Guidelines for the Prevention and Control of Tuberculosis in Nonhuman Primates

I. Introduction

Because tuberculosis is a zoonotic disease that can be devastating and terminal in nonhuman primates (NHPs), and may be transmitted from humans to NHPs, it is necessary to establish guidelines for the prevention and control of this pathogen within the NIH intramural research program. These guidelines apply to all NIH-operated Intramural Research Animal Programs and agencies that lease space from NIH intramural animal programs.

II. Prevention

Preventive measures are required to protect NHPs and personnel who come into contact with NHPs that may be harboring Mycobacterium tuberculosis complex (MTC - *M. tuberculosis*, *M. bovis* and *M. africanum*). Non-tubercle forming, atypical mycobacteria species are also important but primarily because they may confound test results.

A. Quarantine

The entry of NHPs into NIH operated facilities must be in compliance with the NIH NHP Quarantine Policy, Policy Manual 3044-1, "Nonhuman Primate Quarantine." Contact the NIH Animal Center, Division of Veterinary Resources (DVR), Office of Research Services (ORS) (301-402-9862) for further information.

B. Husbandry Practices

The animal husbandry and sanitation practices as applied to NHPs at the NIH are designed to prevent the spread of pathogens including tubercle bacilli. To this end, tuberculocidal detergent disinfectants (the label must read tuberculocidal) must be used in facilities housing NHPs. Periodically rotating the specific disinfectant to prevent anti-microbial resistance should be considered. Cleaning and other in-room equipment must remain in one room unless it is effectively disinfected between rooms. Sanitation schedules and practices must be in compliance with all applicable regulations, policies and guidelines.

NHP holding and procedures rooms must be under negative pressure relative to adjacent corridors. Husbandry practices must minimize the production of aerosols in animal rooms, e.g., sanitizing room surfaces and sanitizing animal cages and litter pans or trays. Other procedures, including research procedures, must be carried out in a manner to prevent the generation of aerosols that potentially contain pathogens. High pressure washing of cages and room surfaces can be performed only after the NHPs have been removed from the room and with proper protection of personnel including protection from splash.

C. Monitoring Procedures

1. Tuberculin Skin Testing - Tuberculin skin testing (TST) is the primary tool used to detect tuberculosis in NHPs.

a) Methods: First the animal should be appropriately restrained. Using a sterile needle for each NHP, inject 0.1 ml. of Mammalian Tuberculin intradermally into one eyelid near the edge or into the abdominal skin or both; 0.05 ml. can be used in small NHPs, e.g., some New World species. Usually the eyelid is preferred as it is relatively easy to observe on awake, unrestrained NHPs. If the abdomen is used, the hair should be clipped without traumatizing the skin and the injection site noted. The abdominal skin test is most commonly used when retesting suspect NHPs. The advantage of using the abdomen is that any induration can be measured and a saline control injection can be used.

b) Reading TST: Observe the animals for reactions at 24, 48, and 72 hours post-injection under good lighting conditions. The readings must be made by a trained technician. Any reactions or suspected reactions of a grade three or higher, or as designated by the clinical veterinarian are to be observed and interpreted by the clinical veterinarian. The following grading systems should be used:

(1) Eyelid injections: When using the following grading system, the actual descriptions or corresponding reaction grade should be entered into the animal's record.[1]

Reaction Grade Description of Changes:

0 - No reaction

1 - Bruise - extravasation of blood in the eyelid associated with the injection of tuberculin.

2 - Varying degrees of erythema of the palpebrum with minimal swelling.

3 - Moderate swelling with or without erythema.

4 - Obvious swelling of the palpebrum with drooping and varying degrees of erythema.

5 - Marked swelling with necrosis and eyelid closed or partially closed.

Interpretation: Grades 0, 1 and 2 are considered negative, grade 3 is suspect and grades 4 and 5 are considered positive.

---

[1] Modified from Fox JG, et al, eds. Laboratory Animal Medicine, 2nd ed. Academic Press, Inc., Orlando FL, 2002.

(2) Abdominal injections:

Induration at widest point	Interpretation
< 5 mm.	Negative
5 to 10 mm.	Suspect
> 10 mm.	Positive

c) Frequency of TST: The following intervals for testing of species or groups of NHPs is recommended during quarantine and post-quarantine holding. Because of a number of variables, the facility veterinarian may elect to test at less frequent intervals. When NHPs are tested at less frequent intervals than these recommendations, the facility veterinarian who is to receive any of those NHPs must be notified of that fact before the animals are transferred.

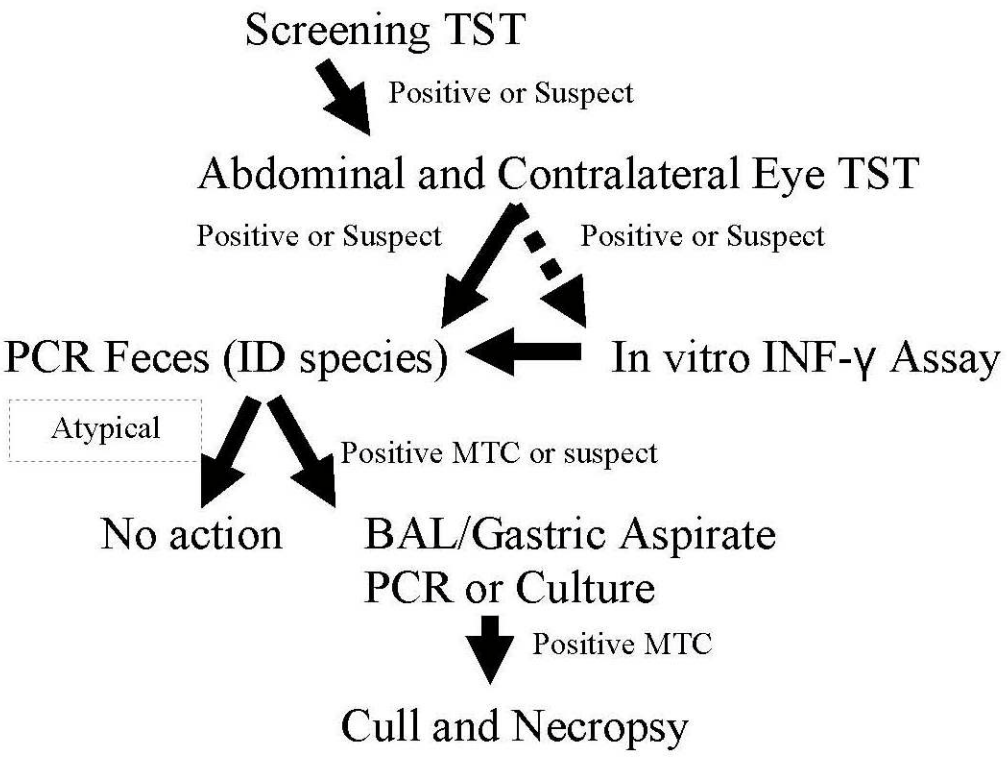
Species or Group	TST Schedule Quarantine as per NIH PM 3044-1	Recommended TST Schedule Post-Quarantine Holding
New World Monkeys	3 times, 2 weeks apart	Semiannually
Macaque species	5 times, 2 weeks apart	Quarterly
Baboons	3 times, 2 weeks apart	Semiannually
Chimpanzees	2 times, 1 month apart	Annually
Patas	3 times, 2 weeks apart	Semiannually
African green	5 times, 2 weeks apart	Quarterly
Prosimians	3 times, 2 weeks apart	Semiannually

2. **Adjunct testing:** Other methods include in-vitro gamma interferon assay (Primagam®) and antibody detection (ESAT-6 and CFP-10) and may be used as adjuncts to TST when investigating TB suspects.

3. **Anergic NHPs:** Tuberculous NHPs infrequently become anergic to TST. Tuberculosis should be considered and further testing performed on animals that have unexplained weight loss or non-healing wounds. Additional testing may include: cytology and culture swabs of non-healing wounds, chest radiographs, acid fast bacillus smear, culture and PCR (polymerase chain reaction) of gastric and/or bronchial lavage, PCR of feces or tissues, and other methods as they are validated. Immunosuppression is known to interfere with cell mediated immunity and may interfere with gamma interferon production and TST results. The clinical vet should consider all factors (immunocopetency, anergy, exsposure to other mycobacteria) that could cause false negatives or false positives.

4. **Suspect NHPs:** Tuberculosis should be considered and further testing performed on animals with a suspect response on palpebral or abdominal tests. Additional testing may include: testing the contralateral eyelid, performing an abdominal test if not already performed, chest radiographs, acid fast bacillus smear, culture and PCR of gastric and/or bronchial lavage, PCR of feces or tissues, in-vitro gamma interferon

assay (Primagam®), antibody detection (ESAT-6 and CFP-10) and other methods as they are validated. The following is a suggested algorithm for testing suspect animals:



5. **Sensitized Nontuberculous NHPs:** NHPs may become reactive to TST when injected with immunologic materials that contain Complete Freund's Adjuvant (CFA) because it contains cell walls of tubercle bacilli. When feasible, other adjuvants should be used to avoid ameliorating the usefulness of the best test available for monitoring NHPs for tuberculosis. If it is necessary to use CFA, the NHP(s) is to be tuberculin tested the week before the CFA is injected. Following the first positive test in a CFA injected NHP, the animal is to be weighed monthly to detect any weight loss, and, at the time the NHP would normally be tuberculin tested, other examinations are to be performed for the detection of tuberculosis. Such testing may include PCR and/or acid fast bacillus smears and cultures of fecal and/or gastric washings for *Mycobacteria* species. If tuberculosis is confirmed in other NHPs in the holding room housing a CFA exposed NHP, the potentially exposed NHP(s) that previously received CFA should be euthanatized.

6. **Necropsy:** A postmortem TB surveillance program is one of the best measures of the effectiveness of our program to exclude the pathogen. Necropsy services are available at NIH to provide postmortem examination for the presence of TB. All NHPs should be considered by the facility and/or IC attending veterinarian for postmortem examination for the presence of TB. Animals may be submitted to the DVR Pathology Service for surveillance necropsy, or alternatively, necropsies may be performed by or under the direction of another veterinarian at the NIH. At a minimum, the lungs, with emphasis on the peripheral lung lobes, and tracheal-bronchial lymph nodes should be

evaluated for signs of disease. Pulmonary tubercles with caseous cavitations, miliary lesions and lymphadenopathy are often found in nonhuman primates with *Mycobacterium* infections. Nonhuman primates traditionally do not demonstrate the extensive calcification and fibrosis found in other species. Suspect or positive results should be reported to the DVR Pathology Service along with tissue(s) for histological (e.g. fixed) and PCR (e.g. fresh and/or frozen) evaluation, as well as fresh samples for Mycobacterial culture.

7. **Radiographs:** Chest radiographs may be used as an additional test procedure but cannot be used as the only screening procedure. Chest radiographs can be difficult to interpret especially in macaque species.

III. Protection of NHPs from Personnel

The procedures mandated in Policy Manual 3044-2, Protection of NIH Personnel Who Work with Nonhuman Primates, to protect personnel from the zoonotic diseases of NHPs, also protect NHPs from being exposed to tubercle bacilli from humans.

IV. Protection of Personnel

Only designated personnel shall be permitted in animal rooms. They shall comply with NIH Policy Manual 3044-2, Protection of NIH Personnel Who Work with Nonhuman Primates and applicable guidelines for the prevention and control of tuberculosis in nonhuman primates in the NIH intramural program.

Biosafety precautions must be taken when dealing with a diagnosed tuberculous NHP, a NHP that is a tuberculosis suspect, and when collecting and handling samples to be cultured for tubercle bacilli.

V. Handling Confirmed Tuberculous Positive NHPs

A. Immediate Euthanasia

When a clinical diagnosis of *M. tuberculosis* complex disease is made in a NHP, it is immediately euthanatized (unless V. B. or C. below applies) and the carcass is taken to the Pathology Section, Diagnostic and Research Services, DVR, ORS for necropsy, or other facilities as discussed under paragraph II.C.6. above, and the Division of Occupational Health and Safety is notified. The DOHS will review and approve the containment requirements for the animals. The cage and room where the tuberculous NHP was held are sanitized and remaining NHPs are placed under quarantine.

Quarantine means:

1) Access to the room is limited to a few essential personnel,

2) Protective clothing (Tyvek® jump suit, shoe covers, head bonnet, mask, latex, nitrile, vinyl or rubber gloves and eye protection) is worn in the room and is not removed from the room except to be autoclaved,

3) Other NHPs are not placed in or removed from the room, and

4) NHPs in the room are tuberculin tested every two weeks until five tests have been performed with negative reactions; the first of these tests is administered about one week after the test that identified the tuberculous NHP.

When 5 tests have been administered with negative reactions, the quarantine may be terminated, except that NHPs are not placed in or removed from the room until a tuberculin test is administered four weeks after the last of the 5 tests with negative reactions being observed. A diligent effort will be made to locate all NHPs that were housed within the last 60 days in the room in which the tuberculous NHP was housed. These NHPs will be tuberculin tested on the same schedule as the NHPs currently housed in the quarantined room.

B. Delayed Euthanasia

The euthanasia of a NHP with M. tuberculosis complex disease can be delayed if the animal is of great value to a research project and can be isolated to minimize the spread of tubercle bacilli to other NHPs or humans. The room in which such a NHP was held when the clinical diagnosis was made will be placed under quarantine as described in V. A. above. The Director, DOHS and the owning IC's APD and Animal Care and Use Committee (ACUC) will be notified. The DOHS will review and approve the containment requirements for the animal.

C. Treatment of Tuberculous NHPs

Normally, NHPs shall not be treated for M. tuberculosis complex disease. However, valuable NHPs may be treated if for scientific reasons. Chimpanzees and other great apes should be treated if deemed appropriate by the clinical veterinarian. The DOHS will review and approve the containment requirements for the animal. If an animal is treated, an ASP approved by the user IC's Animal Care and Use Committee and the Institutional Biosafety Committee (IBC) is also required. A multiple drug regimen based on the most current practice standard must be used in the treatment and the treatment must be for at least 6 months.

VI. Records

It is important that each NHP's tuberculin test be accurately entered into its clinical record. Facility records should include where the animal has been housed including dates. Accurate records are also important in detecting unexplained weight loss or non-healing wounds which may be indications of tuberculosis in NHPs.

References

1. NIH Manual 3044-2, Protection of NIH Personnel Who Work With Nonhuman Primates.
2. NIH Manual 3040-2, Animal Care and Use In the Intramural Program.
3. Institute of Laboratory Animal Resources (ILAR). Laboratory Animal Management: Nonhuman Primates. ILAR News, Vol XXIII: Number 2-3, 1980. Available as a publication from ILAR, NRC.
4. Capuano SV 3rd. Croix DA. Pawar S. Zinovik A. Myers A. Lin PL. Bissel S. Fuhrman C. Klein E. Flynn JL. Experimental Mycobacterium tuberculosis infection of cynomolgus macaques closely resembles the various manifestations of human M. tuberculosis infection. Infec & Immun. 71(10):5831-44, 2003 Oct.
5. Chaparas SD. Good RC. Janicki BW. Tuberculin-induced lymphocyte transformation and skin reactivity in monkeys vaccinated or not vaccinated with Bacille Calmette-Guerin, then challenged with virulent Mycobacterium tuberculosis. American Rev of Resp Dis. 112(1):43-7, 1975 Jul.
6. Corcoran KD, Thoen CO. Application of an enzyme immunoassay for detecting antibodies in sera of Macaca fascicularis naturally exposed to Mycobacterium tuberculosis. J Med Primatol, 1991 20(8):404-408.
7. Garcia MA. Yee J. Bouley DM. Moorhead R. Lerche NW. Diagnosis of tuberculosis in macaques, using whole-blood in vitro interferon-gamma (PRIMAGAM) testing. Comp Med. 54(1):86-92, 2004 Feb.
812. Gormus BJ. Blanchard JL. Alvarez XH. Didier PJ. Evidence for a rhesus monkey model of asymptomatic tuberculosis. J of Med Primatol. 33(3):134-45, 2004 Jun.
9. Hines ME, Kreeger JM, Herron AJ. Mycobacterial infections of animals: pathology and pathogenesis. 1995, Lab An Sci, 45:334-351.
10. Fox JG, Anderson LC, Loew FM, Quimby FC, eds. Laboratory Animal Medicine, 2nd ed., Academic Press, Inc., Orlando Fl, 2002.
11. Rock FM. Landi MS. Meunier LD. Morris TH. Rolf LL. Warnick CL. McCreedy BJ. Hughes HC. Diagnosis of a case of Mycobacterium tuberculosis in a cynomolgus (Macaca fascicularis) monkey colony by polymerase chain reaction and enzyme-linked immunosorbent assay. Lab An Sci. 45(3):315-9, 1995 Jun.
12. Tuberculosis in imported nonhuman primates--United States June 1990-May 1993. Morb Mortal Wkly Rep, 1993 Jul 30: 42(29): 572-6.
13. Vervenne RA. Jones SL. van Soolingen D. van der Laan T. Andersen P. Heidt PJ. Thomas AW. Langermans JA. TB diagnosis in non-human primates: comparison of two interferon-gamma assays and the skin test for identification of Mycobacterium tuberculosis infection. Vet Imm & Immunopath 100(1-2):61-71, 2004 Jul.
14. Institute of Laboratory Animal Resources (ILAR) New Approaches to Tuberculosis Surveillance in NonHuman Primates ILAR Journal Vol 49 (2) Available as a publication from ILAR, NRC.
15. USDA Policy #22, Animal Resource Guide, Issue Date October 13, 1998

Initial approval: 5/08/96
Reapproved - 2/10/99
Revised - 3/27/02, 1/12/05, 9/12/07, 5/12/2010