ASSOCIATION OF PRIMATE VETERINARIANS

GUIDELINES FOR MANAGEMENT OF SARS-CO-2 WITHIN A NONHUMAN PRIMATE COLONY

PURPOSE AND BACKGROUND

During the winter of 2019, a novel coronavirus was identified as the source of a pneumonia outbreak within Wuhan, China. The World Health Organization (WHO) named this new coronavirus SARS-CoV-2 and the resulting disease COVID-19. SARS-CoV-2 quickly spreads by contact with an infected person and their droplet/aerosol production. Nonhuman primates (NHPs) can be infected with SARS-CoV-2, making them both an effective animal model for SARS-CoV-2 research and a population at risk for contracting and spreading the virus.

The purpose of these guidelines is to provide NHP veterinarians, colony managers, and researchers with strategies to manage both personnel and NHPs. Information on the most current research and surveillance methods for both humans and NHPs are included. These guidelines are not designed to be all inclusive or final. Instead, these guidelines are designed to be updated when significant new information or changes in management strategies are identified.

GUIDELINES

A. Colony Management

Protection of NHP colonies from infection with the sudden emergence of SARS-CoV-2 was a rapid priority for facilities with large populations of NHPs. Early experimental data demonstrated that several NHP species (rhesus macaques, cynomolgus macaques, marmosets, baboons, and African green monkeys) are susceptible to experimental infection with varied severity of clinical disease\(^1,9,10,14,19,22\). Irrespective of the level of clinical disease, it is critical to protect NHP colonies from widespread SARS-CoV-2 infection to preserve the supply of animals available for COVID-19 research. As the knowledge about this novel virus increases, colony practices will likely change based on data. There are multiple considerations and approaches to managing NHP colonies which are discussed below.

1. Personal Protective Equipment (PPE)
The use of extensive PPE when working with NHPs is routine among primate facilities. Personnel wear a mask, gloves, and eye protection (safety glasses or face shield) routinely when working with NHPs in research. This level of protection should greatly reduce the risk of transmission of a respiratory virus from personnel to NHPs; however, it requires strict compliance with PPE and other safety measures. Cases of non-COVID respiratory virus transmission from humans to NHPs have been reported. Training and re-training on correct PPE use when working with NHPs is essential practices. Facilities with NHPs can consider the use of N95 respirators to prevent virus transmission. However, widespread use of N95s is problematic given the likelihood of PPE shortages during a pandemic. In addition, facilities utilizing N95s must be able to accommodate fit testing for staff that are new to wearing an N95 respirator. Facilities should consider use of N95 respirators (without an exhaust valve), PAPRs (when available), or surgical masks and face shields for staff in contact with NHPs that are a high risk for COVID-19 (immunosuppressed, geriatric, designated for COVID-19 research).

2. Restricted Access

Animal facilities should consider restricted access of personnel to the animal colonies during a pandemic. It is recommended that access be limited to essential staff only and to reduce as much as possible the number of personnel working closely with NHPs. Limiting the number of people with close interactions with the animals could help mitigate the risk of transmission. This should be universal for other pathogens in addition to SARS-CoV-2. This includes elimination of visitors as well as restrictions to essential researchers working hands on with the animals. It is recommended that all personnel evaluate their personal health before reporting to work and ensure that they are healthy. In addition, facilities should consider taking additional screening precautions for staff working closely with NHPs and ask them to attest daily that they are healthy to report to work.

3. Changes to Food Provision

Preparation of food for NHPs should be performed with personnel wearing a mask and gloves in order to prevent contamination of any food administered to the NHPs. In addition, facilities should ensure produce is appropriately cleaned prior to distribution to NHPs.

4. Animal Quarantine

Facilities should consider creating a barrier colony to protect subsets of the NHP population from SARS-CoV-2 to be used in virus related research. These animals should be screened and relocated to indoor housing in separate rooms with strict quarantine PPE procedures including the use of an N95 respirator. If colonies need to be maintained free of SARS-CoV-2 exposure, they should be isolated under a CDC style quarantine protocol.
5. **Colony Screening**

Facilities should consider developing a screening program for antibody and/or PCR testing. Although screening of animals is essential to monitor for virus within the colony, this strategy can present challenges. Accessing and anesthetizing animals is challenging for facilities with reduced staffing. In addition, sedation for sample collection will increase human contact between animals and staff and may increase risk of disease transmission. The development of screening mechanisms that utilize noninvasive samples is an important tool to continue to explore. In addition to screening the general colony, many NHP facilities are performing SARS-CoV-2 testing on any NHPs with evidence of respiratory disease. Facilities should have protocols or practices in place for screening NHPs that present with evidence of respiratory disease.

More information on colony screening is available under Testing and Surveillance in these guidelines.

6. **Management of a SARS-CoV-2 Positive NHP**

At this time there has been one published case of natural transmission of SARS-CoV-2 to a NHP. NHP facilities should be prepared to manage a NHP population in the event a positive case develops. Preparations should include the ability to do “contact tracing” for the NHP and any other animals that might have been exposed. Positive animals and their contacts should be placed in quarantine conditions (if possible), and the use of additional PPE, such as an N95 respirator, should be considered. The timeline for complete clearance of the virus is unknown at this time, as such there is no official guidance on when/if it would be safe to clear the animal(s) to return to the colony. Repeated testing would be ideal to understand when the risk of transmission to staff and other monkeys is reduced. Facilities should consider evaluation/testing of staff in contact with any NHP that becomes SARS-CoV-2 positive to identify potential carriers. Facilities should be aware of and comply with local, state, and federal public health agencies notification regulations if a SARS COV2 positive NHP is identified.

7. **Assess the overall facility program**

Facilities should review all existing procedures in place and assess their priorities and needs. Examples include cleaning schedules, feeding schedules, and scheduled tasks that can have extended timelines. Consider animal welfare and the regulatory requirements when making decisions in time of hardship and reduced staff. Consider working with regulatory agencies to allow exceptions to the existing regulations.

B. **Animal Care Staff Management**
Ensuring the health of animal care and research staff is critical to safeguarding NHP colony health. There are several measures facilities may want to explore with their staff to protect their NHP colonies. Key considerations include physical distancing, good hygiene, PPE use in both the vivarium and public spaces, and health monitoring such as symptom checks and human virus surveillance.

1. **Physical or social distancing** is one of the best ways to reduce the spread of SARS-CoV-2. The virus spreads mainly from person to person through respiratory droplets produced when an infected person coughs, sneezes, or speaks. Studies show that these droplets can travel 6 feet. Thus, physical distancing strategies typically include maintaining a 6-ft distance from others and avoiding group gatherings. There are several modifications to consider in the biomedical research setting:

   **Meetings:** Many options are available to support virtual meetings with audio and video capabilities. Steps should be taken to ensure security of virtual meetings including one-time PINs or multifactor authentication, announcement when attendees join, and a dashboard to monitor attendees. Staff could also meet outside as weather allows or in large conference rooms that allow 6-ft distancing between participants.

   **Creative staff scheduling** can be an excellent way to promote social distancing as animal care and/or research needs allow. Positions that can support teleworking, or blended schedules of hands-on activities and teleworking, are effective ways to promote physical distancing.

   **Staggered work hours** to reduce the number of staff on campus at one time and/or to create teams of staff that alternate coverage will reduce the impact if one person on that team tests positive for SARS-CoV-2. Rotational teams including staff from all areas of the animal care enterprise (i.e., veterinarians, veterinary technicians, husbandry, behavior staff, etc.) may be a good option depending on staffing numbers needed to maintain animal care and research operations. If isolated rotating teams are not an option, staggering start and end times as well as lunch and break times of staff, is another way to reduce density of staff in common areas.

   **Physical barriers** such as Plexiglas or curtains are useful for staff without dedicated office spaces or in shared open space/cubical type environments. Signage in shared space, such as 6 ft apart floor decals in areas where lines form (e.g., lunchroom) and bathroom signage indicating when the space is occupied, can be helpful in supporting physical distancing. Facilities with elevators may impose limits on the maximum number of riders in the small space, typically recommending no more than 1-3 people on the elevator at one time depending on size.
Minimize number of people present for a task/procedure. Encourage both animal care and research staff to reevaluate the number of people present for procedures like surgeries, necropsies, and other research and animal care-related events. Ensure only essential personnel to accomplish the tasks are present. This also helps conserve PPE by reducing the amount of PPE used by non-critical personnel. Placing signage for maximum capacity on procedure rooms in animal areas can be useful.

Visitor policies should be reassessed, including student and outreach programs, to limit NHP colony and animal care staff exposure to the community and to reduce the number of people entering a facility. Virtual tours may be a suitable replacement for in-person visits. In addition, internal quality assurance teams can serve on behalf of clients/sponsors to perform audits as necessary.

2. **Personal hygiene** is critical to limit exposure to and prevent the spread of SARS-CoV-2. Staff should already be washing their hands or using hand sanitizer after doffing PPE when exiting the vivarium. Encourage additional hygiene practices such as frequent hand washing for at least 20 seconds, especially after having been in a public place/blowing their nose/coughing/sneezing. Hand sanitizer that contains at least 60% alcohol may be used if soap and water are not available. Sanitization stations should be provided around a facility, particularly at all vivarium entrances/exits. COVID-19 prevention supplies, such as soap, alcohol-based hand sanitizers, disinfectant sprays and/or wipes, tissues, and trash baskets, should be provided in common areas. Working closely with facilities, janitorial, and/or administrative staff, shared surfaces should be cleaned and disinfected frequently throughout the day.

3. **Facility modifications and engineering** should be considered to decrease cross contamination by highly touched items, such as toilet flush handles, sink faucets, soap dispensers, etc. Alternatives, such as hands-free options can be installed to decrease touching common areas. Door opening direction can also be reviewed to decrease touch points.

4. **Personal protective equipment (PPE)** must be provided and worn appropriately in the vivarium as well as in public spaces to help prevent SARS-CoV-2 transmission. Acquiring PPE may be challenging, especially since supplies are short and demand has increased. Reuse and conservation strategies are crucial to ensure facilities maintain an appropriate supply. Standard ABSL-2 or higher PPE commonly used for NHP work may be considered adequate to reduce the risk of natural SARS-CoV-2 transmission. However, PPE must be used properly and maintained in good working condition to be effective. Many institutions, especially those associated with human healthcare, have instituted “masks on” policies for the entire facility particularly in any shared or public spaces, requiring all staff to wear face coverings when at work. The CDC recommends wearing face coverings in public settings where social distancing measures are difficult
to maintain. Due to variable availability of several PPE items (e.g., procedure masks, face shields, gloves, Tyvek garments, etc.), the need for flexibility is critical. When modifying PPE practices, consult your occupational health, environmental health and safety, or commensurate department for guidance.

PPE conservation methods that may work for your facility:

a) **Surgical or procedure masks** play an important role in respiratory protection, however with CDC “masks on” guidance, supplies of fluid rated procedure masks typically used in NHP care have been impacted. Options for conserving masks include re-evaluation of scenarios that require masks, reuse of masks where appropriate, and use of cloth or non-fluid rated masks in place of procedure masks. Based on risk assessment, in select scenarios face shields may be adequate for use without a procedure mask (e.g., walking down an animal corridor with no animals present, working outdoors, maintaining greater than 6-ft distance from NHPs). If a procedure mask has been worn in a standard ABSL-2 NHP area and is free of gross contamination and moisture after use, a mask may be removed with clean gloves and stored in a sealable plastic bag (allowing up to 8 hr of reuse) or paper bag (allowing more than 8 hr of reuse). Duration of reuse depends on the mask remaining intact and uncontaminated and should be based on a risk assessment by facility health and safety personnel. Cloth or non-fluid rated masks may replace surgical masks under specific circumstances. These masks are not fluid resistant and should be worn with a face shield for hands-on NHP work or situations where fluid splash is likely. Cloth or non-fluid rated masks are not appropriate for preventing contamination of sterile surgical fields and should not be used during aseptic surgical procedures. Cloth masks used in animal areas should be laundered at the facility rather than being taken home to launder.

b) **Face shields** are another product in high demand and used commonly in NHP facilities. Purchase and distribution of reusable face shields is an excellent way to conserve the disposable face shields that may be difficult to source. Similar to procedure masks, disposable face shields may be reused if they remain undamaged and are disinfected after use in NHP areas.

c) **Boot and shoe covers** are a challenge to source in some areas. Dedicated shoes/boots are an option to reduce dependence on shoe/boot covers in NHP areas. Also, based on risk assessment, reuse of shoe/boot covers for select areas or NHP rooms may be a viable option. If Tyvek shoe covers are used, these may be autoclaved and reused as long as their integrity is regularly checked, and they are discarded when compromised.
d) **Tyvek** brand garments are in limited supply and may be worn by individuals in street clothes entering the animal facility. Thus, requiring scrubs when entering NHP areas may reduce the need for Tyvek. Use of reusable gowns (laundered after each use in-house or with an external contractor) is another option to reduce Tyvek use. Finally, autoclaving Tyvek overalls may be an option.

5. **Monitoring strategies** and testing of animal care personnel are additional measures to prevent symptomatic and/or asymptomatic employees positive for SARS-CoV-2 from entering the vivarium and working with NHPs.

Symptom monitoring and reporting among animal care staff may be as simple as verbally checking in with staff at the beginning of each shift to confirm they are not showing any common symptoms of COVID-19. Digital modalities, including smartphone apps, have been developed that can be utilized as symptom checkers. Frequency of symptom checks is dependent on the comfort level of the facility and local SARS-CoV-2 demographics. A more active monitoring strategy may include temperature checks of all staff when they arrive at work prior to entering the vivarium. Forehead thermometers have been employed for this use, and some facilities have purchased individual thermometers for staff to self-test on a daily basis. Staff presenting with any COVID-19 symptoms, including elevated temperature upon arrival to work, or that become sick during the day, should not be allowed in animal areas, should be separated from other employees, and should be sent home immediately. They should not return to work until CDC return to work criteria are met (see below).

As resources and facility leadership support allow, development of a SARS-CoV-2 testing program for animal care staff can augment NHP surveillance testing and provide a means to proactively identify and appropriately quarantine asymptomatic, virus positive staff thus reducing the risk to the NHP colony.

a) Staff should not come to work if they are experiencing any symptoms associated with COVID-19. Testing of symptomatic individuals, especially critical function staff (such as animal care staff), may be provided for free at those institutions associated with a human health care facility.

b) Unfortunately, most people are infectious before symptoms are present, while others show no symptoms at all, so testing of asymptomatic staff may be warranted. It is imperative to consult facility occupational health personnel, local hospitals, and state boards of health as well as review CDC guidelines when embarking on a testing program for animal care staff.
When developing a program for asymptomatic or pre-symptomatic staff testing, considerations include who to test, how to test, and what the follow-up protocols are in case of positive results.

- Identify why you are testing. Is it to protect the NHP colony or to protect other staff? Is it to identify possible human to NHP and/or NHP to human natural transmission? If the main goal is to protect the NHP colony and other animal care staff, you should start with testing all asymptomatic staff who work hands-on with or within 6 ft of live NHPs in the colony.

- A testing site should be identified that is convenient and readily accessible for staff and allows for social distancing of individuals; this may be an outdoor area or large campus auditorium. Providing signage encouraging social distancing and requiring staff to wear masks when traveling to and while at the testing site will further help reduce the spread of disease.

- To adhere to HIPPA guidelines, reporting of results should come from occupational health or healthcare personnel associated with staff testing. Healthcare personal should have practices in place for human contact tracing in case of positive results. Individuals should be encouraged to notify their managers of their results so managers can make schedule adjustments to accommodate the subsequent required staff quarantine time and follow-up with other key stakeholders. Managers may coordinate with janitorial and facilities staff to decontaminate the positive individual’s office, cubicle, or shared workspace. They should also communicate the positive case to the animal care department head so NHP contact tracing or symptom monitoring can be pursued. If other emergency operations committees or groups have been established during the COVID-19 pandemic, it may be appropriate to communicate positive cases to that group as well for additional tracking and response.

- For asymptomatic staff that test positive for SARS-CoV-2, quarantine processes should be consistent with CDC guidelines. Currently the recommendation is that asymptomatic positive individuals quarantine at home for 10 days from the time of the positive test as long as they remain asymptomatic. Additional quarantine time may be required if symptoms develop, and they should consult their primary care physician. The facility should have policies in place for what they will require of roommates and/or significant others of the positive individual if those people also work with NHPs at the same facility. This may include a concurrent quarantine or active symptom monitoring.

For the most up-to-date information on SARS-CoV-2 and human health recommendations, please visit the Centers for Disease Control and Prevention.
Testing and Surveillance of SARS-CoV-2

Most laboratories that routinely provide testing for nonhuman primate viruses have been designing and validating assays and algorithms to add the detection of SARS-CoV-2 infection in NHP to their testing portfolio. Reports in the research literature suggest that this virus follows a typical pattern: viral RNA is detectable by real time PCR in samples collected by nasopharyngeal or oral swabs soon after the initial infection (days 3-21); followed by the host immune response as indicated by IgM antibody as early as day 7, and IgG antibody as early as day 10. Peak responses have been reported at days 21-28. The duration of antibody response has not been well documented, but other coronaviruses have variable antibody detection at 1 year post initial infection7,10.

A positive PCR reaction means virus is present in the sample and is indicative of infection, but a negative result only means there was no virus in the sample at the time it was collected18. Since a single negative result does not reflect the possibility that virus could have been shed before or after the time of collection, it does not rule out the possibility of infection. There are reports in the literature that multiple samples with no detectable virus are necessary before ruling out infection8,14. Conversely, non-infectious RNA particles may persist for months after infection. The detection of antibody indicates that exposure and infection have occurred. Detection of IgM would indicate a current infection, but detection of IgG does not distinguish current from past infection. Initial reports of whether a positive IgG titer is protective are conflicting1,6,10.

Many large colonies are choosing to use antibody testing for general surveillance. However, if active seroconversion or other signs of an active outbreak are present, PCR testing is an additional important tool to monitor viral transmission18. PCR may also be useful for relocations/introductions of new animals where a more stringent rule out of infection is needed or for investigation of animals with clinical signs or known exposures. A screen and confirm algorithm has been used successfully for most nonhuman primate virus antibodies17. This strategy provides sensitive screening using high throughput platforms, such as enzyme immunoassays or microbead immunoassays, with the smaller number of screen reactive samples tested by more complex, highly sensitive and specific assays such as Western blot or immunofluorescence assay. As more is known about the course of infection, PCR may also be a useful confirmatory test. For smaller numbers of samples or non-laboratory settings, a lateral flow rapid test requiring minimal instrumentation may be a good option.

The National Primate Research Centers’ Pathogen Detection Working Group laboratories have shared a document compiling their experience with various antibody and PCR reagents5. Some laboratories are using spike antigen ELISAs for the primary antibody screen, while others plan to use a panel (spike, nucleocapsid, Receptor Binding Domain (RBD), seasonal coronaviruses) of
ELISAs or multiplex microbeads (Luminex). The overall laboratory workflow, number of tests, and available instrumentation and reagents influence the sequence of assays. Currently, a combination of these immunoassays is used for screening and confirmatory tests until there are confirmatory assays such as immunofluorescence or Western blot ready for use. Once additional assays and more test data are available, a better-defined algorithm will be developed and validated. PCR on nasopharyngeal or oral swabs using nucleocapsid primers and probes may also serve as a confirmatory test, but as explained previously may lack sensitivity and require repeat samplings and testing. Additional work with other gene sequences and sample types (including saliva and feces which can be collected non-invasively) to enhance PCR is in progress. Other genetic amplification methods are also in development.

As with all infections, a single diagnostic test should not be interpreted in isolation. All results should be interpreted considering the clinical signs, exposure history, and colony status. At a minimum testing should be repeated to ensure against possible technical artifacts during identification, collection, testing, and reporting of samples and results.

SARS-CoV-2 NHP Literature Review

A handful of articles have been published describing experimental infection of NHPs with SARS-CoV-2. There is one report of probable community-acquired infection in an African Green monkey13, and a second report of likely community-acquired infection in a troop of gorillas at the San Diego Zoo Safari Park20. The majority of reports describe infection in rhesus macaques, while one article discusses infection in cynomolgus macaques and three reports describe infection in African green monkeys. Animals were challenged with a viral dose of 1.0 - 2.6 x 10^6 TCID₅₀ or 1.1 x 10^4 – 1.1 x 10^6 PFU, which is comparable to the 10^4 – 10^7 viral copies/ml detected in throat swab and sputum human clinical samples12. In all cases, animals were challenged via the intranasal and/or intratracheal routes, and two studies also exposed animals by additional routes including ocular, oral, or aerosol challenge. A new report in pre-print describes infection in all three species challenged via the aerosol route21. Following infection, the most consistent clinical signs were a transient reduced appetite with mild to no associated weight loss, decreased responsiveness, and hunched posture. Transiently increased body temperature was reported in rhesus10, cynomologus22, and African green monkeys16. Two authors reported increased or irregular respirations1,10. Three studies evaluated infection in aged rhesus, cynomolgus, or African green monkeys. The aged African greens presented with severe respiratory distress, necessitating euthanasia at 8 and 22 days after challenge2. In both macaque reports, the geriatric animals did not present with more severe clinical signs than younger animals14,17. However, in studies not yet published older rhesus macaques have demonstrated significant pulmonary disease with clinical impacts.

Viral loads were highest in nasal, pharyngeal, and bronchoalveolar lavage samples and peaked by day 3. Virus was detectable from nasal swabs from 14-28 days after infection4,10. Viral
replication was restricted to the respiratory tract; no virus was detectable in peripheral blood\textsuperscript{10, 15}. Non-infectious viral particles were isolated from rectal swabs, peaking on day 3 and detectable through day 17\textsuperscript{1, 10}.

Histopathologic changes were consistently described as mild to moderate interstitial pneumonia or diffuse alveolar damage in all species. For the aged macaques, only aged rhesus had more severe pulmonary pathology compared to younger animals\textsuperscript{15}. This difference was not seen in the cynomolgus macaques with only 5-10\% of the lung affected in both age groups\textsuperscript{14}. Woolsey et al. commented that radiographic findings did not reflect the severity of the pathology in African green monkeys, and believed the same to be true for rhesus macaques. However, the aged African green monkeys that succumbed to acute respiratory distress syndrome demonstrated a severe alveolar/interstitial pattern on thoracic radiographs\textsuperscript{2}. Histopathology of both animals demonstrated diffuse alveolar damage consistent with their clinical presentation and radiographic findings.

Antibody titer was significantly elevated on day 14 or 21 following infection\textsuperscript{1, 4} with a neutralizing antibody response apparent by day 21 or 28 in rhesus macaques\textsuperscript{2, 10}. In adult African green monkeys, antibody titer peaked at day 15, and neutralizing antibody was evident through the study endpoint at day 21\textsuperscript{15}. Of note, the aged African green monkeys with severe respiratory disease did not mount an antibody response\textsuperscript{2}. Antibody response was not evaluated in the cynomolgus study.

Two studies evaluated reinfection of rhesus at 28 and 35 days after the initial challenge\textsuperscript{1, 4}. Bao et al. reported a transient increase in body temperature, but no weight loss. There was no virus detected in nasal, pharyngeal, or rectal swab samples, and no pulmonary histopathological changes. However, Chandrashekar et al. reported very limited viral RNA detected in bronchoalveolar lavage fluid on day 1 following reinfection with no infectious virus present as determined by plaque assay. They reported minimal to no clinical signs, and did not perform histopathology. Both studies describe a robust immune response including increased virus-specific titers following re-challenge in most animals. One animal did not have enhanced response following re-challenge and maintained the same titer before and after reinfection\textsuperscript{1}.

References


Additional Resources (Publications in Pre-Print):
