4.3 TUBERCULOSIS and its CONTROL

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Tuberculosis (TB) is a major disease of concern in primates. Proper management requires constant testing and vigilance, as its presence can lead to severe clinical disease. Keys to control are accurate diagnosis, sanitation, health programmes for personnel, and quarantine.

PART 1. THE BIOLOGY OF TUBERCULOSIS

Background
Tuberculosis is a major cause of morbidity and mortality in adults worldwide (WHO, 2008). Currently, one-third of the world’s population is infected with Mycobacterium tuberculosis (MTB). Annually, 8–9 million new cases of TB disease occur and more than 2 million people die worldwide (WHO, 2008) and 1.39 million people are infected with both with HIV and TB diseases (Corbett et al., 2003; WHO, 2008). The TB control programs face new challenge with emergency of multi-drug resistant TB (MDR-TB) in many countries and a recent epidemic of extensively drug resistant TB (XDR-TB) (WHO, 2008). Hence TB control strategies are constantly requiring improvement, including paying attention to potential reservoirs that might play a big role in emergency of resistant strains.

This current situation of TB worldwide, presents high risks of TB transmission to primates especially in captive facilities and quarantine centres where there are close interactions with human caretakers.

Transmission and Aetiology

Tuberculosis in humans may result from exposure to any one of the tubercle bacilli included within the Mycobacterium tuberculosis complex (i.e., M. tuberculosis, M. bovis, M. africanum, M. pinnipedii, and M. microti). In non human primates it is a disease that is mainly contracted from humans by inhalation of infectious aerosols expelled from the respiratory tract of infected animals or humans with active disease. Transmission through ingestion is rare but has been reported (Sapolsky and Else, 1987). The occurrence of TB in non human primates is often caused by the same organism that causes TB in humans- M. tuberculosis - and the disease can spread rapidly from non-human primates to humans and vice versa. The second common type of TB, in macaques at least, accounting for about 15% of cases, is due to M. bovis, also a zoonosis, (Bernacky et al., 2002, CDC, 1993; Gibson, 1998 and Garcia et al., 2004). Mycobacterium bovis, unlike M. tuberculosis, has a wide host range, often isolated from tuberculous cattle, and has several wildlife maintenance hosts, including the Eurasian badger (Meles meles), brush-tailed possum (Trichosurus vulpecula), and white-tailed deer (Odocoileus virginianus). Wildlife reservoirs have made M. bovis eradication from national herds in several developed countries, including the United Kingdom, New Zealand, and the United States, particularly difficult. Eradication campaigns in these countries have generally relied on test and removal, slaughterhouse surveillance, movement restriction, and/or wildlife reservoir control strategies.
Although most species of primates are susceptible to tuberculosis, susceptibility varies. Old World monkeys are considered more susceptible than apes, which appear to be more susceptible than new World monkeys (Montali et al., 2001). Experimental studies of TB infection in three species of Old World monkeys revealed different levels of sensitivities to infection. African green monkeys (*Chlorocebus aethiops*) were highly sensitive to infection showing uniformly rapidly progressive disease; Rhesus macaques (*Macaca mulatta*) showed a more variable clinical course, while Cynomolgus macaques (*M.fascicularis*) experienced a more chronic course of infection (Mortzel et al., 2003).

Aetiology Summary

- Primate infection usually results from an association with humans
- *Mycobacterium tuberculosis* (most common), *M. bovis*, *M. avium-intracellulare* (least common)
- All primate species are susceptible

Clinical Signs

Clinical signs are usually chronic and vague unless entering the terminal stages of infection
- Irritability/ change in behaviour, also described in infected humans
- Wasting
- Enlarged lymph nodes or swelling of the abdomen
- Lethargy/exercise intolerance
- Anorexia
- Low grade fluctuating fever
- Sudden death
- Occasionally enlarged lymph nodes, draining tracts, disease of the spine (Pott’s disease)
- Respiratory signs can be minimal. Even in vervet monkeys with gross lung lesions, coughing was rarely observed.
- When lungs are extensively involved, see dyspnea, intermittent, hacking cough. Abdominal breathing is more often observed than coughing.
- Neurological symptoms have been observed in a chacma baboon
- May be asymptomatic and shed organism
- Becomes generalized and progressive disease

Pathology

After primary infection, a range of clinical outcomes is possible from no overt disease, to rapidly progressive disease or more commonly, a chronic debilitating disease course. The general pattern of clinical outcomes following TB infection has been well described in humans into 4 stages of disease progression and resolution (Wallgren, 1948).

In the first stage, approximately 3 to 8 weeks after *M.tuberculosis* contained in inhaled aerosols becomes implanted in alveoli, the bacteria are disseminated by the lymphatic circulation to regional lymph nodes in the lung, forming the so-called primary or Ghon complex. At this time, conversion to tuberculin reactivity occurs. The second stage, lasting about 3 months, is marked by haematogenous circulation of bacteria to many organs including other parts of the
lung. At this time in some individuals, acute and sometimes fatal disease can occur in the form of tuberculosis meningitis or miliary (disseminated) tuberculosis. Pleurisy or inflammation of the pleural surfaces can occur during the third stage, lasting 3 to 7 months and causing severe chest pain, but this stage can be delayed for up to 2 years. It is thought that this condition is caused by either haematogenous dissemination or the release of bacteria into the pleural space from subpleural concentrations of bacteria in the lung. The free bacteria or their components are thought to interact with sensitized CD4 T lymphocytes that are attracted and then proliferate and release inflammatory cytokines (Kamholz, 1996). The last stage or resolution of the primary complex, where the disease does not progress, may take up to 3 years. In this stage, more slowly developing extrapulmonary lesions, e.g., those in bones and joints, frequently presenting as chronic back pain, can appear in some individuals. However, most humans who are infected with TB do not exhibit progression of the disease.

Infected animals with active TB may show no overt signs of the disease for weeks or months, during which time they can transmit infection to other colony animals. However, as in humans, not all primary infections in other primates result in active TB disease and development of latent infections without overt disease is well documented (Capuano et al., 2003; Gormus et al., 2004). These animals with latent infections present a significant risk of reactivation and the development of active TB (Capuano et al., 2003; Flynn and Chan, 2001).

Although animals with latent TB are not infectious, reactivation of latent infections that were not detected using traditional screening methods during primary quarantine is emerging as an important factor in the epidemiology of TB in nonhuman primates. This is a major reason why this disease is so difficult to control.

Focal granulomatous or miliary lesions in any organ are suggestive of Tuberculosis, but lesions are most regularly found in the lung (Figure 1), liver (Figure 2), lymph Nodes (Figure 3) and spleen (Figure 4). In vervet monkeys the mesenterial lymph nodes were mostly affected. Secondary infection by digestion of the bacilli after coughing up the bacilli is common. In one case lesions in the kidney were found.

POST MORTEM EXAMINATION OF TB SUSPICIOUS ANIMALS

TB can affect all organs, but lesions are often hard to found. In early stages of the disease no macroscopic lesions are found at all. In case of TB suspicion it is essential to thoroughly palpate the lungs, liver and spleen for solid structures and to apply as many further diagnostics as possible to reduce a ‘false negative’ PM result. Necrotic abcesses can also be caused by other pathogens. Therefore always take fresh or frozen samples from the edge of lesions to confirm and identify TB infection by culture. Sensitivity testing is recommended to detect (highly) resistant strains, because these strains are very dangerous for humans. Samples in 10% buffered formalin can be used to do histopathology and PCR. When no macroscopic lesions are found, but antemortem tests have produced TB possible results, always take fresh or frozen samples from the mesenteric and mediastinal lymph nodes for culture. Prior to euthanasia take blood to store frozen serum for later analysis (Prima TB STAT-PAK or ELISA).
Figure 1. Granulomatous lesions in the lung in a Tuberculosis case in a Vervet Monkey (M. van Zijll Langhout)

Figure 2. Milary Liver lesions in a TB positive Chimpanzee (L. Mugisha)
Figure 3. Enlarged Lymph Node in a TB positive Vervet Monkey (M. van Zijll Langhout)

Figure 4: Granulomatosus lesions in a vervet monkeys, infected with M.tuberculosis (Photo: M.van Zijll Langhout)
PART 2. THE DIAGNOSIS OF TUBERCULOSIS

Table 1 below provides an overview of TB diagnostic techniques. In-depth notes on the various diagnostics techniques follow this table.

Table 1. Overview of TB testing

<table>
<thead>
<tr>
<th>Testing Based on Detection of Mycobacterial Organisms</th>
<th>Culture and speciation</th>
<th>Infection only found in 50% of human cases confirmed by other means</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8-12 weeks. Improved culture methods such as BACTEC, Septi-Chek, MB/ BacT systems and mycobacterial growth indicator tubes have the potential to reduce this time</td>
<td></td>
</tr>
<tr>
<td></td>
<td>ZN staining</td>
<td>Other acid fast organisms such as Nocardia can produce false positives (reduced specificity)</td>
</tr>
<tr>
<td></td>
<td>1 day</td>
<td>Labeled monoclonal antibodies may confirm acid-fast organisms in tissues as being mycobacteria</td>
</tr>
<tr>
<td></td>
<td>Immunohistochemical staining</td>
<td>Can also do on fixed samples. More information required - may be used to distinguish pathogenic Vs atypical infections</td>
</tr>
<tr>
<td></td>
<td>1 day</td>
<td>In one study in deer, histopath had a PPV of 94%, acid fast 99% and PCR 100%</td>
</tr>
<tr>
<td></td>
<td>PCR and MTb direct test (MTD) and RFLP (Restriction Fragment length polymorphism)</td>
<td>Requires more evaluation</td>
</tr>
<tr>
<td></td>
<td>Combination</td>
<td>Depends</td>
</tr>
<tr>
<td></td>
<td>Secreted antigens (Ag85)</td>
<td>Depends</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Testing Based on Immunologic response to Mycobacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>PPD intradermal testing</td>
</tr>
<tr>
<td>IFN-gamma (Primigam/ Bovigam/ cervigam/ quantoferon)</td>
</tr>
<tr>
<td>ELISA</td>
</tr>
</tbody>
</table>
Multiantigen print immunoassay (MAPIA) - Chembio

Specific antigens are printed as horizontal stripes on a nitrocellulose membrane. Strips can be cut out from this print out and incubated with test serum samples as a Western blot with an anti-immunglobulin conjugate anc colour developer. using this assay, an antibody response to mycobactetrial antigens is observed. Useful in a species where TB has a low prevalence, as appears more sensitive and can detect antibody as early as 4 weeks after infection.

Immunochromatographic multiple antibody screening test (Chembio Stat-Pak) - Lateral flow technology rapid test

Using selected mycobacterial antigens, RT was developed for rapid antibody detection that can use serum, plasma or whole blood. Usually used as a screening test for MAPIA. Sensitivity of 88.9%, specificity 98.5% (Chembio funded research)

Key to acronyms

PPV - positive predictive value = the proportion of all those who test positive who really are infected
PCR - Polymerase Chain Reaction
PPD - Purified Protein Derivative

No single test is 100% effective at diagnosing TB. Test specificity and sensitivity depends on stage of disease, type of mycobacterial infection and a host of other confounding factors. Readers are recommended to stay as up to date as possible, as new diagnostic techniques are developed regularly.

The main challenges in TB diagnostics has been lack of proper tools to detect latent and active TB, pulmonary and extra-pulmonary TB, anergy (overwhelming infection), TB in immunocompromised hosts, immunologic cross-reactivity among mycobacterial species and for humans BCG-induced immune-reactivity.

A broad range of diagnostic tools have been developed over time especially in humans and some of the new approaches have been recently reviewed and are now available for the tuberculosis surveillance in Nonhuman primates (Lerche et al., 2008). The diagnostic tools can be subdivided into

- Immune-response dependents such as Intradermal skin test, Gamma interferon tests or other cytokines tests, antibody detection tests and
- Tests that detect mycobacterium organism including culture of organisms, acid-fast staining of tissues, PCR or nucleic acid detection and immune-detection of mycobacterial specific antigens.

The tests most widely used for the detection of TB in humans and cattle include the measurement of delayed-type hypersensitivity (i.e., skin testing) to purified protein derivatives (PPDs) and/or in vitro assays for gamma interferon produced in response to mycobacterial antigen stimulation (i.e., Bovigam [Prionics AG, Schlieren, Switzerland] and Quantiferon Gold [Cellestis Inc., Carnegie, Victoria, Australia]). These tests rely on early cell-mediated responses, a hallmark of TB immunopathogenesis. Likewise, the intradermal tuberculin skin test (TST)
using tuberculin purified protein derivative (PPD) and mammalian old tuberculin (MOT) has been the main diagnostic tool of NHP tuberculosis surveillance and antemortem diagnosis for more than 60 years (Lerche, et al., 2008 review).

At this stage, for TB screening in primates in sanctuaries and under field conditions, we recommend a combination of the TST and Serological rapid test (StatPak), followed by increasingly specific tests if TB is suspected on the initial screening (Table 2). Prima TB should always be used in combination with the intra-dermal tuberculin tests. Both TST and Prima TB detect infection in different stages and therefore they are supplementing each other. Repeating the Prima TB in 10-14 days after the intra-dermal tuberculin test can in some cases show up positive individuals (Chembio). The higher level of antibodies as a reaction to tuberculin, increases the chance that the Prima TB detects them.

<table>
<thead>
<tr>
<th>ID Skin Test¹</th>
<th>Prima TB Statpak²</th>
<th>Thoracic Radiograph</th>
<th>PCR³</th>
<th>Culture³</th>
<th>AFB⁴</th>
<th>Recommendations</th>
</tr>
</thead>
</table>
| +             | +                 | -                   | -    | -        | -    | Strong suspect positive.  
Quarantine +/-  
Euthanase (once validated) |
| +             | -                 | -                   | -    | -        | -    | Quarantine. Retest in 2 months (full test range)  
Quarantine. Retest in 2 months – OR see MAPIA Protocol if comes back online |
| -             | +                 | -                   | -    | -        | -    | Test for other causes  
(infections, neoplasia)  
but quarantine in case anergic and retest if other cause not found  
PCR is very sensitive –  
CONFIRM WITH LAB – must be able to differentiate between M. tb complex and other mycobacteria.  
Quarantine for 6-12 months and retest.  
Positive – Euthanase |
| -             | -                 | +                   | -    | -        | -    | Quarantine – Retest in 2 months  
Positive – Euthanase |
| -             | -                 | -                   | +    | -        | -    | If one or more of these are positive  
+/- Positive – Euthanase |
| +             | -                 | -                   | +    | -        | -    | If one or more of these are positive  
+/- Positive – Euthanase |
| -             | +                 | -                   | -    | -        | -    | Positive – Euthanase |

Table 2. TB Diagnostic suggestions and recommendations under field conditions for Non human Primates.

¹ Mammalian Old Tuberculin (MOT) +/- Bovine PPD and Avium PPD comparative  
² To be trialled in sanctuaries through 2010, to validate its use in this species as an improved screening test for TB (see above protocol/ recommendations)  
³ Conducted on Tracheal bronchial washes +/- Gastric lavage (10-30ML dependant on animal size)  
⁴ Dr Wendi Bailey (Liverpool School of Tropical Medicine) is investigating a new, more specific AFB test utilising fluorescence. More information pending.
1. The Intradermal Tuberculin Skin test (TST) and its interpretation.

The common method of monitoring TB in primates as approved by USDA is delayed hypersensitivity in response to an intradermal injection of mammalian old tuberculin (MOT) and comparative bovine and avian purified proteins into the skin of the eyelid (Kaufmann and Anderson, 1978).

The underlying principal of the TST is the detection of delayed-type hypersensitivity to tuberculin antigens. This has been the primary tool for detection of tuberculosis in primates since the 1940s and is currently the only ILAR/CDC-approved method for tuberculosis testing of animals in primary import quarantine (Kennard et al., 1939; NRC 1980; Roberts and Andrews, 2008). In NHPs, the delayed-type hypersensitivity to tuberculin antigens develops as part of the adaptive immune cascade within 3-4 weeks following 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 TST readings may correlate with the number of (replicating) tubercle bacilli and is influenced by 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 screening. Mammalian Old Tuberculin (MOT) is the most sensitive but least specific with 135000 Tuberculin Units (TU)/0.1mL, Avium Purified Protein Derivative (PPD) is 25000 TU/0.1mL and bovine PPD has 1500TU/0.1mL. 1500 is at the very bottom of the range to illicit a response in non-human primates. Human PPD, at just 5TU/0.1mL is the most specific but least sensitive and should NEVER be used in non-human primates. Of the three tuberculins, Bovine is the least sensitive in picking up Mycobacteria tuberculosis, but in combination with avium, would at least be able to eliminate cross reactors. We recommend Using MOT, as well as Bovine and Avian Tuberculin

Composition

Tuberculin purified protein derivative (PPD) used for TST is prepared from culture filtrate of MTB and contains numerous antigens, most of which are homologous to vaccine strains of bacillus Calmette–Guérin (BCG), and environmental non-tuberculous mycobacteria (NTM). On the other hand, MOT is a poorly defined preparation composed of various mycobactetarial antigens that are known to be highly cross-reactive. MOT is less purified but contains more tuberculin units than PPD.

Method of TST delivery

- Carefully clean the eyelid of any dirt or debris with an alcohol swab, to prevent inadvertently injecting dirt and creating a local reaction separate to the tuberculin reaction looked for. Inject 0.1mL MOT intradermally into upper eyelid near the margin using a 27g needle, or a demarcated site on the abdomen (Richter et al., 1994). The eyelid is a preferred site as it is relatively easy to observe in NHPs. The practice has been to use abdominal skin test when re-testing NHPs suspected of TB infection after the first TST. The abdominal skin test has an advantage in that the indurations can be easily measured, especially in very small species where the eyelid is difficult to use. However, there is accumulating evidence that the abdominal TST in NHPs may be significantly less sensitive and may therefore be of limited utility in TB
surveillance programs (Capuano et al., 2003; Motzel et al., 2003). In great apes, abdominal TST may not be of great value as it may not be easy to read as measurement often requires a further anaesthesia, an unnecessary risk.

- The batch number & expiry date of the tuberculin and which eyelid was injected should always be recorded.
- When using Avium and Bovine intradermally, by convention the Avium is injected intradermally into the right upper eyelid (0.1mL), and the Bovine into the left.
- If you are using all three, find an alternative site for the MOT that can easily be read (e.g flank or abdomen)
- Diagnosis is based on results of delayed hypersensitivity to intradermal tuberculin testing. The animals should be observed under good lighting for hypersensitivity reactions at 24, 48 and 72 hours post injection. The delayed hypersensitivity reaction will be most prominent at 72 hours, but the animal needs to be checked at 24 hour intervals, so local reactions are not misinterpreted as a positive delayed hypersensitivity. Any reactions or suspected reactions are to be observed and interpreted by the attending veterinarian. This is done according to established standardized scoring system for intrapalpebral reactions developed jointly by the California and Oregon Primate Research Centres; 0 to 5 grading system (Ritcher, et al., 1984) and abdominal skin reactions are scored on a similar 0 to 5 scale (Stanley et al., 1995).

Interpretation will be subjective, especially with an indefinite or suspicious result. In a known outbreak of TB in a population, grade 3 reactions should be considered TB positive. Table 2 and 3 should help with interpretation of the intradermal skin test.

<table>
<thead>
<tr>
<th>Reaction at 72hrs</th>
<th>Grade</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No reaction observed</td>
<td>0</td>
<td>Negative</td>
</tr>
<tr>
<td>Bruise only</td>
<td>1</td>
<td>Negative</td>
</tr>
<tr>
<td>Erythema without swelling</td>
<td>2</td>
<td>Negative</td>
</tr>
<tr>
<td>Erythema with minimal swelling, or slight swelling without erythema</td>
<td>3</td>
<td>Suspicious</td>
</tr>
<tr>
<td>Obvious swelling with drooping of eyelid and erythema</td>
<td>4</td>
<td>Positive</td>
</tr>
<tr>
<td>Swelling +/or necrosis with eyelids closed</td>
<td>5</td>
<td>Positive</td>
</tr>
</tbody>
</table>

Table 2. Interpretation of the tuberculin intradermal skin test

<table>
<thead>
<tr>
<th>Score</th>
<th>Description</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>No reaction</td>
<td>negative</td>
</tr>
<tr>
<td>1</td>
<td>Moderate swelling, height of duration 3-5mm</td>
<td>Negative</td>
</tr>
<tr>
<td>2</td>
<td>Moderate swelling, height of induration 5-10mm</td>
<td>questionable</td>
</tr>
<tr>
<td>3</td>
<td>Obvious swelling, height of induration &lt;10mm</td>
<td>positive</td>
</tr>
</tbody>
</table>

Table 3. Interpretation of the tuberculin intradermal skin test when used on abdomen

Figures 4 to 6 demonstrates the TST procedure and positive hypersensitivity reactions to tuberculin, to assist interpretation.
Figure 4: injection site for Avian Tuberculin PPD in the right eyelid (M. van Zijl Langhout)

Figure 5. Positive if see erythema or swollen lid (Source, Internet)

Figure 6. Positive - complete closure of lid with necrosis and purulent discharge (Source, Internet)
Limitations
TST has a number of limitations with regard to sensitivity and specificity that reduce its ability to be used as a standalone test.

1. TST tends to give only intermittently positive results in a serial testing of infected animals (Garcia et al., 2004b; Walsh et al., 1996). TB infections were found in imported nonhuman primates to USA in 0.4% of cynomologus and rhesus monkeys of the 22,913 NHPs, representing 7% of the 249 shipments between 1992 and 1993, CDC, 2003. All animals had received routine tuberculin skin tests (TSTs) (three tests, with 2-week intervals between tests) by experienced personnel in well-established quarantine facilities using accepted methods and a U.S. Department of Agriculture-licensed skin-test antigen.

2. TST has a tendency of giving negative results on TST-positive infected animals (even those with radiographic evidence of lung disease) on serial testing due to the development of latent infections, anergy associated with progressive disease or other mechanisms that are poorly understood (Heywood et al., 1970, Mayall et al., 1981; Motzel et al., 2003).

3. Lack of specificity of the mycobacterial antigens that constitute MOT, results in false positive TST reactions in animal sensitized to other, nontuberculous environmental mycobacteria (Brammer et al., 1995; Goodwin et al., 1988; Soave et al., 1981)

4. Unfortunately, the concentration of antigens required to elicit a positive TST reaction is higher in NHPs than in humans. MOT meets this requirement and has a greater reactivity than PPD and is therefore preferred to PPD as the regent to use in a TST to identify infected animals. However, MOT is a crude culture of filtrate preparation that contains antigens common to many mycobacterial species including those not associated with TB. Because of this antigenic cross reactivity, TST suffers low specificity and false positive reactions are common.

5. Commercial production of MOT is cumbersome and hence there is only one manufacturer in USA (Synbiotics, Inc) and thus MOT is not readily available.

Outbreaks of TB continue to occur in established colonies of primates and can have severe consequences due to the loss of animals, transmission to human caretakers, disruption of re-introduction programmes and costs associated with disease control (Otto et al., 2004)).
Detection of latent TB infections is therefore a high priority in the control and prevention of disease in nonhuman primates. Limitations of TST described above and especially its inability to detect animals with latent TB infections, makes it an unsuitable tool to use as a single, standalone test for TB surveillance in non human primates. While the incidence of false positives and false negatives varies from batch to batch of tuberculin, false results have tended to be a problem with all skin tests aimed at diagnosing primate TB [Kaufmann and Anderson 1978, Walsh et al 1996]. None of the existing TB tests alone is sufficient to diagnose disease. Therefore, new TB diagnostic algorithms are being developed, in which serological assays may play an important role [Lyashchenko et al 1998, 2000, Brusasco et al 2003]. One of these that shows promise, to be used in conjunction with the TST, is the Prima-TB Statpak, a rapid test lateral flow immunoassay.

2. Rapid Test Lateral Flow Immunoassay

The lateral flow assay utilizes a membrane impregnated with selected antigens combined with a sample pad, a conjugate pad, and a sink pad in individual plastic cassettes.

**CHEMBIO'S PRIMA TB STAT-PAK®, FOR DETECTION OF TB ANTIBODIES**

US Veterinary License No. 645  
US Patent No. 2006057621  
Distributed in North America by:  
Tel: 1-800-236-6180  
Fax: (913) 390-5907  
E-mail: CentaurUNAVET@aol.com  
3661 Horseblock Road, Medford, New York 11763  
Tel: (631) 924-1135  
Fax: (631) 924-6033  
E-mail: info@chembio.com  
Web: www.chembio.com

**Principle of Test**

The PrimaTB STAT-PAK Assay is a qualitative, single use, two-step, immunochromatographic (lateral flow technology) screening test for the detection of antibodies to *Mycobacterium tuberculosis* and *Mycobacterium bovis* in primate serum, plasma or whole blood. The rapid test does not require laboratory equipment or specific technical training. This assay uses a “cocktail” of three immunodominant TB-specific antigens (ESAT-6, CFP-10, and MPB83), a combination that was sufficient to correctly identify 25 of 27 (93%) macaques (20 rhesus and 7 cynomolgus) experimentally infected with *M. tuberculosis* or *M. bovis*. Testing of 195 uninfected macaques produced 3 (1.5%) false positive results (Greenwald et al. 2007). The test is used for the diagnosis of active tuberculosis (TB) in conjunction with other diagnostic methods. If specific antibodies are present in the sample, the expected test result is reactive. A reactive result is suggestive of active TB. In the absence of antibodies, the expected test result is nonreactive.

The test employs a unique cocktail of recombinant *M. tuberculosis* proteins that are bound to the membrane solid phase. Blue latex particles conjugated with protein are used as the detection system. Once a test sample is applied to the SAMPLE (S) well followed by the addition of a diluent, it flows laterally through the membrane strip. When it reaches the conjugate pad,
antibodies, if present, bind to protein-latex conjugate and then the migrating immune complex binds to the antigens on the solid phase in the TEST (T) area producing a blue line. In the absence of antibodies there is no line in the TEST (T) area. The sample continues to migrate along the membrane and produces a blue line in the CONTROL (C) area demonstrating that the reagents are functioning properly. Results can be obtained in 20 minutes and require a small volume of serum, plasma, or whole blood.

The PrimaTB STAT-PAK Assay should be stored at 8 to 30°C in the original sealed pouch. The diluent should be stored in the original vial at 8 to 30°C. The kit is stable until the date imprinted on the box label and/or pouch.

**Test Procedure**

1. If test samples are refrigerated, remove them from the refrigerator and allow them to come to a temperature of 18 to 30°C before testing.
2. Remove the required number of PrimaTB STAT-PAK. Assay devices from their pouches and place the devices on a flat surface area. It is not necessary to remove the desiccant from the package. NOTE: If desiccant packet is missing, DO NOT USE, discard the test device and a new test device should be used.
3. Label test units with sample names and/or identification numbers.
4a. For Venous Whole Blood, Serum or Plasma: Using a laboratory pipette, obtain 30 uL of the specimen to be tested.
4b. For Fingerstick Blood:
   - Step 1: Prepare to perform the fingerstick blood collection procedure. Clean the finger with an antiseptic wipe. Allow the finger to dry thoroughly or wipe dry with a sterile gauze pad. Using a sterile lancet, puncture the skin just off the center of the finger and wipe away the first drop with sterile gauze. Avoid squeezing the fingertip to accelerate bleeding as this may dilute the blood with excess fluid. Collect the sample from the second drop as explained in Step 2.
   - Step 2: Once the drop of blood has formed on the finger, hold the tube horizontally and touch the tip of the pipette to the sample. Capillary action will draw the sample to the black fill line and stop. Test immediately.
   - **CAUTION!** Filling is automatic. Never squeeze the pipette while sampling.
   - Step 3: To expel the 30 4L sample, align the tip of the pipette with the sample well and squeeze the bulb.
   - **ONLY IF THE SAMPLE DOES NOT COME OUT OF THE TUBE,** hold the pipette vertically and slide the finger over the vent hole. Then align the tip with the sample well and squeeze the bulb.
5. Once the specimen has been applied to the SAMPLE (S) well, invert the diluent bottle and hold it vertically (not at an angle) over the SAMPLE well. Add the diluent slowly dropwise; add 3 drops (~100 4L) into SAMPLE (S) well.
6. Read results at 20 minutes after the addition of diluent. Do not read any results after 30 minutes. Refer to **INTERPRETATION OF RESULTS** section below.
7. Discard the used disposable pipette, test device and any other test materials into a biohazard waste container.

**Quality Control**

A blue colored line should always appear in CONTROL (C) area if the test has been performed correctly and the device is working properly. It serves as an internal test procedural control. Good Laboratory Practice (GLP) recommends the use of control materials along with the test
samples to ensure proper performance of the test kit. Positive and Negative serum or plasma based commercial controls should be used for this purpose. Use controls as per the TEST PROCEDURE instructions of this insert.

**Interpretation of Results**

**Nonreactive Result**

One blue colored line in the CONTROL (C) area, with no visible colored line in the TEST (T) area indicates a nonreactive result. A nonreactive result at 20 minutes means that neither *Mycobacterium tuberculosis* nor *Mycobacterium bovis* antibodies were detected in the specimen. A nonreactive result does not preclude the possibility of TB infection.

**Reactive Result**

Two blue lines - one in the TEST (T) area and one in the CONTROL (C) area - indicate a reactive result (Figure 7). Intensities of the TEST and CONTROL lines may vary. Even a very faint line in the TEST (T) area of the device within 20 minutes is indicative of a reactive result. A reactive result means that *Mycobacterium tuberculosis* and/or *Mycobacterium bovis* antibodies were detected in the specimen.

![Figure 8. Comparing a non-reactive (A) and reactive (B) test result for the Prima-TB statpak](image)

**Invalid Results**

A blue line should always appear in the CONTROL (C) area, whether or not a line appears in the TEST (T) area. If there is no distinct blue line in the CONTROL (C) area, the test is invalid and should be repeated using a new device.

**Limitations of the Procedure**

1. The assay is designed for detecting antibodies against *M. tuberculosis* and *M. bovis* only from macaque plasma, serum or whole blood, although it has been used effectively in many other
primate species, and is currently being validated in at least chimpanzees, gorillas, orangutans and vervet monkeys. Any result from the testing of other body fluids or of pooled serum or plasma samples should not be used.

2. A reactive result suggests the presence of antibodies to *M. tuberculosis* and/or *M. bovis*.

3. For a reactive result, the intensity of the test line does not necessarily correlate with the titre of antibody in the specimen.

4. Reading nonreactive results earlier than 20 minutes or any results later than 30 minutes may yield erroneous results.

5. Do not use hemolysed blood samples.

6. Be careful to add only 30 uL of specimen and 3 drops of diluent after applying the specimen to the SAMPLE (S) well.

7. Do not open the sealed test pouch until just prior to use.

8. Do not use kit contents beyond labelled expiration date.

9. Read results in a well-lit area.

**Performance Characteristics of the Prima TB statpak, based on the manufacturers won trials, can be found in an appendix at the end of this chapter.**

One preliminary study in sanctuary chimps (Ngamba Island) using the PrimaTB statpak shows promise (Figure 8).

<table>
<thead>
<tr>
<th>Status</th>
<th>Skin test result</th>
<th>Culture result</th>
<th>PrimaTB STATPAK</th>
<th>Necropsy result</th>
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</thead>
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<tr>
<td>Healthy (5)</td>
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<td>ND</td>
<td>Negative</td>
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<td>BCG vax (1)</td>
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<td>ND</td>
<td>Neg</td>
<td>ND</td>
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</tbody>
</table>

*Figure 9. Comparing the Prima TB Statpak with Intradermal Skin Testing and TB culture results in a group of chimpanzees*

Ngamba Island also did a small trial comparing the Elephant TB Statpak and the Prima TB Statpak results. Note that the Primate version correctly identified the TB positive chimp (Figure 9 (arrow), while the elephant kit remained negative.
FURTHER DIAGNOSTICS (Provides information on other diagnostic tools especially applied in human TB diagnostics as a useful guide to new tools being developed for NHPs)

1. Thoracic radiographs and CT Scans

Chest radiographs may be used as an additional test procedure but cannot be used as the only screening procedure. 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 cavitations. 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. 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 cavitations may be appreciated radiographically.
Infiltrates and pulmonary fibrosis can be seen in advanced cases (Figure 10). Hilar lymph nodes are early site of disease (cardiac silhouette makes diagnosis difficult). However, changes are not specific to TB, and are similar to other diseases, such as pneumonia due to another bacterial infection. Computed tomography scanning gave detailed real-time imaging of disease progression but is very expensive and not readily available.

2. Cytology and culture of bronchial wash.

This should always be taken at the same time as the initial TB testing (weather using intradermal testing, rapid test, or a combination). As culture can take up to 3 months, it is worth getting the sample as soon as possible, so if the screening tests do come up as reactive, the culture can be started sooner rather than later. Culture samples can be stored at room temperature for 7 days, allowing time to read the screening tests which will indicate if further tests are required. 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* (e.g. *Nocardia spp*). 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 Lowenstein–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 and laparoscopy.

3. 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 (bronchial wash and gastric lavage samples being the most
useful). 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.

4. Interferon-γ Assays

IFN-γ is a critical cytokine in the cell-mediated immune response to tuberculin antigens,
including the DTH response measured by the TST and in the host immune response to infection
with tuberculosis mycobacteria (Collins and Kaufmann, 2001; Fletcher, 2007, Flyn et al., 1993;
Lin et al., 2006). Hence assessing the IFN-γ response to TB antigens in vitro provides an
alternative method for screening and diagnosis.

Recently a whole blood WB-IFN-γ based assay has become commercially available as
PRIGRAM® TEST (Product number 63301, Prionics, Va Vista, Nebras ka, USA), received
provisional USDA licensure for use in nonhuman primates. The assay is based on the response
of memory T-cells to stimulation with either tuberculin or TB-specific antigens, resulting in the
release of IFN-γ. Briefly, within 24 hours of blood collection, 0.5 to 1.0ml aliquots of blood in
24-well culture plates are stimulated with a nil antigen control, purified protein derivative
(PPD) of Mycobacterium bovis (bPPD), or M. avium PPD (aPPD). Although not included in the
commercial version, some users of the assay add a mitogen-stimulated well as a positive
control. After 24hrs of incubation at 37 oc and 5% CO2 in a humidified atmosphere, the
concentration of IFN-γ in the supernant plasma of each aliquot is determined by enzyme
immunoassay.

The interpretation of WB IFN-γ assay is based on the difference in IFN-γ response to bPPD
and aPPD; the suggested criterion for a positive test is OD bPPD-ODaPPD ≥ 0.05 OD units. A
positive reaction to bPPD is interpreted as an indication of sensitization to antigens of either
M. tuberculosis or M. bovis. Incorporation of the aPPD antigens addresses the specificity issue of cross reactivity among
mycobacterial PPD antigens. A stronger response to aPPD than to bPPD is often
Interpreted as sensitization to M. avium or other non-tuberculous environmental mycobacterial
species. This interpretation, however, should be based on test results from more than a single
time point, as a small subset of nonhuman primates in the early stages of M. tuberculosis or M.
bovis infection may show a stronger IFN-γ response to aPPD than to bPPD. Subsequent testing of
these animals shows conversion to a dominant IFN- response to bPPD (Lerche, unpublished
data; Vervenne et al. 2004). To avoid misdiagnosis, animals showing high avian PPD responses
should be retested more than 2 weeks after the original test (Vervenne et al. 2004)
A whole-blood IFN-γ-test, QuantiFERON®-TB Gold (QFT-G; termed QFT-2G in Japan; Cellestis
Limited, Carnegie, Australia), measuring cellular immune responses to two of these TB-specific
proteins ESAT-6 and CFP-10, has recently been developed and received both US Food and Drug
Administration and Japanese MHLW regulatory approval for use in humans. QFT-G-test has a
high sensitivity and specificity for diagnosis of active MTB infection, and importantly, is unaffected by BCG vaccination. More recently, it has been showed that QFT-G detects those likely to have latent TB infection (LTBI). (Arend et al., 2000; Scarpellin et al., 2004; Funayama et al., 2005; Haranda et al., 2006; Kang et al., 2005; Pai et al., 2005)

The ELISPOT Assay

New in vitro tests that are based on detecting the interferon-γ (IFN-γ) released by activated T lymphocytes (T cells), and whose antigens are a number of secretory proteins coded by the RD1 genes of the *M. tuberculosis* complex but are absent from the majority of environmental isolates, including BCG strains. Two of these proteins, early secretory antigenic target 6 (ESAT-6) and culture filtrate protein 10 (CFP-10), and synthetic overlapping peptides corresponding to the full length of each were initially tested and elicited a strong T-cell response in subjects with active TB infection or LTBI. The in-house assay is based on a restricted and highly selected pool of synthetic peptides containing multiple epitopes derived from ESAT-6 and CFP-10 proteins. (Brock et al., 2004; Diel et al., 2006).

The ELISPOT uses a known concentration of Ficoll-separated peripheral blood mononuclear cells rather than whole blood for incubation with stimulating antigens. The ELISPOT assay enables enumeration of the cells releasing IFN-γ by counting the “spots” of labeled IFN-γ through a stereomicroscope or automated ELISPOT plate reader (Lin et al. 2006; Wang et al. 2007). The ELISPOT IFN-γ assay has been used primarily for research purposes in experimental models, but a commercially available version (T SPOT-TB, Oxford Immunotec Ltd., Oxford, UK, commercial T-SPOT. TB™ assay) and In-house ELISPOT-IFN-assay has been licensed for use in human testing (Wang et al. 2007); to date, there is no commercial ELISPOT-based assay for use in nonhuman primates.

5. In vitro assays of humoral immune responses to TB antigens

Recent advances in the sequencing of the *M. tuberculosis* and *M. bovis* genomes (Cole et al. 1998; Garnier et al. 2003) have facilitated the identification of a number of secreted proteins that are unique to mycobacterial species of the *M. tuberculosis* complex (Amor et al. 2005; Harboe et al. 1996). Two of these proteins, ESAT-6 and CFP-10, are highly immunogenic and have been evaluated as antigen targets for detection of TB-specific antibodies in nonhuman primates. Gennaro and colleagues (Brusasca et al. 2003) reported that sera from 100% of 17 nonhuman primates from three species (cynomolgus, rhesus, and African green monkeys) experimentally infected with *M. tuberculosis* had detectable ESAT-6 antibodies, and 90% were reactive against two additional proteins, CFP-10 and-crystallin. Although animals tended to be positive by palpebral TST earlier than in the antibody test, the levels of specific antibody remained elevated over the course of infection, whereas TST reactivity was intermittent or waned (Brusasca et al. 2003).

In a naturally occurring outbreak of *M. bovis* infection in rhesus and cynomolgus macaques, 22 of 25 animals with tuberculosis lesions identified at necropsy had detectable immunoglobulin G (IgG) ESAT-6-specific antibodies, and the sensitivity and specificity of the antibody ELISA (88% and 84%, respectively) were comparable to the sensitivity (84%) and specificity (84%) of the TST (Kanaujia et al. 2003). Three confirmed positive cases in this outbreak did not have detectable antibody in early serum samples, and 5 of 32 animals (16%) without lesions were antibody-test positive (Kanaujia et al. 2003). Although seroconversion to ESAT-6, the earliest recognized antigen, occurs 1 to 2 months after infection, the persistence of detectable antibody over the
course of infection suggests that a significant improvement in TB surveillance programs may result from the addition of antibody testing for ESAT-6 and other specific antigens. In addition to ELISA, several additional formats have been proposed for TB-specific antibody testing and may have applications for TB surveillance of nonhuman primates.

6. Multiantigen Print Immunoassay (MAPIA)

This assay allows for the simultaneous evaluation of the pattern of reactivity to multiple TB specific antigens is the multiantigen print immunoassay (MAPIA) (Lyashchenko et al. 2000). In this assay multiple antigens are immobilized in solid phase on nitrocellulose membranes as narrow bands using a semi-automated airbrush printing device (Linomat IV, CAMAG Scientific, Wilmington, NC). The antigen-coated nitrocellulose membrane is cut into 4 mm-wide strips that are then used for antibody detection by Western blot–like chromogenic immunodevelopment. Presence of a visible band is interpreted as a positive result (Lyashchenko et al. 2000). Use of multiple antigen arrays such as MMIA and MAPIA on sequential serum samples enables the assessment of changing patterns of immunoreactivity over the course of infection. This format can also serve as a confirmatory test for ELISA or rapid test reactive sera. In addition, multiple antigen arrays provide a powerful research tool for identifying novel immunodominant proteins, and may identify reactivity patterns that are predictive of disease progression or reactivation. MAPIA reactivity patterns have also been used to monitor response to therapy in animals undergoing treatment for tuberculosis (Lyashchenko et al. 2006).

**Blood collection for Rapid Test (Chembio Stat Pak) and MAPIA.** At 72 hours, repeat MAPIA (so needs another blood sample). Repeat the MAPIA again at 3 weeks. If there is an increasing antigen stimulation, this will be indicative of an active infection, as opposed to using the tests independently. NOTE - MAPIA is currently off the market (2009).
PART 3. GUIDELINES FOR THE PREVENTION AND CONTROL OF TUBERCULOSIS IN HUMANS WORKING WITH NON HUMAN PRIMATES. (to be read in conjunction with the relevant sections of the PASA Operations Manual and Section 1 of this vet manual)

Introduction

Tuberculosis is zoonotic with the potential to be transmitted from Humans to NHPs and Vice-versa with grave consequences in both scenarios, it is important to have guidelines for prevention and control of tuberculosis in sanctuaries managing primates and other captive facilities.

Personnel Prevention procedures

All individuals (including visitors) entering areas where non-human primates are housed may be at risk of acquiring *M. tuberculosis* infection or pose a risk of transmitting *M. tuberculosis* to non-human primates.

**Step 1:** All new staff should provide any past medical documents related to TB diagnosis and treatment if any. They should undergo baseline screening for *M. tuberculosis* exposure during the hiring process. TST or Rapid Test Lateral Flow Immunoassay (In-House ELISAPOT) and preferably a combination should be performed by the medical service provider chosen by the organization for easy follow up.

A positive TST or a history of a previous positive reaction to a tuberculin skin test should be followed by a chest radiograph.

A chest radiograph is obtained if:

a. the employee cannot provide documentation of a normal chest radiograph within the last three month following the discovery of the positive reaction, or

b. the employee's responses to the questions suggest active pulmonary tuberculosis, or

c. the employee did not receive appropriate chemoprophylaxis or treatment, as determined by the Health service provider

If there is clinical or radiographic evidence of active pulmonary tuberculosis, the person should not be hired to work with non human primates. He/she should be advised/assisted to get medical attention. Conditions of hire after treatment should include provision of documentation establishing that there is no clinical or radiographic findings attributed to active pulmonary tuberculosis.

**Step 11:** Prospective employee with no history of a prior positive reaction to a tuberculin skin test will receive a “two step” tuberculin skin test (Purified Protein Derivative, PPD test) and Rapid Test Lateral Flow Immunoassay on enrollment. The second tuberculin test should be given 1-3 weeks after the first. If the participant has documented negative PPD tests within the previous 6 months, only a single tuberculin skin test is administered.

Note: Persons who have received Bacillus Calmette-Guerin (BCG) immunization will be given PPD tests. Interpretation of a reaction will be based upon the size of the reaction, length of time since BCG administration, and risk of prior exposure to tuberculosis. A positive skin test may result from either exposure to *M. tuberculosis, M. bovis*, BCG injection, or exposure to non-tuberculous strains of mycobacteria. The American Thoracic Society has published guidelines
for the interpretation of intradermal testing. If inoculation with BCG occurred more than 10 years ago, a positive PPD test should not be considered a reaction due to BCG, but should instead be considered as positive for exposure to TB.

If the tuberculin skin tests are both negative and there are no other medical contraindications, the employee is medically cleared for work.

**Step III:** All employees should undergo annual tuberculin skin testing or medical screening for active tuberculosis infection. However, for persons working in quarantine and those working with suspected risky animals, the duration should be reduced to 3 to 6 months.

If the prior tuberculin skin test was negative, but the current test indicates positive, this is considered a recent conversion and the individual will undergo evaluation as in step one above. The employer should have a policy well documented on how to deal with cases TB acquired while on job as this is part occupational health and safety. Employees with acid-fast positive sputum smears should be removed from animal contact until it is determined whether this represents infection with an organism of the *M. tuberculosis* complex (*M. tuberculosis* or *M. bovis*). Treatment guidelines and recommendations for contact with animals and humans are available through state public health departments.

**Step IV:** All volunteers and visitors likely to come into close contact with the primates, should present evidence of recent TB screening within the last three month either a TST or Chest radiograph before admitted into the facility.

**Step V: Implementation**
The resident Veterinarian/director has the responsibility to ensure all staff adhere to the TB screening guidelines and arrange follow up checks with each individual and medical personnel assigned with screening responsibility.

All entrants (including all visitors and volunteers) into non-human primate areas must be verified and certified free from of active tuberculosis.

**Step VI: Personal Protective Equipment (PPE)** – Items of clothing (i.e. lab coats, shoe covers, face masks, gloves, etc.) or equipment (i.e. face shields, eye goggles, etc.) designed to prevent or limit exposure to potentially harmful agents should be provided to staff throughout the entire period of employment.

PART 4. GUIDELINES ON THE PREVENTION AND CONTROL OF TUBERCULOSIS IN NON HUMAN GUIDELINES (also refer to the relevant sections of the PASA Operations Manual)

Preventive measures are required to protect NHPs from acquiring Mycobacterium tuberculosis complex (MTC - *M. tuberculosis*, *M. bovis* and *M. africanum*) through interactions with humans and other species. Non-tubercle forming, atypical mycobacteria species are also important but primarily because they may confound test results. Below are guidelines to follow.

A. Quarantine
All recently rescued primates must undergo a quarantine period of 90 days. During this period, three TST and/or PrimaTB STAT-PAK Assay should be carried out. The first test should be performed 1 week after arrival and thereafter 6 weeks interval for the rest of the tests. The reactions should be read at intervals of 24, 48 and 72 hours and interpreted as already described. It is important to accurately record the results of TST for each animal in addition to any other information that may be relevant like the social animal partners and any movements. A digital image and exact measurements of a suspect skin reaction is a useful tool to compare test results in repeated tests.

B. Post Quarantine Husbandry Practices
The animal husbandry and sanitation practices within sanctuaries should be designed to prevent the spread of pathogens including tubercle bacilli. Use of appropriate disinfectants is recommended. For rooms with primates with reactive TST, tuberculocidal detergent disinfectants (the label must read tuberculocidal - e.g. 5% cresylic compound or phenol derivative such as sodium orthophenyl phenate) 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. 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. 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.

**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.
<table>
<thead>
<tr>
<th>Species or Group</th>
<th>TST Schedule Quarantine as per NIH PM 3044-1</th>
<th>Recommended Schedule Post-Quarantine Holding</th>
</tr>
</thead>
<tbody>
<tr>
<td>New World Monkeys</td>
<td>3 times, 6 weeks apart</td>
<td>Semiannually</td>
</tr>
<tr>
<td>Macaque species</td>
<td>5 times, 6 weeks apart</td>
<td>Quarterly</td>
</tr>
<tr>
<td>Baboons</td>
<td>3 times, 6 weeks apart</td>
<td>Semiannually</td>
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<td>Chimpanzees, gorillas and bonobos</td>
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<td>Annually</td>
</tr>
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<td>Patas</td>
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</tr>
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<td>African green</td>
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<td>Quarterly</td>
</tr>
<tr>
<td>Prosimians</td>
<td>3 times, 6 weeks apart</td>
<td>Semiannually</td>
</tr>
</tbody>
</table>

Special attention should be taken for 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 faeces 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.

Managing 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 Chart below provides a guide on the steps to follow. It is recommended that initial screening for TB include the TST and Prima TB Statpak, with additional samples taken as is possible in each situation (see table 2).

Further comments on interpretation

- A case with two questionable test results should be regarded as positive.
- A negative reaction may indicate that no disease is present or that the disease has progressed to such an advanced state that the primates has become anergic
- A viral infection such as measles, a debilitating illness, corticosteroids, or immunizations may all depress sensitivity to the test.
- When retesting primates with questionable reaction observations should be made between 2 and 8 hours after the test is performed as well as daily, since primates that are anergic may demonstrate a "flash" reaction, which quickly recedes.
- False positive reactions: Contaminated equipment, too frequent testing, sensitization to the adjuvant, trauma, cross reaction with other mycobacteria
- False negative reactions: Subcutaneous injection, anergy, steroid therapy, measles vaccines, inadequate dose, isoniazid therapy
- Suspicious reaction?
o Isolate the animal
o Retest after 60 days
o Use a different site to where the positive reaction was shown
o If positive to second test, animal should be considered infected

Handling Tuberculous NHPs
A. Immediate Euthanasia
When a clinical diagnosis of *M. tuberculosis* complex disease is made in a NHP, it is immediately euthanased and the carcass is taken for post mortem and histopathology, with associated further testing as necessary. 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 six 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 about one week after the test that identified the tuberculous NHP.

B. Delayed Euthanasia
The euthanasia of a primate 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 primates or humans. The room in which such a primate was held when the clinical diagnosis was made will be placed under quarantine as described. Most sanctuaries do not have required housing conditions to contain spread of tubercle bacilli, hence euthanasia should be carried out immediately.

C. Treatment of Tuberculous primates
As positive animals can never be considered disease free, usually elect for euthanasia. ANIMALS DESTINED FOR RELEASE SHOULD BE REMOVED FORM THE REINTRODUCTION PROGRAMME. DO NOT TREAT POSITIVE ANIMALS AND THEN RELEASE.

- Reserve treatment only for most valuable animals
- If decide to treat you need to understand the consequences
- Animal can never be considered free of the disease
- Reaction to skin test will be reduced
- May shed organisms after therapy is stopped
- Positive animal must be removed from all contact with other primates
- Vigilant against the possibility of transmission to other primates and humans
• Combination of isoniazid, ethambutol, and rifampin is recommended treatment
• Dose of isoniazid in great apes is 10-30 mg/kg per day
• May need to treat for 6-12 months
• Consult with experts, especially human physicians

<table>
<thead>
<tr>
<th>1st Line of drugs</th>
<th>2nd Line</th>
<th>3rd Line</th>
<th>New drugs</th>
</tr>
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<td>Streptomycin</td>
<td>Linezolid</td>
<td>Etambutol Congeners (SQ-109)</td>
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<td>Rifampin</td>
<td>Cycloserine</td>
<td>Nebulised interferon-γ</td>
<td>Diarylquinolines (DARQ)</td>
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<td>P-Aminosalicylic acid</td>
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<td>Amikacin/Kanamycin</td>
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<td>Cepreomycin</td>
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<tr>
<td></td>
<td>Gatifloxacin</td>
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</tr>
</tbody>
</table>

Table 4: Shows generation of drugs that have been used/being used in treatment of TB in humans

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.

SUMMARY

• TB continues to be a worldwide health threat for humans and animals
• TB in non-human primates is mostly transmitted from humans. Personnel health programmes and limiting contact by strict rules for visitors and staff are crucial to prevent infection.
• Application of new diagnostic technology may allow more rapid and accurate detection of TB in non-human primates
• No diagnostic method is accurate on its own and as many techniques as possible must be combined to reduce ‘false negative’ results.
• Availability and cost will continue to be major obstacles for diagnostic technology for sanctuaries
• Partnering with research, zoos, and industry may advance knowledge and tools available
REFERENCES


**APPENDIX.**

Performance Characteristics of the Prima TB statpak (Source, Chembio clinical trials).

Highly specific antibody binding proteins are used in the PrimaTB STAT-PAK Assay. The PrimaTB STAT-PAK Assay was compared to the standard USDA approved methods and the diagnostic performance was found to be superior.

**Sensitivity and Specificity**

Clinical trial studies were performed on 372 well-characterized samples to evaluate the PrimaTB STAT-PAK Assay. No cross reactivity was found in Rhesus macaques experimentally infected with *M. avium* or *M. kansasii*. The sensitivity of the PrimaTB STAT-PAK was determined by testing 106 samples. Of these samples 92/106 samples were reactive by the Chembio PrimaTB STAT-PAK antibody test kit (Figure 11).

<table>
<thead>
<tr>
<th>Infection</th>
<th># of + results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus macaque</td>
<td></td>
</tr>
<tr>
<td>M. tb</td>
<td>45/55</td>
</tr>
<tr>
<td>M. bovis</td>
<td>13/13</td>
</tr>
<tr>
<td>Cynomologus macaque</td>
<td></td>
</tr>
<tr>
<td>M. tb</td>
<td>15/16</td>
</tr>
<tr>
<td>M. bovis</td>
<td>19/22</td>
</tr>
<tr>
<td>Total</td>
<td>92/106</td>
</tr>
</tbody>
</table>

*Figure 12. Sensitivity trial for the Prima TB Statpak*

The specificity of the PrimaTB STAT-PAK was determined by testing 266 samples. Of these samples 263/266 samples were nonreactive by the Chembio PrimaTB STAT-PAK antibody test kit (Figure 12).

<table>
<thead>
<tr>
<th>Status</th>
<th># of neg results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rhesus macaque</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>221/224</td>
</tr>
<tr>
<td>M. avium or M. kansasii</td>
<td>6/6</td>
</tr>
<tr>
<td>Cynomolgus macaque</td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>36/36</td>
</tr>
<tr>
<td>Total</td>
<td>263/266</td>
</tr>
</tbody>
</table>

*Figure 13 Specificity trial for the Prima Tb Statpak*

**REPRODUCIBILITY STUDIES**

Reproducibility was tested at three independent laboratories using three serials of PrimaTB STAT-PAK Assay. A reference panel of 20 blinded samples representing negative, weakly reactive and reactive were tested 3 different times on 3 different days. The compiled results from the 3 laboratories demonstrated 98.3% accuracy.