OCCUPATIONAL PRIMATE DISEASE SAFETY GUIDELINES FOR
ZOLOGICAL INSTITUTIONS

Introduction: Zoonotic diseases are a concern in a variety of taxa that are maintained in zoological facilities. Concerns for employee health can have implications in the management of these animals. This document addresses one taxonomic group, nonhuman primates. This document includes all primate taxa, including prosimians and callitrichids, when it refers to nonhuman primates.

Nonhuman primates (NHPs) and humans share a number of diseases. A few NHP diseases have serious consequences for humans and an even greater number of human diseases can cause serious or even fatal illness in NHPs. Transmission of diseases from nonhuman primates to humans and vice versa can be avoided or reduced by following precautionary procedures. These guidelines provide a framework for developing specific institutional policies to minimize the risks of disease transmission under each unique situation.

While we realize and accept that there is no such thing as zero risk, the goal is to provide zoo personnel with information that they may use to make informed animal and personnel management health risk decisions. Unfortunately, current knowledge does not allow quantitative risk assessments to be performed in many zoological settings. The level of risk associated with working with NHP is dependent on numerous factors that will vary between and within institutions, necessitating a programmatic approach to developing and implementing an effective health and safety program (for more detailed information on risk management, the reader is referred to Chapter 7 of Occupational Health and Safety in the Care and Use of Research Animals and Section V of CDC’s Biosafety in Microbiological and Biomedical Laboratories, 4th ed.-see appendix 6).

Ascribing institutional risk may be done in several ways: first, all at-risk animals in a collection can be tested for pathogens of concern on a regularly scheduled basis and the biosecurity plan built based on the results; alternatively, an institution can choose not to test, assume infection in the collection, and use minimum standards recommended based on likelihood of diseases present. A combination of methods to assess risk based on scientific data and epidemiological principles is considered the best approach when information is limited.

Purpose: These guidelines provide a standardized framework of recommendations for managing nonhuman primates in zoological collections in a manner that minimizes the risk of exposing employees to zoonotic diseases as well as human-to-animal disease transmission while maintaining the animals’ quality of life. These guidelines are based on the current state of knowledge and accepted professional practices regarding zoonotic diseases and nonhuman primate management. Each institution should develop and implement its individual occupational nonhuman primate safety policy. Individual animal, species, and collection health status should be used to modify these guidelines based on risk assessment by each institution.
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I. Personnel Responsibilities

All persons having direct or indirect contact with nonhuman primates (NHPs), and/or their bodily fluids or waste should be informed of the risks. It is the responsibility of each individual institution to assess its own risk in order to create specific NHP safety protocols. Those potentially at risk and in need of education may include staff, volunteers, and students in animal care, veterinary, education, research departments; construction, maintenance, horticulture personnel or contractors, and special visitors to NHP areas.
II. Personal Protective Equipment

Personal protective equipment should be available in all nonhuman primate areas. Items commonly included:

- Rubber boots, dedicated shoes or boots for area, and/or heavy-duty plastic shoe covers
- Disposable or reusable disinfected gloves
- Full or elbow-length leather restraint gloves for capture/restraint
- Disposable face masks and plastic face shields
- Long-sleeved clothing
- Disposable head covers
- Phenolic disinfectant, sodium hypochlorite or other broad-spectrum disinfectant (effective against mycobacteria and viruses); foot bath, spray bottle, and instructions for proper dilution of concentrate and appropriate use of disinfectant
- Nonhuman primate bite kit with instructions and eyewash (see appendix 1 for suggested contents of bite/wound kit)
- Other first aid supplies as deemed appropriate

III. Definition of Nonhuman Primate Areas

A nonhuman primate area is any building, enclosure, vehicle, or designated space in which NHPs are present or that may be contaminated with NHP body fluids or waste. This includes primate kitchen facilities, areas where soiled transport cages or wastes are stored, areas of the hospital, quarantine, and nursery housing NHPs, primate exhibit and bedroom areas, and necropsy area. Any exhibit or area housing NHPs, including mixed exhibits, should be considered a primate area when NHPs are present. Although the clinical pathology laboratory should be considered to be a potentially contaminated area when NHP samples are being handled, appropriate precautions would likely only include the use of personal protection equipment rather than the complete set of recommendations listed when handling animals.

Relatively little is known about NHP individual species susceptibility to many emerging zoonotic pathogens. However, research has been conducted on some species and some infectious agents. Where knowledge exists, it should not be ignored; where knowledge does not exist, we must realize that this is often due to a lack of research and be careful about assumptions of risk. Each institution should take it upon itself to assess the level of knowledge about a given infectious agent in a given species when creating institutional guidelines. (See bibliography for additional references.) Species of nonhuman primates (e.g. macaques, langurs) that are identified as having a higher likelihood of carrying and transmitting serious zoonotic diseases (e.g. herpes B or herpes B-like virus) or those individuals with evidence of previous exposure to or infection with potentially serious zoonotic diseases (based on serologic or other laboratory tests) should
be handled with increased precautions as outlined in section XV. Individual institutions should develop and continuously assess their nonhuman primate guidelines based on new scientific information as well as the status of their primate collection.

IV. Procedures for Entering a Nonhuman Primate Area

Any person entering a nonhuman primate area should protect their mucous membranes from exposure to, or release of, infectious agents. The best way to protect from any respiratory disease transmission is to wear a facemask; all people entering an enclosure should be tuberculin test negative (or physician evaluated if positive and determined non-infectious) within the previous 12 months. If the person is recovering from an infectious illness, wearing a facemask should be required to minimize the risk of NHP exposure, regardless of tuberculin test status (see section X. Procedures for Human Illness).

When entering a nonhuman primate area, a footbath (changed at least daily), a spray bottle with an appropriate disinfectant, or plastic disposable shoe covers may protect against tracking contamination outside of the area on shoes or boots (note: this prevents contamination from entering the area). Shoes/boots should be disinfected when leaving a NHP area; shoe covers must be disposed of in the primate area. The use of a uniform or outerwear that can be disposed of or laundered at the workplace if it becomes soiled while in the primate area is an important biosecurity measure that is easily implemented. Guests or staff that enter without the intention of working in the area, should wear a facemask (or have a current negative tuberculin test), use a footbath or shoe covers, and avoid contact with the animals or areas contaminated with body fluids. Gloves are recommended if staff or guests will have contact with animals or areas that may be contaminated. Long pants are highly recommended for guests or staff that are not working in the primate area.

Hand washing must be performed (whether or not glove have been worn) upon leaving the primate area.

V. Procedures for Working in a Nonhuman Primate Area

“Working” includes feeding, handling, training, and restraining nonhuman primates. This definition also includes cleaning (due to high likelihood of aerosolization of bodily fluids and/or urine/feces) and maintaining enclosures, handling NHP biological samples, or any other activity that could lead to contact with materials contaminated by a NHP. Staff that will be performing construction, maintenance or repair work in a cleaned and disinfected enclosure are most likely at a lower level of risk but should still follow “procedures for entering a nonhuman primate area” to further reduce risk of human-NHP transmission.
All persons working in a nonhuman primate area must have evidence of a negative tuberculin test within the previous 12 months (or if test positive, physician evaluated and found to be non-infectious).

Historical trace-back of exposure to infectious agents is a principle of sound zoo veterinary medicine. For the same reason, serum banking for employees (working with NHPs and/or NHP tissues) by their physician can be a highly valuable tool for retrospective health evaluation should infectious agent exposure occur. In addition, good health monitoring should include a fecal parasite screen and culture every 12 months. This program could be monitored through the institution’s occupational health department or left for self-evaluation. Nevertheless, it is important to both educate personnel on this point and make this option available through the institution’s health coverage. Veterinary staff should not be involved in testing or monitoring of humans but should be involved as a consultant to the institution’s occupational health program.

Recommended minimal attire for persons working in a nonhuman primate area include the following:

- Uniform with long pants and long sleeves (work clothing that can be laundered at the work place) can minimize the risk of exposure to humans. If climatic conditions preclude long sleeves/long pants, shower or hosing facilities should be available for personnel to thoroughly wash if contaminated. If dermatitis or other conditions that compromise skin integrity of the worker is present, long sleeves and/or pants should be more strongly recommended in order to reduce the risk during this period. Long-sleeved coveralls can be substituted. When working with NHP biological samples, a lab coat is sufficient if it will prevent contamination of the uniform. Soiled coveralls, lab coats, or uniforms should not be worn outside the primate area.

- Disposable or disinfectable rubber gloves. For enclosure maintenance work, gloves are recommended but may not be necessary if the animals are removed from the work area and the area has been cleaned and disinfected prior to work commencing.

Hand washing must be performed upon leaving the primate area.

VI. Procedures for Cleaning, Feeding, Handling, Training and Restraint of Nonhuman Primates

Additional personal protective attire may be recommended when performing specific activities, depending on the degree of contact with animals or contaminated materials and the health status of the NHPs.
When collecting urine, feces, or other bodily fluids or tissue samples from a cage, staff should wear gloves and an appropriate uniform or other additional clothing to minimize contact. A face shield or protective goggles should be considered if splash back is likely.

Good thorough cleaning often results in aerosolization of bodily fluids. In order to decrease the risk of exposure to these airborne particles when cleaning an enclosure, gloves and a facemask can be worn. For maximum risk reduction, any time there is the potential for aerosolization, splash-back, or poor ventilation of the enclosure during cleaning, rubber boots, a face shield or eye protection (e.g., safety glasses) and face mask, disposable gloves, and long-sleeved coveralls/uniform and head cover should be recommended. In cases where the recommended personal protection equipment above is not used, personnel should have the ability to shower after cleaning/hosing to remove potential aerosolized material from skin surfaces. All fecal material, remaining food, enrichment items and bedding should be removed prior to hosing. Surfaces should be wetted with low pressure prior to being hosed to decrease aerosolization.

All people working in the food preparation industry are required by law to wear gloves when preparing food for human consumption – this was a public health measure implemented nearly 100 years ago after the spread of Salmonella by “typhoid Mary” in New England. In order to protect the health of NHP in captivity, disposable or designated washable rubber gloves are recommended when preparing NHP food or food pans. Frequent hand-washing and good hygiene should be practiced at all times as well.

To reduce the risk of NHP to human disease transmission, disposable or designated rubber gloves can be worn when cleaning used food pans or hand feeding. Long sleeves and a face shield can be worn to further minimize contact with saliva or other body fluids. A more biosecure alternative to direct hand feeding is the use of tongs, spoons, or other remote delivery methods for feeding. Certain species with documented higher levels of risk, such as macaques, should not be hand-fed without adequate protection from potential contact with bodily fluids and trauma from scratches or bites (i.e. thick gloves).

By definition, animal handling and restraint increases the likelihood of NHP-human contact, thereby increasing the potential risk of zoonotic disease transmission should it occur in either party. Personal protection should be strongly considered when indirectly manipulating the nonhuman primate (i.e., crating, using a squeeze cage, shifting). Minimal recommendations include the use of disposable gloves and facemasks, while additional protection such as long sleeves and pants, and eye protection (e.g., safety glasses) should be discussed during policy development. In addition, hearing protection should be considered if working in an area containing large numbers of animals in an enclosed space.

Procedures requiring personnel to enter the same space as the non-anesthetized NHP (ex. netting, hand-restraint, or routine care in “free contact” enclosures) should be performed by or under the direct supervision of qualified individuals. Non-anesthetized macaques
should not be manually restrained. The following recommendations also apply when carrying or examining nonhuman primate. Minimum protective attire includes:

- Disposable gloves - gloves should be worn when handling any nonhuman primate and double gloves should be worn when working with macaques or other primates with known zoonotic diseases, to prevent accidental exposure due to tears in gloves.

- Long-sleeves/long pants, face mask, head cover, eye protection or face shield may be recommended depending on species and health status and should be required by institutional policy if working with macaques or other primates with known serious zoonotic diseases.

- If heavy leather gloves are used, disposable gloves should be worn inside the leather gloves to minimize risk of exposure since thorough disinfection of leather gloves is difficult.

During a veterinary procedure, the veterinarian may request additional protective attire be worn as necessary for the particular situation. Like humans, NHP infants have a greater susceptibility to infectious diseases. Therefore, similar recommendations apply to handling, feeding, and caring for infant NHPs. Recently (or currently) sick caretakers should avoid close contact with infants. Regardless of recent medical history, the caretaker should consider wearing disposable gloves, a facemask or shield and long-sleeved outerwear (i.e., surgical gown or lab coat). The personal NHP protection protocol used should be developed with a human occupational consultant and reviewed with the veterinary staff.

**VII. Procedures for Transport of Nonhuman Primates and Biological Samples**

Whenever NHPs need to be moved (whether within the zoo or between institutions), with the exception of anesthetized animals or neonates, they should be transported in crates or other devices that are appropriate for that species. Anesthetized animals or neonates are exceptions (since these animals may be transported safely outside a crate according to the risk assessed by the husbandry and veterinary staff). The transport enclosure should permit safe transfer out of the crate into a secondary enclosure. Crates for moving strong primates or those over approximately 10 kg should have sliding or guillotine-type doors rather than hinged doors (i.e. not airline kennels).

In any case, design of the transport crate should minimize the likelihood of the NHP reaching through to make contact with personnel. Ideally, crates should be constructed out of materials that allow for disinfection (i.e. no unsealed wood). Crates should be cleaned and disinfected immediately following use. Crates that cannot be disinfected should be disposed of as biohazardous waste immediately after transport. Crates used to ship animals outside of the institution should be designed to minimize or eliminate direct
contact with humans and to prevent the loss of any bedding or waste (check specific requirements of transporter). Crates used to transport NHPs by air must adhere to the applicable IATA container requirements.

During transport in a vehicle, crates containing nonhuman primates should be separated by a physical or spatial barrier from all other animals and cargo. Access to animals during transport should be restricted to authorized personnel only. Crates should be in an area separate from the driver/passenger area, such as the cargo area of a van or bed of a pickup. The contact surface of the vehicle should be impervious and easily disinfected. If the NHP is being moved outside the institution, the crate must be in an enclosed compartment or secondary containment with adequate ventilation and temperature control. Vehicles used to transport NHPs should be cleaned and disinfected immediately after use.

Dead NHPs should be double-bagged or transported to the necropsy area in such a manner as to minimize the possibility of leakage of contaminated fluids in route. All animals should be clearly identified with species and individual identification. Personal protective equipment recommended for all NHP necropsies includes disposable gloves, facemask, face shield and/or eye protection, long-sleeved shirt and long pants or disposable or cloth coveralls, and hair cover. See the section on “General guidelines for nonhuman primate necropsies” for more details.

NHP biological samples (i.e., urine, blood, semen, feces) should be placed in containers within clearly labeled (i.e., species and identification) leak-proof secondary containers (e.g. sealed plastic container or bag) for transport to the clinical pathology laboratory. This is necessary to prevent contamination of surfaces during transport and handling. Persons preparing these samples should wear gloves to prevent contaminating the outside of the container or should wipe the outside of the container with appropriate disinfectant. Specimens being sent to outside laboratories should have additional labeling to identify them as “nonhuman primate” samples.

VIII. Procedures for Personnel and Equipment Hygiene and Disinfection

Good hygiene of personnel may often be taken for granted and therefore not included in biosafety protocols. Obviously, any person entering or working in a nonhuman primate area should adhere to general good hygiene practices; a list of common points to consider follows:

- Eating, drinking, smoking, applying cosmetics, or handling contact lenses should not be permitted in a nonhuman primate area. Any food intended for human consumption should not be stored in a NHP area. Persons should never share food, drink, or other personal items with a NHP.
• Hand-washing should be performed before handling animal food items, after removing gloves, before leaving a primate area, and before eating, drinking, applying cosmetics, handling contact lenses, or smoking after working in a primate area.

• Disposable gloves should be changed often and when going from one activity to another (i.e., from cleaning to feeding), and when they become soiled or develop tears or holes.

• Work clothing should be changed when visibly soiled or contaminated.

• Persons should shower when contamination of skin or hair has occurred.

• Nets, protective leather gloves, and other equipment should be cleaned and disinfected following each use. Since complete disinfection may be difficult, leather gloves should be dedicated for nonhuman primate use.

IX. Procedures for Waste Disposal

Waste disposal provides the perfect opportunity for breakdown in a carefully planned biocontainment protocol and should therefore be carefully considered when developing an institutional plan. Normally, solid waste from nonhuman primates can be disposed of through the sanitary sewer or bagged and disposed of through the general trash. These wastes should not be composted unless it is done in an enclosed system inaccessible to vermin. However, wastes from macaques or any NHP with the high risk of spreading zoonotic disease(s) should be given further consideration.

Biodegradable wastes from nonhuman primates with potential zoonotic diseases can be disposed of through the sanitary sewer or double-bagged and handled as biohazardous waste if a sanitary sewer connection is not available. Biohazardous (known infectious diseases) or medical wastes must be disposed of in compliance with applicable regulations.

Wastes can be sprayed with an appropriate disinfectant before disposal (although it should not be assumed that pathogens have been eliminated by this treatment). If wastes are disposed of through the sanitary sewer, the drain should be treated with an appropriate disinfectant after disposal. If double-bagged, waste should be transported in such a manner as to minimize potential contamination. The dumpster or other holding container should be secured to prevent accidental exposure to waste materials.
X. Procedures for Human Illness

Each institution should develop a policy that addresses contagious or infectious diseases of humans that may enter the nonhuman primate area. For example, before entering a nonhuman primate area, an employee with any febrile illness (fever), cold sores, skin rashes, prolonged diarrhea, or exposure to chicken pox, measles or any other highly contagious disease must report these symptoms to a supervisor. In addition, persons with the above signs or those of a cold, the “flu”, or moderate to severe dermatitis, or diarrhea should avoid entering primate areas. Affected individuals should not enter NHP areas unless absolutely necessary. If this is absolutely necessary, the person must wear a surgical facemask and gloves. All visitors should be questioned prior to entering a non-public primate area; a standardized recent health history questionnaire may help provide consistency in this area.

A clinically ill individual should not, under any circumstances, perform the following procedures:

- Prepare food for nonhuman primates
- Perform any procedure that involves close contact with a nonhuman primate such as restraint or handling ill or infant primates.

XI. Procedures for Nonhuman Primate Related Injuries or Contamination by Bodily Fluids

Any person that receives a nonhuman primate related injury must report this immediately to their supervisor, or designated staff member. The supervisor will follow the institution’s on-the-job injury protocol in regards to additional reporting, evaluation, and treatment of the injured person.

Nonhuman primate injuries include any break in the skin resulting from direct or indirect contact with a nonhuman primate such as bites (intentional or accidental), cuts, or scratches inflicted by a NHP. Indirect contact injuries may occur when skin breaks are caused by contact with a primate enclosure or items (e.g. training tools, waterers, etc.) potentially contaminated with bodily fluids or direct contamination of the person with nonhuman primate bodily fluids.

Additionally, any person that is injured by a nonhuman primate should:

- Immediately cleanse the injury site (even minor injuries) with large amounts of water and disinfectant soap for 10-15 minutes.
• Follow procedures as outlined in the institution’s nonhuman primate bite kit (see appendix 1 and 2 for suggested procedures).

• After washing, be transported to the institution’s designated health services provider (this could be First Aid, etc.) for further evaluation, according to the institution’s animal injury policy.

• Complete an accident investigation report according to the institution’s protocol.

• Notify the veterinarian on duty.

Other contamination by nonhuman primate bodily fluids (e.g. urine, feces, saliva, or blood splashed in contact with open cuts, mouth or eyes) or cuts and scratches from enclosure hazards should be thoroughly cleansed and reported to the supervisor immediately.

XII. Staff Training

All employees working in a nonhuman primate area should receive training on a regular basis on all aspects of the institution’s nonhuman primate safety policy. Training in up-to-date information regarding blood borne pathogens and zoonotic diseases, as well as safe handling of biohazardous materials should be provided as required by law and institutional policies, and is highly recommended for all employees.

XIII. Policy Development and Enforcement

Each institution is responsible for developing its own nonhuman primate safety policy; this document seeks to provide standardized guidelines for points to consider and risk management measures that may be implemented for different levels of risk. Most importantly, due to the rapidly changing nature of available information in this area, the policy should be reviewed regularly and revised as needed by animal management and veterinary staff. Methods for staff training and enforcement of policy should be included in development of individual protocols. While the creation of this document DOES NOT seek to impose guidelines upon any given institution, it DOES seek to encourage a given institution to have a policy and provides points for consideration in this light.
XIV. Public Protection

The most reliable way to minimize the risk of spreading transmissible diseases between humans and animals is to prevent direct or close indirect contact. Because the most prevalent form of disease transmission is often through aerosolized droplets containing disease particles, the prevention of airborne transmission is imperative. As a result, enclosures/exhibits should be designed and maintained to minimize the possibility of physical contact between the public and nonhuman primates or their feces, fluids, and tissues.

XV. Procedures for Nonhuman Primates Infected with Potential Zoonoses

Because the gaps in our knowledge of emerging diseases are large, this document was designed to conservatively minimize risks associated with all types of known and, as yet, unknown diseases. However, there are some cases where the state of existing knowledge demands a high level of precaution such as in the case of macaques. Working with macaques poses the additional risk of being exposed to Herpes B virus. Herpes B has also been documented to cause disease in other NHPs [1,2]. In addition, other species of Asian primates (i.e., langurs) have the potential to carry Herpes B-like virus. Even those animals that have previously tested negative may potentially shed the virus. The basic procedures outlined in this section of the guidelines, when properly performed, should provide adequate precautions when working with macaques and these other species. Each institution should investigate and determine which species should be handled following the guidelines for macaques. It will be assumed that additional identified primate species can be substituted for “macaques” in the recommendations that follow.

- Always have face protection (face mask and shield or eyewear) when working with macaques. Strictly avoid procedures that require manual restraint of non-anesthetized macaques.

- If injured by a macaque or exposed to bodily fluids, follow the instructions for wound cleansing and steps outlined in the section on nonhuman primate injuries. Those institutions housing macaques should develop a separate policy for dealing with these injuries or exposures. Immediately notify the supervisor and veterinarian on duty.

Exposure of mucous membranes (e.g., eyes, mouth) from urine or feces of macaques has resulted in fatal Herpes B virus infections in humans. Therefore, avoiding these exposures is as important as avoiding bite and scratch wounds. If any exposure occurs, it is imperative that the institution’s procedures are followed. Herpes B can remain infectious for 48 hours in secretions left to dry on surfaces [3]. Therefore, items in contact with macaques should be disinfected thoroughly.
Other more common zoonotic pathogens include *Salmonella, Campylobacter, Shigella,* and *Yersinia,* as well as influenza, Herpes simplex and other viruses. Although these may cause mild or asymptomatic infection, the risks associated with transmission from or to NHP should be considered and appropriate precautions addressed in the institution’s primate policy.

When nonhuman primates have been diagnosed with a known or potential zoonotic disease, it is imperative that the veterinary staff communicates any additional specific precautions to the animal care staff. While veterinarians are not human medical doctors (MD’s), they are thoroughly trained in the diagnosis, prevention and treatment of animal diseases that can be transferred between humans and animals (zoonoses).

Protocols should be developed on a case-by-case basis in conjunction with the institution’s occupational health department and public health officials. The veterinary staff’s responsibilities should be restricted to communication of the diagnosis to the appropriate persons and development of procedures to minimize risks to the animals and contamination of the environment. Human screening, monitoring, and training programs should be developed and implemented by the institution’s occupational health and safety departments in conjunction with the curatorial staff and consultation with the veterinary staff.

At the time of the writing of these recommendations, every effort has been made to provide precautions for known zoonotic diseases of nonhuman primates. However, there may be other zoonoses that we are not aware of at this time that would require revision of the recommended precautions.

**XVI. General Guidelines for Nonhuman Primate Necropsies**

*Purpose:* To determine the cause of death and other contributory or incidental health problems, to determine if other nonhuman primates in the collection are at risk, if there is a specific zoonotic risk, and/or if management of the primate collection needs modification.

*Objective:* To obtain the maximum information while minimizing health risks to the prosector.

*Where:* The post mortem examination should be performed in a room designated for necropsies. The work surface and floor should be impervious (e.g. metal, not wood) and easily disinfected. It is advisable to perform the post mortem examination in a laminar flow or similar biological safety cabinet if available and if the size of the primate permits.

*Personnel:* The necropsy should be performed by a veterinary clinician or pathologist if at all possible, or by a trained technician under the direct supervision of a veterinarian.
The number of people participating in the examination should be limited to those necessary for prosecution, documentation of findings, collection and labeling of tissues and other samples, and clean-up.

Personal protection: Individuals directly involved in the necropsy or in handling samples collected should wear personal protective equipment including: coveralls or surgical gown made of Tyvek or similar water (and blood) impermeable material, mask or respirator, face shield, hair (and beard) covering, double gloves, boot or shoe covers. All observers should also wear masks, some form of eye protection, uniforms or lab coats, and disposable shoe covers or boots that must be disinfected upon exiting the necropsy facility.

The necropsy: TAG, SSP or other protocol documents concerning performance of the necropsy and data and samples to be collected should be obtained and read prior to commencing the examination. It is advisable to label all needed containers before the examination to minimize contamination. See attached example of the Ape TAG necropsy protocol.

Disposal of the carcass: The carcass should be disposed of in a manner that will prevent spread of disease. Incineration is the recommended method, but the exact method of disposal should be consistent with local regulations and is left to the veterinarians and health officers of each institution. If the carcass is to be given to a museum or research institution for anatomic dissections or preparation, it is highly recommended that the carcass be double bagged and frozen until the pathology report is finalized and results from cultures and other ancillary diagnostics are obtained. The museum or other institution recipient should be apprised of the cause of death and whether any specific zoonotic disease was present. The carcass should then be transported in a plastic box or cooler or enclosed in an additional clean plastic bag to minimize leakage of bodily fluids during transport.

Clean up: All instruments, work surfaces and the floor should be flushed with water and washed with a lipolytic detergent followed by an appropriate disinfectant such as dilute bleach. The outside of specimen containers should be cleaned with similar disinfectant. Specimen containers should be placed in sealed bags for transport to a laboratory. Disposable clothing should be placed in a bag that is then sprayed with disinfectant, and enclosed in a clean bag (double bagged). Incineration is recommended. All reusable protective clothing should be disinfected directly upon leaving the necropsy room.

See Appendix 3 for “Nonhuman primate post mortem examination” and “Standardized necropsy report for Great Apes and other primates”.

For specific questions on SSP/TAG necropsy protocols or other veterinary information, contact the SSP or TAG Veterinary Advisor(s). See Appendix 5 for contact information.
APPENDIX 1

Suggested Contents of a Bite/Wound Kit for Use in Nonhuman Primate Areas

1. Cleansing materials
   a. Detergent or soap (povidone-iodine or chlorhexidine scrub)
   b. Sterile surgical scrub brushes
   c. Sterile basin for soaking large wounds
   d. Sterile 4x4 inch gauze pads for soaking and dressing of wounds
   e. Sterile saline solution (1 liter bottles) for irrigation of contaminated eyes, nose, or mouth
   f. Sterile large (60 cc) syringe for saline irrigation of mucosa
   g. Paper or cloth tape for dressing of wounds
   h. Sterile exam gloves (various sizes for persons assisting with cleansing and specimen collection)

2. Specimen collection and culture materials
   a. Sterile cotton or Dacron swabs (without metal shafts)
   b. Sterile vials of viral transport media (check with local human lab for preferred medium)

3. Copy of institutional standard operating procedures and nonhuman primate safety guidelines; names, mailing addresses, and telephone numbers of reference laboratories, local physicians and other health professionals to contact in case of exposure

4. Bite/wound log to record details of any exposures
APPENDIX 2

General Steps to Follow for Evaluation and Management of a Nonhuman Primate Bite/Wound

1. First aid – clean the wound
   a. Immediately soak or scrub wound/exposure site with soap or detergent for at least 15 minutes; rinse well with water
   b. Rinse eyes and mucus membranes with sterile saline/flowing water for at least 10 minutes.
   c. Contact supervisor as soon as possible to report injury.

2. Post-cleansing specimen collection (for possible Herpes B virus exposure from macaques)
   a. Swab wound for viral culture after rinsing wound with water (contact local hospital or clinical pathology laboratory for appropriate media specific handling instructions).

3. Contact appropriate health services personnel for physician evaluation.

4. Identify animal or enclosure where exposure occurred.

5. Notify veterinarian. Veterinarian to review animal or group medical records and provide relevant information to physician.

6. Veterinarian and animal management to decide on possible testing procedures for animal.
APPENDIX 3

STANDARDIZED NECROPSY REPORT FOR NON-HUMAN PRIMATES WORK SHEET

Pathology # _____________ Species_________________ Date___________

Animal #/Name ________________ Sex ______ Age(DOB)___________

Date of Death/Euthanasia _____________ Time _______ (am/pm)

Method of euthanasia _______________

Time and date of necropsy ___________ Duration of necropsy _____

Post mortem state _________________ Nutritional state _________

Pathologist or prosector and

institution:_______________________________________________________

________________________________________________________________________

_______________

Gross diagnoses:

___________________________________________

___________________________________________

Abstract of clinical history:
Please check tissues submitted for histopathology.

External Examination (note evidence of trauma, exudates, diarrhea etc):

_____Hair coat:

_____Skin:

_____Scent glands:

_____Mammary glands and nipples:

_____Umbilicus (see neonatal/fetal protocol):

_____Subcutis (note: fat, edema, hemorrhage, parasites):

______Mucous membranes (note: color, exudates):

Ocular or nasal exudate?:

_____Eyes and ears:

_____ External genitalia:

_____Oral cavity, cheek pouches and pharynx:
Dentition (see attached dental form):

_____ Tongue:

Musculoskeletal System:
Note fractures, dislocations, malformations?:

_____ Bone growth plate (rib, distal femur, sternabra)

_____ Muscles:

_____ Bone marrow (femur):

_____ Joints (note any exudates or arthritis):

_____ Spinal column (examine ventral aspect when viscera removed)

Examination of the neck region:
_____ Larynx:

_____ Laryngeal air sac (see protocol for great apes):

_____ Mandibular and parotid salivary glands:
_____Thyroids and parathyroids:

_____Cervical/cranial lymph nodes:

_____Esophagus:

Thoracic Cavity:

Note any effusions, adhesions, or hemorrhage:

Note amount, color and any lesions in mediastinal and coronary fat:

_____Thymus:

_____Heart (see attached protocol):

_____Great vessels:

_____Trachea and bronchi:

_____Lungs:

_____Esophagus:
Abdominal Cavity:

Note any effusions, adhesions, or hemorrhage?:

Note amount, color or lesions in omental, mesenteric and perirenal fat:

Liver and gall bladder:

Stomach:

Pancreas:

Duodenum:

Jejunum:

Ileum:

Cecum and (in apes) appendix:

Colon and rectum:

Lymph nodes:
Kidneys and ureters:

Adrenals:

Gonads:

Uterus:

Bladder and urethra:

Male accessory sex glands (prostate and seminal vesicles):

Umbilical vessels, round ligaments of bladder in neonates:

Abdominal aorta and caudal vena cava:

Nervous System:

Meninges:

Brain:

Pituitary:
Trigeminal (gasserian) ganglia:

Spinal cord (please note to which lumbar segment the cord extends):

Brachial plexus and sciatic nerves:

Is there an identifiable pineal gland?

WEIGHTS AND MEASUREMENTS (in grams, kilograms, and cm, please):

Body weight:_____________________________________________________

Lymphoid tissue:
R. axillary LN _________ L. axillary LN _________
R. inguinal LN _________ L. inguinal LN _________
Jejunal LN _________
Spleen _____________ Thymus ______________

Abdominal Organs:
Liver _______________
R. kidney ____________ L. kidney ____________
R. adrenal ____________ L. adrenal ____________
R. ovary ____________ L. ovary ____________
uterus __________________________________________
placenta (weigh and measure disc(s)): 
Thoracic Organs:

Heart wt.__________________
Thymus (above)

Height:__________________
Circumference at coronary groove:_______

Left Vent.thickness__________
Rt. vent.thickness__________

Septum__________________

Lt. AV valve circ.________
Rt. AV valve circ._______

Aortic valve circ._______
Pulmonary v. circ._______

R. lung ______________
L. lung ______________

Other:

Brain ________________
Tumors? ______________

R. testes (wt.)__________
L. Testes _____________

Length x dia.__________

Penis (length x diameter)__________________________

STANDARDIZED BODY MEASUREMENTS FOR NONHUMAN PRIMATES INCLUDING APES:

crown rump length (linear)__________________________

crown rump length (curvalinear)__________________________

cranial circumference (above brow ridge)______________

Length of head (tip of jaw to top of crest)______________

width of brow ridge________________________________

chest circumference (at nipples)______________________

abdominal circumference (at umbilicus)________________

Left arm: Shoulder-elbow:___________________________
elbow-wrist:____________________________________
wrist-tip of middle finger:_______________________
pollex:__________________________________________

Right arm: Shoulder elbow:__________________________
elbow- wrist:____________________________________
wrist-tip of middle finger:_________________________
pollex:___________________________________________

Left leg: hip-knee:________________________________
knee-ankle:____________________________________
ankle-tip of big toe:______________________________

heel-tip of big toe:______________________________
hallux:__________________________________________

Right leg: hip-knee:_______________________________
knee-ankle:____________________________________
ankle-tip of big toe:______________________________

heel-tip of big toe:______________________________
hallux:__________________________________________

ANCILLARY DIAGNOSTICS (CHECK IF PERFORMED, GIVE RESULTS IF AVAILABLE, NOTE LOCATION IF STORED, OR TO WHOM SENT):

Cultures:

bacterial:
fungal:

viral:

Heart blood:

serum:

filter paper blot:

Parasitology:

feces:

direct smears:

parasites:

Tissues fixed in 10% formalin (list tissues or specific lesions other than those checked above):

Tissue fixed for EM:______________ Tissue frozen:_______
Impression smears:____________________________________________

Comments (interpretation of gross findings):

NONHUMAN PRIMATE POST MORTEM EXAMINATION

Collection of tissues

Tissues to be fixed in 10% neutral buffered formalin should be less than 0.5 cm thick to ensure penetration of formalin for fixation.

Initial fixation should be in a volume of fixative 10 times the volume of the tissues. Agitation of the tissues during the first 24 hrs is helpful to prevent pieces from sticking together and inhibiting fixation. Once fixed tissues may be transferred to a smaller volume for shipment.

Labeling of specimens

If pieces are small or not readily recognizable (e.x. individual lymph nodes) they can be fixed in cassettes or embedding bags or wrapped in tissue paper labeled with pencil or indelible ink. Another alternative is to submit lymph nodes with attached identifiable tissue, e.x. axillary with brachial plexus, inguinal with skin, bronchial with bronchus, etc.

Sections from hollow viscera or skin can be stretched flat on paper (serosal side down) and allowed to adhere momentarily before being placed in formalin with the piece of paper. The paper can be labeled with the location from which the tissue came.

The formalin container should be labeled with the animals name or number, the age and sex, the date and location, and the name of the prosector.

Tissues to be preserved

From the skin submit at least one piece without lesions, a nipple and mammary gland tissue, scent gland, any lesions and subcutaneous or ectoparasites.

Axillary and or inguinal lymph nodes may be submitted whole from small animals and should be sectioned transversely through the hilus in large primates.

Mandibular, and/or parotid salivary glands should be sectioned to include lymph node with the former and ear canal with the latter.
Thyroids, if it is a small primate, may be left attached to the larynx and submitted with the base of tongue, pharynx, esophagus as a block. In larger primates, take sections transversely through the thyroids trying to incorporate the parathyroids in the section.

Trachea and esophagus and laryngeal air sac sections may be submitted as a block.

Cervical lymph nodes may be submitted whole if small or sectioned transversely.

A single sternebra should be preserved as a source of bone marrow. A marrow touch imprint may be made from the cut sternebra and air dried for marrow cytology.

Section of thymus or anterior pericardium should be taken perpendicular to the front of the heart.

Heart: weigh and measure heart after opening but before sectioning. Please fix longitudinal sections of left and right ventricles with attached valves and atria in large animals and the whole heart opened and cleaned of blood clots in smaller animals. In tiny animals the heart may be fixed whole after cutting the tip off the apex.

Lungs: if possible inflate at least one lobe by instilling clean buffered formalin into the bronchus under slight pressure. Fix at least one lobe from each side and preferably samples from all lobes. In little animals the entire "pluck" may be fixed after perfusion and sampling for etiologic agents.

Gastrointestinal Track: Take sections of all levels of the GI track including: gastric cardia, fundus and pylorus (or presaccus, saccus, tubular stomach and pylorus in colobines); duodenum at the level of the bile duct with pancreas attached; anterior, middle and distal jejunum; ileum; ileoceccolic junction with attached nodes; cecum and (in apes) appendix; ascending, transverse and descending colon. Open loops of bowel to allow exposure of the mucosa and allow serosa to adhere momentarily to a piece of paper before placing both bowel section and paper in formalin; or gently inject formalin into closed loops.

Liver: Take sections from at least two lobes, one of which should include bile ducts and gall bladder.

Spleen: Make sure sections of spleen are very thin if the spleen is congested; formalin does not penetrate as far in very bloody tissues.

Mesenteric (jejunal) nodes: section transversely; colonic nodes may be left with colon sections.

Kidneys: Take sections from each kidney: Cut the left one longitudinally and the right one transversely so they will be identifiable (or label). Please make sure the sections extend from the capsule to the renal pelvis.
Adrenals: small adrenals may be fixed whole but larger ones should be sectioned (left - longitudinal and right transversely) making sure to use a very sharp knife or new scalpel blade so as not to squash these very soft glands.

Bladder: sections should include fundus and trigone. Please make sure to include round ligaments (umbilical arteries) in neonates.

Male gonads and accessory sex glands: Section the prostate with the urethra and seminal vesicles transversely. Section testes transversely. If testes are being collected perimortem for sperm retrieval, try to arrange to take small sections before the gonads are manipulated.

Female reproductive organs: Fix the vulva, vagina, cervix, uterus and ovaries from small and medium sized primates as a block (after making a longitudinal slit to allow penetration of formalin). Rectum and bladder (opened) can also be included in this block. In somewhat larger animals make a longitudinal section through the entire track. In great apes make transverse sections of each part of the track and the ovaries. (See reproductive track protocols from the contraception advisory group if animals are to be included in their database).

Gravid females: weigh and measure placenta and fetus. Perform a post mortem examination of the fetus. Take sections of placental disc(s) from periphery and center and from extraplacental fetal membranes. Take sections of major organs and tissues of fetus (see fetal protocol).

Nervous system: The brain should be fixed whole, or, if too large for containers, may be cut in half longitudinally (preferred) or transversely through the midbrain. It should be allowed to fix for at least a week before sectioning transversely (coronally) into 0.5-1.0 cm slabs to look for lesions. Submit the entire brain if possible and let the pathologist do the sectioning, otherwise submit slabs from medulla, pons and cerebellum, midbrain, thalamus and hypothalamus, prefrontal, frontal, parietal and occipital cortex including hippocampus and lateral ventricles with choroid plexus. (Note: limited sectioning is advised if the brain is to go to the great ape brain project).

Pituitary and pineal gland: Fix the pituitary whole. Put pituitary in an embedding bag if it is small. If the pineal gland is identifiable, fix it whole. Also remove and fix the Gasserian (trigeminal) ganglia.

Spinal chord - if clinical signs warrant, remove the cord intact and preserve it whole or in anatomic segments (eg. cervical, anterior thoracic etc.) (Please note to which lumbar vertebra the cord extends)

Bone marrow: Take bone marrow by splitting or sawing across the femur, to get a cylinder and then make parallel longitudinal cuts to the marrow. Try to fix complete cross sections or hemisections of the marrow.
Additional sections for fixation: Take sections of any and all lesions, putting them in embedding bags if they need special labeling.

NOTE: It is better to save "too many" tissues than to risk missing essential lesions or details.

THANK YOU for taking the time to perform this necropsy.

CARDIAC EXAMINATION FOR GREAT APES (AND OTHER PRIMATES IN WHICH CARDIAC DISEASE IS PRESENT)

Examine heart in situ. Check for position, pericardial effusions or adhesions. Collect for culture or fluid analysis if present.

Remove heart and entire thoracic aorta with "pluck".

Examine heart again. Check the ligamentum (ductus) arteriosus for patency. Check position of great vessels. Open pulmonary arteries to check for thrombi.

Remove heart and thoracic aorta from the rest of the "pluck". Examine for presence of coronary fat. Examine external surfaces especially coronary vessels. Note relative filling of atria and state of contracture (diastole or systole at death) and general morphology. (The apex should be fairly pointed.)

Measure length from apex to top of atria. Measure circumference at base of atria (around coronary groove).

Open the heart:

Begin at the tip of the right auricle and open the atrium parallel to the coronary groove continuing into the vena cava. Remove blood clot and examine the AV valves. Cut into the right ventricle following the caudal aspect of the septum and continuing around the apex to the anterior side and out the pulmonary artery. Remove postmortem clots and examine inner surface. Open left atrium beginning at the auricle and continuing out the pulmonary vein. Remove any clots and examine valves. Open the left ventricle starting on the caudal aspect and following the septum as for the right ventricle. When you reach the anterior aspect, clear the lumen of blood and identify the aortic outflow. Continue the incision around the front of the heart and into the aorta, taking care to cut between the pulmonary artery and the auricle. Open the entire length of the thoracic aorta. Remove all postmortem clots. You may gently wash the heart in cool water or dilute formalin to better visualize the internal structures and valves. Sever the thoracic aorta from the heart just behind the brachiocephalic arteries. Examine intima and adventitia and section aorta for formalin. Sever the pulmonary vessel and vena cava close to the heart.
Weigh and measure the heart and record.

Measure thickness of right and left ventricular free walls and the septum. (On the left side, do not measure directly through a papillary muscle.)

Measure the circumference of the right and left AV valves and the aortic and pulmonary valves using a pliable measuring tape (or use a piece of string and measure the string on a straight ruler).

Take sections for histopathology:

Sections should include:

longitudinal sections of left and right ventricles AV valves and atria.

Sections of myocardium from left and right ventricles including coronary vessels.
Sections of papillary muscles. Sections from the septum at the vace of the AV valves (area of conduction system).

In small animals like callitrichids, you may fix the heart whole.

Fix the entire heart, if possible by immersion in 10% buffered formalin for more detailed examination by a cardiac pathologist.

Other vessels:

Make sure to examine the abdominal aorta, iliac arteries and popliteal arteries (frequent sites of aneurysms in humans).

Note the location and severity of fibrous plaques, fatty streaks and atherosclerotic plaques and presence of mineralization or thrombosis.

POSTMORTEM EXAMINATION OF NONHUMAN PRIMATE FETUSES AND NEONATES

External examination of the fetus:

Weigh the fetus and make body measurements.

Measure the placental disc(s) and weigh the placenta. Note umbilical length and vascular patterns on the placenta.

Note presence of hair, freshness of the carcass (if dam is dead, is the decomposition of the fetus consistent with that of the dam) and any evidence of meconium staining.
Internal examination of the fetus:

Follow the general nonhuman primate necropsy protocol.

Make sure to note whether ductus arteriosus and foramen ovale are patent. Note also whether the lungs are aerated and to what extent.

Note dentition / erupted teeth.

Identify umbilical vein and arteries and check for inflammation. Make sure to save umbilicus and round ligaments of the bladder (umbilical arteries) for histology.

Make sure to save a growth plate (e.g. costochondral junction or distal femur) in formalin.

Cultures:

Take as many of the following as possible: Stomach content or swab of the mucosa; lung; spleen or liver; placental disc and extra-placental membranes. Do both aerobic and anaerobic cultures if possible.

POST MORTEM EXAMINATION OF THE AIR SACS OF ORANGUTANS AND OTHER NONHUMAN PRIMATES

Examine the skin over the air sac for signs of fistulae or scars. Note thickness of the skin and presence of fat or muscle overlying the air sac.

Incise the air sac through the skin on the anterior aspect.

Note color and texture of air sac lining.

Note presence or absence of exudate.

Note presence or absence of compartmentalization by connective tissue and presence of diverticulae.

Note extent of air sacs (e.g. under clavicle, into axilla, etc.)

Identify and describe the opening(s) from the larynx into the air sac (e.g. single slit-like opening, paired oval openings etc). Note any exudate.

Note the location, size and shape of the opening in the larynx (e.g. from lateral saccules or centrally at the base of the epiglottis). Note length of any connecting channel between larynx and air sac and direction a probe must take to go from inside the larynx to the air sac.
Cultures:

Please culture several different sites within the air sacs (we need data to determine normal flora and if infections are "homogeneous" or compartmentalized).
APPENDIX 4

Retrovirus Information Sheet

This document is intended to provide both information about and guidelines for the care of captive nonhuman primates infected with retroviruses. These viruses may be significant for the health of individual primates and collections as a whole. These viruses present an extremely low, but documented risk of transmission to humans. No human disease has been associated with infection with NHP retroviruses at the time of the writing of these guidelines.

What are retroviruses, and what types are found in nonhuman primates?

Retroviruses are a large group of RNA viruses that replicate in a unique way, using an enzyme called reverse transcriptase. They are divided into 3 groups: the oncornaviruses, the lentiviruses, and the spumaviruses. Retroviruses are found in all animal species tested to date, and do not always cause disease. The NHP retroviruses that may represent significant zoonotic concerns are listed below:

Oncornaviruses

*Simian T-lymphotropic virus (STLV)*

- Closely related to Human T-cell leukemia virus (HTLV), which is prevalent in many human populations in Asia, Africa and the Americas. HTLV can cause adult T-cell leukemia or lymphoma in a small proportion of infected humans and has also been associated with rare neurologic disorders. There is evidence that HTLV originated from ancient cross-species transmission of STLV.

- There are several distinct but related viruses in this group.

- Seroreactivity has been seen in more than 33 species of Old World primates, both captive and wild. Mode of transmission is thought to be through sexual contact and from dam to infant in breast milk.

- Usually does not cause clinical signs, but has been associated with disease in baboons, African green monkeys, and gorilla.

- A related virus has been found in spider monkeys, and is the only STLV-like virus found in New World primates, but no disease has been associated with it at this time.
**Gibbon ape leukemia virus (GaLV)**

- Isolated from many captive gibbons (in Asia, USA and Europe) with leukemia.
- Virus is shed in urine and feces, and sexual transmission is also suspected.
- Chronically infected, apparently healthy, antibody negative, virus positive gibbons have been reported.
- The host range for GaLV has not been well explored.

**Simian sarcoma virus**

- Known from a single isolate from a fibrosarcoma in a woolly monkey which was housed with a gibbon (suspect mutant of GaLV)

**Simian retrovirus Type D (SRV)**

- Several different serotypes, all unique to macaques.
- Causes acquired immune deficiency and is associated with opportunistic infections and cutaneous and retroperitoneal fibromatosis in captive macaques.
- Transmitted readily through sexual contact, bite wounds and from dam to infant, both pre- and post-natally.
- Apparently healthy carrier animals have been recognized, particularly in cynomolgus macaques. These virus positive animals may be seronegative, making their identification by serology alone difficult.

Antibodies to type D retrovirus have been reported in 2 of 247 persons tested who were occupationally exposed to nonhuman primates. No disease has been identified in these individuals.

**Lentiviruses**

**Simian immunodeficiency virus (SIV)**

- Very closely related to human immunodeficiency virus (HIV); in fact HIV-1 originated from a strain of SIV in chimpanzees. HIV-2 originated from SIV of sooty mangabeys.
A large percentage of African monkeys, both wild and captive that have been tested, are seropositive for SIV. Each species appears to be infected with its own strain of SIV.

Clinical signs of immunosuppression due to SIV is rare in African species, but have been recognized in some individuals.

Asian primates are not natural hosts of SIV and are very susceptible to immunodeficiency disease when they contract SIV.

Susceptibility of New World primates and prosimians is unknown.

Natural transmission is thought to be through sexual contact, although bite wounds are also suspected.

2 of 3123 (0.06%) samples from humans with occupational exposure to NHPs have tested positive for SIV. These tests, however, represent an unknown number of repeat tests for some of the same individuals, so the prevalence may actually be a bit higher. One of those persons has since reverted to seronegative status. No clinical disease has been noted in either positive person.

**Spumaviruses**

*Simian foamy virus (SFV)*

- Complex retroviruses that have been identified with high prevalence in many Old and New World primate species. Foamy viruses have not yet been identified in prosimian species but are suspected to exist.

- A foamy virus genetically closely related to chimpanzee foamy virus has been isolated from a human. No disease association with foamy virus infection in humans has been established.

- No known disease associated with these viruses in their natural NHP hosts.

- 11 of 296 (3.7%) blood samples from humans with occupational exposure to NHPs have tested positive for SFV. At least 4 of these were associated with deep bite wounds. No disease has been noted in any of these individuals.

**Nonhuman Primate Testing and Collection Management Recommendations**

Although at this time there is little retroviral associated disease in NHPs and no apparent disease from NHP retroviruses in humans, it is recommended that the retroviral status of nonhuman primate collections be determined for reasons of animal health and occupational safety. This can be accomplished by initial serial serologic screening of all
animals for antibodies to the retroviruses discussed on the retrovirus information sheet above at two time points, one year apart. Serologic testing alone is sufficient for detection of SIV and SFV-infected animals. For STLV, a prolonged interval to seroconversion may require repeated testing - over several years, or use of molecular techniques for viral detection at initial screening. For SRV, initial testing by both serology and virus detection methods are required to identify all infected animals. Testing for GaLV is currently not routinely available, but may be available in the near future. Both serology and virus detection methods will need to be employed to detect all infected gibbons.

Once an individual nonhuman primate has been confirmed to be test-positive for any retrovirus, it should be considered infected for life, and retesting for that virus is not necessary. (It should be emphasized that an initial positive test result should be confirmed through follow-up testing by repeating the same or alternative methodology and laboratory used in the first test before considering the animal “positive”.) Serum banking at the time of annual examination is still recommended, for surveillance of other diseases. If an animal is test-negative, but housed with positive animals, retesting on an annual basis is recommended. If all animals in the collection are negative after repeat testing, and no new animals are introduced, alternate or every third year testing, with serum banking in the off years, is justifiable.

The retroviral status of new acquisitions should be determined prior to introduction. Whenever possible, positive animals should only be introduced into groups with positive animals. Introduction of positive animals into known all-negative groups may result in retrovirus-related disease in the naïve animals. The documented differential pathogenicity of some retroviruses between Asian and African species should reinforce the standard practice of preventing direct contact between members of these two groups of nonhuman primates. The pathogenic potential of variants of these viruses among different species of African primates is largely unknown. There is currently insufficient information to make recommendations for individual risk assessment for movement of NHPs infected with retroviruses. The primate TAG and SSP veterinary advisors should be consulted for specific advice. Labs that test for SIV, STLV, SRV and SFV are listed below. Contact the laboratories to find out whether both antibody (serology) and virus testing (PCR or culture) are available.

Pathogen Detection Laboratory
California National Primate Research Center,
University of California, Davis, CA
http://pdl.primate.ucdavis.edu/
(530) 752-8242

BioReliance,
14920 Broschart Rd.
Rockville, MD 20850
http://www.bioreliance.com/assays.aspx
(800) 756-5658
It should be recognized that different laboratories use different assays and reagents. Unusual or unexpected results, particularly in highly endangered species or when breeding groups are being established, should be confirmed in two different laboratories.

**Guidelines for Working with Retrovirus Positive Animals**

Any retrovirus positive or untested animal should be handled using practices similar to the CDC’s Biosafety Level 2, which include use of personal protective equipment (gloves, clothing, mask and eye protection, or face shield) (see CDC’s website for additional information on biosafety guidelines[4]). All materials in contact with infected nonhuman primates should be properly disposed of and/or decontaminated. These viruses are inactivated by 70% ethanol, 10% household bleach, formalin, and most lipolytic detergents, provided adequate contact time is allowed (minimum 10 minutes).

The institutional occupational health program may include serum banking of personnel who work with nonhuman primates. The Centers for Disease Control is currently conducting serosurveys of animal care personnel who work with nonhuman primates for several of these viruses. If your institution is interested, contact Bill Switzer, Centers for Disease Control and Prevention, 1600 Clifton Rd, MS-G19, Atlanta, GA 30333, (404) 639-0219.

There have been attempts in the lab animal community to develop Specific Pathogen Free (SPF) colonies for several of these viruses, including SIV and SRV/D in macaques, and
these have been successful. These colonies are developed through strict serial serologic testing and culling over several years, and then maintaining closed colonies. Pulling infants at birth or delivering them by cesarean section to avoid virus contamination has also been useful for some of these colonies. For some of the African species for which SIV is relatively prevalent in wild and captive animals, and captive numbers are small, establishing SIV-free breeding groups might prove to be quite difficult.
APPENDIX 5

AZA Veterinary Advisor Group (SSP/TAG Veterinary/Pathology Advisors)

A current list of clinical and pathology advisors can be found at:

http://www.aazv.org/?page=A12

APPENDIX 6

Excerpt from “Biosafety in Microbiological and Biomedical Laboratories-Section V Risk Assessment”

"Risk" implies the probability that harm, injury, or disease will occur. In the context of the microbiological and biomedical laboratories, the assessment of risk focuses primarily on the prevention of laboratory-associated infections. When addressing laboratory activities involving infectious or potentially infectious material, risk assessment is a critical and productive exercise. It helps to assign the biosafety levels (facilities, equipment, and practices) that reduce the worker's and the environment's risk of exposure to an agent to an absolute minimum. The intent of this section is to provide guidance and to establish a framework for selecting the appropriate biosafety level.

Risk assessment can be qualitative or quantitative. In the presence of known hazards (e.g., residual levels of formaldehyde gas after a laboratory decontamination), quantitative assessments can be done. But in many cases, quantitative data will be incomplete or even absent (e.g., investigation of an unknown agent or receipt of an unlabeled sample). Types, subtypes, and variants of infectious agents involving different or unusual vectors, the difficulty of assays to measure an agent's amplification potential, and the unique considerations of genetic recombinants are but a few of the challenges to the safe conduct of laboratory work. In the face of such complexity, meaningful quantitative sampling methods are frequently unavailable. Therefore, the process of doing a risk assessment for work with biohazardous materials cannot depend on a prescribed algorithm.

The laboratory director or principal investigator is responsible for assessing risks in order to set the biosafety level for the work. This should be done in close collaboration with the Institutional Biosafety Committee (and/or other biosafety professionals as needed) to ensure compliance with established guidelines and regulations.

In performing a qualitative risk assessment, all the risk factors are first identified and explored. There may be related information available, such as this manual, the NIH Recombinant DNA Guidelines, the Canadian Laboratory Biosafety Guidelines, or the WHO Biosafety Guidelines. In some cases, one must rely on other sources of information such as field data from subject matter experts. This information is interpreted for its tendency to raise or lower the risk of laboratory-acquired infection.\(^\text{1}\)

The challenge of risk assessment lies in those cases where complete information on these factors is unavailable. A conservative approach is generally advisable when insufficient information forces subjective judgment. Universal precautions are always advisable.
The factors of interest in a risk assessment include:

The *pathogenicity* of the infectious or suspected infectious agent, including disease incidence and severity (i.e., mild morbidity versus high mortality, acute versus chronic disease). The more severe the potentially acquired disease, the higher the risk. For example, *staphylococcus aureus* only rarely causes a severe or life threatening disease in a laboratory situation and is relegated to BSL-2. Viruses such as Ebola, Marburg, and Lassa fever, which cause diseases with high mortality rates and for which there are no vaccines or treatment, are worked with at BSL-4. However, disease severity needs to be tempered by other factors. Work with human immunodeficiency virus (HIV) and hepatitis B virus is also done at BSL-2, although they can cause potentially lethal disease. But they are not transmitted by the aerosol route, the incidence of laboratory-acquired infection is extremely low for HIV, and an effective vaccine is available for hepatitis B.

The *route of transmission* (e.g., parenteral, airborne, or by ingestion) of newly isolated agents may not be definitively established. Agents that can be transmitted by the aerosol route have caused most laboratory infections. It is wise, when planning work with a relatively uncharacterized agent with an uncertain mode of transmission, to consider the potential for aerosol transmission. The greater the aerosol potential, the higher the risk.

*Agent stability* is a consideration that involves not only aerosol infectivity (e.g., from spore-forming bacteria), but also the agent's ability to survive over time in the environment. Factors such as desiccation, exposure to sunlight or ultraviolet light, or exposure to chemical disinfectants must be considered.

The *infectious dose* of the agent is another factor to consider. Infectious dose can vary from one to hundreds of thousands of units. The complex nature of the interaction of microorganisms and the host presents a significant challenge even to the healthiest immunized laboratory worker, and may pose a serious risk to those with lesser resistance. The laboratory worker's *immune status* is directly related to his/her susceptibility to disease when working with an infectious agent.

The *concentration* (number of infectious organisms per unit volume) will be important in determining the risk. Such a determination will include consideration of the milieu containing the organism (e.g., solid tissue, viscous blood or sputum, or liquid medium) and the laboratory activity planned (e.g., agent amplification, sonication, or centrifugation). The volume of concentrated material being handled is also important. In most instances, the risk factors increase as the working volume of high-titered microorganisms increases, since additional handling of the materials is often required.

The *origin* of the potentially infectious material is also critical in doing a risk assessment. "Origin" may refer to geographic location (e.g., domestic or foreign); host (e.g., infected or uninfected human or animal); or nature of source (potential zoonotic or associated with a disease outbreak). From another perspective, this factor can also consider the potential of agents to endanger American livestock and poultry.

The *availability of data from animal studies*, in the absence of human data, may provide useful information in a risk assessment. Information about pathogenicity, infectivity, and route of transmission in animals may provide valuable clues. Caution must always be exercised, however, in translating infectivity data from one species of animal to another.
species.

The established availability of an effective prophylaxis or therapeutic intervention is another essential factor to be considered. The most common form of prophylaxis is immunization with an effective vaccine. Risk assessment includes determining the availability of effective immunizations. In some instances, immunization may affect the biosafety level (e.g., the BSL-4 Junin virus can be worked on at BSL-3 by an immunized worker). Immunization may also be passive (e.g., the use of serum immunoglobulin in HBV exposures). However important, immunization only serves as an additional layer of protection beyond engineering controls, proper practices and procedures, and the use of personal protective equipment. Occasionally, immunization or therapeutic intervention (antibiotic or antiviral therapy) may be particularly important in field conditions. The offer of immunizations is part of risk management.

Medical surveillance ensures that the safeguards decided upon in fact produce the expected health outcomes. Medical surveillance is part of risk management. It may include serum banking, monitoring employee health status, and participating in post-exposure management.

Risk assessment must also include an evaluation of the experience and skill level of at-risk personnel such as laboratorians and maintenance, housekeeping, and animal care personnel (see Section III). Additional education may be necessary to ensure the safety of persons working at each biosafety level.

The infectious agents whose risk is evaluated often will fall into the following discrete categories.

**Materials containing known infectious agents**

The characteristics of most known infectious agents have been well identified. Information useful to risk assessment can be obtained from laboratory investigations, disease surveillance, and epidemiological studies. Infectious agents known to have caused laboratory-associated infections are included in this volume's agent summary statements (see Section VII). Other sources include the American Public Health Association's manual, *Control of Communicable Diseases*. Literature reviews on laboratory acquired infections also may be helpful.

**Materials containing unknown infectious agents**

The challenge here is to establish the most appropriate biosafety level with the limited information available. Often these are clinical specimens. Some questions that may help in this risk assessment include:

1. Why is an infectious agent suspected?
2. What epidemiological data are available? What route of transmission is indicated? What is the morbidity or mortality rate associated with the agent?
3. What medical data are available?

The responses to these questions may identify the agent or a surrogate agent whose existing agent summary statement can be used to determine a biosafety level. In the absence of hard data, a conservative approach is advisable.
Materials that may or may not contain unknown infectious agents

In the absence of information that suggests an infectious agent, universal precautions are indicated.

Animal studies

Laboratory studies involving animals may present many different kinds of physical, environmental, and biological hazards. The specific hazards present in any particular animal facility are unique, varying according to the species involved and the nature of the research activity. The risk assessment for biological hazard should particularly focus on the animal facility's potential for increased exposure, both to human pathogens and to zoonotic agents.

The animals themselves can introduce new biological hazards to the facility. Latent infections are most common in field-captured animals or in animals coming from unscreened herds. For example, monkey b-virus presents a latent risk to individuals who handle macaques. The animal routes of transmission must also be considered in the risk assessment. Animals that shed virus through respiratory dissemination or dissemination in urine or feces are far more hazardous than those that do not. Animal handlers in research facilities working on infectious agents have a greater risk of exposure from the animals' aerosols, bites, and scratches. Section IV describes the practices and facilities applicable to work on animals infected with agents assigned to corresponding Biosafety Levels 1-4.1

References

5. Sewell, David L. Laboratory Associated Infections and Biosafety. Clinical Microbiology Reviews, 8:389-405, 1995
APPENDIX 7

SELECTED BIBLIOGRAPHY

Publications, texts:


Guide to the Care and Use of Laboratory Animals. (www.aalas.org)

General disease information on nonhuman primates:


Other references/resources:

Proceedings of the annual conference of the AAZV

www.primatevets.org - website of the Association of Primate Veterinarians

http://primatelit.library.wisc.edu - excellent website for references on primate literature in general

www.nal.usda.gov/awic - animal welfare information center website

www.cdc.gov/ - information on various infectious diseases that affect humans and zoonoses

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References


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