

ORIGINAL ARTICLE

Guidelines for the prevention and control of tuberculosis in non-human primates: recommendations of the European Primate Veterinary Association Working Group on Tuberculosis

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Abstract

Background Effective tuberculosis (TB) control requires accurate diagnostic methods but the tuberculin skin test has serious limitations. Both false-negative and false-positive reactions are common, resulting in the spread of the infection and devastating TB outbreaks. Results of questionnaire surveys concerning TB testing practices in primate housing facilities showed great differences in testing practices. Although there was some uniformity regarding the sites of application, the amounts of tuberculin used and the time intervals for retesting, a great deal of variety was revealed considering the types of tuberculin preparations, the interpretation of tests and the susceptibility of animals.

Conclusion Here, we summarize the most common practices as regards TB control and prevention for non-human primates, and attempt to establish a uniform guideline based upon our experience with primate husbandry and care programmes as well as recent developments in the literature. The present guideline represents a consensus recommendation intending to harmonize the existing protocols.

Introduction

Simian tuberculosis (TB) is one of the most important bacterial diseases of non-human primates because of its ubiquitous and insidious nature and its ability to spread rapidly. It is caused by two aerobic facultative intracellular bacillus species, *Mycobacterium tuberculosis* and *M. bovis* [1]. Tuberculosis in non-human primates is a disease most often contracted from humans. Although it usually becomes manifest as a respiratory disease, tuberculosis can disseminate to almost any organ and it should be regarded as a systemic infection

[1, 2, 10]. Outbreaks of tuberculosis have been reported in non-human primate colonies almost as long as primates have been used as experimental animal models or kept in zoological gardens. Despite the significant reduction in the incidence of tuberculosis among captive non-human primates since 1970, tuberculosis remains a serious threat to the health of non-human primates and their caretakers. Outbreaks of tuberculosis still occur in established colonies and can have economic consequences because of the loss of animals, disruption of research and costs associated with disease control. Infected animals with active

tuberculosis may show overt signs of disease for weeks or months [3, 11, 21]. The tuberculous monkey is a health hazard especially to other monkeys in the group, but (re)transmission of the infection to man has been reported as well. For this reason, an effective preventive tuberculosis screening programme is essential to reduce the risk of infection with this classical anthroponosis.

Guidelines for the prevention and control of tuberculosis in non-human primates

Before acquiring new animals in a facility, efficient preventive measures and procedures have to be in place, so that the spread of TB from the new animals to the colony is prevented. Specific examination of animals including TB testing at the supplier facility and careful retracing of the source/breeder will help in preventing the introduction of mycobacterially infected individuals into the colony. Preventive measures also require the protection of non-human primates as well as the personnel in contact with these primates, possibly harbouring *Mycobacterium* spp. Efficient quarantine procedures, husbandry practices, and monitoring procedures, and protection of personnel and non-human primates are useful for preventing of TB outbreaks in a colony. A quarantine procedure at the time of the animal's arrival in a country or facility is mandatory for controlled animal health assessments. The basic components of quarantine include isolation of imported animals, veterinary health observations, physical examinations, diagnostic testing for important zoonotic diseases and protection of the personnel involved. In general, the quarantine programme must take into account the zoonotic potential of non-human primates and depends on the information available on individual animals undergoing the quarantine procedures. The life history and available documentation on the health status of an animal may influence the duration and extensiveness of the quarantine process. As a rule, the less the information available on an animal and for animals originating from an infected source, the longer and more stringent the quarantine procedures. For all aspects of this guideline the legal requirements which could be differing from country to country, should be followed.

Quarantine and testing

As it takes a minimum of 3 weeks for infected animals to develop a delayed hypersensitivity reaction to tuberculin testing, a series of consecutive testing is recommended during any quarantine period to

increase the possibility of detecting TB positive animals upon importation. The duration of quarantine has to be a minimum of 42 days (6 weeks) with three tuberculin skin tests (TSTs) incorporated into the routine at 2-week intervals and starting 1 week after arrival. Whenever feasible, it is recommended to extend the quarantine to a length of 3 months (13 weeks). In case of a positive TB test, the respective animal(s) will be isolated and/or killed, while for all other animals the quarantine procedure will be restarted at that point.

Performing pre-transport TB testing on animals from controlled breeding or supplier facilities with good documentation is desirable, as this testing can be performed in a controlled manner under veterinary care. This pre-transport testing will reduce the risk of importing animals with TB and may allow for reduced testing requirements during the post-import quarantine.

Because of the enormous economic interests and possible ethical impact, some dedicated primate centres routinely comply with a 3-month (13 weeks) quarantine period, with four skin tests at four weekly intervals, regardless of the origin of the animals. Moreover, two PRIMAGAM[®] tests are incorporated at the start and the end of the quarantine period for an optimal diagnostic coverage.

Animals from an approved source or the so-called good-quality animals are those which are captive bred and conditioned in their country of origin and exported for research. Animals that are wild-caught and kept in captivity for a variable amount of time before their export to another country bear a higher risk of disease. In cases of pre-export tuberculosis testing, three negative tests at 2-week intervals, with the last test not more than 10 days prior to shipment are required. The supplier should provide health certificates including dates of all previously performed TB tests and the parameters tested [purified protein derivatives (PPD), mammalian old tuberculin (MOT), etc.]. These documents should accompany all primate shipments (from suppliers to research institutions or zoos, between institutions, etc.). Animals should not be accepted from suppliers who have reportable diseases or significant losses in animal numbers. All animals have to be physically examined by a veterinarian after arrival. Very valuable animals diagnosed with TB may be treated; others are to be qualified as non-acceptable and should be killed or returned to the supplier.

Quarantine groups should be established in consideration of the species, disease status (if known), source and date of arrival in quarantine. Juvenile macaques are the most susceptible to TB. Animals should be

housed together by shipment and not mixed with different shipments or species. If groups are housed together, the start of the quarantine is defined as the day when the last animal enters the quarantine room.

At no time should different species be housed together during quarantine. To minimize the risk of contamination, movement of animals should be avoided and is acceptable only with prior approval of the attending veterinarian. Any movement of animals during quarantine should be strictly recorded to allow tracing in case of disease. Other factors that have to be considered when establishing quarantine groups include the age of animals and social history. The quarantine facility should contain a minimal number of animals per room and have good and efficient environmental conditions.

Loss of weight is an important indicator for tuberculosis. Therefore, weight of the animals should be carefully monitored periodically and animals that have lost more than 10% of their body weight from the start of the quarantine procedure or young animals which do not gain weight after 42 days should be re-examined and carefully evaluated.

Prior to release of animals from quarantine, all information pertinent to the group should be reviewed, including shipping documents, applicable permits, health certificates and records from the supplier (originator), tuberculin test records for each animal, individual health records containing the results of all surveillance and diagnostic procedures and all prophylactic measures performed during quarantine. The animals should be subjected to a final physical examination, preferably including a thoracic radiograph. Release from quarantine should be possible only with the permission of the attending veterinarian after all physical examination records have been checked.

Unexpected deaths in quarantine should be handled with caution. Animals should carefully be double-bagged, weighed and submitted for necropsy to a competent pathology section. The veterinarian and/or veterinary pathologist should be notified immediately. The remaining animals of the group should be kept totally isolated in the quarantine until the cause of death is identified.

Husbandry practices

The animal husbandry and sanitation practices applied to non-human primates are designed to prevent the spread of pathogens including tubercle bacilli. Optionally, tuberculocidal detergent disinfectants can be used in facilities housing non-human primates. Cleaning

equipment must be kept in one room unless it will be effectively disinfected between rooms. Sanitation schedules and practices must be in compliance with all applicable regulations, policies and guidelines.

Non-human primate holdings and procedure 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 lifter 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 non-human primates have been removed from the room and with proper protection of personnel.

Monitoring procedures

TST, the primary tool used to detect tuberculosis in nonhuman primates

Antemortem diagnosis of tuberculosis is based upon the intradermal TST using MOT or PPD. They are injected intradermally into an eyelid near its edge or into the abdominal skin, or both. The test relies on the development of a delayed hypersensitivity response against a mycobacterial antigen. In non-human primates this delayed-type hypersensitivity develops as part of the adaptive immune cascade within 3–4 weeks post-infection. The protein fraction of the tuberculin is recognized by sensitized T lymphocytes causing release of lymphokines, local oedema and local cellular infiltration. The amplitude of the hypersensitivity response and therefore the accuracy of the TST reading may correlate with the number of (replicating) tubercle bacilli and depend on various factors including the amount of circulating, primed, antigen-specific T cells and the amount of specific antigen in the tuberculin preparation that is used for the screening. MOT is a poorly defined preparation composed of various mycobacterial antigens that are known to be highly cross-reactive. In comparison, the PPD are not only a precipitated fraction of culture filtrates, but also comprise a mixture of mycobacterial antigens. MOT is less purified, but it holds more tuberculin units than PPD. In non-human primates, MOT has a greater reactivity than PPD and is therefore preferred to PPD as the reagent to use in a TST to identify infected animals.

The TST is limited in its efficacy as animals with early or advanced infection may give false-negative reactions because of a latency period upon infection or immunosuppression in case of progressive TB disease,

respectively. Concomitant diseases like measles or fungal infections may also result in a false-negative TST reaction because of immunosuppression [17]. Vaccination for polio, measles or yellow fever may have the same effect [15, 19]. Therapy with isoniazid or treatment with immunosuppressive drugs including corticosteroids will also negate the value of the tuberculin test. Furthermore, false-negative reactions may result from incorrect injection, subjectivity in the interpretation of the skin test, or a suboptimal concentration of antigen in the applied preparation.

False-positive reactions may result from previous (experimental) exposure to complete Freund's adjuvant (CFA), trauma because of improper installation of the tuberculin test substance, or non-specific reactions [5, 14, 16]. As regards non-specific reactions, contaminants like phenolic components may lead to (transient) allergic reactions, which can present within 30 minutes after injection. Furthermore, cross-reactivity after exposure to atypical or saprophytic mycobacteria like *M. goodii* may result in a false-positive TST [18].

Methods

Using a sterile 25–27 gauge needle for each non-human primate, inject 0.1 ml of MOT (2 500 IU), intradermally into one eyelid near the margin or into the abdominal skin or both; 0.05 ml can be used in small non-human primates, e.g. marmosets. Usually, the eyelid is preferred as it is relatively easy to observe. An injection of the solvent into the second eye lid or the abdominal skin can be considered as a control. If the abdomen is used, the hair should be shaved off without traumatizing the skin and the injection site should be marked with ink or edding to make the reading easy. The abdominal skin test is most commonly used when retesting non-human primates suspected of TB infection after a first TST. The advantage of using the abdomen is that any indurations can be measured and a saline control injection can be used. In marmosets and tamarins, this site is recommended as injection into the eyelid may be difficult.

Reading TST

Observe the animals for reactions at 24, 48 and 72 hours after injection under optimal lighting conditions. Animals which are suspected to be anergic should be observed after 2–8 hours and everyday as 'flash' reactions can quickly recede. The initial readings may be made by a trained technician. Any reactions or suspected reactions are to be observed and interpreted by the attending veterinarian. The following grading systems should be used:

Eyelid injections

When using the following grading system, the actual results should be recorded for every animal:

Reaction: scoring of changes

0 no reaction

1 bruise: extravasation of blood in the eyelid associated with the injection of tuberculin: negative

2 varying degrees of erythema of the palpebrum with minimal swelling: negative

3 moderate swelling with or without erythema: positive

4 obvious swelling of the palpebrum with drooping and varying degrees of erythema: positive

5 marked swelling with necrosis and closed eyelid: positive

Abdominal injections

Reaction: scoring of changes

0 no reaction

1 moderate swelling, height of induration 3–5 mm: negative

2 moderate swelling, height of induration 5–10 mm: questionable

3 obvious swelling, height of induration < 10 mm: positive

Cave: a case with two questionable test results should be regarded as positive!

Finally, again, the visual scoring of the skin test is largely subjective promoting confusion between negative and positive results. Therefore, alternative diagnostic approaches for antemortem TB testing are needed.

Frequency of TST

Before importation

Three negative tests at 2 week intervals are required. The last test should be performed 10 days before shipment. The supplier should provide data on tests conducted before importation (Fig. 1).

In quarantine

The minimal duration of quarantine is 42 days. During this time period, three tests are required. The first test should be performed 1 week after arrival to ensure the animals a short acclimation period (Fig. 1). In case there were any reports of TB infections in the origin/source of the monkey within the past year, a prolonged quarantine period of 60 days including two additional tests with bovine PPD is advisable. As

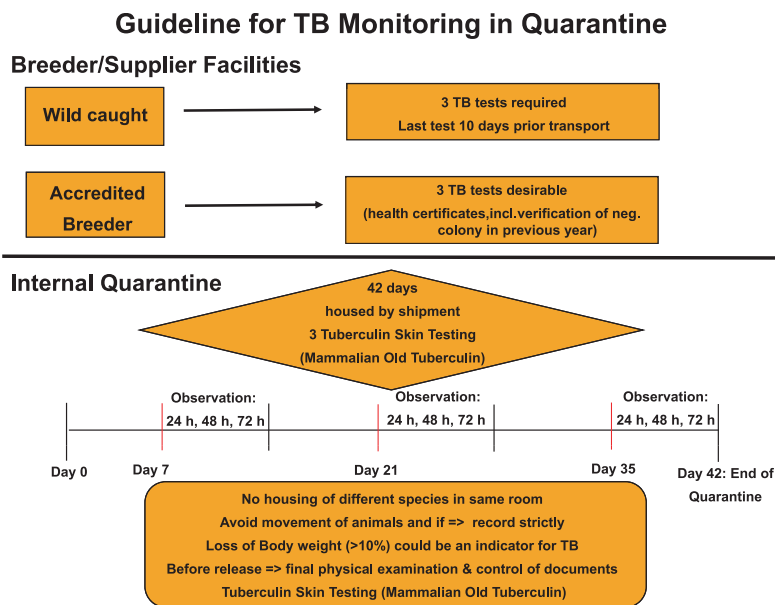


Fig. 1 Interpretation of Tuberculin Skin Test.

stated above, some dedicated primate centres (e.g. Bio-medical Primate Research Centre (BPRC), Rijswijk) follow a 3-month (13 weeks) quarantine procedure regardless of the origin/history of the animals.

Post-quarantine

The following interval for TST of species or groups of non-human primates is recommended during the post-quarantine housing period for institutes with frequent animal transfer and where humans work in close contact with animals: macaques and vervets quarterly; baboons, prosimians and New World monkeys semiannually; and great apes annually. A so-called closed institute holding monkeys with limited exposure to humans may have a routine of TB testing every 12 months, depending on the policy of the institute.

Because of a number of variables, the facility veterinarian may opt for TST at less frequent time intervals. When non-human primates are shipped from a facility with less frequent TB testing than that advised in these recommendations, the veterinarian, to whose institute the animals are shipped, must be notified about the deviation in the procedure before the animals are transferred.

It is important that the tuberculin test is accurately recorded for each animal. An animal’s clinical record should also include information about the building and/or room in which it was kept, its social housing

partners, and any movement (when it applies) during quarantine. A digital image and exact measurement of a suspect skin reaction is a useful tool to compare test results in repeated tests.

Tuberculous non-human primates can become anergic to mycobacterial antigens and appear negative in a TST. At present, it is unclear to what extent a condition of TB-associated immunosuppression or anergy affects other immunodiagnostics. Therefore, TB should be considered and further tests performed if an animal displays unexplained weight loss, non-healing wounds or any signs of distress or unexplained deviation from normal behaviour. Additional tests may include culture swabs of non-healing wounds, chest radiographs, and selective culturing of a gastric lavage.

Non-human primates display a positive TST upon experimental injection of CFA, because it contains cell wall components of tubercle bacilli. Ideally other, non-mycobacterium-based adjuvants should be selected such that the diagnostic power of the TST is not compromised. If the use of CFA is unavoidable, the individual animal has to be tuberculin-tested the week before the CFA is injected or it has to be isolated from the colony. Because CFA application will induce a positive TST, other indirect measures like weight monitoring, chest X-ray or bacterial culturing of biofluid might indicate TB infection. If TB is diagnosed in any other animal in the holding room housing a CFA-exposed non-human primate, the non-human

primate(s) that previously received CFA should be regarded as potentially infectious and monitored carefully. Because of the economic risk and possible ethical implications of a case of TB remaining unidentified, some dedicated primate facilities insist on a strict regime and do not allow animals to be in contact with their regular colony facilities at all after the injection of CFA.

Chest radiographs

A posterior–anterior chest radiograph can be used to detect chest abnormalities. Lesions may appear anywhere in the lungs and may differ in size, shape, density and caviations. These abnormalities may suggest TB, but cannot be used for definitive differential diagnosis. Therefore, chest radiographs may be used as an additional test procedure only for screening TB. Moreover, considering the limited sensitivity, this additional test, however, is very valuable for the identification of animals that have a negative TST reaction because of immunosuppression associated with fulminant disease. Chest radiographs can be difficult to interpret and ideally are presented to an experienced radiologist or lung physician for interpretation. Because of the rareness of calcified tubercles, TB may present with a weak radiological contrast in non-human primates compared with other animals. There are no pathognomonic lesions, but enlargement of the bronchial lymph nodes may be an early sign of pulmonary mycobacteriosis. Larger tubercles or caviations may be appreciated radiographically. Abdominal radiography may aid in the identification or confirmation of splenomegaly or mesenteric lymphadenopathy. Computed tomography scanning gave detailed real-time imaging of disease progression [12].

Diagnostic microbiology

The presence of acid-fast bacilli (AFB) on a sputum smear or other specimen may indicate TB. Acid-fast microscopy is easy and quick, but it has a limitation in that some AFBs are not *M. tuberculosis*. Therefore, a bacterial culture is required on all initial samples to confirm the diagnosis. A positive culture of *M. tuberculosis* confirms a positive acid-fast staining and thereby the diagnosis of TB infection. Culture examinations should be completed on all specimens regardless of AFB smear results.

- Culture of mycobacteria from clinical materials using Löwenstein–Jensen agar or other suitable cultivation media;

- gastric lavage with acid-fast cytology and culture of gastric mucus;
- tracheal or bronchoalveolar wash with acid-fast cytology and culture of tracheal mucus
- faecal examination with acid-fast staining and culture
- biopsy of altered organs with acid fast stain and culture
- laparoscopy.

PCR

Polymerase chain reaction (PCR) can be used to detect mycobacterial DNA in any biological samples and intrinsically has the advantage of being much quicker than the conventional culture methods of diagnosis. Detection of infection by screening faeces or sputum by PCR for mycobacterial DNA may therefore be considered as a rapid and alternative diagnostic tool for tuberculosis.

PRIMAGAM-IFN- γ test

In vitro immune stimulation assays have been reported to distinguish between TB-infected and TB-non-infected macaques. Recently, a whole-blood stimulation assay has become commercially available as the so-called PRIMAGAM[®] (Product number 63301, Prionics, Va Vista, Nebraska, USA) test. This test detects cellular immune reactivity to PPD antigen through the measurement of interferon-gamma (IFN- γ) production in whole-blood samples [4]. IFN- γ is an important cytokine involved in the cell-mediated immune response to mycobacteria. The assays may discriminate between *M. tuberculosis* complex and *M. bovis* infection by including avian and bovine PPD separately in the test. It is a quantifiable diagnostic test with good sensitivity and specificity compared with disease status determined by pathological examination [6, 7, 20]. Recent results suggest that the IFN- γ response to tuberculin antigen may not be reliable in cynomolgus macaques and that another cut-off should be used to read the test [6]. Moreover, a more formal validation of this test kit would wait extensive testing in larger cohorts of naturally TB-exposed cohorts of monkeys. Thus, in general, the parallel use of TST and PRIMAGAM[®] test is advisable for an optimal coverage in a diagnostic regimen.

The PRIMAGAM-IFN- γ test is an additional test which can be used for gorillas, chimpanzees, orangutans, gibbons, colobids, baboons, mandrills, macaques, vervets, guenons, squirrel monkeys, langurs and marmosets. As both tests can suffer from intermittent

positive and negative reactions on repeated testing, the parallel use of skin testing while performing a PRIMAGAM[®] assay is recommended for maximal overall sensitivity in TB screening programmes, particularly also for cynomolgus monkeys.

PrimaTB STAT-PAK assay

Recent studies suggest that the serological detection of antibody against Mycobacterium-specific early secretory antigenic target-6 (ESAT-6) by enzyme-linked immunosorbent assay may be a useful tool for the development of an immunodiagnostic tool for TB [9]. There is a strong association between TB in non-human primates and an immune response against ESAT-6, a protein secreted by the metabolically active, virulent tubercle bacilli.

In a comprehensive approach, ESAT-6 is one of several antigens used in the PrimaTB STAT-PAK[®] assay – as a new lateral flow test that has been developed as a TB-specific serodiagnosticum. This test was evaluated in comparison with the intradermal palpebral tuberculin test on non-human primates of three different species. Some of the animals were experimentally infected with *M. tuberculosis*. Serological evaluation demonstrated high diagnostic sensitivity (90%) and specificity (99%) [13].

This commercial test is very easy to use, and can be used to analyse serum, plasma or possibly any other antibody-containing body fluid. It may therefore be an attractive option for institutes with more limited capacity or facilities. This serodiagnostic test employs a selective array of recombinant *M. tuberculosis* proteins covering several immunodominant mycobacterial antigens for immune detection. A combination of PrimaTB STAT-PAK[®] assay and the skin test seems to be a sensitive and reliable diagnostic approach for detection of TB in non-human primates.

Shipment of material to the laboratory

If specimens have to be shipped they should be packed in compliance with regulations covering the transportation of aetiological agents. See Guideline: Diagnostic Specimens, Category B, assigned to UN identification number 3373. A triple packaging system must be used (see Appendix 7.1.).

General remark: Intradermal testing alone is not sufficient to adequately diagnose tuberculosis. Limitations of the TST, particularly its inability to identify latent TB infections reliably makes it unsuitable for use as a single, stand-alone test for TB surveillance. The use of

a combination of intradermal testing and PRIMAGAM-IFN- γ test or PrimaTB STAT-PAK assay is advisable. In doubtful cases, a range of additional test methods like chest radiographs and microbiology should be applied and the results evaluated critically. The use of several tests in combination will help to increase the overall sensitivity and specificity of screening and surveillance programmes for non-human primates.

Handling of tuberculous non-human primates

In case of a suspicious TB test

The animal has to be taken into quarantine. Repeated testing according to the quarantine procedures is necessary. The use of alternative sites (other eyelid or abdomen) and possibly more potent tuberculin preparation (see above) are advisable to compare the results of the skin test. Other diagnostic tools like chest X-rays, bacterial culturing and/or immune assays like the PRIMAGAM[®] test or PrimaTB STAT-PAK[®] may be considered. All animals in the group of the TB suspected animal should be regarded as possibly infected and therefore be tested up to five times. The quarantine period should be extended to 90 days (Fig. 2).

In case of positive tuberculin test

Immediate killing

When a clinical diagnosis of TB is made in a non-human primate, it has to be killed immediately and the carcass has to be taken to the pathology section for necropsy. The cage and room, where the tuberculous non-human primate(s) was/were held, have to be sanitized and the remaining non-human primate(s) should be placed under a whole period of quarantine or be killed. In case an animal tests positive for TB during quarantine, the quarantine period should be extended to 90 days. During this time five TST should be performed (Fig. 2).

Quarantine sanctions

Notification to all workers, to government authorities and a clear indication of the infected room are essential.

- Access to the room is limited to assigned personnel only,
- Protective clothing (Tyvek[®] overall, DuPont, France, N95 respirator masks, shoe covers, head bonnet, vinyl or rubber gloves and eye protection)

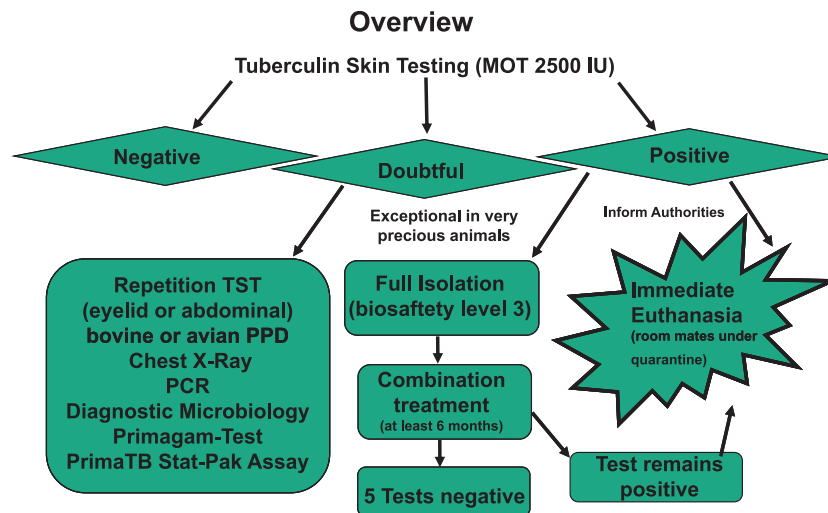


Fig. 2 Frequency of Tuberculin Skin Test.

is worn in the room and is not removed except for autoclaving,

- no other non-human primates are placed in the room; no animals are removed from the room,
- the non-human primates in the room are tuberculin-tested every 2 weeks until five tests have been performed with no reactions. The first of these tests is administered 2 weeks after the test that identified the tuberculous non-human primate.

All previously negative contact personnel should be tuberculin-tested again and personnel with access to the primates should be kept to a minimum.

If all animals are negative the quarantine may be terminated after the 90-day period except that non-human primates are not placed in or removed from the room until a tuberculin test is administered 4 weeks after the last of the five tests with no reactions. A diligent effort should be made to locate all non-human primates that were housed in the room in which the tuberculous non-human primate was housed over the last 60 days. These non-human primates should be tuberculin tested on the same schedule as the non-human primates currently housed in the quarantined room.

Delayed killing

Killing of a tuberculous non-human primate can be delayed if the animal is of great value for a research project and can be isolated to minimize the spread of tubercle bacilli to other non-human primates or humans. The room in which such a non-human primate is held at the time of clinical diagnosis has to be placed under quarantine. Biosafety level 3 prac-

tices and facilities are recommended for animal studies using non-human primates that are naturally or experimentally infected with *M. tuberculosis* and *M. bovis*. Therefore, animals that are tuberculin-positive should be killed if this level of containment is not available. Multidrug treatment may be considered, but only if appropriate isolation and containment facilities can be provided, if such containment and isolation is ethically justified, and if accurate detection of infection is possible. Every single animal that could have been exposed (via direct contact, air supply, etc.) has to be handled according to quarantine conditions. All previously negative contact personnel should be tuberculin-tested again and the personnel with access to the primates should be kept to a minimum.

Treatment of tuberculous non-human primates

Valuable non-human primates of those species that are not highly susceptible to tuberculosis or belonging to endangered species may be treated to free them of tubercle bacilli, e.g. chimpanzees. A multiple drug regimen based upon the most current information must be used for treatment. Effective drug combinations include isoniazid and streptomycin, isoniazid and *p*-aminosalicylic acid, or isoniazid, ethambutol and rifampin [8, 22, 23]. In all cases of successful treatment, the organism had to be isolated and antibiotic sensitivity determined beforehand. Therapy must be maintained for at least 6 months. The room, in which such a non-human primate was kept when the clinical diagnosis was made, has to be placed under quarantine. An animal under therapy has to be kept under quarantine

conditions for the whole duration of therapy. In general, treatment is only advisable for very valuable animals considering costs and ethical issues involved with containment.

Protection of non-human primates and personnel

Protection of personnel

Biosafety precautions must be taken when dealing with a suspected or diagnosed tuberculous non-human primate (a non-human primate suspicious of tuberculosis) and when collecting and handling samples to be cultured for tubercle bacilli. Access to quarantine is limited to authorized personnel, who should have participated in seminars about the risks of handling non-human primates. They have to wear safety clothing. Serum samples of workers have to be stored as reference serum.

Protection of non-human primates

All humans working with primates should have negative skin tests or be non-infectious. The skin test should be repeated every 6 months. People with positive reaction (>10 mm) (some due to BCG vaccination) have to undergo chest X-ray or any alternative TB diagnostic test like the QuantiFERON®-TB Gold test. These complementary diagnostic tests may be used in an alternating fashion, each of them being repeated every 12 months. Tuberculin test (Teinte test or Goldmann Mantoux)-positive personnel should be monitored medically, whenever they have respiratory symptoms that persist longer than those usually seen with transient viral upper respiratory infections. Human tuberculin converters should be referred to a physician for follow-up with radiographs, cultures, or other diagnostic procedures and treatment. TB-positive people should not be allowed to work with primates. People whose jobs require contact with animals should be assigned other duties until all diagnostic tests are completed and they are considered to be free of infection and not shedding tubercle bacilli.

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Appendix

Guideline: Packing Diagnostic Specimens for Transport

(Diagnostic Specimens, Category B, assigned to UN identification number 3373)

Triple packaging system

Primary Packing

- Primary receptacle(s) must be water tight, e.g. screw cap seal with parafilm or adhesive tape or similar.
- Multiple primary receptacles must be wrapped individually to prevent breakage.
- When determining the volume of diagnostic specimens being shipped, include the viral transport media.
- Primary receptacle(s) must not contain more than 500 ml or 500 g.
The entire content of the primary receptacle is the diagnostic specimen.

Secondary Packing

- Use enough absorbent material in the secondary container to absorb the entire contents of all primary receptacles in case of leakage or damage.
- Secondary packaging must meet the IATA packaging requirements for diagnostic specimens including 1.2 meter (3.9 feet) drop test procedure. Since infectious substance packaging surpasses the requirements for diagnostic specimen packaging stated in the IATA Packing Instruction 602, it can be used.
- Infectious substance packaging must have the required specification markings on packaging.
Secondary packaging must be watertight. Follow the packaging manufacturer or other authorized party's packing instructions included with the secondary packaging.
Secondary packaging must be at least 100 mm (4 inches) in the smallest overall external dimension, must be large enough for shipping documents, e.g. air waybill.

Outer Packing

- The outer packaging must not contain more than 4 l or 4 kg.
- Both dry ice and wet ice must be placed outside the secondary packaging.
- Dry ice: packaging must permit the release of carbon dioxide gas and not allow a build-up of pressure that could rupture the packaging.
- Wet ice: the packaging must be leak-proof.
- Each package and the air waybill must be marked with the following exact wording:
 - UN 3373 Diagnostic Specimen

- Packed in compliance with IATA Packing instruction 650
- An itemized list of contents must be enclosed between the secondary packaging and the outer packaging.
- Place in a sealed plastic bag to protect from moisture.

- A shippers declaration for dangerous goods is NOT required.

This document was compiled using the expertise of the members of the working group and their personal literature resources. For further reading, we refer to the following articles and documents.