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SARS-CoV-2 surveillance for a non-human primate breeding research facility

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Abstract

Background: The emergence of SARS-CoV-2 and the ensuing COVID-19 pandemic prompted the need for a surveillance program to determine the viral status of the California National Primate Research Center non-human primate breeding colony, both for reasons of maintaining colony health and minimizing the risk of interference in COVID-19 and other research studies.

Methods: We collected biological samples from 10% of the rhesus macaque population for systematic testing to detect SARS-CoV-2 virus by RT-PCR and host antibody response by ELISA. Testing required the development and validation of new assays and an algorithm using in laboratory-developed and commercially available reagents and protocols.

Results and Conclusions: No SARS-CoV-2 RNA or antibody was detected in this study; therefore, we have proposed a modified testing algorithm for sentinel surveillance to monitor for any future transmissions. As additional reagents and controls become available, assay development and validation will continue, leading to the enhanced sensitivity, specificity, accuracy, and efficiency of testing.

KEYWORDS
antibody, COVID-19, management practices, RNA PCR, testing algorithm

1 | INTRODUCTION

The COVID-19 pandemic brings new challenges to the management of non-human primate (NHP) colonies for breeding, research, conservation, or other settings. The increasing human prevalence of the novel coronavirus, SARS-CoV-2, and evolving understanding of its transmission, particularly from asymptomatic carriers,[1-3] requires that the risk of human-to-NHP, NHP-to-NHP, and potentially NHP-to-human transmission be mitigated (Figure 1). Non-human primates (including *macaca*, *cercopithecus*, and *papio* species) can be infected with SARS-CoV-2.[4-6] Conversely, experimental trials to infect marmosets (*Callithrix* spp.) have not shown any signs of active infection.[7] The potential for SARS-CoV-2 to cause clinical disease in NHPs has been variable under experimental conditions.[8,9] Studies to determine whether certain populations (age, co-morbidities) are at greater risk of infection and/or clinical disease are in progress.[10,11] Although early data indicate that macaques that are experimentally infected are immune to reinfection,[12] the durability

of this protection is not known. There are still many uncertainties about the short- or long-term health effects of SARS-CoV-2 on NHP. Thus, it is imperative to reduce the risk of a SARS-CoV-2 outbreak at a NHP research facility for several reasons, including (a) its potential harmful effects on general health of the breeding colony, (b) possible interference with specific research projects, and (c) suitability of infected and/or immune animals for future SARS-CoV-2 research.

Given the susceptibility of NHPs and presumed lack of pre-existing immunity, the California National Primate Center (CNPRC) at the University of California, Davis (UCD), adopted management practices to reduce the risk of virus introduction and transmission and initiated surveillance testing to monitor for SARS-CoV-2 in the colony. The CNPRC had a measles outbreak in 1987 that resulted in extensive colony morbidity and mortality.[13] This historical event heightened concern at CNPRC about the possible impact of any SARS-CoV-2 introduction.

Following identification of SARS-CoV-2 in a few individuals in Northern California, with support of the UCD administration, on March 4, 2020, the CNPRC implemented practices to reduce risk, including denying admission to visitors, interns, students, and non-employees. All public tours, demonstrations, classes, and any vendor- or employee-sponsored visits to CNPRC were canceled. In addition, on-site staffing was reduced from approximately 300 people to essential personnel only, who were required to modify their practices to incorporate new CDC guidelines for hygiene, personal protective equipment (PPE), and physical distancing. Access to both the indoor vivarium and outdoor vivarium was restricted to employees with work-related activities. New signage reinforcing entry restrictions and PPE requirements was posted. Upon issuance of shelter-in-place directives from the Yolo County public health department, on March 18 UCD suspended normal campus operations and moved to remote student instruction and employee work. All non-critical, on-site research activities were ramped down. From February 22 to May 18, 2020, 1794 human cases of COVID-19 were confirmed in Yolo, Sacramento, and Solano counties surrounding the CNPRC.[14-16] Human-to-human transmission including community spread beyond travel or household contact acquisition has been documented in this region, where the majority of CNPRC employees reside. Therefore, there is risk of infection in, and transmission between, facility staff and captive non-human primates.

The CNPRC drafted a sentinel surveillance plan including laboratory testing to detect both the presence of the virus and the

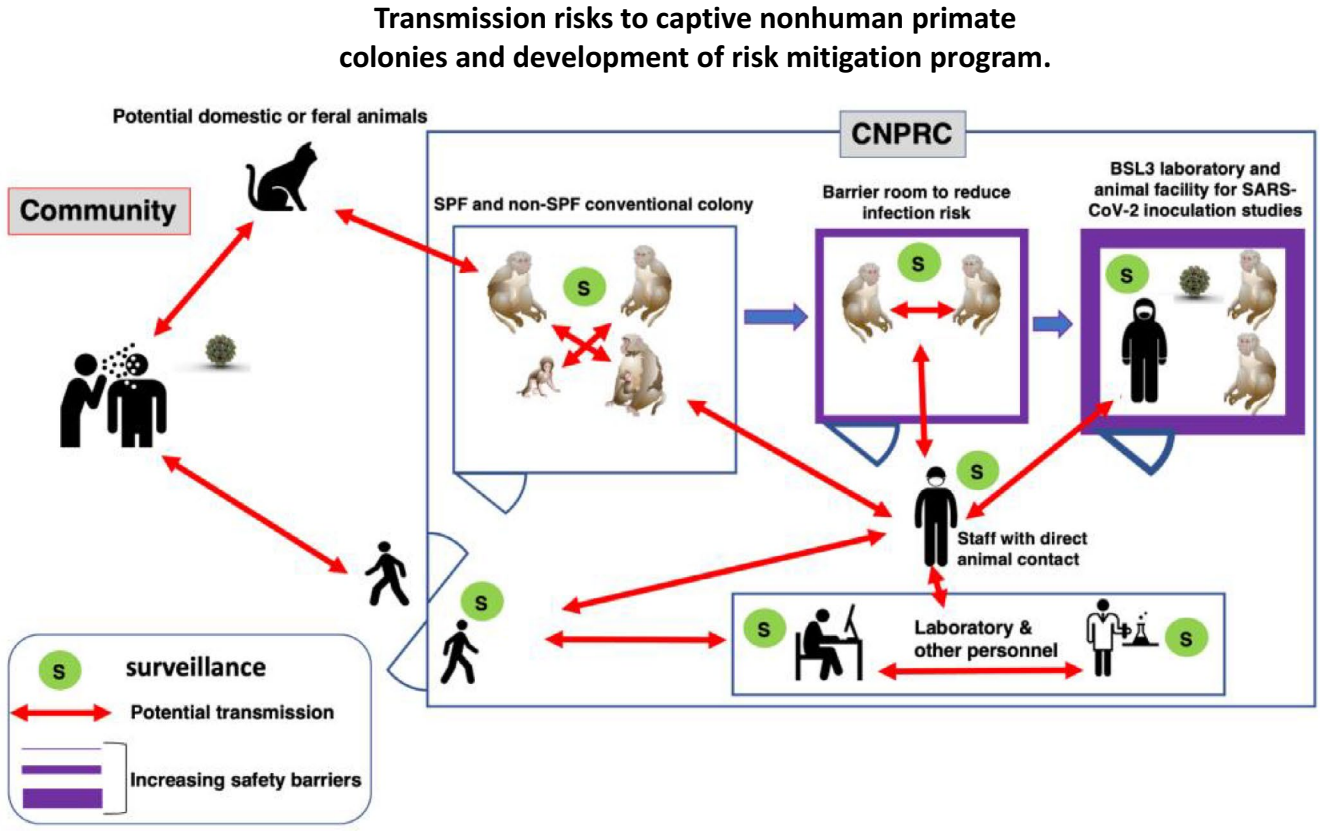

FIGURE 1 Non-human primates at CNPRC are at risk of SARS-CoV-2 infection from humans primarily, but also from susceptible feral animals (such as cats [35]). Reducing entry of people to CNPRC, reduced operations, limiting inter-individual (human-to-animal, human-to-human, animal-to-animal) contact, proper PPE and distancing, and the setup of a surveillance testing program for humans and animals are important components of the infection control program. Special barrier rooms have been set up to house animals screened and removed from either the conventional non-SPF or SPF colonies with the goal of keeping them uninfected, so that they can subsequently be used for SARS-CoV-2 inoculation studies in biosafety level 3 (BSL3) setting (icons by Adioma and Iconfinder)

development of antiviral antibodies in the non-human primate colony. In addition, we endeavored to develop testing methods that would not add pressure to the already overloaded supply chain used in human diagnostic medicine. When it becomes more widely available, accurate testing of humans with occupational exposure to the non-human primate colony for past or present SARS-CoV-2 infection will be an additional, adjunct surveillance tool to reduce the risk of NHP infection.

Our Primate Assay Laboratory (PAL) developed the capacity to conduct both serology testing for antibody to SARS-CoV-2 and RT-PCR testing for the virus. Antibody responses may be observed as early as 14 days post-infection.[4,17] Optimally, a longitudinal change in titer should be demonstrated. A positive antibody test indicates that an infection has occurred but unless testing includes both IgG and IgM, an ongoing infection cannot be distinguished from the convalescent stage of an infection or a past infection. In contrast, the PCR assay to detect viral RNA provides an indication of possible viral shedding at the time the sample is collected. Although the PCR assay provides a real-time result, recently published data suggest that PCR results can be variable in humans known to be infected with SARS-CoV-2.[18-20] This finding suggests a possible need for multiple repeat tests before ruling out infection. A positive PCR result confirms an infection, but a negative PCR result only suggests that a swab collected from the animal at a specific location and specific time did not have detectable viral RNA, taking the limit of detection into consideration. Ideally, both serology and PCR would be incorporated into a surveillance program, as has been successful in the development of specific pathogen-free non-human primate colonies for other agents.[21,22]

2 | MATERIALS AND METHODS

2.1 | Study design

A sentinel surveillance program to test at least 10% of the CNPRC macaque colony was instituted in March. Excluding the current season's birth cohort, there are approximately 2450 animals housed outdoors and 1300 indoors. The goal was to collect biological samples from the rhesus population for conducting systematic testing for both antibodies and nucleic acid. To minimize additional animal contact and manipulation, the samples were opportunistically collected from all animals being admitted to one of the conventional or specific pathogen-free hospitals for various reasons or being immobilized for an outpatient examination by a CNPRC veterinarian. In addition, samples from colony clinical cases or necropsies with suspect pathology that demonstrated evidence of a respiratory component and any animals entering a SARS-CoV-2–free barrier facility (set up with the aim of keeping animals free for SARS-CoV-2 research) were also collected. Due to the scarcity of available resources for antibody and PCR testing, this initial study was limited to these animals.

2.2 | Animals and sample collection

All animals were maintained in fully AAALAC-accredited facilities in accordance with the Animal Welfare Act, Regulations, and the Guide for the Care and Use of Laboratory Animals.[23,24] The following samples were collected: (a) nasal and oropharyngeal swabs using either a Universal Viral Transport Collection Kit (Becton Dickinson, Franklin Lakes, NJ) or sterile polyester tipped 6" applicator sticks (Puritan, Guilford, ME) submerged in 1 mL of TRIzol Reagent (Thermo Fisher Scientific); (b) approximately 1 g feces placed in a sterile plastic vial; and (c) 5 mL whole blood (without anticoagulant). TRIzol is a monophasic solution of phenol and guanidine isothiocyanate commonly used to fix and preserve blood and body samples for nucleic acid isolation. The whole blood was centrifuged at 1000 g for 15 minutes to separate serum for antibody testing. The samples were stored frozen at ≤−80°C until tested. A subset of surveillance samples collected were selected and tested on a weekly basis to provide the widest distribution possible, that is, to maximize the number of indoor and outdoor housing types and locations represented. Any reactive samples were rechecked by repeat sampling and testing.

2.3 | Antibody

SARS-CoV-2 strain Wuhan-Hu-1 recombinant spike antigen-coated microtiter plates, commercially available from Xpress Bio, were used for antibody screening. The manufacturer describes the antigen as a mixture of recombinant spike S1 and spike S2 glycoproteins produced in HEK293 cells and purified with Protein G chromatography. The S1 protein contains the amino acids 1-674 with a C-terminal sheep Fc-tag, and the S2 protein has amino acids 685-1211 with a C-terminal sheep Fc-tag. The spike S1 protein contains the receptor-binding domain that has affinity to the angiotensin-converting enzyme 2 region, and the spike S2 protein contains the fusion machinery and is anchored to the virus membrane.[25,26] The plate also includes wells coated with an uninfected cell control antigen. A protocol based on other ELISAs already in use by PAL was established: Briefly, serum samples were diluted 1:50 in phosphate-buffered saline with goat and bovine serum for blocking. After 45 minutes of incubation at 37°C, the wells were washed five times with a Tris-buffered saline containing surfactant. Peroxidase-conjugated anti-simian IgG (Xpress Bio) or anti-monkey IgM (KPL) was then added and incubated for an additional 45 minutes at 37°C. Wells were washed again and finally incubated with 2'2'azino-di[3-ethyl-benzothiazoline sulfonate (ABTS) for 30 minutes at room temperature. The reaction was stopped by adding 2N sulfuric acid to change the pH and then read at 405 nm with a 600 nm blank in a Tecan Sunrise Reader. The reagents and protocol were validated using sera from experimentally infected or vaccinated macaques graciously provided by Emmie de Wit, PhD, NIAID, NIH, and Dennis Hartigan O'Connor, MD, PhD., at our institution and PAL-archived normal sera collected from 2017 to 2019 as controls. Vaccination against RBD was accomplished by DNA prime and adenovector boost.

A subset of serum samples was used to format additional ELISAs using commercially available antigens: spike protein trimers from SARS-CoV-2 (BetaCoV/Wuhan/IVDC-HB-05/2019) (aa 1-1208; GIAID# EPI_ISL_402121) and nucleocapsid protein (NP) from coronavirus SARS-CoV-2 (COVID-19/Wuhan) with 6xHis tags at C-terminus (Immune Technology Corp); and SARS-CoV-2 (2019-nCoV) spike S1 + S2 ECD and nucleocapsid His tag recombinant proteins expressed in insect cells (Sino Biological). Following titrations to optimize antigen, blocking, primary dilution, and conjugate conditions (concentrations, times, and temperatures), a protocol was developed and validated using the previously described controls. Briefly, 96-well Nunc MaxiSorp microtiter plates (Thermo Fisher) were coated overnight at 4° with 10 ng/well antigen diluted in PBS and blocked for 2 hours at room temperature with PBS containing 1% bovine serum albumin and 1% dried nonfat milk powder. Sera were diluted 1:100 in the blocking buffer and incubated at 37° for 2 hours. Following five washes with PBS containing 0.1% Tween 20, SARS-CoV-2–specific antibodies were detected by subsequent incubation at 37° for 1 hour with horseradish peroxidase–conjugated anti-monkey IgG (KPL); washing; and finally incubation with 3,3',5,5'-tetramethylbenzidine (TMB) for 10 minutes at room temperature. The reaction was stopped by adding 2N sulfuric acid to change the pH and read at 450 nm with a 600 nm blank.

2.4 | RNA extraction

Viral RNA was extracted from the oral and/or nasopharyngeal swabs using viral total nucleic acid purification reagents on the semi-automated Maxwell RSC Instrument (Promega) following the manufacturer's instructions. 200 µL of swab eluate was lysed and proteinase K treated for 10 minutes at 56°C, before being added to a reagent cartridge and loaded into the instrument for automated extraction of nucleic acid using paramagnetic particles as a mobile solid phase for sample capture, purification, washing, and elution in 50 µL final volume.

2.5 | PCR

5 µL of viral RNA was transcribed to cDNA and subsequently amplified using the TaqPath 1-Step RT-qPCR Master Mix (Thermo Fisher) in a 15 ul reaction. Cycling conditions were 2 minutes at 25°C, 15 minutes at 50C, 2 minutes at 95°C, 45 cycles of amplification for 3 seconds at 95°C, and 30 seconds at 55°C. The reactions were carried out in a QuantStudio 12K Flex Thermocycler (Thermo Fisher). Singleplex PCRs used the CDC-designed oligonucleotide primers and FAM-labeled probes for the N1, N2, and N3 virus nucleocapsid gene segments[27] and the single-copy human RNase P gene as a control for amplifiable DNA (IDT). Multiplex reactions used the same N1 and N2 primers and probes duplexed with diploid oncostatin M (OSM) primers [28] and VIC-labeled probe as an internal control for the presence of amplifiable DNA (Thermo Fisher). A plasmid for the SARS-CoV-2 (Wuhan-Hu-1) nucleocapsid gene (IDT) and TRIzol-inactivated SARS-CoV-2/human/USA/CA-CZB-59X003/2020 MT394531 tissue culture RNA generously provided by Dr Timothy Carroll and Joseph Dutra at our institution were used as positive controls. [Correction added on 20 July 2020, after first online publication: in the preceding sentence, 'SARS-CoV-2 (USA-WA-1)' was corrected to 'SARS-CoV-2/human/USA/CA-CZB-59X003/2020 MT394531'.] Amplification was measured as cycle threshold (Ct) reflecting the linear phase of the amplification curve as the fluorescence intensity increases due to the reporter dye molecules being cleaved from the probes.

3 | RESULTS

3.1 | Samples

385 animals housed in 73 different indoor rooms or outdoor enclosures were tested in March, April, and May 2020. 256 paired blood and swab samples were tested for SARS-CoV-2 antibody and RNA. Sera from 120 additional monkeys were collected and tested for antibody without parallel PCR testing due to a lack of collection swabs. PCR testing was performed on nine monkeys for which serum could not be collected. Repeat samples from the same animals were collected on later dates for 20 PCR and 33 antibody determinations. No changes in reactivity were observed in the repeat testing. The study population included 157 males and 228 females ranging from 1 to 24 years of age. Fecal samples were archived frozen at −80°C for future studies.

3.2 | Antibody

No SARS-CoV-2 antibody was detected. The data generated using the Xpress Bio Spike S1, S2 antigen are shown in Figure 2. The delta (spike—control antigen) OD values for the 13 samples that were used as positive controls were all > 0.359. These samples were collected at multiple post-inoculation timepoints from five animals that were either experimentally infected or vaccinated. The values for the historical negative samples collected and banked prior to 2020 ranged from −0.235 to 0.222 with the exception of one outlier at 0.543. The 2020 surveillance sample values were similar to the historical samples (collected 2-3 years earlier) with delta OD values ranging from −0.246 to 0.225, excluding three animals with high outlier values at 0.548, 0.964, and 0.693, respectively. Repeat ELISA testing with IgM conjugate yielded negative delta OD values for the outliers, thus ruling out a current or recent infection. Aliquots of these four outlying serum samples were sent to Dr William Boteler (Xpress Bio) for further workup with a panel of coronavirus antigens. Dr Boteler and colleagues found no reactivity to either recombinant His-tag–labeled receptor-binding domain prepared in insect cells, or His-tag–labeled nucleocapsid prepared in *E coli*. In addition, no reactivity to seasonal coronaviruses 229E and NL63 was detected.

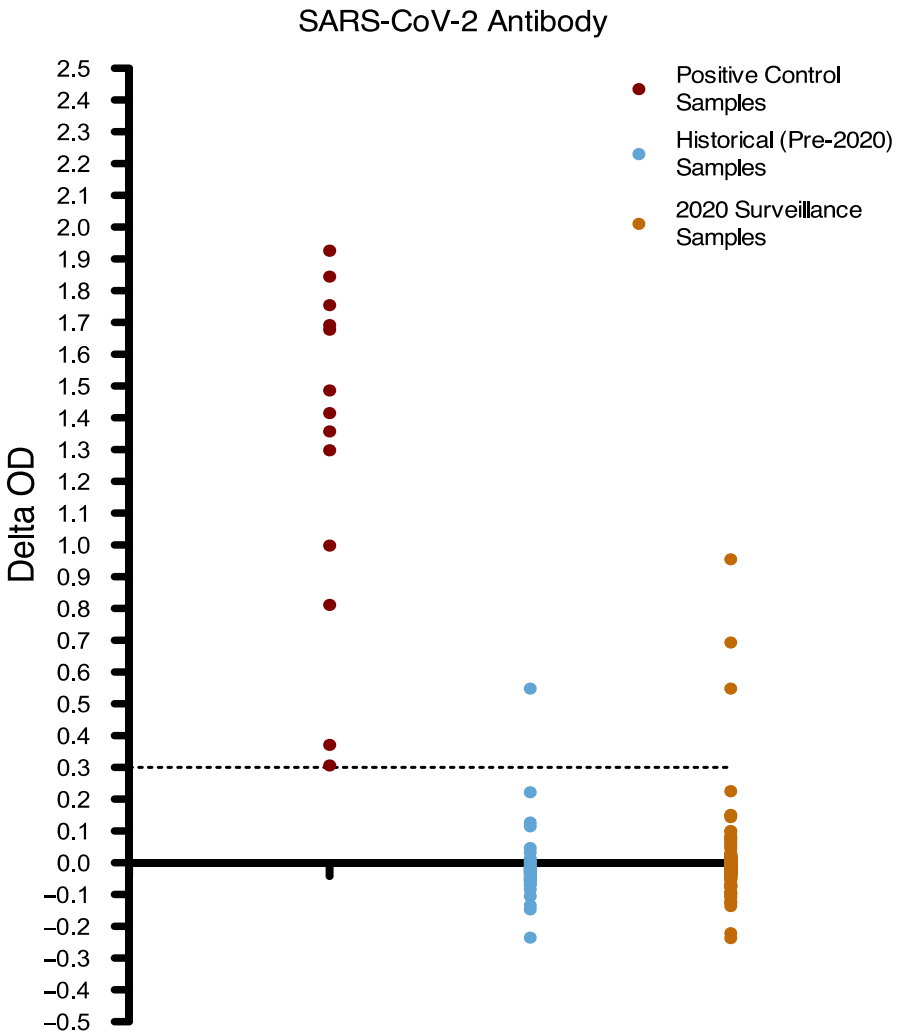

FIGURE 2 Xpress Bio antigen ELISA results for positive control, historical (collected pre-2020), and current 2020 surveillance samples. The delta OD value represents the difference in reactivity between SARS-CoV-2 S1,2 antigen and an uninfected cell control. A dashed line below the lowest positive control value highlights the range of negative values and outliers in the historical and surveillance groups

By comparing reactivities to a panel of antigens prepared in different cell lines, they were able to conclude that the observed reactivity to spike S1, S2 antigen was due to reactivity to either the HEK cell line or the sheep Fc purification tag used in its production. Figure 3 displays additional, parallel data obtained from the ELISAs developed in the PAL laboratory. Although the data are limited, differences in antigen preparations were observed and not all performed acceptably. Despite titrations to optimize antigen concentrations, blocking protocols, sample and conjugate dilutions and buffers, incubation times, and temperatures, we could not clearly distinguish positive from negative samples with the SB spike antigen. We speculate that this could be caused by non-SARS-CoV-2–specific reactivity due to this recombinant protein epitope or its production method. Assay development and validation work are continuing with the other antigens. The antibody data are summarized in Table 1.

3.3 | RNA extraction and PCR

No SARS-CoV-2 RNA was amplified from any of the monkeys. As it became difficult to acquire the Universal Viral Transport Collection Kit (UVT) used for initial sample collection, later samples were collected using sterile polyester tipped 6" applicator sticks submerged in 1 mL of TRIzol Reagent. Similarly, TRIzol fixed RNA from cultured coronavirus, spiked into swab samples, and was used as an extraction control to test equivalence of recovery from both types of collection materials as well as storage stability. When spiked into the swab eluate and extracted, the tissue culture RNA Ct values for N1, N2, and N3 were 18.8, 19.6, and 19.4 as compared to 17.9, 18.6, and 19.0 when the same amount of tissue culture RNA was added directly to the PCR without extraction, thus showing an average of 96.0% recovery (Figure 4A). Two samples collected by both UVT and polyester swab in TRIzol systems yielded RNase P Ct values of 26.8 and 22.9 in UVT as compared to 25.6 and 22.7 in polyester/TRIzol. The mean RNase P Ct value for UVT samples was 27.0 (ranging from 20.9 to 31.0) as compared to a mean of 23.6 (ranging from 20.0 to 28.3) for TRIzol samples. Similarly, the OSM values for UVT samples were 23.0 (ranging from 20.31 to 26.) as compared to 21.1 (ranging from 17.1 to 28.9) for TRIzol samples (Figure 4B). The broader range correlates with more samples tested. All values met the acceptable CDC criterion of less than 35.0.[29] These ranges indicated comparable recovery of RNA from samples collected in either system. As compared to processing of a fresh sample, Ct values for N1, N2, and OSM Ct did not change significantly following 1-week storage at room temperature, 4°C, and −80°C (Figure 4C). The loss of reactivity to N1

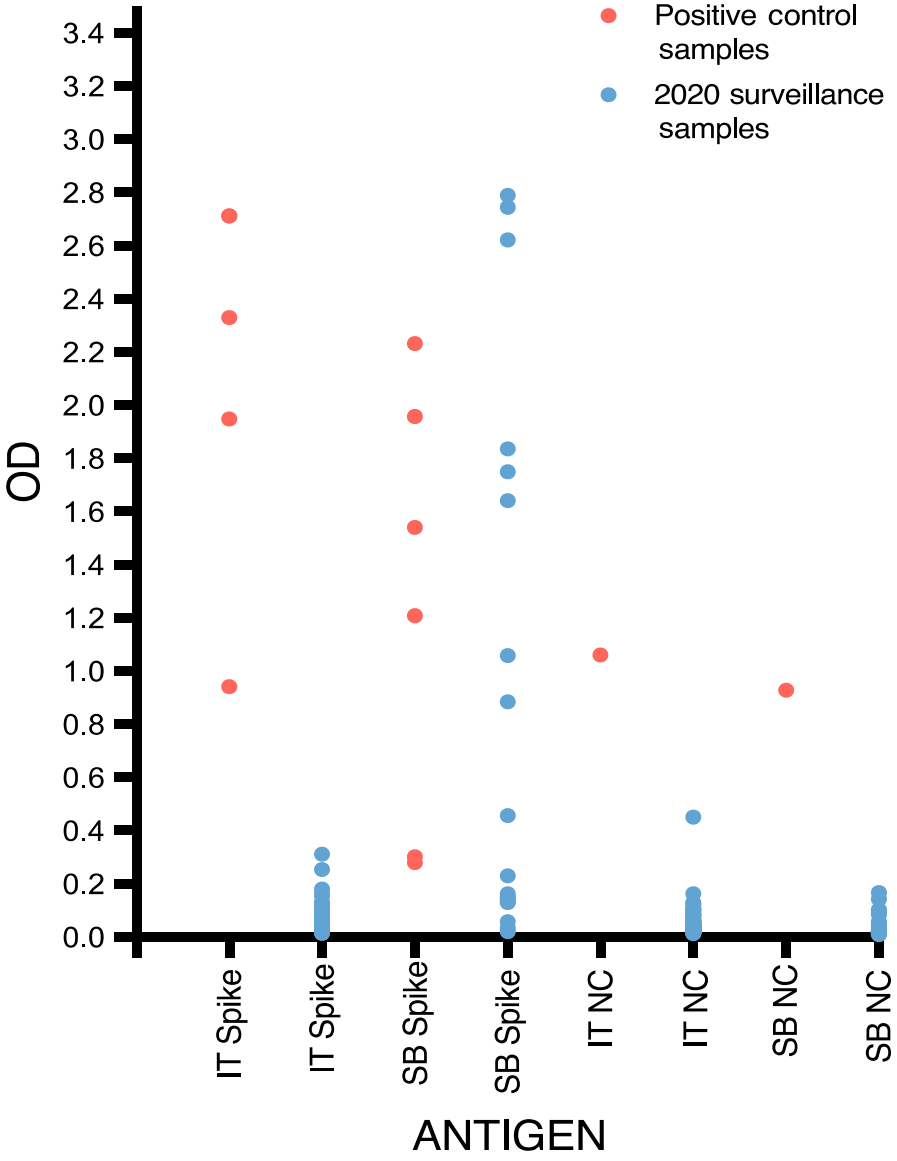

FIGURE 3 Performance of ELISAs formatted with commercially available SARS-CoV-2 spike and nucleocapsid antigens on a subset of current 2020 surveillance and positive control samples. The IT spike antigen is aa1-1208 from BetaCoV/Wuhan/IVDC-HB-05/2019 expressed in 293 cells; the SB spike antigen is a DNA sequence encoding the 2019-nCoV spike S1 + S2 expressed in baculovirus-insect cells. The IT NC is the nucleocapsid protein from COVID-19/Wuhan expressed in E. coli; the SB NC antigen is a DNA sequence encoding the 2019-nCoV nucleocapsid protein expressed in baculovirus-insect cells

TABLE 1 Summary of surveillance samples tested using different SARS-CoV-2 antibody ELISAs

Source	Antigen	# Tested	# Reactive
Xpress Bio	Spike S1 + S2	409	3
Sino Biological	Spike S1 + S2	18	9
Immune Technology	Spike trimer	69	0
Sino Biological	Nucleocapsid	18	0
Immune Technology	Nucleocapsid	69	1

Note: In addition, 40 samples collected and archived prior to 2020 were tested using the Xpress Bio spike antigen S1 + S2. 1 was reactive.

and N2 was only slight over 3 freeze-thaw cycles (Figure 4D). Initially, samples were amplified in 4 singleplex reactions for N1, N2, N3, and RNase P in separate wells. In accordance with changes in the CDC recommendations, after 04/03/2020, N3 reactions were not performed. Following validation of VIC-labeled OSM primers as a duplexed internal control gene for amplifiable DNA as shown by the Ct values in Figure 5, all remaining surveillance samples were tested in the duplex format.

4 | DISCUSSION AND CONCLUSIONS

No detection of SARS-CoV-2 antibody or RNA in this 10% sentinel surveillance of the CNPRC macaque colony suggests that the current colony management practices, including restricted access and PPE, are sufficient. However, it is difficult to adequately assess the extent of risk reduction achieved because the prevalence of infection in facility staff could not be clearly determined under the current conditions of limited human testing. Currently, PPE in routine use at the CNPRC includes standard surgical masks and face shields, which can reduce respiratory pathogens but may not be as effective as NIOSH-approved respirators.[30] Until we better understand the epidemiology of SARS-CoV-2 in the human population, it is prudent to assume that non-human primate

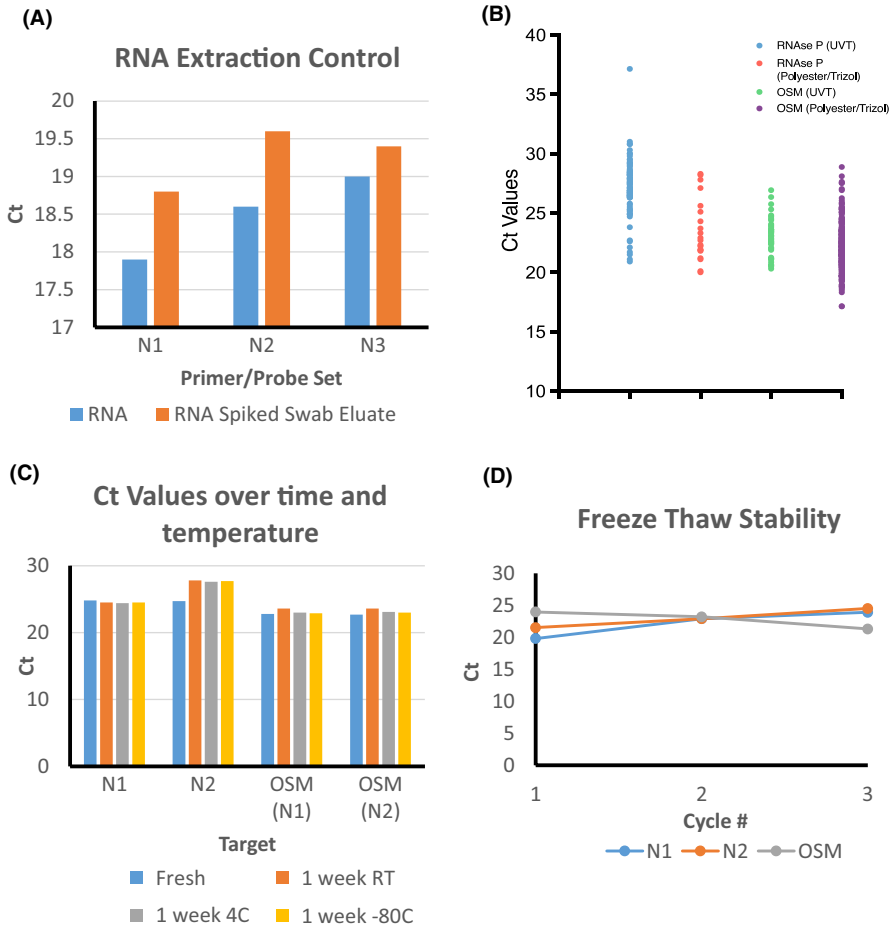

FIGURE 4 Validation of collection, elution, and extraction protocols for SARS-CoV-2 RNA. A. Extraction control: Ct values for N1, N2, and N3 values for the recovery of TRIzol fixed tissue culture RNA spiked into swab eluate and extracted as compared to the same dilution of TRIzol fixed tissue culture RNA added directly to the PCR. B. Comparison of amplifiable DNA control Ct values for samples collected using the Becton Dickinson Universal Viral Transport system and a polyester swab submerged in TRIzol. C. Stability: N1, N2, and OSM Ct values for samples tested fresh as compared to after storage for 1 week at room temperature, refrigerated (4oC), and frozen (-80oC). D. Stability: A single aliquot of TRIzol fixed tissue control RNA spiked swab eluate was frozen at -80o and then thawed for PCR testing 3 times to compare changes in Ct values

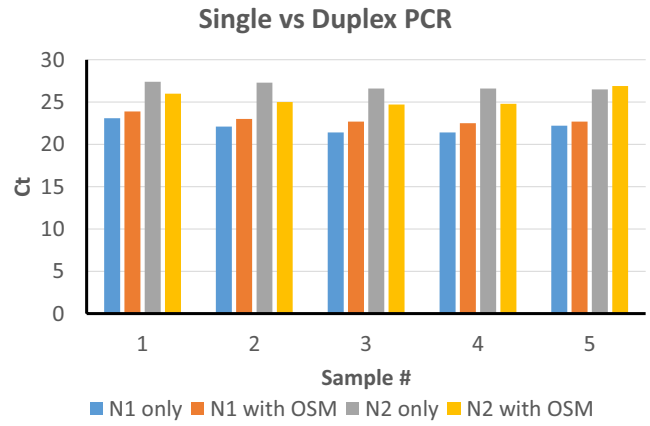

FIGURE 5 Comparison of singleplex vs duplex PCR: 5 individual swab eluates were spiked with TRIzol fixed tissue culture RNA prior to extraction. The resulting RNA was then amplified with either N1 and N2 primers and probe alone or duplexed with the internal cell gene OSM used to control for amplifiable DNA

populations are at risk of infection from humans. That risk makes the development of an active surveillance program essential. The collection and testing of these samples provide valuable baseline data for ongoing monitoring. With continued prevalent cases in the human population (including those who have potential contact with the non-human primate colony), continued testing of at least a representative sample of the colony on a regular basis will be necessary. Given the logistical and resource limitations and lack of active infection, antibody testing will be used for general ongoing surveillance, with RNA detection reserved for animals showing respiratory signs, relocation to sensitive locations, and pre-research study screening. Increased, widely available human testing to determine the accurate prevalence and transmission rates will allow for better informed risk assessment and refinement of the surveillance program for the CNPRC non-human primate colony. Thus, surveillance testing for employees is being proposed to complement the NHP colony surveillance efforts.

Important experience with various commercially available and laboratory-developed reagents has been gained from this study. Using the currently available assays, we will apply the algorithm described in Figure 6 to ongoing testing at the CNPRC. This algorithm considers the purpose of testing and prioritizes the use of resources by using antibody only for general surveillance and reserving RT-PCR testing for more critical situations including supporting COVID-19 research and ruling out respiratory-related clinical signs or pathology findings. Currently, there are no validated confirmatory antibody tests to facilitate a traditional screen and confirm sequence.[21] In the interim, we have substituted repeat sampling and testing using a battery of supplemental immunoassays targeting

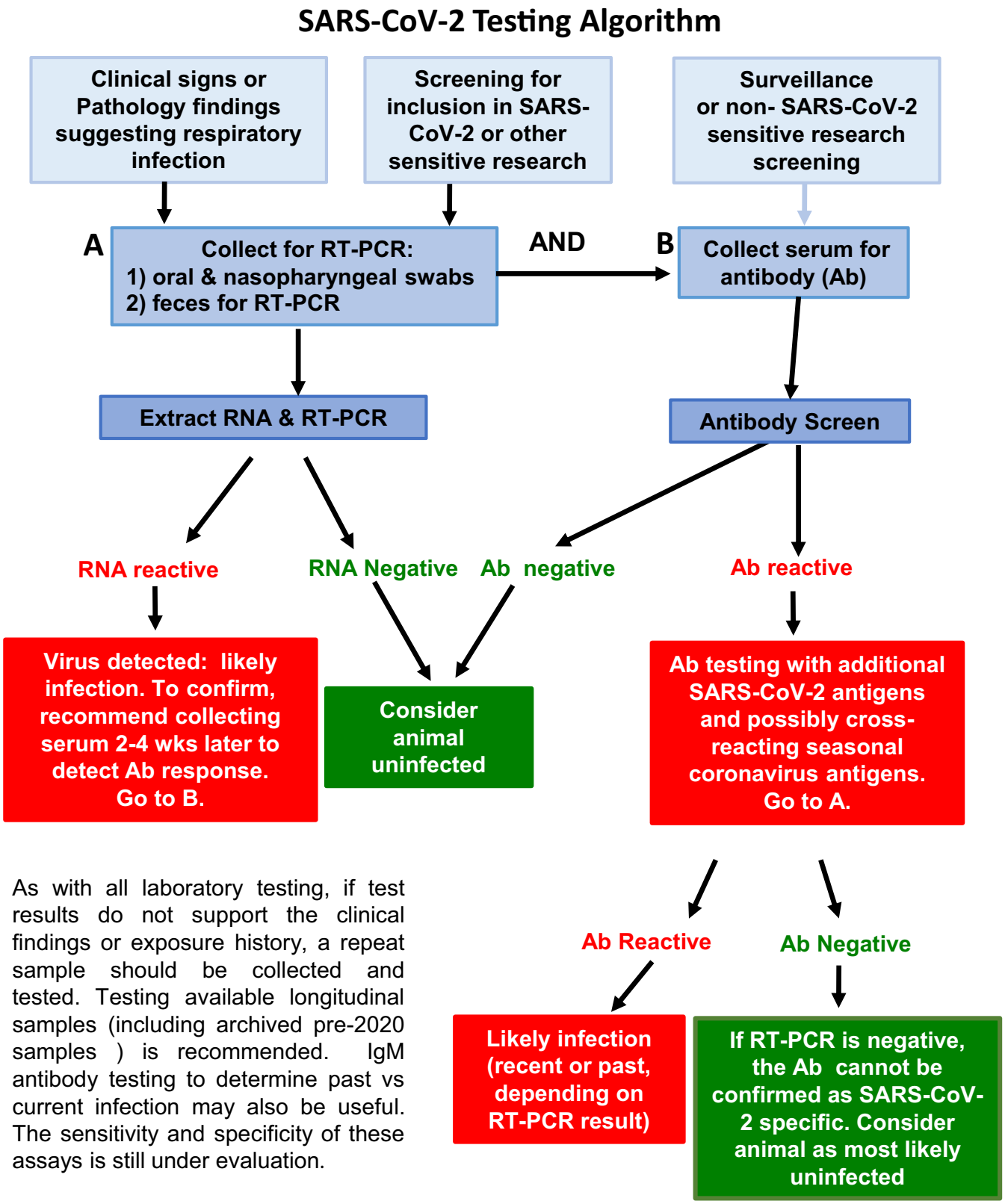

FIGURE 6 SARS-CoV-2 testing algorithm

various spike, nucleocapsid, receptor-binding site, whole virus, and possibly cross-reacting seasonal coronavirus antigens. As additional antigens are validated, we will re-evaluate the assays and combine them in a configuration that provides the most accurate and efficient screening and confirmatory algorithm. The availability of archived frozen samples collected before 2020 will also provide useful specificity controls. We have other research studies in progress developing primer and probe sets for additional gene sequences. We will

be able to evaluate which and how many are necessary to further enhance the accuracy and efficiency of virus detection.

The National Primate Research Centers Pathogen Detection Working Group (PDWG) has shared testing guidance and outlined a general testing algorithm on their website.[31] We will use this information and work collaboratively with the PDWG to efficiently validate assays and a testing algorithm that ensures high quality, accurate, rigorous, and reproducible results appropriate to the questions being asked. Group members have already generously shared suggestions, critiques, protocols, reagents, controls, and testing experience to begin testing across NHP colonies. The PDWG has successfully developed testing algorithms and shared protocols, reagents, controls, and proficiency testing for a panel of specific pathogen-free agents. Similar collaborative effort will lead to the development of a testing algorithm for SARS-CoV-2 that effectively supports management of non-human primate colonies.

The identification of a reactive test for either PCR or antibody in the non-human primate colony would necessitate a rapid management response to control possible spread. The standard response for a respiratory outbreak in the colony is to isolate the affected room, limit access to essential animal care and veterinary personnel, stop all animal moves, and place appropriate quarantine signage. These steps are complicated if a case is diagnosed in a larger outdoor enclosures that houses 100-150 animals. In those large outdoor locations, quarantine measures would be aimed at confining infection to that particular enclosure, where, as herd immunity builds up, infection is expected to eventually die out. The open-air environment and distance of more than 12 feet between outdoor caging units would possibly reduce the risk of aerosol transmission between outdoor enclosures.[32] Each NHP facility should develop appropriate preventative steps to formulate a plan for control of SARS-CoV-2 in their colony. It is evident that rhesus macaques and other NHP free of the SARS-CoV-2 pathogen are going to be essential for the development of immunomodulators and vaccines. The supply of these valuable research animals has decreased with the temporary reduction in NHP importation.[33,34] The ability of the biomedical research community to end the COVID-19 pandemic will depend not only on the availability of NHP, but also more importantly on the availability of NHP that are specific pathogen-free for SARS-CoV-2. Thus, it is important for NHP research facilities to develop a robust surveillance program and a plan for a response to the detection of SARS-CoV-2 in their colony. The CNPRC has established a barrier colony of SARS-CoV-2–negative animals to ensure availability of SPF animals for future COVID-19 research.

Colonies of outdoor housed primates are potentially at risk from exposure to other species of wildlife that may be susceptible to SARS-CoV-2 infection. However, the most likely route of introduction of SARS-CoV-2 into a non-human primate colony remains to be from employees and visitors from the community. Thus, the success of surveillance and emergency response plans to prevent and control SARS-CoV-2 infection in NHP colonies also depends on our ability to integrate these NHP facility control measures with medical and public health programs that target SARS-CoV-2 in human communities. The programs and ideas described in this manuscript can help to bridge this gap and promote the One Health approach to society's effort to defeat the COVID-19 pandemic.

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