraction Control: CoV2 positive control synthetic RNA and trizol inactivated RNA from tissue spiked into macaque specimen controls at time of extraction were recovered at >90%.

- Extraction Control: CoV2 positive control synthetic and trizol inactivated tissue culture RNA spiked into macaque specimen controls before extraction were recovered at >90%.

- Stability over time and temperature shows minimal loss:

  These are examples of internal cell control OSM and Control RNA Ct values under different conditions:

Total RNA from swabs (OSM Ct)

Time (hr)/ Temp	RPMI rayon	RPMI cotton	AL rayon	AL cotton
1 hr/RT	28.66	26.97	27.68	26.22
18 hr/4C	29.05	27.75	27.64	26.3
18hr/-80C	30.61	28.3	27.42	26.54

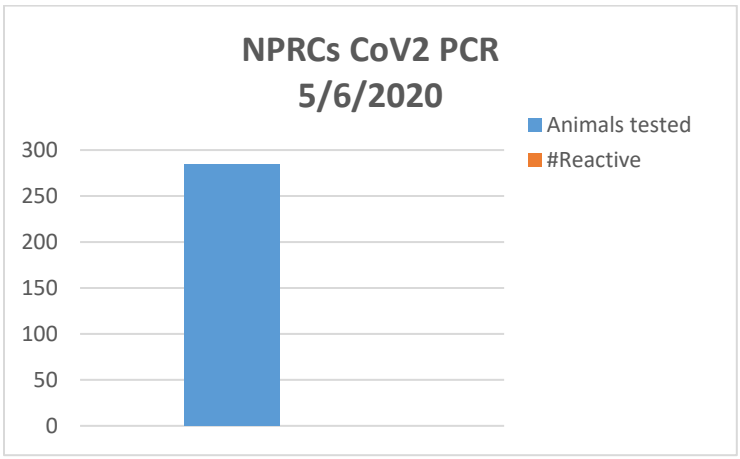
SARS-CoV-2 RNA Ct / OSM Ct

Time (wk)/ Temp	N1 Ct / OSM Ct	N2 Ct / OSM Ct
Fresh	24.8/22.8	24.7/22.7
1 week RT	24.5/23.6	27.8/23.6
1 week 4C	24.4/23.0	27.6/23.1
1 week -80C	24.5/22.9	27.7/23.0

- The following Extraction reagents have been used successfully
  - Total Viral Nucleic Acid- Promega
  - Qiagen Min Elute, Viral RNA, RNAEasy
  - Zymo RNA

3. PCR:

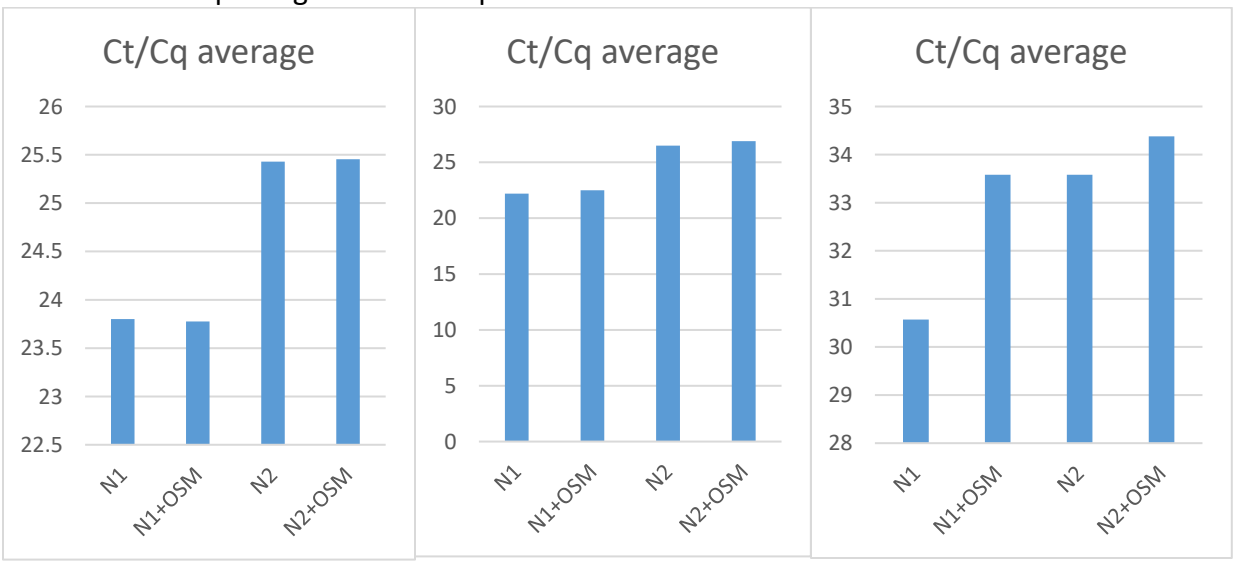
284 samples have been tested as of 5/6/2020- all were housekeeping gene positive and CoV2 nucleocapsid negative.



The following reagents are in use in the various PDWG laboratories:

- Primers and Probes:
  a. Nucleocapsid Primers (as recommended by CDC).
     - N1-FAM
     - N2-FAM
     - N3-FAM (this primer was originally included by is no longer recommended)
  b. Internal Amplifiable DNA Controls
     - RNaseP-FAM
     - OSM (Oncostatin M)-HEX or VIC

  c. Multiplexing- 3 labs have been able to increase throughput and efficiency by multiplexing OSM with N primers:



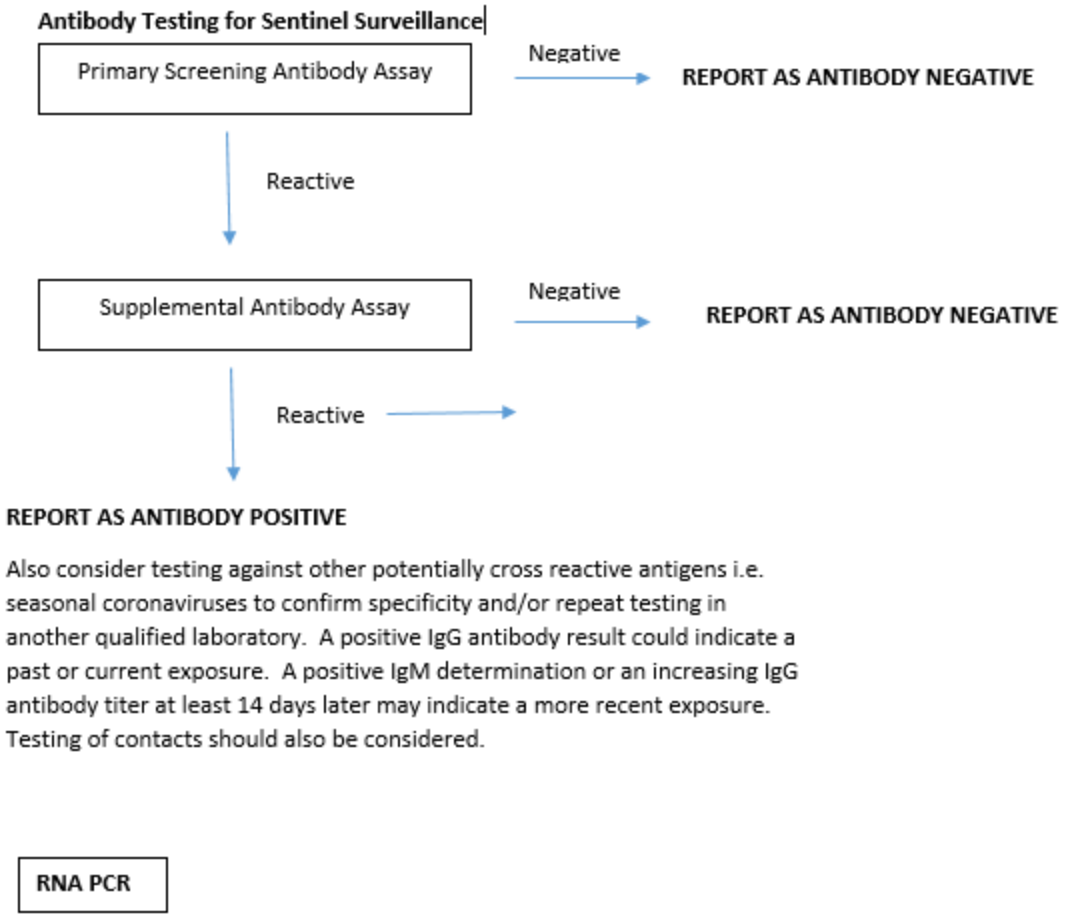
- Master mixes:
    - Thermo Fisher TaqPath 1-Step RT-qPCR
    - Thermo Fisher TaqMan™ Fast Virus 1-Step Master Mix
    - Bioline OneStep RTPCR kit.
    - Promega 1 Step PCR

- Positive controls for validation have included plasmid (IDT), trizol fixed tissue culture RNA, synthetic RNA (ATCC), RNA from experimentally infected animals.

- Additional Observations:
    - LOD 18 copies/uL for N1, N2. Accuracy variable below 1800 copies/uL.
    - Two animal swab samples can be pooled (two animal swabs extracted together and eluted in same final volume as one sample) without affecting LOD.

4. Testing Algorithms

Some laboratories are using Spike antigen ELISAs for the primary antibody screen; while others plan to use a panel (Spike, Nucleocapsid, RBD, seasonal coronaviruses) of ELISAs or Multiplex Microbeads (Luminex). Others are choosing to run in the reverse order, dependent on overall laboratory workflow, number of tests, and available instrumentation and reagents. Currently there are no Immunofluorescence or Western Blot assays ready for use as confirmatory assays. Once these and other additional assays and more test data are available a better defined algorithm will be developed and validated. Based on our experience with other agents and our limited experience with SARS-CoV-2, the following interim algorithm is under consideration:



As warranted by known exposures, clinical signs, relocations (i.e. COVID 19 research pre-screens, barrier colony formation) RNA PCR may also be performed in parallel with antibody testing. There is not yet enough data to determine the sensitivity and specificity of PCR results- unless confirmed by other clinical signs or diagnostic testing a repeat determination is recommended. If reactivity is confirmed, public health testing and reporting requirements must be met and an expanded surveillance program should be implemented.