

Guideline for the Prevention and Control of Tuberculosis in Nonhuman Primates

Introduction: 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 to agencies that lease space from NIH Intramural Animal Programs.

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

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." For further information, contact the NIH Animal Center, Division of Veterinary Resources (DVR), Office of Research Services (ORS) (301-402-9862) if animals will be quarantined at the DVR- managed/operated Primate Quarantine Facility (NPQF) in Poolesville or the IC Animal Program Director (APD) if animals will be quarantined in an IC-managed/operated quarantine facility.

Husbandry Practices: The animal husbandry and sanitation practices as applied to NHPs at the NIH are designed to prevent the spread of pathogens including *Mycobacterium* spp. 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. Caging and other in-room equipment must remain in one room unless it is appropriately disinfected between rooms. Sanitation schedules and practices must be in compliance with all applicable regulations, policies and guidelines.

NHP holding and procedure rooms must be under negative pressure relative to adjacent corridors. Husbandry practices should minimize the production of aerosols in rooms housing NHPs (e.g., sanitizing room surfaces, animal cages, litter pans or trays, etc.). Other procedures, including research procedures, must be carried out in such a manner as to prevent the generation of aerosols that potentially carry pathogens. Husbandry practices which generate aerosols (i.e. high pressure washing of cages and room surfaces) should ideally be performed only after the NHPs have been removed from the room and with proper protection of personnel including protection from splash and respiratory hazards.

Monitoring Procedures:

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

Methods:

Eyelid Injection: In most situations the eyelid is the standard, preferred testing site because it is sensitive and relatively easy to observe on awake, unrestrained NHPs. First the animal should be appropriately restrained. Using a new, 27-gauge or smaller sterile needle for each NHP, inject 0.1

ml of USDA licensed and approved Mammalian Old Tuberculin intradermally into one upper eyelid. When conducting consecutive TB tests, the eyelids should be alternated between each testing period. In accordance with ILAR guidelines, the CDC recommends an injection volume of 0.1 ml for all animals during quarantine. It is believed that this standardized volume ensures the presence of sufficient antigens to elicit a delayed Type II hypersensitivity reaction in positive animals. Many believe that this volume is too large for the eyelid of smaller NHPs and may lead to needless tissue trauma and false positive results. Therefore, a smaller volume, 0.05 ml, maybe used in the eyelid of small New World primates (e.g. marmosets, squirrel monkeys, etc.) that have been processed through a CDC approved quarantine facility or born/maintained in a domestic TB negative colony. To further reduce the trauma associated with TST intradermal injections, consider using a 30-gauge needle for the small New World primates. Avoid inserting the small gauge needles through the rubber stopper of the Mammalian Old Tuberculin bottle. Instead change to a new needle after drawing up the tuberculin to avoid needle damage that would potentially cause tissue trauma thus leading to false positive skin reactions.

Skin Injection: Abdominal skin testing is most commonly used when retesting suspect NHPs or as an alternative route when testing small New World primates (e.g. marmosets, squirrel monkeys, etc.). The advantage of using the abdomen is that any induration can be measured, and a saline control injection can be used. Once the animal is appropriately restrained, the hair should be clipped without traumatizing the skin over the proposed injection site. Using a new, 27-gauge or smaller sterile needle for each NHP, inject 0.1 ml of USDA licensed and approved Mammalian Old Tuberculin intradermally. The 0.1 ml injection volume has been used successfully for abdominal testing in all sizes of NHPs. Care should be used when identifying the injection site of some animals (e.g. marmosets), because the animal may traumatize the injection site trying to remove the markings.

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. Evaluation of eyelid injections can be made visually. With abdominal skin testing, the evaluator must, at a minimum, palpate the 72-hour skin test. 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. For both systems the grades and descriptions should be recorded in the animal's record. The following grading systems should be used:

Eyelid injections: *This grading system is a modified from Fox JG, et al, eds. Laboratory Animal Medicine, 3rd ed. Academic Press, Inc., Orlando FL, 2015.*

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
3	Moderate swelling with or without erythema
4	Obvious swelling of the palpebrum with drooping with or without erythema
5	Necrosis of the eyelid with varying degrees of swelling, including eyelid partially or completely closed

Interpretation: Grades 0, 1 and 2: considered negative; Grade 3: is suspect; Grades 4 and 5: considered positive

Abdominal injections:

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

Frequency of TST: Animals born into TB free colonies are routinely tested after six (6) months of age. All animals born into TB free colonies must be tested by one (1) year of age. The following intervals for testing of species or groups of NHPs are recommended during quarantine and post-quarantine holding. If the facility veterinarian elects to test at less frequent intervals, they must notify any facility receiving animals from their program of this information prior to the transfer of animals.

The exemption of an animal from testing for scientific purposes must be justified in the investigator's approved animal study protocol. In addition, the facility veterinarian/ACUC overseeing the holding facility for the exempted animals must also be in agreement with the exemption. In these situations, one or more of the alternative/adjunct testing methods outlined below should be considered.

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	4 times, 2 weeks apart	Semiannually
Baboons	3 times, 2 weeks apart	Semiannually
Chimpanzees	2 times, 1 month apart	Annually
Patas	3 times, 2 weeks apart	Semiannually
African green	4 times, 2 weeks apart	Semiannually
Prosimians	3 times, 2 weeks apart	Semiannually

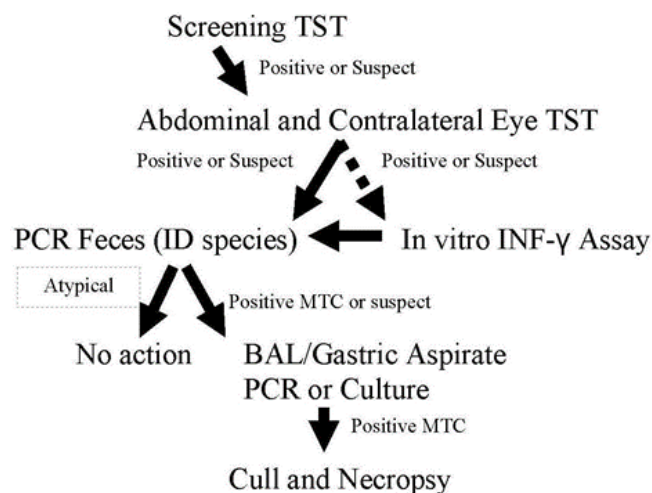
Adjunct testing: When available, other methods including in-vitro gamma interferon assays, PCR (polymerase chain reaction) testing for *Mycobacterium* spp., gamma-interferon stimulation assays, and antibody detection platforms may be used as adjuncts to TST when investigating TB suspects or animals exempt from TST. Chest radiographs or other imaging modalities such as computed tomography 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.

Necropsy: A postmortem TB surveillance program is one of the best measures of the effectiveness of a program to exclude this pathogen. Necropsy services are available at NIH to provide postmortem examinations 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 NHPs with *Mycobacterium* infections. NHPs 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.

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 of gastric and/or bronchioalveolar lavage (BAL), 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 (immunocompetency, anergy, recent history of measles or measles vaccination, exposure to other mycobacteria) that could cause false negative or positive reactions.

Suspect NHPs: Tuberculosis should be considered, and further testing performed on animals, with a suspect response on palpebral or abdominal tests. When retesting a suspect animal, the full 0.1 ml volume of Old Mammalian Tuberculin should be used for used for both eyelid and skin injections. 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 bronchioalveolar lavage (BAL), PCR of feces or tissues, in-vitro gamma interferon assay, antibody detection, and other methods as they are validated. The following is a suggested algorithm for testing suspect animals:



Sensitized Nontuberculous NHPs: NHPs may, in rare situations, become reactive to TST due to nonspecific reactions to mammalian tuberculin tests or 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 reducing the usefulness of the best available test for monitoring NHPs for tuberculosis. If it is necessary to use CFA, the NHP is to be tuberculin tested the week before the CFA is injected. If compatible with the ASP under which the animal is being held, the CFA exposed NHP should continue to be tested with tuberculin until a suspect reaction is noted. If not compatible with the animal's ASP, alternative testing strategies as outlined below should be implemented. Unpublished data has demonstrated that many animals exposed to CFA do not become reactors and TST may remain a useful monitoring tool in these animals. Following the first suspect tuberculin skin test in a CFA injected NHP, the animal should be weighed frequently (i.e., monthly) to detect any weight loss, and alternative monitoring strategies should be implemented at the time the NHP would normally be tuberculin tested. Such testing strategies may include PCR and/or acid-fast bacillus smears and cultures of fecal and/or gastric washings for Mycobacteria species. Chest radiographs can be taken, but it is difficult to distinguish between tuberculosis and other diseases of the lung. 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 and became reactive to TST should be relocated to an appropriate isolation/quarantine facility or euthanatized.

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.

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 NHPs within the NIH intramural program. Additional biosafety precautions must be taken when dealing with a diagnosed tuberculous NHP, an NHP that is a tuberculosis suspect, and when collecting and handling samples to be cultured for tubercle bacilli.

Handling Confirmed Tuberculous Positive NHPs:

Immediate Euthanasia:

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

Quarantine Means:

- Access to the room is limited to a few essential personnel,
- Protective clothing (Disposable coveralls/jump suit, shoe covers, head bonnet, N-95 face mask and eye protection (goggles) or a Powered Air-Purifying Respirator (PAPR), and latex/nitrile/vinyl/rubber gloves) is worn in the room and is not removed from the room except to be decontaminated (items that will not be disposed of) or autoclaved and properly disposed of,

- Other NHPs are not placed in or removed from the room, and NHPs in the room with a confirmed positive animal are tuberculin tested every two weeks until five tests have been performed with negative results; the first of these tests is administered about one week after the test that identified the tuberculous NHP. When retesting animals housed in a room with a confirmed positive animal, the full 0.1 ml volume of Old Mammalian Tuberculin should be used for both eyelid and skin injections.

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.

Delayed Euthanasia: The euthanasia of an 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 an NHP was held when the clinical diagnosis was made will be placed under quarantine as outlined above. The DOHS Director 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.

Treatment of Tuberculous NHPs: Normally, NHPs shall not be treated for *M. tuberculosis* complex disease. However, valuable NHPs may be treated 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 and the treatment duration must be for at least 6 months.

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.

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