VETERINARY MEDICAL MANAGEMENT

Rita McManamon, D.V.M.

HEALTH EVALUATIONS AND PREVENTIVE MEDICINE

General Comments Regarding Health Evaluations

It is the position of the Orangutan SSP© that a regular schedule of thorough physical examinations under anesthesia – combined with regular un-anesthetized observations and health checks -- should be performed on an individualized basis (see below for guidance and definition) for every orangutan in the SSP©. Routine physical examinations identify health and welfare issues that can be corrected and/or treated early, thus minimizing discomfort and pain to the animal. The medical data taken during these examinations also contributes to the international knowledge database, and informs health decisions for that individual, for others in the captive population, and potentially for caretakers and managers of the wild population(s).

Excellent training programs have been developed and applied in several institutions (Reichard et al, 1993). These training programs cannot fully substitute for examinations under anesthesia, but are extremely helpful and complementary. Some training is absolutely essential for some treatments (like effective nebulization). Training, and the trusting relationship between keeper and animal, allows the animal some choice and participation in its own health care, and facilitates many diagnostic and treatment manipulations without anesthesia. These include measuring blood pressure, hand-syringe administration of anesthetic agents and other medications, conducting vaginal swabs and ultrasound examinations, blood collection, dental health monitoring, and many other valuable procedures. However, some procedures cannot be thoroughly accomplished (or may actually involve more time, distress or pain to the animal) without general anesthesia.

The Orangutan SSP© recommends that every orangutan receive a complete physical examination, ideally every year but at least every 3-4 years. From consultation with other zoo veterinarians, it appears that every 2-3 years is within acceptable standard of practice. If more than 4 years elapses between exams, this frequency is probably below the accepted standard of practice and is too infrequent to identify and address common health problems. More frequent examination allows anesthetic protocols to be safely “fine-tuned” for the individual, and evaluation of potential “quality of life” issues (dental and periodontal disease, heart disease, respiratory disease, degenerative arthritis, recurrent urinary infections) to maintain quality of life, without waiting for potentially catastrophic illness.

Obviously, "risk versus benefit" assessments are essential for each individual animal, and case-by-case individual exemptions may be judged necessary by the attending veterinarian. If an SSP© animal's medical condition warrants an exemption from these guidelines, this fact should be established in consultation with the Veterinary Advisor and the SSP© Coordinator, Lori Perkins, lori410@mindspring.com.

It is the responsibility of any orangutan-holding facility to make sure that it is adequately equipped, staffed, and supported so that it can perform whatever anesthetic procedures are required to stay current with acceptable standards of practice, on a 24 hour, 7 day-per-week schedule. Current published reports and zoo veterinary experience do not indicate that orangutan anesthesia is unacceptably risky or stressful to the animal. In 1990, the MedARKS anesthetic survey in "Medical Management of the Orangutan" (Wells et al 1990) demonstrated that "clinicians are satisfied with their anesthetic regimens" and that "the number of complications (7.6%) seems reasonable" with "one abnormal recovery and no deaths" out of 131 anesthetic episodes. Since that publication, the affordability and availability of gas (isoflurane or sevoflurane) anesthesia units and portable anesthesia monitoring equipment have greatly improved, additional anesthetic agents and protocols (Horne, 2001) have enhanced safety factors in orangutan anesthesia, and a large number of zoo veterinarians have developed significant expertise in orangutan and great ape anesthesia. These resources (and the Orangutan SSP© Veterinary Advisor) are available for consultation and help – in person, over the phone, or through digital/analog video imaging – to less experienced facilities and staff. Prior planning, adequate instrumentation, and appropriate expertise will reduce anesthetic risk to an acceptable level. Some hints for managing anesthetic risk, are offered below.

Anesthesia and Physical Examinations

Anatomical considerations/positioning. Certain anesthetic risks must be considered, and prepared for, with orangutans. Jones (1982) presented an excellent species-specific review of anesthesia, and Horne's summary (2001) is very useful. Attention to careful positioning (in particular, maintaining an open airway) is essential. Some individual orangutans (especially obese animals, and some adult males) have anatomic challenges that complicate anesthetic management. In these individuals, "the flaccid soft palate and walls of the pharynx can occlude the laryngeal opening during inspiration, and adult male orangutans are especially at risk from asphyxia" (Jones, 1982). An elongated soft palate and/or epiglottis, mobile larynx, excessive salivation, and a propensity for laryngospasm under

ketamine anesthesia alone, can make intubation difficult (Wells et al, 1990). Spontaneous laryngospasm has also rarely been observed with unanesthetized youngsters under psychological stress, such as when the mother is being immobilized. Intravenous diazepam administration was effective in reversing the situation, in these cases. Animals with concurrent airsacculitis are also at risk for inadvertent aspiration of air sac exudate during anesthesia, through the communication (ostia) between the air sac and the larynx.

One unusual incident of acute respiratory distress syndrome (ARDS) and pulmonary edema in an orangutan, has been described (Kenny et al 2003). This syndrome is a complication occasionally seen in humans, particularly in those who experience temporary airway obstruction. No specific incident of airway obstruction was identified in this case, but it was presumed to have occurred. The authors reiterated the need to anticipate, monitor, and quickly correct any respiratory obstruction during anesthetic procedures.

With prior planning, anesthesia risks in orangutans can be anticipated and greatly reduced. These include: 1) weight control, to reduce obesity and consequent obstruction by the soft palate and pharyngeal walls; 2) the use of anesthetic protocols which include benzodiazepines or other agents that will reduce laryngospasm; 3) a squeeze cage or poles/restraint ropes available during induction, to hold the animal in positions which maintain a patent airway; 4) placement of the animal immediately into lateral recumbency, to maintain potential air sac exudates below the level of the ostia; 5) maintenance of non-intubated animals in lateral recumbency whenever possible; 6) having a laryngoscope with long blade, endotracheal tubes, suction apparatus, and oxygen close by during induction; 7) consideration of the use of IV lidocaine (0.5 – 1mg/kg) just before intubation to reduce the potential of laryngospasm; 8) having a stent or bougie available in the endotracheal tube, to facilitate placement; 9) being prepared to try alternative positions and other methods to extend the neck for intubation; 10) being familiar with, and ready to use, retrograde intubation techniques if deemed necessary; 11) considering the use of atropine (or suction), to control excessive salivation; 12) maintaining a cuffed endotracheal tube in place until swallowing reflexes have returned; 13) having doxapram readily available at all times, and consider its potential use to facilitate recovery; 14) consider the potential use of flumazenil as a reversal agent for benzodiazepines, in cases of prolonged recovery.

Intubations . In the author's experience, infants and juveniles require endotracheal tubes of 1-4 mm; adult females require sizes from 4 – 9 mm, and adult males require 9-14mm. As with all primates, the trachea is fairly short. Caution should be exerted to avoid intubation

of one mainstem bronchus; auscultation and/or radiographs may confirm proper intubation positioning. Cuffed endotracheal tubes are recommended. Please be aware that routine intubation for every procedure is controversial among veterinarians with orangutan experience – the removal of the tube at the end of the procedure can occasionally precipitate laryngospasm, or regurgitation and consequent aspiration. For long or complicated procedures, however (e.g., when air sac exudate is present and the ostia have not been surgically closed) the benefit of intubation usually outweighs the risk. Anecdotally, some clinicians give metaclopromide (to reduce regurgitation), IV furosemide and/or corticosteroid injections (to reduce laryngotracheal inflammation). Ondansetron (Zofran) and closely-related medications are effective anti-emetics which have been used in humans and in other great apes; they are administered as oral premedication, or intravenously during the procedure.

Monitoring and recovery notes. The most effective instruments for anesthetic monitoring are the clinician's eyes, ears, and hands – combined with an alert brain that accurately analyzes and acts on the information. Other valuable instruments include a thermometer, heart rate and EKG monitor, pulse oximeter, end-tidal capnography, and blood pressure monitoring equipment. Mucus membrane color, and pulse oximetry frequently indicate lower oxygenation values in apparently-stable, non-intubated orangutans, than are ideal with most other nonhuman primates. Anesthetic manipulations to improve ventilation (repositioning, "bagging", and/or mechanical positive pressure ventilation) may improve oximeter readings.

Some clinicians have noted that some orangutans exhibit "athetoid" (spontaneous, nondirected) movements during anesthesia (B. Swenson, E. Strobert personal communication). These movements do not indicate inadequate anesthetic level, and must be distinguished from actual arousal.

Appropriate attention must be paid to positioning and airway patency during the recovery period, just as in the induction period. Techniques that maintain an endotracheal tube in place, provide additional oxygen, and/or administer doxapram IM or IV, may provide additional safety and facilitate quicker recovery. Flumazenil (Romazicon ™) has been used intravenously (in incremental 0.1mg doses, per human protocols) to obtain partial or full reversal of benzodiazepine agents.

Clinical Examination Database

A good individual clinical database (especially body weight) improves the accuracy of dosing routine antiparasiticals and antibiotics, dosing anesthetic agents in routine or emergency situations, and monitoring of vital organ function to assess prognosis, and risk of side effects from other medications. On a species management basis, wild and rehabilitant orangutan managers rely on captive animal database managers for information on normal and abnormal health trends, and to help predict the risk/benefit ratio of medical intervention in the wild.

At least every 4 years – and ideally every 1-2 years -- the following information should be acquired on every orangutan (highest priority items marked with *):

*Accurate Body Weight

*Complete physical examination including eyes, ears, mouth, heart and lung auscultation, abdominal palpation, flexion and examination of joints, rectal palpation (prostate exam in males, vaginal/cervical exam in females if reachable)

*Dental examination, including periodontal probing if possible, prophylactic cleaning, and documentation of any intra-oral or extra-oral abnormalities.

*Urinalysis including sediment examination
Urine culture/sensitivity whenever possible (especially in adult males)

*Complete blood count and routine chemistry panel (also consider insulin levels especially in obese individuals)

*Hepatitis A, B, C serology (minimum one full viral screening panel per lifetime; recommend Hepatitis B surface antigen and Hepatitis C antibody tests every 2-4 years minimum)

Viral serology panel (*Herpesvirus hominis,* measles, SIV) serology (minimum once per lifetime; or more often based on risk analysis of potential animal and human exposure). Also consider running serology for other respiratory viruses (Parainfluenza, etc.) and Mycoplasma, in order to contribute to upcoming epidemiological study of airsacculitis.

Thyroid panel at least once per lifetime; more frequently if possible in adult years

*Indirect blood pressure measurement (every 4-5 years minimum during adult years)

*Electrocardiogram (every 4-5 years minimum during adult years)

*Cardiac ultrasound (recommend one during infant period, recommend every 4-5 years during adult period)

*Abdominal ultrasound (recommend every 4 years minimum, esp. liver/kidneys)

*Gynecological ultrasound (ovaries, uterus minimum) for adult females (recommend every 4 years minimum)

*Chest radiograph (heart and lungs)

*Tuberculin skin test (or equivalent screening, in previously "atypical reactor" animals)

Recommended Vaccinations

An institution-specific vaccination protocol should be established by the attending veterinarian. The protocol should be based on the current CDC vaccination recommendations for humans, combined with an appropriate institutional risk-versus-benefit assessment (see CDC website, and Loomis, 1990.) Also refer to the (attached) Ape TAG Veterinary Advisors' Preventative Medicine Programs for Apes vaccination recommendation. The Orangutan SSP© Veterinary advisor highly recommends annual influenza vaccination, and recommends that each institution consider and choose an appropriate Hepatitis B colony management strategy (vaccination, and/or serologic monitoring) for each situation.

Parasite Screening Tests

Regular fecal examinations (every 3-4 months), using direct smears, flotation and ideally Baermann techniques, should be performed. Examinations should seek to identify and quantitate *Balantidium coli*, amoebae (many nonpathogenic amoebae are found in orangutans, but *Entamoeba histolytica* is of clinical concern), other protozoans, nematodes, trematodes, and tapeworms. *Balantidium coli* is a frequently-noted and often clinically insignificant commensal parasite. However, it can occasionally cause significant clinical

diarrheal disease (especially in infants) and treatment (with metronidazole and/or iodoquinol) is warranted in some instances. See the Health concerns section for notes on Strongyloidiasis due to *Strongyloides spp*; this organism is an extremely serious pathogen in orangutans.

Zoonotic Concerns

Refer to AZA Occupational Primate Safety Guidelines (attached) and to Roberts, 1995.

DISEASE CONCERNS IN ORANGUTANS

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Introduction

The most comprehensive clinical reference regarding patterns of medical problems, diagnoses, and treatments for orangutans in the North American SSP© remains *The Medical Management of the Orangutan* (Wells et al, 1990). This Institute of Museum and Library Sciences (IMLS)-funded, extensive survey of the captive population was a daunting task, and no similar systematic survey has been conducted since that time. Most of the information summarized in that publication – combined with the addition of some important references published since 1990 -- is still current and relevant. Therefore, this section of the husbandry manual is not written to cover or to supplant all of that information. It is intended to provide a general overview, and to emphasize major issues of current concern using a holistic (clinical-pathological-management) approach which is based on review of commonly-reported clinical cases and available necropsy reports.

Animal managers (including veterinarians) working with orangutans are strongly urged to read "Medical Management of the Orangutan" and to refer to the primate medicine chapter(s) in the new (2003) version of M.E Fowler and R.E. Miller's Zoo and Wild Animal Medicine for detailed descriptions of specific diagnoses and current treatment recommendations for orangutans. Standard human medical references – particularly *Harrison's Internal Medicine* (Dienstag et al, 1998 and later editions) – are also extremely helpful in addressing disease concerns common to both orangutans and humans. Finally, the reference list at the end of "Medical Management of the Orangutan" and this chapter, provide additional valuable publications.

Morbidity/Mortality Trends

A statistical exhaustive review of all orangutan necropsies, with a listing of common pathological findings, has not been possible at the date of this writing. However, all available necropsy forms have been reviewed by Dr. Linda Lowenstine, and she has listed her general impressions of the most common lesions found, below. We have also listed associated clinical recommendations, and areas for potential future research, where appropriate.

Please note: we believe that some management and enrichment manipulations might reduce some of the incidents, or slow the exacerbation of, some of these conditions.

CARDIOVASCULAR DISEASE

Myocardial fibrosis continues to be a cause of cardiogenic death, in all of the great apes and in some of the Old World primates. The level of significance in orangutans cannot be determined, until all necropsy forms are received and thoroughly analyzed. The etiology of myocardial fibrosis has not yet been determined, but several potential areas are being studied. Some postmortem tissues have been collected and will be analyzed for trace minerals (especially iron and copper levels).

Based on the necropsy reports surveyed thus far, aortic dissection has not been noted as a significant morbidity problem in the captive orangutan population.

Based on the necropsy reports surveyed thus far, atherosclerosis does not appear to be a significant morbidity problem in most orangutans. However, atherosclerosis has been noted at a significant level in the lower extremities of older, obese male animals. In humans, this situation occurs under conditions of inadequate leg exercise, and consequent poor circulation.

Clinical Recommendations: These issues warrant careful monitoring of cardiovascular function during anesthesia. Please continue to conduct full cardiovascular assessments (including cardiac ultrasound examinations whenever possible) during physical examinations. Transesophageal ultrasound techniques are recommended, but not required. Include blood pressure measurements if at all possible. We do not have any current evidence that anesthetic protocols or physical examination recommendations need to be modified.

Necropsy Recommendations: Please continue to take the measurements of heart and great vessels, as described in the Great Ape Necropsy Protocol. We realize this is extremely time-consuming. However, this information is critically needed, in order to establish normal and abnormal values, for reference during ultrasound examinations on living animals. Please take extra fixed pieces of liver and heart and kidney for iron and copper levels, and submit to Dr. Lowenstine at UC Davis.

Possible upcoming research: Dr. Hayley Weston is conducting a survey to collect cardiovascular examination data in gorillas. Gathering comparative information in orangutans would be helpful as well, once that study is completed.

Management Recommendations: At this time, we do not have hard statistical evidence to support the recommendation of any specific management efforts to encourage exercise and arboreality. However, it appears logical that efforts to reduce obesity and to encourage regular exercise, especially in obese males, will facilitate good health and promote good circulation in the lower extremities.

RENAL/URINARY DISEASE

Evidence of recurrent and hidden/occult urinary infections, including ascending bladder infections that cause pyelonephritis, are frequently noted in necropsy reports. This appears to be especially prevalent in older males, especially obese males. It is hypothesized that urine retention due to matted perineal fur, poor hygiene and lack of exercise contribute to this problem. Glomerulonephritis and chronic interstitial nephritis are also frequently noted on necropsy reports. Glomerulonephritis may be associated with chronic airsacculitis cases.

Clinical Recommendations: A high index of suspicion is warranted in this species, toward occult urinary bladder infections. Accurate diagnosis and early effective treatment of cystitis are important in reducing the likelihood of pyelonephritis. An anecdotal association between airsacculitis and glomerulonephritis is presumably due to chronic antigen-antibody complex formation.
It is very important to collect urine during routine physical examinations (using cystocentesis if possible) and to conduct complete urinalyses, with culture and sensitivity when appropriate. Ultrasound examination of kidneys (to detect early evidence of pyelonephritis, for example) is also recommended. Treatments should follow standard veterinary and human protocols. Clinicians are encouraged to inform the SSP© Veterinary Advisor of unusual or successful treatment protocols.

Necropsy Recommendations: Please follow Great Ape Necropsy protocol.

Management Recommendations: In humans, ascending urinary infections and pyelonephritis are often associated with poor hygiene, and sedentary lifestyles. At this time, we do not have any hard statistical evidence to support the recommendation of any specific management efforts to encourage exercise and arboreality. However, it appears logical that efforts to reduce obesity, to encourage regular exercise, and to assure adequate water intake and urination, will facilitate better hygiene in the perineal area.

DEGENERATIVE ARTHRITIS

Degenerative arthritis is frequently noted during necropsies. The knee joints are particularly affected, and the incidence of arthritis is higher in older obese males. Some of these animals were clinically asymptomatic, and were not receiving analgesic medication.

Clinical Recommendations: There is some evidence that stoic, inactive, or aging orangutans may not demonstrate clinical symptoms until joint changes are quite advanced. The SSP© Veterinary Advisor recommends that joint mobility be assessed, and occasional radiographs be taken, especially in aging animals, during routine physical examinations. Particular attention is warranted toward the lower spine, hip joints, and knees.

Necropsy recommendations: Please examine and assess joints; we know this is time-consuming, but the data is essential for providing accurate morbidity/mortality assessments. See Great Ape Necropsy protocol. (Note: orangutans normally lack a round ligament of the head of the femur.)

Management Recommendations: There is insufficient empirical evidence to absolutely prove (but common sense and human medicine recommendations support the idea) that encouraging moderate exercise and arboreality, would improve the quality of life for arthritic animals. Gentle exercise opportunities could also assist caretakers in identifying affected patients earlier, so that effective analgesics, at lower doses, can be prescribed. Glucosamine supplements have been used by several institutions, as well as NSAIDS, with reported clinical success. Please inform the Orangutan SSP© Veterinary Advisor of any successful clinical treatments and dosages, so that she can assist other institutions.

RESPIRATORY DISEASE COMPLEX (EXAMPLES: RHINITIS, AIRSACCULITIS, BRONCHITIS, BRONCHIOLITIS, AND CHRONIC PNEUMONIA)

The prevalence of these diseases in captive orangutans (and recently in rehabilitant orangutans) is well-documented. Airsacculitis has also been noted in wild orangutans, and other great apes living in the wild. Updated general reviews are included in this chapter. Published references can be obtained from the SSP© Veterinary Advisor.

Clinical Recommendations: As in humans, cause(s) of respiratory system disease can be complex. Many of these diseases are chronic, require early diagnosis and long term therapy which controls (but does not cure) symptoms, and long term support of respiratory function. It is especially important that all handling and anesthetic protocols recognize the potential for hidden respiratory system disease in orangutan patients. Fortunately, many chronically-infected individuals have been successfully anesthetized, positioned, treated, and maintained comfortably for many years. Skilled keeper training techniques for monitoring and nebulization have been especially helpful in maintaining patient comfort.

Necropsy recommendations: Please be sure to examine and assess the air sac and associated respiratory system, and please submit samples, during necropsy examinations. See Great Ape Necropsy protocol.

Management Recommendations: In humans, some (but not all) respiratory diseases are associated with, or exacerbated by poor hygiene, poor hydration, sedentary lifestyles, and/or lack of regular exercise. At the time of this writing, a full epidemiological survey has not been conducted. But the SSP© Veterinary Advisor currently believes that regular increased exercise (especially for obese animals), regular exposure to fresh air and sunlight, decreasing any exposure to fecal aerosolization during cage cleaning, and other similar enrichment opportunities will improve quality of life and prognosis for some of these animals.

OBESITY AND THE POTENTIAL OF TYPE II DIABETES

It was previously believed that captive orangutans had a high incidence of diabetes (Type I or Type II), but current surveys do not support this conclusion at this time. Through the leadership of Dr. Joseph Kemnitz, the AZA Contraceptive Advisory Task Force, and several institutions, several individuals were assessed using the glucose tolerance test. To date, no association between MGA implants and the development of diabetes in orangutans has been documented. In an article published in the Journal of Zoo and Wildlife

Medicine (see references), Dr. Kemnitz' results indicated that while diabetes was not common in the tested population, a limited number of individuals were diabetic or potentially pre-diabetic.

Fructosamine assays have also been used to assess animals for diabetes. Diabetic orangutans have been managed using injectable insulin protocols, as well as oral agents.

Clinical Recommendations: A current effort is underway to systematically collect data on body weights and condition (as assessed during anesthetic examinations). The human literature (medical journals and lay press articles) are reporting dramatic trends in the increase in obesity, and in Type II diabetes in humans. Obesity is associated with many serious metabolic and anatomical problems (including heart disease, respiratory disease, anatomical respiratory blockages, and joint disease).We encourage any efforts to monitor and control weight, and increase exercise in orangutans.

When evaluating animals for potential diabetes under anesthesia, clinicians are advised to be especially vigilant in choosing anesthetic protocols that will not produce elevations in blood glucose level.

STRONGYLOIDIASIS

One "old" problem which has not been a cause of frequent mortality in recent years, but which requires extreme vigilance, is strongyloidiasis (= parasitic infection by ***Strongyloides spp***.). This can be a "hidden" disease with difficult diagnostic timing, and it is especially devastating/fatal in infants and juveniles. Published reports indicate that serial, daily fecal examinations for up to 17 days may be necessary to detect ***Strongyloides spp*** *ova*. Free-living forms of the parasite exist, so self-reinfection is possible. It can be difficult to eradicate the organism from the environment. Regular prophylactic parasite control is advised, especially with pregnant, lactating, and infant animals. Ivermectin has been the most commonly effective antiparasitical used, but other drugs are possible. The Medical Management of the Orangutan publication, as well as Fowler and Miller, present good reviews of this subject. Please contact the SSP© Veterinary Advisor with any current updates, concerns or questions.

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ORANGUTAN AIR SACCULITIS/RHINITIS/BRONCHITIS - SUMMARY OF SUGGESTED DIAGNOSTIC AND THERAPEUTIC METHODS

Lucy Spelman, D.V.M. and Rita McManamon D.V.M., July 2003

1. General description: Infection of the upper and lower respiratory tract including the laryngeal (submandibular) air sac by multiple bacteria, usually a mixture of enterics which includes Pseudomonas aeruginosa. The "syndrome" may present as acute/subacute illness (pneumonia, obvious air sac distension) or chronic disease (rhinitis, bronchitis, dermatitis).

Airsacculitis is a fairly common complaint in this species, but it is not exclusive to the captive orangutan population. Some young rehabilitant orangutans in Indonesia have developed the disease, and Dr. Birute Galdikas (personal communication to McManamon) has reported seeing a wild orangutan with airsacculitis. A wild mountain gorilla was also diagnosed with this disease. Other species affected by airsacculitis include owl monkeys, baboons, and chimpanzees.

2. Suspect Clinical Signs based upon history/visual exam:

Acute/subacute form: lethargic, anorexic, moist cough, possibly also laryngeal air sac swelling, and, apparently febrile; usually there is a history of intermittent nasal discharge.

Chronic form: Obvious signs include a history of recurring nasal discharge and upper airway congestion (rhinitis) with minimal signs of illness otherwise, or, intermittent cough (may have previously been antibiotic-responsive but recurrent.) Less obvious signs include halitosis, chronic dermatitis along the ventral neck and axilla which is exacerbated by self-picking (may improve temporarily with parenteral or topical antibiotic therapy), and intermittent diarrhea.

3. Anesthetic considerations:

a. Positioning: If the laryngeal air sac contains "fluid" secretions (more likely with the acute form), the orangutan is at risk for aspiration of the fluid via the ostia which connect the air sacs to the trachea. This risk increases with anesthesia and recumbency. Be prepared to suction, drain the airsac, and/or maintain upright positioning until intubation.
b. Laryngospasm/tracheal sensitivity from chronic cough: topical or iv lidocaine (0.5 mg/kg) works well to facilitate intubation.
c. Delayed anesthetic recovery following isoflurane may occur due to airway disease and decreased clearance of the inhalant anesthetic

4. Diagnostics:

a. General approach:

General diagnostics include physical examination, radiographs, complete blood count, serum chemistry analysis, bronchoscopy, bronchio-alveolar lavage for cytology and culture, air sac examination (via aspirate, endoscopy, exploratory) and biopsy. Consider bronchoscopy of all "normal" orangutans as well. This procedure may reveal airway disease in an individual with no clinical signs.

b. Physical exam findings:

Acute/subacute: mucoid upper airway congestion; moist rales on auscultation, possibly with tachypnea; febrile; dehydration; palpable "fluid" in the laryngeal air sac

Chronic: mucoid upper airway congestion; inflamed larynx/arytenoids if chronic cough; often auscultation is normal; superficial ulcerative dermatitis in a "ring" around the neck skin overlying the air sac; palpable "pasty material" in the laryngeal air sac

c. Radiographic findings (thorax)

Acute/subacute: bronchial to alveolar pattern (bronchitis, pneunomia); may see fluid line in air sacs; cardiac size/shape usually normal

Chronic: mild to severe bronchial pattern with areas of bronchiectasis and possibly consolidation; may see R ventricular hypertrophy secondary to pulmonary hypertension

Consider also skull radiographs to evaluate rhinitis/sinusitis.

d. Bloodwork:

Acute/subacute or Chronic: variable, may see leukocytosis, hyperfibrinogenemia

e. Bronchoscopy:

Acute/subacute or chronic: obvious accumulation of mucopurulent material present throughout the airways. If cough has been present, the mucosal surface of the arytenoids, larynx and proximal trachea may appear inflamed.

f. Bronchio-alveolar lavage:

Acute/subacute or chronic: Cytology and aerobic culture typically reveal neutrophilic inflammation associated with numerous bacteria including pseudomonas arugenosa , miscellaneous gram negative enterics (e.g., klebsiella), as well as gram positives (e.g., staphylococcus, B-streptococcus.

g. Air sac examination and biopsy:

Method: Aspiration using a 14 g needle and flushing-and-aspiration of nonbacteriostatic saline is an option for recovery of a sample for examination/culture/sensitivity. But if material is palpable or identified, it is far simpler to make a stab incision into the air sac and proceed with endoscopy or exploration using a larger incision (ultimately necessary for ostia surgery or marsupialization.) It is best to explore the entire layrngeal air sac including its lateral extensions into the axilla, as significant amounts of fluid can be "sequestered" in those areas.

Acute/subacute: typically, the skin over the air sac appears normal and the cavity itself consists of one or two compartments lined by normal appearing mucosa but filled with a tan-green "fluid". This discharge has cytology and culture characteristics similar to that collected via bronchio-alveolar lavage. On biopsy, the air sac mucosa is usually intact and consists of hyperplastic epithelial lining cells with abundant goblet forms and ciliated superficial epithelium; neutrophils and mononuclear cells are found transmigrating the epithelial layers and infiltrating the subepithelial connective tissue. When separate compartments are identified, the exudates from each should be cultured separately. Separate populations with differing culture sensitivities can be present.

Chronic: typically, there is hyperkeratosis and superficial ulceration of the overlying skin. The air sac cavity consists of multiple compartments divided by fibrous bands of scar tissue and is filled with "pasty" thick secretion. Cytologically, there is amorphous, proteinaceous material containing cocci and bacilli with a flora similar to that of the lavage. Biopsy reveals ulceration of the epithelium with loss of cilia and fewer goblet cells; mononuclear cells predominate with some transmigrating and a lymphoplasmacytic infiltrate in the subepithelial layer.

5. Treatment

Acute/subacute:
1. IV fluids as needed.
2. Begin with parenteral antibiotics under anesthesia and then continue orally for 2 weeks. Pending sensitivities, consider starting with fluoroquinolones as they are often the choice for pseudomonas; acceptance may be a problem. Long term antibiotics have been used, but nebulization as "maintenance" therapy may be more effective (see below).
3. Close both ostia surgically (advised to use a double layer closure with scarification to encourage scarring and purse-string suture) in order to prevent future aspiration of air sac contents. Surgeons encourage the use of long-lasting suture material, such as PDS (polydiaxone), to retard premature digestion of suture material and breakdown of the incision. Other surgical techniques are being explored.
4. Breakdown and biopsy the fibrous bands separating any compartments, to encourage effective drainage. Flush and suction all areas of the air sac to remove exudate. Marsupialize the air sac by making a large vertical or inverted T incision (consider steel skin sutures to deter picking) in order to promote drainage.
5. Begin nebulization with tobramycin (reconstitute to 200 mg/ml) or gentamicin (100 mg/ml large animal preparation) at 5 mg/kg via nebulizer bid for one month, then consider "maintenance therapy" with one week of nebulization per month or as symptoms recur.
6. Exert caution during extubation – ensure a patent airway to minimize larygospasm and regurgitation.
7. Some clinicians have allowed an acutely affected air sac to close spontaneously, following surgery. This approach bears some risk, but may be successful in some cases. Such air sacs must be monitored closely, as recurrence of infection, and breakdown of the surgical closure of the ostia, are possible.

Chronic:
1. Give fluids, close ostia and marsupialize air sac as above.
2. Exert caution during extubation – ensure a patent airway to minimize laryngospasm and regurgitation.
3. Begin with 2 week course of nebulization therapy followed by "maintenance" therapy
4. If marsupialized sac closes (more likely with chronic skin infection and ulceration of the air sac lining), consider removal of the air sac entirely.

5. Other considerations: antihistamines are generally not recommended as they are likely to dry the secretions and promote airway plugging. Mucolytic agents (acetylcystine nebulization, guaifenesin orally) are similarly not recommended by some, but have been used effectively by others.
6. In one case with extremely thick and tenacious bronchial exudates, Pulmozyme ™ (dornase alfa) was used successfully. However, this drug is extremely expensive and availability is limited.
7. Ensure adequate hydration, as with all chronic respiratory patients.
8. Develop a plan to re-evaluate regularly and ensure that the ostia closure remains intact (ideally through bronchoscopy).
9. Consider surgical extirpation (= removal) of the entire air sac. This time-consuming (2-3 hrs for female; 5-6 hours for adult male) surgery has been performed at several institutions (National Zoo, Gladys Porter Zoo, Louisville Zoo, Los Angeles Zoo) fairly recently (2001-2003). Assistance from MD surgeons with neck/throat experience is essential. Early results indicate success; evaluation of long term results will require additional time. This procedure has been elected in cases where chronic airsacculitis/bronchitis/pneumonia exist, and where initial ostia closure has broken down and risk of pulmonary aspiration has returned. It has the advantage of removing the source of the infection (if limited to the air sac), closing the ostia/communication to the lungs, and eliminating the draining air sac fistula.

Prognonsis
Acute/subacute: generally fair to poor for long term survival, especially if it occurs in a young animal. At best antibiotic therapy suppresses the Pseudomonas, and the eventual result will include bronchiectasis, pulmonary hypertension, and recurring pneumonia. However, many orangutans have been managed successfully with this condition for years

Chronic: generally good, especially if diagnosed early and the air sac marsupialization is permanent and the ostia are closed.

Etiology
Possibilities include poor ventilation, poor hygiene during fecal aerosolization, immune system dysfunction, genetics

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GUIDELINES FOR APE PREVENTATIVE HEALTH PROGRAM

Routine health monitoring should be performed on a regular basis. The following protocol advises that specific baseline laboratory tests be performed for the purpose of evaluating current health status. Additional tests are recommended to increase baseline information on other diseases to determine their significance to ape health. The final decision for specific procedures and their frequency should be made by the institutional animal care and veterinary staff based on individual circumstances.

Minimum Database:

1. Signalment - age, sex, origin, studbook #, ISIS #

2. Anamnesis – Previous medical history (including previous health screens, medical problems, diagnostic test results, treatments, contraception, anesthetic data and diet information

3. Complete physical examination should be performed by a veterinarian familiar with ape health issues, including complete review of systems (ophthalmic, otic, dental, lymphatic, cardiovascular, respiratory, abdominal palpation, musculoskeletal, urogenital, neurologic).

4. Body weight. Morphometric data if requested by the SSP©.

5. Verification of permanent identification – transponder, tattoo

6. Radiographs - thoracic and abdominal, VD and lateral. Include limbs of geriatric individuals to screen for arthritic changes. Oblique views of teeth taken with jaws open to screen for dental pathology is recommended.

7. Negative tuberculin skin test: 0.1 ml of mammalian tuberculin, human isolates (Colorado Serum Co., Synbiotics Corp.) administered intradermally and visually evaluated by veterinary staff for reaction at 24, 48 and 72 hours. Concurrent testing with avian tuberculin may be useful.

8. Cardiovascular status, using electrocardiogram, echocardiogram, and blood pressure measurements, is recommended to screen apes for high blood pressure and cardiac disease. If possible, these tests should be performed whenever apes are immobilized for routine and diagnostic procedures. Zoos are also encouraged to train apes for voluntary measurement of these parameters. Transthoracic echocardiography is usually sufficient to obtain complete cardiac examination in all but the largest animals. However, transesophageal echocardiography can give improved cardiac views in deep-chested animals and is necessary for complete imaging of the aorta, especially the descending portion. Due to reports of aortic dissection in gorillas, transesophageal echocardiography is recommended in this species whenever possible. A complete cardiac study includes 2-dimensional images to assess the anatomic relationship and motion of the heart structures, M-mode unidimensional images for cardiac measurements, Doppler analysis to assess blood flow through the heart, and echophonocardiography for evaluation of heart sounds and murmurs. Recommended measurements include (but are not limited to): aortic cusp separation, aortic root dimension, left atrium, right ventricle, interventricular septum, left ventricle in diastole, left ventricle in systole, left ventricular posterior wall in diastole, left ventricular posterior wall in systole, fractional shortening percentage, ejection fraction percentage, mitral valve, aortic valve, tricuspid valve and pulmonic valve. If your zoo is willing to contribute measurements for the development of reference ranges, please send blood pressure measurements, echocardiographic measurements, echocardiograms,

and concurrent anesthesia information to the SSP© Veterinary Advisor.

9. Blood collection
    - Complete blood count
    - Serum chemistry panel, including cholesterol, triglycerides, HDL, LDL and VLDL, and protein electrophoresis. Consider adding baseline thyroid testing (free and total T3, free and total T4 and TSH), Vitamin B12 and folate.
    - C reactive protein is a strong marker for cardiac disease risk in humans and may be effective in apes, but this has not been proven. Measuring this protein in healthy and ill animals may provide information for determining whether it is also a marker for cardiac disease in the different ape species.
    - Serologic testing appropriate for species:

Gibbon and Siamang (SSP© recommendations)
Herpes simplex 1 and 2, Herpes B, Cytomegalovirus, Epstein-Barr virus, Varicella-zoster, Hepatitis A, B and C, Simian T-cell lymphotropic virus, Simian retrovirus, Simian immunodeficiency virus, Simian foamy virus, and measles

Chimpanzee (SSP© recommendations)
Parainfluenza I, II and III, Influenza A and B, Respiratory syncytial virus, Hepatitis A, B and C, Herpes B, Herpes simplex 1 and 2, Human immunodeficiency virus, Simian immunodeficiency virus, Simian T-cell lymphotrophic virus (for STLV, recommend using the Simian Retrovirus Laboratory, Nicholas W. Lerche, DVM, Director, California Regional Primate Research Center, Road 98 at Hutchison, University of California, Davis, CA 95616 USA, 530-752-8242), Cytomegalovirus, Epstein-Barr virus, Varicella-zoster, measles

Bonobo (SSP© recommendations)
Parainfluenza I, II and III, Influenza A and B, Respiratory syncytial virus, Hepatitis A, B and C, Herpes B, Herpes simplex 1 and 2, Human immunodeficiency virus, Simian immunodeficiency virus, Simian T-cell lymphotrophic virus (for STLV, recommend using the Simian Retrovirus Laboratory, Nicholas W. Lerche, DVM, Director, California Regional Primate Research Center, Road 98 at Hutchison, University of California, Davis, CA 95616 USA, 530-752-8242), Cytomegalovirus, Epstein-Barr virus, Varicella-zoster, measles.

Orangutan (SSP© recommendation)
Parainfluenza I, II and III, Influenza A and B, Hepatitis A, B and C – contact SSP© Advisor regarding positive individuals

Gorilla (SSP© recommendation)

Respiratory syncytial virus, Hepatitis B, Varicella-zoster, Herpes simplex 1 and 2, measles

- Bank at least 2 ml of serum.
- Bank genetic material as recommended by BBAG protocol.

10. Fecal analyses
    - Negative parasite screen – direct, flotation and sedimentation of feces for detection of endoparasites.
    - Negative fecal culture for enteric pathogens (Salmonella sp., Shigella sp., Campylobacter sp., pathogenic E.coli).

11. Verify contraceptive method (if any). See AZA Contraceptive Advisory Group recommendations (http://stlzoo.org/downloads/CAGrecs2004.pdf). Send in contraceptive survey annually for reversible methods.

12. Vaccinations
    - Killed rabies 1 ml intramuscular every 1-3 years, where applicable based on local rabies epidemiology and status.
    - Tetanus toxoid 1 ml intramuscular every 1-10 years.
    - Pneumococcal vaccination once in childhood with geriatric booster for bonobos and orangutans.
    - Influenza vaccination – Bonobo: consider yearly in early fall prior to influenza season. Consider annual vaccination of animal care staff to enhance biosecurity.
    - Measles vaccination – optional; colony specific decision. Measles is now considered a foreign disease in humans in the USA. Attenuvax modified live vaccine has been given to apes and primates in the past with no reported adverse effects, but risk of shedding live virus and susceptibility of pregnant females and fetus is unquantified.
    - Consider childhood vaccination based on human schedule for all apes, including killed polio series and Haemophilus vaccination (http://www.cdc.gove/nip/child-schedule.htm). Recommended by Orangutan SSP© Vet Advisor.

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GUIDELINES FOR PRESHIPMENT TESTING, TRANSPORT AND QUARANTINE OF APES

Preshipment testing should be performed within 30-45 days of the anticipated shipping date. Any increase to this time interval should be approved by the veterinary staff of the receiving institution. The following protocol advises that specific baseline laboratory tests are

performed for the purpose of evaluating the animal's current health status. Additional tests are recommended to increase baseline information to determine their significance to individual animal health. The final decision for specific preshipment testing procedures should be made in partnership between the shipping and receiving institutions. To facilitate this, communication between the veterinary staffs of the shipping and receiving institution should occur prior to initiation of preshipment testing. Any abnormal findings discovered during preshipment testing should be communicated to the veterinary staff of the receiving institution in a timely manner.

Minimum Database:

1. Signalment - age, sex, origin, studbook #, ISIS #

2. Anamnesis – Previous medical history (including previous health screens, medical problems, diagnostic test results, treatments, contraception, anesthetic data and current diet information) should be provided to the veterinary staff of the receiving institution for review prior to preshipment testing.

3. Complete physical examination should be performed by a veterinarian familiar with ape health issues, including complete review of systems (ophthalmic, otic, dental, endocrine, lymphatic, cardiovascular, respiratory, abdominal palpation, musculoskeletal, urogenital, neurologic).

4. Body weight. Morphometric data if requested by the SSP©

5. Verification of permanent identification – transponder, tattoo.

6. Radiographs - thoracic and abdominal, VD and lateral. Recommend taking both oblique views of the teeth taken with the jaws open to screen for dental pathology.

7. Negative tuberculin skin test: 0.1 ml of mammalian tuberculin, human isolates (Colorado Serum Co., Synbiotics Corp.) administered intradermally and visually evaluated by veterinary staff for reaction at 24, 48 and 72 hours. Concurrent testing with avian tuberculin may be useful.

8. Electrocardiogram, blood pressure measurements and echocardiography are recommended to assess cardiovascular status, screen for high blood pressure or cardiac disease, and to collect information necessary for the development of species specific reference ranges. See Section 8 of the Guidelines for Ape Preventative Health Programs for more detailed information.

9. Blood collection
   - Complete blood count.
   - Serum chemistry panel including cholesterol, triglycerides, HDL, LDL, VLDL and protein electrophoresis.
   - Serologic testing appropriate for species. For detailed recommendations, see Section 9 of the Guidelines for Ape Preventative Health Programs. Routine retesting of an animal previously determined to be serologically positive for a given antigen may not be warranted. The final decision for specific serologic testing should be based on the serologic status of the previous, shipping, and receiving troops.
   - Bank at least 2 ml of serum at the shipping institution. If space does not permit, offer serum to receiving institution before discarding.

10. Fecal analyses
   - Negative parasite screen – direct, flotation and sedimentation of feces for detection of endoparasites. If negative parasite screen cannot be achieved, update receiving instituion on status of parasite load.
   - Negative fecal culture for enteric pathogens (Salmonella sp., Shigella sp., Campylobacter sp., pathogenic E.coli, Yersinia sp.).

11. Verify contraceptive method, if any. See AZA Contraceptive Advisory Group recommendations (http://www.stlzoo.org/downloads/CAGrecs2004.pdf).

12. Vaccinations
   - Killed rabies 1 ml intramuscular within 3 yr prior to shipment
   - Tetanus toxoid 1 ml intramuscular within 5 yr prior to shipment
   - Previous Pneumococcal vaccination – Bonobo
   - Influenza vaccination – Bonobo (yearly in late fall) prior to shipment if shipment occurs during October - April

13. Transport Guidelines – The USA has adopted International Air Transport Association (IATA) Live Animal Regulations. Some airlines have specific nonhuman primate requirements or restrictions.
   - Animals should be crated for shipment to the receiving institution.
   - Animals should be trained for voluntary crating whenever possible.
   - Zoo staff should accompany an ape in transit whenever possible.
   - Certificate of Veterinary Inspection, medical records, and appropriate permits should accompany ape in transit.

- Animals should be shipped in climate-controlled manner, with temperatures optimally held between 60-85 F.

14. Quarantine – should follow AZA guidelines. Recommended:
    - Review previous health records.
    - Gradually switch animal to new diet while monitoring food intake, stool consistency and weight.
    - Weigh animal at time of quarantine entry, examination and exit.
    - Minimum of 3 consecutive negative fecal examinations for parasites (direct, float and sedimentation) and 3 consecutive negative fecal or rectal cultures for enteric pathogens (Salmonella sp., Shigella sp., Campylobacter sp., pathogenic E.coli, Yersinia sp.).
    - Complete physical examination by veterinarian familiar with ape health issues, including complete review of systems (ophthalmic, otic, dental, endocrine, lymphatic, cardiovascular, respiratory, abdominal palpation, musculoskeletal, urogenital, neurologic).
    - Verify permanent identification – transponder, tattoo
    - Body weight, and morphometric data if requested by SSP
    - Thoracic, abdominal and dental radiographs
    - Negative tuberculin skin test: 0.1 ml of mammalian tuberculin (human isolates) administered intradermally and visually evaluated by veterinary staff for reaction at 24, 48, and 72 hours. Test must be administered a minimum of 14 days after preshipment testing.
    - Electrocardiogram, echocardiogram and blood pressure measurements to screen for high blood pressure and cardiac disease. Send echocardiogram results and blood pressure measurements to SSP© Veterinary Advisor for development of normal reference ranges.
    - Complete blood count
    - Serum chemistry panel, including cholesterol, triglycerides, HDL, LDL, VLDL, and protein electrophoresis. Consider baseline thyroid testing, and Vitamin B12 and folate levels.
    - Serologic testing appropriate for species.
    - Bank at least 2 ml of serum.
    - Bank genetic material as recommended by BBAG protocol.
    - Complete any vaccinations not administered by shipping institution.

Revised August 2004

OCCUPATIONAL PRIMATE DISEASE SAFETY GUIDELINES FOR ZOOLOGICAL INSTITUTIONS

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.

Table of Contents:

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 areosolization of bodily fluids. In order to decrease the risk of exposure to these air born 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 allows 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.

Information on known and potential zoonotic diseases of concern in nonhuman primates can be found at: www.cdc.gov/ncidod/dastlr/Retrovirology/aboutretrovirology.htm, www.cdc.gov/od/ohs/biosfy/bmbl4/bmb14s7f.htm, http://primatelit.library.wisc.edu/, and texts on laboratory animal and zoological medicine.

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 prosection, 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: cover-alls 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.

XVII. Appendices

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 and 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.

STANDARDIZED NECROPSY REPORT FOR APES AND OTHER PRIMATES
(APE TAG – L.J. Lowenstine, Pathology Advisor)

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/ institution:_____

_____

Gross diagnoses:

_____

Abstract of clinical history:

_____

Please check tissues submitted for histopathology.

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

Fractures or malformations ?:

_____Muscles:

_____Bone marrow (femur):

_____Joints:

_____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:

Effusions, adhesions, or hemorrhage?:

Mediastinal and coronary fat:

_____Thymus (are there cervical portions as well as antermediastinal?):

_____Heart (see attached protocol):

_____Great vessels (see attached):

_____Trachea and bronchi:

_____Lungs:

_____Esophagus:

_____Lymph nodes:

Abdominal Cavity:

Effusions, adhesions, or hemorrhage?:

Omental, mesenteric and perirenal fat:

_____Liver:

_____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:

_____Gasserian ganglia:

_____Spinal cord:

_____Brachial plexus and sciatic nerves:

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 in and measure disc(s)):

Thoracic Organs:
Heart _____ Thymus (above)

Height _____ Circumference at coronary groove_____
Left Vent._____ Rt. vent._____ Septum_____

Lt. AV valve_____ Rt. AV valve_____

Aortic valve_____ Pulmonary valve._____

R. lung _____ L. lung _____

Other:
Brain _____ Pituitary _____

Thyroids (wt) Left _____ Right _____
Thyroids (3 dimensions) Left_____ Right _____

Testes (wt.) Left _____ Right _____
Testes Length x dia. Left _____ Right_____

Penis (length x diameter) _____
Tumors(?) Measurements (3 dimensions) _____ Weight_____

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 allow for adequate 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.

Labeling of specimens

If pieces are small or not readily recognizable (eg. 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, eg. 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, and 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. 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. Take sections of all levels of the GI track including: gastric cardia, fundus and pylorus; duodenum at the level of the bile duct with pancreas attached; anterior, middle and distal jejunum; ileum; ileocecocolic 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: One section should include bile ducts and gall bladder and take sections from at least one other lobe.

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 should be sectioned transversely; colonic nodes may be left with colon sections.

Take sections from each kidney: cut the left one longitudinally and the right one transversely so they will be identifiable.

Fix small adrenals whole and section larger ones (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. Make sure to include round ligaments (umbilical arteries) in neonates.

Section the prostate with the urethra and seminal vesicles transversely. Section testes transversely.

In small females fix the vulva, vagina, cervix, uterus and ovaries 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 large primates make transverse sections of each part of the track and the ovaries.

If gravid: weigh and measure placenta and fetus. Perform a post mortem examination of the fetus. Take sections of disc from periphery and center and from extraplacental fetal membranes. Take sections of major organs and tissues of fetus.

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.
Institutions may elect to send brains to the Great Ape Aging Project.

Fix the pituitary whole. Put pituitary in an embedding bag if it is small. 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.)

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 hemi-sections of the marrow.

Take sections of any and all lesions, putting them in embedding bags if they need special labeling.

Remember, it's better to save "too many" tissues than to risk missing essential lesions or details.

This represents a lot of work on the part of the prosector, often under less than comfortable conditions. But the effort expended at the time of the gross post mortem is much appreciated by the histopathologist, and is crucial to our investigations of the causes of morbidity and mortality of free-living nonhuman primate **THANK YOU !!!!!**

CARDIAC EXAMINATION FOR APES AND OTHER PRIMATES

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 contraction (diastole or systole at death) and general morphology. (The apex should be fairly sharp.)

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 and foramen ovale. 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 atrium. 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. Examine the foramen ovale for patency.

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 (see work sheet).
Measure height of heart and circumference.
Measure thickness of right and left ventricles and septum.
Measure the circumference of the right and left AV valves and the aortic and pulmonary valves.

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 vase of the AV valves (area of conduction system).
Section of the ascending aorta just above the valves (the most common site of dissecting aneurysms in great apes) as well as sections of descending thoracic aorta and abdominal aorta.
Sections from any lesions noted.

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

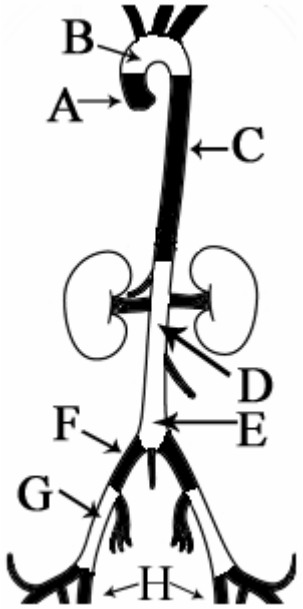
Other vessels:
Make sure to open and examine the entire aorta, iliac arteries and popliteal arteries (frequent sites of aneurysms in humans).

Note the location and severity of fibrous or fatty streaks and overt atherosclerosis. (see diagram)

Branches of the aorta:

A. Aortic root and ascending aorta
B. Aortic arch
C. Thoracic aorta/ descending aorta
D. Abdominal aorta
E. Bifurcation of abdominal aorta to iliac arteries
F. Common(internal) iliac arteries
G. External iliac and common femoral
H. Superficial femoral artery

Please note location of atherosclerosis, aneurysms, dissections or other abnormalities



POSTMORTEM EXAMINATION OF PRIMATE FETUSES, NEONATES and PLACENTAS

Follow the general primate necropsy protocol.

Make sure to **weigh** the fetus and **make morphologic measurements.**

In addition, **measure the placental disc(s) and weigh the placenta.**

Describe the placental discs and membranes and the vascular pattern.

Measure umbilical length and diameter . If possible, please photograph the placenta.

Internal examination:

Note dentition/ erupted teeth and carefully examine the palate.

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.

Before removing the heart from the pluck, open the pulmonary artery to check for patency of ductus arateriosus. Open the lateral side of the right atrium and examine the foramen ovale for patency.

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

POST MORTEM EXAMINATION OF THE AIR SACS OF APES AND OTHER PRIMATES

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

Incise the air sac through the skin on the anterior (ventral) aspect.

Note color and texture of air sac lining.

Note presence of absence of exudates, and character of exudate.

Note presence or absence of comparmentalization by connective tissue.

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

Is there a central compartment?

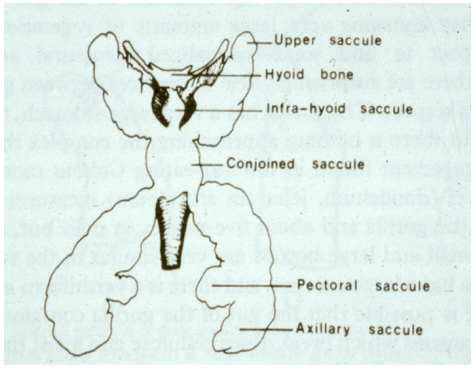
Are the lateral sacs symmetrical (they may vary in size in chimpanzees and bonobos)

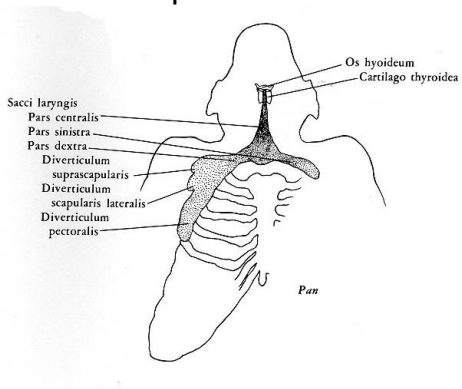
Identify and describe the opening(s) from the larynx into the air sac (e.g. single slit-like opening or paired oval openings). Are the openings parallel or perpendicular to the long axis of the larynx and trachea. Note any exudate.

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).

Cultures: Please culture several different sites within the air sacs (we need data to determine if infections are "homogeneous" or compartmentalized).

Diagrams of air sacs to aid in measurements and descriptions.





Gorilla air sacs (From Dixon) Chimpanzee air sacs (From Swindler & Wood)

Information on air sac anatomy is especially important for bonobos as there is nothing in the literature about their air sacs.

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.

Simian Retrovirus Laboratory
California National Primate Research Center,
University of California, Davis, CA
(530) 752-8247

BioReliance,
14920 Broschart Rd.
Rockville, MD 20850
(800) 553-5372

Esoterix Infectious Disease Center
Simian Diagnostic Laboratory
7540 Louis Pasteur Dr.
San Antonio, TX 78229
(210) 614-7350

Bill Switzer
Centers for Disease Control and Prevention
1600 Clifton Rd, MS-G19
Atlanta, GA 30333
(404) 639-0219

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

SSP Veterinary/Pathology Advisors

Baboon Hayley Murphy, Zoo New England, hweston@zoonewengland.com

Bonobo Vickie Clyde, Milwaukee County Zoo, Vclyde@execpc.com

Callimico Jennifer Langan, Brookfield Zoo jalangan@brookfieldzoo.org

Colobus Cornelia Ketz-Riley, Topeka Zoo, cketz@topeka.org

Chimpanzee Kathryn Gamble, Lincoln Park Zoo,, kgamble@lpzoo.org

Gibbon P.K. Robbins, Disney's Animal Kingdom, Pk.robbins@disney.com

Gorilla Tom Meehan, CZS (Brookfield Zoo), tomeehan@brookfieldzoo.org
Linda Lowenstine (pathol), UC Davis, ljlowenstine@ucdavis.edu

Langur Donna Ialeggio, Philadelphia Zoo, Ialeggio.Donna@phillyzoo.org

Lemur Randy Junge, St. Louis Zoo, Rejunge@aol.com

Black & Blue-eyed Lemur
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Ring-tailed black Lemur
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Ruffed Macaque
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Mangaby Shirley Llizo, Houston Zoo, sllizo@houstonzoo.org

Orangutan Don Neiffer, Disney's Animal Kingdom, Donald.L.Neiffer@disney.com

Cotton-top Tamarin
Scott Terrell (pathol), Disney's Animal Kingdom,
Scott.Terrell@disney.com

TAG

New World Primate:
Old World Primate: Jan Ramer, Indianapolis Zoo, jramer@indyzoo.com
Apes: Linda Lowenstine (path), UC Davis, ljlowenstine@ucdavis.edu
Prosimian: Randy Junge, St. Louis Zoo, Rejunge@aol.com
Ilse Stalis (path), San Diego Zoo, istalis@sandiegozoo.org

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.[(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*.[(2)] Literature reviews on laboratory acquired infections also may be helpful.[(3)(4)(5)(6)(7)(8)]

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]

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Proceedings of the annual conference of the AAZV
http://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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