

Guideline for the Prevention and Control of Tuberculosis in Nonhuman Primates

Introduction:

Tuberculosis (TB) 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 (IRP). These guidelines apply to all NIH-operated IRP Animal Programs and to agencies that lease space from NIH IRP Animal Programs. Practices and recommendations are drawn from a variety of sources as indicated in the references at the end of this guideline.¹⁻²³

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. While they often do not cause as serious a disease as MTC, they may confound experimental results. There are several methods used by animal programs to prevent tuberculosis in NHPs.

1. **Protection of NHPs from Personnel:** One of the primary goals to prevent TB disease is to eliminate its entry into facilities. Procedures mandated in NIH Policy Manual Chapter (MC) 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. This includes enrollment in the Animal Exposure Program (AEP), or a contractor equivalent, that mandates testing for tuberculosis for staff members, contractors, maintenance staff, facility contractors, visitors, or emergency personnel with NHP contact or anyone who enters NHP facilities. The required testing frequency is up to the IC Animal Program(s), but must be at least annual to remain a part of the Animal Exposure Program. Use of personal protective equipment as outlined in MC 3044-2 and in the ARAC [Guidelines for Personal Protective Equipment in Animal Facilities](#) can also help to protect both staff and animals.
2. **Quarantine:** The entry of NHPs into NIH operated facilities must be in compliance with the NIH Policy MC 3044-1: Nonhuman Primate Quarantine. Most animals quarantine at the NIH Animal Center in Poolesville, MD and the Division of Veterinary Resources (DVR) can be contacted for information about the quarantine procedures there. Further information can also be obtained from the IC Animal Program Director (APD).
3. **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 detergents/disinfectants (the label must read tuberculocidal) must be used in facilities housing NHPs. 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. Facilities should consider methods for sanitizing room surfaces, animal cages, litter pans or trays, and other equipment that will limit aerosol production. 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. Research procedures should also be carried out in such a manner as to prevent the generation of aerosols that potentially carry pathogens.

For interstate or international shipments, refer to APHIS testing requirements prior to shipment.

Monitoring Procedures for Routine Screening:

Despite practices to limit NHP exposure to TB, infection is still possible and animals must still be screened for tuberculosis. The primary screening method is tuberculin skin testing, but other methods may prove useful in defined situations.

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

- a. **Methods:**

- i. Eyelid Injection in Old World Species: 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 (MOT) 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.
 - ii. Eyelid Injection in New World Species: Many believe that 0.1 mL of MOT 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, may be 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 MOT 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.
 - iii. 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 MOT intradermally. The 0.1 ml injection volume has been used successfully for abdominal testing in all sizes of NHPs. One can use a black/blue surgical skin marker and draw two circles to mark locations of both test (MOT) and control (saline). 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.

b. Reading TST:

- i. Eyelid injections: Animals should be observed for skin reactions to the MOT at 24, 48, and 72-hours post-injection for eyelid tests. Visual assessment of eyelid tests at 24 and 48 hours may provide useful information as to trauma and other considerations. But the 72-hour time is most important for the actual diagnosis. The readings must be made by a trained technician or veterinarian.
- ii. Abdominal Skin Test: With abdominal skin testing, the evaluator must, at a minimum, palpate the 72-hour skin test and cannot only observe the reaction visually.

- c. **Determining Reaction**: Any reactions or suspected reactions of a grade three or higher based on the tables below are to be observed and interpreted by the clinical veterinarian. For eyelid tests, the grades should be recorded in the animal's record along with descriptions for grades 3-5. For abdominal tests, a measurement of the induration should be included in the record for any suspect or positive tests. The following grading systems should be used:

Eyelid Injections: *This grading system is modified from Fox JG, et al, eds. Laboratory Animal Medicine, 3rd ed. Academic Press, Inc., Orlando FL, 2015 and Abee CR, et al, Nonhuman Primates in Biomedical Research: Biology and Management, 2nd ed. Academic Press, Inc, 2012.*

Reaction Grade Description of Changes:	
0	No Reaction (negative result)
1	Bruising of eyelid (negative result)
2	Varying degrees of erythema of the eyelid without swelling (negative result)
3	At least minimal swelling with/without any degree of erythema (indeterminate result)
4	Obvious swelling of the palpebrum with drooping of eyelid (positive result)
5	Marked swelling and/or necrosis of the eyelid; the eyelid may be partially or completely closed (positive result)

Abdominal Injections:

Induration at Widest Point	Interpretation
< 5 mm	Negative
5 to 10 mm	Suspect
> 10 mm	Positive

2. **Testing Frequency:** All species should undergo routine screening semiannually (every 6 months) after clearing quarantine. Any animal born into a colony at NIH should begin testing between 6 months of age and 1 year of age.
3. **Testing Exemption:** Animals may be exempt from testing due to experimental or scientific needs. This 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.
4. **Adjunct Testing:** While the tuberculin skin test should be the primary screening method, there are some instances when an alternative may be necessary. This may include, for example, protocol specific requirements to avoid TST or animals given Complete Freund's Adjuvant. Potential adjunct testing strategies may include PCR of bronchoalveolar lavage (BAL) +/- gastric fluid and, in the past, have included gamma-interferon stimulation assays and antibody detection platforms. The requirement to perform an adjunct method to augment or replace a TST strategy for individual animals should be evaluated on a case-by-case basis by the Facility Veterinarian, Animal Program Director and/or Attending Veterinarian.

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.

5. **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 tracheo bronchial lymph nodes should be evaluated for signs of disease. Histopathology may not be necessary but a gross observation of tissue (especially lungs) for lesions can help ensure the absence of subclinical 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., formalin fixed) and PCR (e.g., fresh and/or frozen) evaluation, as well as fresh samples for Mycobacterial culture.
6. **False Negative/Positive Reactions:** Some animals display false negative or false positive results intermittently. 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.
 - a. **False Negatives:** 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 BAL fluid, 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 TST results as well as some adjunct methods such as gamma interferon testing.

- b. **False Positives:** Testing strategies will often result in suspect or false positive reactions. Animals should undergo repeat testing (described below) and the room should be quarantined until the issue is resolved. Animals receiving CFA may demonstrate false positive TST reactions and it is important for records to clearly indicate any animal that has received CFA. However, 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.

Managing Suspect NHPs:

Tuberculosis should be considered, and further testing performed, on animals with a suspect response on palpebral or abdominal tests. Animals are not considered to be confirmed as positive without confirmatory testing results, as described below:

1. **Retesting:** When retesting a suspect animal, the full 0.05 mL (New World) or 0.1 mL (Old World) volume of Old Mammalian Tuberculin should be used for both the alternate (contralateral) eyelid from the original test and/or abdominal skin injections (0.1 mL regardless of species).
2. **Adjunct Testing:** Additional testing may include chest radiographs, acid fast bacillus smear, culture and PCR of gastric and/or BAL fluid, PCR of feces or tissues, in-vitro gamma interferon assay, antibody detection, and other methods as they are validated.

Managing Confirmed TB Positive NHPs:

An animal is considered confirmed positive when a positive tuberculin skin test has been confirmed by another diagnostic method such as acid-fast smear, PCR testing, culture, or histopathology of biopsy or necropsy samples. The following steps should be implemented:

1. **Notification:** OACU, DS, and OMS must be informed of the positive test result.
2. **Euthanasia:** Animals with a positive TST and positive confirmatory test should be euthanized immediately to confirm the result and reduce the risk to other animals in the facility, as well as caretakers. The carcass can be taken to the Pathology Section, Diagnostic and Research Services, DVR, ORS for necropsy, or other facilities as discussed above.
3. **Cleaning:** The cage and room where the tuberculous NHP was held must be sanitized.
4. **Quarantine:** Any remaining NHPs in the affected holding room must be placed under quarantine. The DS, working with the facility staff, will review and approve the containment requirements for the animals. An effort should also be made to identify other animals that may have been housed with the identified animal since the most recent negative TB test and those animals and their rooms should also be placed under quarantine.
 - a. Access to the room is limited to a few essential personnel.
 - b. 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 disposed properly.
 - c. Other NHPs are not placed in or removed from the room (except for euthanasia).

- d. NHPs in the room with a confirmed positive animal are tuberculin tested every two weeks until each of the NHPs has received five tests with negative results since the last confirmed positive animal was removed from the room. 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 that held a confirmed positive animal, the 0.1 mL volume of Old Mammalian Tuberculin should be used for both eyelid and skin injections for all species.
- e. If the original animal is euthanized and the confirmatory testing from necropsy reveals that the diagnosis was not MTB then the quarantine may be lifted early.

Recordkeeping:

It is important that each NHP's tuberculin test is 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. **Requirements for importers of nonhuman primates.** 42 CFR 7153.
2. [Internet]. USDA APHIS. Available at: <https://www.aphis.usda.gov/>.
3. 1980. Laboratory Animal Management: Nonhuman Primates. Washington, DC: The National Academies Press.
4. **Abee CR.** 2012. Nonhuman primates in biomedical research, Second edition. ed. Amsterdam: Academic Press, an imprint of Elsevier.
5. **Capuano SV, 3rd, Croix DA, Pawar S, Zinovik A, Myers A, Lin PL, Bissel S, Fuhrman C, Klein E, Flynn JL.** 2003. Experimental Mycobacterium tuberculosis infection of cynomolgus macaques closely resembles the various manifestations of human M. tuberculosis infection. *Infect Immun* **71**:5831-5844.
6. **Centers for Disease C, Prevention.** 1993. Tuberculosis in imported nonhuman primates--United States, June 1990-May 1993. *MMWR Morb Mortal Wkly Rep* **42**:572-576.
7. **Chaparas SD, Good RC, Janicki BW.** 1975. Tuberculin-induced lymphocyte transformation and skin reactivity in monkeys vaccinated or not vaccinated with Bacille Calmette-Guerin, then challenged with virulent Mycobacterium tuberculosis. *Am Rev Respir Dis* **112**:43-47.
8. **Corcoran KD, Thoen CO.** 1991. Application of an enzyme immunoassay for detecting antibodies in sera of Macaca fascicularis naturally exposed to Mycobacterium tuberculosis. *J Med Primatol* **20**:404-408.
9. **Fox JG, Anderson LC, Otto GM, Pritchett-Corning KR, Whary MT, American College of Laboratory Animal Medicine.** 2015. Laboratory animal medicine, Third edition. ed. Amsterdam: Elsevier/Academic Press.
10. **Garcia MA, Yee J, Bouley DM, Moorhead R, Lerche NW.** 2004. Diagnosis of tuberculosis in macaques, using whole-blood in vitro interferon-gamma (PRIMAGAM) testing. *Comp Med* **54**:86-92.
11. **Gormus BJ, Blanchard JL, Alvarez XH, Didier PJ.** 2004. Evidence for a rhesus monkey model of asymptomatic tuberculosis. *J Med Primatol* **33**:134-145.
12. **Hines ME, 2nd, Kreeger JM, Herron AJ.** 1995. Mycobacterial infections of animals: pathology and pathogenesis. *Lab Anim Sci* **45**:334-351.
13. **Lerche NW, Yee JL, Capuano SV, Flynn JL.** 2008. New approaches to tuberculosis surveillance in nonhuman primates. *ILAR J* **49**:170-178.
14. **OD/OIR/OACU.** NIH Manual 3044-2: Protection of NIH Personnel Who Work with Nonhuman Primates.
15. **OD/OIR/OACU.** NIH Manual 3040-2: Animal Care and Use in the Intramural Program.

16. **Pereira AHB, Lopes CAA, Pissinatti TA, Pinto ACA, Oliveira DRA, Leal GM, Oliveira LCM, Redner P, Barbosa BEP, Moreira SB, Pissinatti A, Maruyama FH, Nakazato L, Dutra V, Ubiali DG.** 2022. Pulmonary Granuloma Is Not Always the Tuberculosis Hallmark: Pathology of Tuberculosis Stages in New World and Old World Monkeys Naturally Infected with the Mycobacterium tuberculosis Complex. *J Comp Pathol* **199**:55-74.
17. **Rock FM, Landi MS, Meunier LD, Morris TH, Rolf LL, Warnick CL, McCreedy BJ, Hughes HC.** 1995. Diagnosis of a case of Mycobacterium tuberculosis in a cynomolgus (*Macaca fascicularis*) monkey colony by polymerase chain reaction and enzyme-linked immunosorbent assay. *Lab Anim Sci* **45**:315-319.
18. **Staley EC, Southers JL, Thoen CO, Easley SP.** 1995. Evaluation of tuberculin testing and measles prophylaxis procedures used in rhesus macaque quarantine/conditioning protocols. *Lab Anim Sci* **45**:125-130.
19. **Tanner R, Hoogkamer E, Bitencourt J, White A, Boot C, Sombroek CC, Harris SA, O'Shea MK, Wright D, Wittenberg R, Sarfas C, Satti I, Verreck FAW, Sharpe SA, Fletcher HA, McShane H.** 2021. The in vitro direct mycobacterial growth inhibition assay (MGIA) for the early evaluation of TB vaccine candidates and assessment of protective immunity: a protocol for non-human primate cells. *F1000Res* **10**:257.
20. **Vervenne RA, Jones SL, van Soolingen D, van der Laan T, Andersen P, Heidt PJ, Thomas AW, Langermans JA.** 2004. TB diagnosis in non-human primates: comparison of two interferon-gamma assays and the skin test for identification of Mycobacterium tuberculosis infection. *Vet Immunol Immunopathol* **100**:61-71.
21. **Warit S, Billamas P, Makhao N, Jaitrong S, Juthayothin T, Yindeeyoungyeon W, Dokladda K, Smittipat N, Kemthong T, Meesawat S, Kongsombat N, Kraitat C, Prammananan T, Palaga T, Chaiprasert A, Malaivijitnond S.** 2020. Detection of tuberculosis in cynomolgus macaques (*Macaca fascicularis*) using a supplementary Monkey Interferon Gamma Releasing Assay (mIGRA). *Sci Rep* **10**:16759.
22. **Yee JL, Prongay K, Miles B, Smedley J, Hansen SG, Axthelm MK, Ardeshir A, Van Rompay KKA, Timmel G, Roberts JA.** 2019. Interferon-Gamma test for the detection of Mycobacterium tuberculosis complex infection in *Macaca mulatta* and other non-human primates. *J Med Primatol* **48**:260-263.
23. **Yee JL, Prongay K, Van Rompay KKA, Meesawat S, Kemthong T, Halley B, Carpenter A, Nham P, Rogers K, Hasselschwert D, Villinger F, Jay AN, Warit S, Malivijitnond S, Roberts JA.** 2021. Tuberculosis detection in nonhuman primates is enhanced by use of testing algorithms that include an interferon-gamma release assay. *Am J Vet Res* **83**:15-22.

Initial Approval – 05/08/1996

Re-Approved – 02/10/1999

Revised – 03/27/2002, 01/12/2005, 09/12/2007, 05/12/2010, 09/11/2013, 3/22/2017, 02/26/2020, 06/28/2023, 08/26/2026