

GUIDELINES FOR THE CONTROL OF TUBERCULOSIS IN ELEPHANTS 2012

**UNITED STATES ANIMAL HEALTH ASSOCIATION (USAHA)
ELEPHANT TUBERCULOSIS SUBCOMMITTEE
20 April 2012 DRAFT REVISION**

TABLE OF CONTENTS (topics are bookmarked)

1. [Introduction](#)
 2. [Definitions](#)
 3. [Annual Testing](#)
 4. [Culture Collection Procedure](#)
 5. [ElephantTB STAT-PAK[®] and MAPIA[™] Collection Procedure](#)
 6. [Ancillary Diagnostic Tests](#)
 7. [TB Management Groups](#)
 8. [Principles of Anti-tuberculosis Therapy](#)
 9. [Anti-tuberculosis Drugs](#)
 10. [Dosages and Routes of Administration](#)
 11. [Blood Levels](#)
 12. [Postmortem Examination](#)
 13. [Employee Safety and Health](#)
 14. [Reporting](#)
 15. [Appendices](#)
- Appendix 1. [References](#)
- Appendix 2. [Acknowledgments](#)
- Appendix 3. [A Trunk Wash Technique for the Diagnosis of TB in Elephants](#)
- Appendix 4. [Testing Laboratories](#)
- Appendix 5. [Contacts for Questions](#)
- Appendix 6. [Sources for Anti-tuberculosis Drugs](#)
- Appendix 7. [SSP Serum Banking Form](#)

These guidelines are available on the Internet at the following sites:

1. http://www.aphis.usda.gov/animal_welfare/index.shtml
http://www.aphis.usda.gov/animal_welfare/downloads/elephant/elephant_tb.pdf (2008 guidelines available to the public)
2. www.aazv.org (available to AAZV members by password)
3. www.elephantcare.org (2008 guidelines available to the public)
4. www.usaha.org (Look under Tuberculosis committee for 2010 guidelines)

1. INTRODUCTION

Tuberculosis (TB) is caused by bacteria in the genus *Mycobacterium*. Over 100 species comprise this genus. Mycobacteria infect a broad range of species including humans, non-human primates, carnivores; marine mammals, psittacine birds, reptiles, fish, artiodactylids, pachyderms, and domestic and non-domestic ungulates. Species susceptibility to specific mycobacteria varies (Montali 2001).

In mammals, the term “tuberculosis” is used to define disease¹ caused by *Mycobacterium tuberculosis* (*M. tb*) complex organisms. The *M. tb* complex includes *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. canetti*, *M. caprae*, and *M. pinnipedii*. A vaccine strain derived from *M. bovis* (*M. bovis* BCG) is sometimes included as a separate member of this complex.

The term “mycobacteriosis” refers to infection with any mycobacteria but is generally used to define disease caused by non-tuberculous mycobacteria (NTM). “Atypical mycobacteria” or “mycobacteria other than TB” (MOTT) are other terms used to describe this group. Most NTM are saprophytes found in soil or water but they may occasionally cause disease in humans and animals, including elephants.

Mycobacterium tuberculosis is the predominant infection-causing agent in elephants although cases caused by *M. bovis* have occurred. *Mycobacterium szulgai*, an uncommon NTM species, was associated with fatal disease in two African elephants (Lacasse 2007) and *Mycobacterium elephantis*, a rapidly growing mycobacterium, was isolated from a lung abscess of an elephant that died of chronic respiratory disease (Shojaei 2000). *Mycobacterium avium* is commonly isolated from elephants (Payeur 2002) and is not generally associated with disease although a single case has been reported (Yong 2011).

The National Tuberculosis Working Group for Zoo and Wildlife Species has been monitoring TB in elephants since 1996. The original Guidelines for the Control of Tuberculosis in Elephants were released in 1997 and modified in 2000, 2003, 2008, and 2010. The Guidelines include recommendations for the testing, treatment, and surveillance of TB in elephants and are revised as new information becomes available. The 2012 guidelines include updated information on diagnostic tests and add further clarification to TB management groups.

2. DEFINITIONS

Ancillary diagnostic test: A subordinate or auxiliary test to be used in support of a primary test to diagnose disease.

Airborne transmission. Airborne transmission occurs by dissemination of either airborne droplet nuclei or small particles in the respirable size range containing infectious agents that remain infective over time and distance (e.g., spores of *Aspergillus* spp, *Mycobacterium tuberculosis* bacilli). Microorganisms carried in this manner may be dispersed over long distances by air currents and may be inhaled by susceptible individuals who have not had face-to-face contact with (or been in close proximity to) the infectious animal or person (Siegel 2007).

Aerosol transmission: Aerosol transmission occurs when pathogens travel through the air to

enter a host. Aerosols may be large droplets that are deposited on the mucous membranes or smaller particles that are inhaled. For most pathogens transmitted by this route, specific data defining risk of infection are limited; in general, risk of aerosol transmission increases with proximity to the source and duration of exposure. Aerosols can contain environmentally persistent pathogens that serve as a source for indirect contact transmission (NASPHV, 2010).

Attending veterinarian: A person that is licensed in the state in which they practice and has graduated from a veterinary school accredited by the American Veterinary Medical Association's Council on Education, or has a certificate issued by the American Veterinary Medical Association's Council on Education Commission for Foreign Veterinary Graduates; has received training and/or experience in the care and management of the species being attended; and who has direct or delegated authority for activities involving animals at a facility subject to the jurisdiction of the Secretary (i.e. a USDA licensed facility).

Atypical mycobacteria: See non-tuberculous mycobacteria

Contact:

Direct contact - Any situation in which an individual is present in the same barn, travel/transport compartment, outdoor enclosure, or is located in an adjacent enclosure that allows physical reach between animals, and **at the same time** there is an elephant present.

Indirect contact - Any situation in which an individual is present in the same barn, travel/transport compartment, outdoor enclosure, but the elephant occupies the space **at different times** or is located in any adjacent elephant enclosure. This may include shared feed and water sources and possible exposure by aerosol transmission.

Culture positive for *M.tb* complex: Isolation and identification of *M. tuberculosis* complex organisms from any site using standard mycobacterial methods.

Culture positive (*M.tb* complex) elephant: An elephant from which a *M. tuberculosis* complex organism has been isolated from any bodily specimen. A culture positive elephant is considered positive until it has met the treatment requirements as outlined in the current Guidelines.

Disease: A disordered or incorrectly functioning organ, part, structure, or system of the body resulting from the effect of infection; any abnormal condition that interferes with its vital physiological processes, caused by pathogenic microorganisms.

Dual Path Platform (DPP®) VetTB Assay: A new generation screening kit for the rapid detection of IgG antibodies to *M. tuberculosis* or *M. bovis* in elephant serum, plasma, or whole blood. The DPP® has shown 100% correlation with MAPIA™ (Greenwald et al. 2009).

ElephantTB STAT-PAK® Assay: A qualitative screening kit for the detection of antibodies to *M. tuberculosis* and *M. bovis* in elephant sera, plasma, or whole blood (Lyashchenko 2005, 2006, Greenwald 2009).

ELISA: Enzyme-linked immunosorbent assay; a test used to detect and measure either antigen or antibody.

Exposure to *Mycobacterium tuberculosis* complex – Any situation in which an individual is in direct or indirect contact with *Mycobacterium tuberculosis* complex organisms, or an *M. tuberculosis*-infected animal (e.g., *M. tuberculosis* infected elephant, human, or other animal).

Fomite: An inanimate object or material on which infection-producing agents may be conveyed.

Gamma-interferon test: A whole blood *in vitro* assay that can be used as an ancillary diagnostic test for TB (not currently available for use in elephants).

GEN-PROBE® AMPLIFIED™ Mycobacterium Tuberculosis Direct (MTD) Test: a target-amplified nucleic acid probe test for the *in vitro* diagnostic detection of *Mycobacterium tuberculosis* complex rRNA.

Genotyping assay: A technique for the identification and analysis of polymorphism in certain types of repeat units in DNA. Restriction fragment length polymorphism (RFLP) and variable number tandem repeat (VNTR) are examples of genotyping techniques.

Herd: A group or groups of elephants, maintained on common ground. Alternatively, two or more groups of animals under common ownership or supervision that are geographically separated, but may have an interchange or movement of animals or personnel without regard to health status.

Incidence: The rate at which a certain event occurs, for example, the number of new cases of a specific disease occurring during a certain period.

Index animal: The animal in which infection is first diagnosed.

Infected elephant: an elephant from which *Mycobacterium tuberculosis* complex has been identified through culture, PCR or other molecular techniques.

Infection: Invasion and multiplication of microorganisms in body tissues, causing local cellular injury.

Intradermal tuberculin test (skin test): The injection of purified protein derivative (PPD) tuberculin into the skin for the purpose of detecting exposure to tuberculosis. In cattle, the test site is either the caudal fold (CFT) or cervical region (e.g. comparative cervical test, CCT) and the test is read by observation and palpation at 72 hours (plus or minus 6 hours) following injection. In humans, the test site is the forearm and the test is read at 48-72 hours. The intradermal tuberculin test is not a reliable test in elephants (Mikota 2001, Lewerin 2005).

Licensed veterinarian: A person who has graduated from an accredited school of veterinary medicine or has passed the ECFVG or PAVE and who has a valid license to practice veterinary medicine in the U.S.

MultiAntigen Print ImmunoAssay (MAPIA™): A confirmatory test to the Elephant TB

STAT-PAK[®] Assay for detection of antibodies to *M. tuberculosis* and *M. bovis* in elephant sera or plasma (Lyashchenko 2000, 2006, Greenwald 2009).

Mycobacteria other than TB (MOTT): See non-tuberculous mycobacteria.

Mycobacteriosis: A disease caused by non-tuberculous mycobacteria (NTM).

***Mycobacterium*:** A genus in the family Mycobacteriaceae.

***Mycobacterium avium* (*M. avium*):** A non-tuberculous mycobacteria that is the primary causative agent of tuberculosis in birds. *M. avium* may be isolated from non-clinically affected elephants and is usually considered an environmental contaminant.

***Mycobacterium bovis* (*M. bovis*):** The primary causative agent of tuberculosis in cattle, bison, and cervids; may also affect a variety of mammals including pigs, humans, primates, and non-domestic ungulates.

***Mycobacterium tuberculosis* (*M.tb*):** The primary causative agent of tuberculosis in humans; may also affect a variety of animals, including primates, pigs, cattle, dogs, parrots, elephants, and rhinos.

***Mycobacterium tuberculosis* complex (*M.tb* complex):** A group of mycobacteria which includes *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, *M. canetti*, *M. caprae*, and *M. pinnipedii*. A vaccine strain derived from *M. bovis* (*M. bovis* BCG) is sometimes listed as a separate member of this complex.

No isolation: Absence of growth of *M. tb* complex organisms from trunk wash, feces, tissue or other samples using standard mycobacterial culture methods. Failure to isolate organisms may be due to the following reasons:

1. The animal is not infected
2. The animal was not shedding at the time of sample collection
3. Sampling error (culture overgrowth by contaminating organisms, inadequate sample, or laboratory error)
4. Improperly handled or shipped sample

Non-reactive: Absence of response; in the context of serological testing for TB in elephants, a non-reactive result indicates that an antigen-antibody reaction has not occurred in the presence of an appropriate positive control response.

Non-tuberculous mycobacteria (NTM): Mycobacteria that generally do not cause the formation of granulomas. Most NTM are saprophytes found in soil or water. They are typically non-pathogenic but may occasionally cause infection in humans and animals, including elephants. Also referred to as “atypical” mycobacteria or “Mycobacteria Other Than TB” (MOTT).

Nucleic acid amplification test: A technique that amplifies entities such as DNA or RNA.

PCR (polymerase-chain reaction): A nucleic acid amplification technique in which specific sequences of nucleic acid (DNA or RNA) are replicated, allowing for detection of target sequences.

Premises: A parcel of land containing elephants, administered by a person, government entity (city, county, state, region) or organization (zoological society, corporation).

Prevalence: The total number of cases of a specific disease in a given population at a given time.

Public contact: any situation in which an elephant is touched or the public is present in the same space as the elephant without solid barriers that would prevent potential aerosol or fomite transmission of microorganisms.

Rapid Test: see ElephantTB STAT-PAK[®] Assay

Reactive: Presence of response; in the context of serological testing for TB in elephants, a reactive result indicates that an antigen-antibody reaction has occurred.

Report date: The date the laboratory reports the results.

Spoligotyping: A genotyping assay

Variable number of tandem repeats (VNTR): A genotyping assay

Submission date: The date the sample is received at the laboratory.

Test date: The date the sample is collected.

Tested elephant: An elephant that has been tested for tuberculosis according to the protocol established in these guidelines.

Triple sample method: A method of culture collection whereby 3 samples are obtained on separate days.

Trunk wash: A procedure used in elephants to obtain a sputum sample using one of the approved methods outlined in Section 4 – Culture Collection Procedure.

Sensitivity: A measure of the ability of a test to identify infected animals. Sensitivity is the frequency of a positive or abnormal test result (e.g. a test that is outside of the reference interval) when infection is present (i.e. the percentage of true positive results). Sensitivity = $[\text{TP} \div (\text{TP} + \text{FN})] \times 100$ where TP = true positive; FN = false-negative).

Specificity: A measure of the ability of a test to identify non-infected animals. Specificity is the frequency of a negative or “normal” test result when infection is absent (i.e. the percentage of true-negative (TN) test results). Specificity = $[\text{TN} \div (\text{TN} + \text{FP})] \times 100$.

Untested elephant: An elephant is considered “untested” if it has not had three trunk washes obtained by the method outlined in this protocol within a 12 month period; or if fewer than three valid culture results are obtained; if it has not been tested with the ElephantTB STAT-PAK[®] Assay performed by a USDA-employed veterinarian trained and certified to perform the test; or a reactive Stat-Pak test that has not been followed by a MAPIA.

3. ANNUAL TESTING

To adequately address the concerns of TB in the general elephant population, all captive elephants must be tested annually by culture and with the ElephantTB STAT-PAK[®] Assay (a blood test. Currently samples for official testing must be collected under the supervision of a USDA, Animal Care employee or State Employed veterinarian. The test must be performed by a trained USDA or State employed veterinarian or sent to a certified laboratory. See further information below under ElephantTB STAT-PAK[®] Assay. It is required that elephants with a reactive ElephantTB STAT-PAK[®] Assay result be tested using the confirmatory MultiAntigen Print ImmunoAssay (MAPIA[™]). See item 5 below.

Elephants should be tested within \pm 30 days of the established annual test date. Blood for ElephantTB STAT-PAK[®] Assay and culture should be collected within a 2 week period. All elephants must be tested every calendar year. Note that the date the sample is collected is the “test date,” the date the sample is received at the laboratory is the “submission date,” and the date the laboratory reports the results is the “report date.”

Record keeping of TB testing and treatment by the attending veterinarian is of utmost importance. It is recommended that attending veterinarians maintain open communication with the United States Department of Agriculture (USDA) and State Veterinarian, particularly concerning elephants under treatment for TB or in cases of exposure to TB positive elephants. It is recommended that at least a 1 ml aliquot of sera collected at the time of TB testing be sent to the elephant serum bank (See appendix 7).

4. CULTURE COLLECTION PROCEDURE (also see Appendix 3)

Samples for culture must be collected by or under the supervision of a licensed veterinarian using the “triple sample method.” This method consists of obtaining three samples from the trunk on separate days. If possible, collect samples within a seven-day period. Do not pool samples. Samples should be taken after water has been withheld for at least two hours to reduce sample dilution and contamination. Light exercise prior to collection may facilitate obtaining secretions from lower in the respiratory tract, which is desirable. Of the following methods, the trunk wash with bag seems to provide the most effective way to collect samples at this time. **Samples collected by swab are not acceptable.** As there is a risk of human exposure to sputum produced during this procedure, personal protective measures are recommended for personnel during sample collection. These should include gloves, eye protection, and N-95 respirators (or superior respiratory protection) certified by the National Institute for Occupational Safety and Health (NIOSH) to protect against TB (see Employee Health and Safety). Respirator users must be properly fit-tested and trained on the use of respirators.

A. Trunk wash with bag (or other suitable container) - Using a catheter tip syringe, instill 60 ml sterile saline into the trunk. Raise the trunk as high as possible to distribute the fluid deeper into the trunk. Lower the trunk and place a clean, one-gallon plastic bag over the end of the trunk and hold in place until the elephant exhales into the bag. Transfer at least 20 ml of the sample to a sterile leak proof, screw-top container. Sterile 50-ml conical screw-top plastic centrifuge tubes are preferred and are available free of charge from the National Veterinary Services Laboratories (NVSL) – call 515-337-7388.

B. Trunk wash - Using a 14 French feeding tube, introduce 60 ml of sterile saline into the trunk then aspirate. Transfer at least 20 ml of the sample into sterile leak proof, screw-top container. Methods A and C are preferable to this method.

C. Forcible exhalation – Mucous collected without instilling saline into the trunk is acceptable if elephants are trained to forcibly exhale into a clean plastic collection bag and the volume collected is at least 20 ml. This may allow sampling of secretions from other areas of the respiratory tract and may be a preferable sample. Transfer the sample into sterile, leak proof, plastic screw-top container.

Storage

Do not expose samples to sunlight or heat. Consult receiving laboratory to determine whether samples should be refrigerated or frozen prior to shipment. As standard frost-free freezers undergo cyclic freeze-thaws to limit frost, freezers that do not have this feature are preferred. Freezing at -80°C (ultra-low temperature freezer) is also acceptable. **Frozen samples must be shipped within 2 weeks of sample collection to the testing lab.**

Packaging and Shipping

All three refrigerated or frozen samples may be submitted together. **Label containers with the animal ID and date of collection** and put the same information on the submission form. Place screw-top containers in double zip-lock bags with an absorbent pad in case of leakage. **Do not send samples in glass containers or packaged only in plastic bags.** Sterile 50-ml conical plastic centrifuge tubes with lids sealed with parafilm or electrical tape are preferred.

Place samples on ice packs or dry ice and ship overnight via Federal Express, Airborne, or other overnight carrier. **Do not ship by U.S. mail** as samples may be irradiated which will render them unacceptable. Packaging and shipping should be in accordance with the International Civil Aviation Organization Technical Instructions for the Safe Transport of Dangerous Goods by Air 2009-2010 (<http://www.icao.int/publications/pages/publication.aspx?docnum=9284>). (Also helpful is the WHO document “Guidance on Regulations for the Transport of Infectious Substances 2011-2012 (WHO/HSE/IHR/2010.8) (http://whqlibdoc.who.int/hq/2010/WHO_HSE_IHR_2010.8_eng.pdf).

Packaging and shipping samples and cultures should be in accordance with Department of Transportation regulations – 49 CFR Parts 171, 172, 173 and 175- Hazardous Materials: Infectious Substances; Harmonization with the United Nations Recommendations; Final Rule, published June 2, 2006 in the Federal Register.

http://isu1.indstate.edu/terc/infectiousmaterial/pdf/Hazardous%20Materials_Infectious%20substances%20harmonization%20with%20the%20UN%20final%20rule.pdf

http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&tpl=/ecfrbrowse/Title49/49cfrv2_02.tpl

Send samples to NVSL or other laboratory facility offering comparable procedures for identification of mycobacteria species. When submitting samples to NVSL, use VS Form 10-4, Specimen Submission Form. This form is available online in Word or pdf format:

http://www.aphis.usda.gov/animal_health/lab_info_services/forms_publications.shtml.

Request mycobacterial culture with species differentiation.

Positive cultures from laboratories that do not have the capability to differentiate *M. tuberculosis* complex organisms must be forwarded to NVSL or other qualified laboratories for speciation. Culture of mycobacteria requires a minimum of eight weeks. **Laboratory reports that do not provide a definitive result due to contamination/overgrowth or other causes are considered invalid.** Additional samples should be collected and resubmitted to replace those reported as contaminated.

Note: Other mycobacteria species such as *M. avium*, *M. kansasii*, *M. elephantis*, and *M. fortuitum* have been isolated from elephants. At this time, there is no substantive evidence that these organisms are pathogenic for elephants with the exception of one reported case of *M. avium* (Yong 2011). *Mycobacterium szulgai*, an unusual non-tuberculous mycobacterium, has also been associated with pathology in elephants (Lacasse 2007).

5. ELEPHANT TB STAT-PAK[®] ASSAY SAMPLE COLLECTION PROCEDURE

Blood collection for the Guideline-required elephant TB STAT-PAK Assay must be collected under the supervision of a USDA, Animal Care veterinarian or ACI or State Employed veterinarian. The test must be performed by a trained USDA or State employed veterinarian or sent to a certified laboratory. It is advisable to also bank a serum sample. Blood from elephants with reactive Elephant TB STAT-PAK[®] Assay results must be submitted for MAPIA[™] /DPP[®] testing to:

Chembio Diagnostic Systems, Inc.

3661 Horseblock Road

Medford, NY 11763

Tel: 631-924-1135 Press 2

Fax: 631-924-6033

Email: customerservice@chembio.com

Contact Chembio for shipping instructions.

The USDA-employed veterinarian is responsible for shipping the sample but the owner must pay for shipping and must contact Chembio to arrange payment for the MAPIA[™] or DPP[®] test.

6. ANCILLARY SCREENING / DIAGNOSTIC TESTS

A number of other ante mortem tests have been under investigation to diagnose TB in elephants. Following is a summary of those tests and current recommendations for their use.

Intradermal Tuberculin Test

A correlation between the intradermal tuberculin test (skin test) and culture results has not been established (Mikota 2001, Lewerin 2005). Therefore, intradermal tuberculin testing cannot be deemed reliable for screening or diagnosis and is not recommended.

Enzyme Linked Immunosorbent Assay (ELISA)

A multiple antigen ELISA was developed at the Animal Population Health Institute at Colorado State University (Larsen 2000). This test was used for detecting the presence of elephant serum antibodies to mycobacteria and investigations showed high sensitivity and specificity for detecting infected elephants and monitoring elephants over time. However, ELISA testing is not currently available.

Acid Fast Smears

Acid fast stains of trunk wash smears or other tissue are not reliable indicators of tuberculosis when used as a sole diagnostic test.

7. TB MANAGEMENT GROUPS (1-4)

All elephants will fall into one of four management groups (1-4) based on test results or will be untested (group 5). A culture positive elephant is defined as an elephant from which *Mycobacterium tuberculosis* or *Mycobacterium bovis* has been isolated from any bodily site or specimen. **A culture positive elephant is considered positive until it has met the treatment requirements as outlined for Group 4.** Exposure history has been incorporated into the Guidelines as ongoing data collection has indicated that it is an important risk factor.

GROUP 1: Culture negative; ElephantTB STAT-PAK[®] non-reactive; no known exposure with culture positive elephant in past 12 months.

Monitor annually by culture (triple sample method) and ElephantTB STAT-PAK[®] (single serum sample collected concurrently).

- No treatment or travel restrictions.
- No elephant should move into a facility where there is an untested elephant.
- If an elephant has had contact with other untested elephants in the previous 6 months, then a STAT-PAK[®] test should be repeated in 6 months to confirm non-reactive status. If the ElephantTB STAT-PAK[®] remains non-reactive, the elephant continues in Group 1.

GROUP 2: Culture negative; ElephantTB STAT-PAK[®] non-reactive; exposure to culture positive animal within the last 12 months*.

Monitor by culture (triple sample method) and ElephantTB STAT-PAK[®] every 6 months for three years following cessation of exposure.

- No travel or public contact until 1 additional non-reactive ElephantTB STAT-PAK[®] test

is performed at 6 months post-exposure (6 month restriction).

- If non-reactive at 6 months, travel/public contact restrictions removed as long as additional testing can be performed as outlined above.
- If the results during any of the follow-up testing become positive/reactive, the individual elephant will change group (see table).
- If the elephant remains culture negative, ElephantTB STAT-PAK non-reactive after three years of testing, the elephant moves to group 1.
- No elephant should move into a facility where there is an untested elephant.
- *As long as this individual elephant is with a culture positive elephant that has not been treated, the start date for the 12 months of exposure has effectively not started.

Follow-up status for Group 2 elephants after testing

Culture	STAT-PAK	MAPIA/DPP	New Group Status
Negative	Non-reactive	N/A	1 (after 3 years)
Negative	Reactive	Non-reactive	3B
Negative	Reactive	Reactive	3C
Positive			4

Individual elephant risk assessment can be requested from USDA since circumstances vary between facilities and herds.

GROUP 3: Culture negative; ElephantTB STAT-PAK[®] reactive

It is required that blood from elephants with reactive ElephantTB STAT-PAK[®] results be submitted for MAPIA[™] / DPP[®] testing (see item 5 above). Based on MAPIA[™]/DPP[®] results and exposure history, the elephant will fall into one of the following subgroups:

A. Culture negative; STAT-PAK[®] reactive, MAPIA[™]/DPP[®] non-reactive, no known TB exposure

Monitor by culture (triple sample method) and STAT-PAK every 6 months for the first 3 years after becoming ElephantTB STAT-PAK[®] reactive. Perform MAPIA[™] / DPP[®] if any STAT-PAK[®] test is reactive. If all cultures, STAT-PAK, and MAPIA[™]/DPP[®] remain negative/non-reactive during the three year test period, the elephant moves to group 1.

- No treatment or travel restrictions.
- If the culture becomes positive or the STAT-PAK remains persistently reactive during the 3 years of follow-up testing the individual elephant will change category (see table).
- No elephant should move into a facility where there is an untested elephant.

Follow-up status for Group 3A elephants after testing

Culture	STAT-PAK	MAPIA	New Group Status
Negative	Non-reactive	N/A	1 (after 3 years)
Negative	Reactive	Non-reactive	R (after 3 years)
Negative	Reactive	Reactive	3C
Positive			4

B. Culture negative; STAT-PAK® reactive, MAPIA™/DPP® non-reactive, known exposure to TB culture positive elephant (no time limit on exposure history)

Monitor by culture (triple sample method) and STAT-PAK every 6 months for three years post-exposure. Perform MAPIA™/DPP® if any STAT-PAK® test is reactive. If all cultures, STAT-PAK, and MAPIA™/DPP® are negative/non-reactive after three years, the elephant moves to group 1.

- No travel or public contact restrictions UNLESS the exposure to the TB culture positive elephant occurred within the last 12 months.

If contact within the last 12 months:

- Travel and public contact is restricted for the first year after exposure until 2 additional cultures and MAPIA™/DPP® are performed at 6 month intervals. If results are unchanged after the first year (i.e., STAT-PAK reactive, MAPIA™/DPP® unreactive, culture negative), travel and public contact restrictions are removed but elephant must complete additional 2 years of testing at 6 month intervals as outlined above.
- If the culture or MAPIA™/DPP® results change during any of the follow-up testing and become positive/reactive, the individual elephant will change group (see table).

Follow-up status for Group 3B elephants after testing

Culture	STAT-PAK	MAPIA	New Group Status
Negative	Non-reactive	N/A	1 (after 3 years)
Negative	Reactive	Non-reactive	R (after 3 years)
Negative	Reactive	Reactive	3C
Positive			4

Previously-treated previously culture positive elephants

- Culture positive elephants that have completed a course of anti-tuberculosis therapy may remain ElephantTB STAT-PAK® ± MAPIA reactive and fall into this group. They will not have travel/public contact restrictions unless there is a change in their results during follow-up testing (i.e., become culture positive and/or a change in MAPIA™/DPP® reactivity).
 - If USDA approved appropriate treatment has been documented, supported by a declining MAPIA reaction, and completed within the last 12 months, the elephant must follow the testing guidelines for group 3B as outlined above. Once three years of testing has been completed, the elephant may change group status according to the table above.
 - If USDA approved appropriate treatment has been documented and completed greater than 12 months ago, if STAT-PAK reactive, MAPIA non-reactive, the animal will be moved to group R. The elephant's group status may change if there is a positive culture and/or reactive MAPIA™/DPP® result during follow-up testing.

C. Culture negative; STAT-PAK® reactive, MAPIA™/DPP® reactive

Monitor by culture (triple sample method) and STAT-PAK by pooling daily trunk

wash for one week each month for the first year, then quarterly the following 2 years. Serological testing should be done every 6 months for 3 years; perform MAPIA™/DPP® if STAT-PAK® is reactive. If the elephant shows any signs compatible with TB disease (weight loss, increased respiratory sounds, respiratory discharges), daily trunk washes are highly recommended. Contact NVSL for additional advice on methods on pooling of samples.

- No travel or public contact until the first year of testing has been completed after initial detection of reactive serology.
- Treatment should be considered. If serological conversions are demonstrated to be recent (within the past 12 months) then prophylactic treatment can be used. If serological conversions are longer standing or unknown, then full treatment may be advisable. Individual cases should be evaluated in conjunction with USDA. If treatment is performed, the elephant may be able to travel and have public contact after 6 months of successful documented USDA approved treatment.
- If culture, STAT-PAK, or MAPIA™/DPP® results change during the testing the individual elephant will change group status (see table).

Follow-up status for Group 3C elephants after testing

Culture	STAT-PAK	MAPIA	New Group Status
Negative	Non-reactive	N/A	1 (after 3 years)
Negative	Reactive	Non-reactive	R (after 3 years)
Negative	Reactive	Reactive	3C (repeat for 3 years)
Positive			4

Note: The STAT-PAK® and MAPIA™/DPP® tests have been shown to be early indicators of TB infection. Retrospective studies have identified elephants that were serologically reactive months to years in advance of detection by culture (Greenwald 2009). Changes in patterns and intensity of antigen recognition by MAPIA can be used to detect waxing and waning of mycobacterial antibodies (Lyashchenko 2006). This may be useful in determining whether more frequent culture monitoring should be considered. While elephants may be seroreactive for years, there is now substantial evidence that frequent culture surveillance is required to detect elephants that are shedding. Contact NVSL if considering more frequent culture collection regarding pooling of samples to reduce costs.

Previously-treated previously culture positive elephants

- Culture positive elephants that have completed a course of anti-tuberculosis therapy may remain ElephantTB STAT-PAK®/MAPIA™ reactive and fall into this group. They will not have travel/public contact restrictions unless there is a change in their results during follow-up testing (i.e., become culture positive).
 - If USDA approved appropriate treatment has been documented, with evidence of declining antibody response on MAPIA, and completed within the last 12 months, the elephant must follow the testing guidelines for group 3C as outlined above. Once three years of testing has been completed, the elephant may change group status according to the table above.
 - If USDA approved appropriate treatment has been documented, with evidence

of declining antibody response on MAPIA, and completed greater than 12 months ago, these animals will be moved to group R. The elephant's group status may change if there is a positive culture and/or reactive MAPIA™/DPP® result during follow-up testing.

Group R. Persistently ElephantTB STAT-PAK® reactive elephants.

Monitor annually by culture (triple sample method) and ElephantTB STAT-PAK® (single serum sample collected concurrently).

- No treatment or travel restrictions.
- No elephant should move into a facility where there is an untested elephant.
- If the ElephantTB STAT-PAK® is reactive, a MAPIA™/DPP® must be performed.
- If the elephant remains STAT-PAK® reactive, culture negative, MAPIA™/DPP® non-reactive, the elephant stays in group R and continues annual testing as outlined above. If any test results change, the group status will change according to the table below.

Follow-up status for Group R elephants after testing

Culture	STAT-PAK	MAPIA	New Group Status
Negative	Non-reactive	N/A	1 (requires 3 years of negative results)
Negative	Reactive	Non-reactive	R
Negative	Reactive	Reactive	3C
Positive			4

Elephants may develop antibodies to mycobacterial antigens months to years prior to detection by culture, however, the time intervals between exposure, seroconversion, and shedding are not precisely known. Numerous variables such as age, genetics, immune status, nutritional condition, other concurrent health problems, and other factors influence the development of infection in an individual animal following exposure to a pathogenic agent. Results of MAPIA™/DPP® testing are useful in helping determine potential risk categories as defined above and determine which animals require more frequent surveillance or should undergo prophylactic treatment (Greenwald 2009). In elephants that have undergone treatment for tuberculosis, it has been shown that the MAPIA™/DPP® will decline and may indicate a response to treatment so on-going annual monitoring with STAT-PAK® and MAPIA™/DPP® if STAT-PAK® is reactive, is required for life as changes in serology may detect relapse (Lyashchenko 2006).

It is important to review history for possible contact to a culture positive animal or previous treatment for TB since this may also affect results. Nonetheless, it is important to monitor these elephants for possible development of infection and disease. Retrospective analyses of banked serum samples are strongly encouraged to provide a more complete serological history.

Elephants that are culture negative, ElephantTB STAT-PAK® reactive and MAPIA™/DPP® reactive are at increased risk of TB infection. Factors to consider in the decision to administer treatment vs. increased monitoring include exposure history, age, whether the elephant travels, potential exposure of personnel or public, side effects of treatment, concurrent health problems, etc. Increased monitoring and travel/public contact restrictions is required based on risk. If culture results during any of the follow-up testing become positive, the individual elephant will

move to Category 4.

Consideration should be given to minimizing or eliminating contact with the public that would result in exposure by contact or aerosol transmission and to providing personal protective equipment such as a NIOSH certified N95 respirator /N95 face mask for staff when working in close proximity to elephants that are under enhanced surveillance. Employees must be respirator fit tested and trained in the use of the respirator before they use the N95 respirator.

Based on a history of exposure to a culture positive animal, or other considerations, the attending veterinarian may elect to administer prophylactic or full treatment after consultation with USDA. Effective prophylactic therapy is defined as the administration of a specific number of doses of two anti-TB drugs within a specified time. It must be demonstrated that adequate anti-TB drug levels are achieved in the blood of the elephant under treatment. Acceptable anti-tuberculosis drugs include isoniazid (INH), rifampin (RIF), pyrazinamide (PZA), ethambutol (ETH), or a fluoroquinolone such as levofloxacin, moxifloxacin, ciprofloxacin, or enrofloxacin. Isoniazid is recommended as one of the two drugs if a known exposure case isolate is INH sensitive. PZA should not be given if *M. bovis* infection is suspected since this organism is inherently resistant to PZA.

Prophylactic therapy is for 9 months can be administered using either of the following schedules:

Prophylactic Treatment Schedule 1 (preferred):

Administer two anti-TB drugs daily for 9 months (270 total doses). The first 60 doses should be administered within a period of 90 days (i.e. no more than 30 days of “refused medication” should occur. It must be documented that the elephant received 270 total doses at a dosage level sufficient to achieve adequate drug serum levels. First line drugs should be used unless there has been an exposure to an elephant with a drug resistant strain.

Prophylactic Treatment Schedule 2:

Administer the two anti-TB drugs daily for two months (as above, the first 60 doses should be administered within a period of 90 days). Adequate levels of both drugs must be demonstrated in two serum samples collected approximately two weeks apart. Serum samples should be collected as soon as the elephant is accepting medication reliably. If acceptable levels (see below) are not achieved, the dosage should be adjusted and serum levels tested again (two samples collected approximately two weeks apart). It must be documented that the elephant received the first 60 doses at a dosage level sufficient to achieve adequate drug serum levels. Once this has been demonstrated, administer the two drugs every other day but at twice the previous dosage level for an additional 9 months (105 total doses of every other day dosing plus the initial 60 doses for a total of 165 doses). It is not necessary to repeat serum drug levels when changing to the every other day schedule.

Note: Pyridoxine 50 mg is administered to humans receiving INH for treatment of active or latent tuberculosis to prevent the development of peripheral neuropathy. Although this side effect has not been reported in elephants, it may be possible. At the discretion of the attending veterinarian, Vitamin B6 (pyridoxine) can be given prophylactically at a dose of 0.8-1 mg/kg

daily.

Concomitant use of INH, rifampin, and PZA with other hepatotoxic drugs should be done with caution.

Refer to TB Drugs section for starting dosages, routes of administration, side effects, blood levels, and other information.

Monitoring of Prophylactically Treated Elephants

During the 9 months of treatment, elephants should be closely observed for changes in appetite, behavior, and any other signs that may be attributable to adverse drug effects. Monthly blood tests (CBC and serum chemistry profile) are recommended to monitor general health and possible drug effects on the liver. Liver tests (AST, ALT, LDH, bile acids, and bilirubin) should be included in the serum chemistry panel. Elevations in these enzymes have been associated with treatment (Dumonceaux 2011). Isoniazid may cause hepatitis and anemia. In addition, leukopenia has occurred in at least one elephant apparently due to INH toxicity).

GROUP 4: *M. tuberculosis* complex positive culture

Animals that have had *Mycobacterium tuberculosis* complex isolated from any sample (sputum, stool, tissue, etc.) are considered culture positive for TB. A culture positive elephant is defined as an elephant from which *Mycobacterium tuberculosis* complex organism has been isolated from any bodily site or specimen.

The ElephantTB STAT-PAK[®] and MAPIA[™]/DPP[®] tests must be performed on blood from culture positive elephants. Serum for MAPIA[™]/DPP[®] testing must be submitted regardless of ElephantTB STAT-PAK[®] results.

Positive cultures must be submitted to NVSL for genotyping.

A culture positive elephant is considered positive until it has met the treatment requirements as outlined below. These elephants must be separated from the public for the duration of the treatment period. Separation from previously non-exposed elephants is also recommended until treatment is completed. Precautions to safeguard personnel health and safety should be instituted immediately (see Employee Safety and Health section). Elephants with cultures that yield non-tuberculous strains of mycobacteria are not considered infected and are not a risk to other animals or humans. Options for Category 4 elephants include:

Options:

A. Treatment: This is the preferred option for culture positive elephants whenever possible.

1. If the organism was isolated at a laboratory other than NVSL and they do not perform mycobacterial species differentiation and genotyping, the owner must request that the laboratory submit the isolate to NVSL or other qualified laboratory for mycobacterial species differentiation and DNA fingerprinting.
2. Antimicrobial sensitivity testing should be performed on all positive isolates. Sensitivities should be requested for the following drugs: isoniazid, rifampin, pyrazinamide, ethambutol,

ciprofloxacin (or other fluoroquinolone), and amikacin. (Antimicrobial susceptibility testing for *M. tuberculosis* complex organisms is now available at NVSL).

3. Perform ElephantTB STAT-PAK[®] and MAPIA[™] every 6 months during treatment then every 6 months for 3 years according to the group that the elephant falls into post-treatment. Serological monitoring of treated elephants with MAPIA[™] has shown changes that may indicate successful treatment or recrudescence of infection (Lyashchenko 2006).

4. Beginning with the onset of treatment, cultures should be collected by the triple sample method every 2 months for the first 6 months of treatment, then every 6 months for the remainder of the elephant's life. This culture schedule aids in determining if the animal is shedding during the treatment period and after treatment has ended, although a negative culture does not guarantee that it would not pose a risk to animals or humans. More frequent monitoring by culture is highly recommended and contacting NVSL to work out an appropriate and cost-effective monitoring program is suggested.

5. Pending antimicrobial susceptibility results, initiate empiric therapy with 3 or 4 of the following drugs: isoniazid, rifampin, pyrazinamide, and ethambutol or a fluoroquinolone (moxifloxacin is preferred). Following the human model, initiating empiric treatment with four drugs is considered "ideal." However, the difficulties associated with training an elephant to accept medications are acknowledged. After determining sensitivities, continue treatment using one of the following schedules:

Schedule 1 (preferred): Administer 3 drugs to which the isolates are susceptible daily for 2 months. The first 60 doses should be administered within a period of 90 days (i.e. no more than 30 days of "refused medication" should occur). Adequate blood levels of all 3 drugs must be demonstrated in two samples collected approximately two weeks apart. Serum samples should be collected as soon as the elephant is accepting medication reliably. If acceptable levels (see below) are not achieved, the dosage should be adjusted and serum levels tested again (two samples collected approximately two weeks apart). It must be demonstrated that the elephant received the first 60 doses at a dosage level sufficient to achieve adequate drug serum levels. Treatment is then continued daily for an additional 10 months with 2 drugs to which the isolate is susceptible for a total number of doses (with two drugs) of 300. As above, the inclusion of INH is recommended. The total number of doses for the entire treatment is 360. The entire treatment should be completed within 15 months (this allows for "refused medicine" days and periods of interruption that may be needed if side effects are noted).

Schedule 2: Administer 3 drugs to which the isolate is susceptible daily for 2 months. The first 60 doses should be administered within a period of 90 days (i.e. no more than 30 days of "refused medication" should occur). Adequate levels of all drugs must be demonstrated in two samples collected approximately 2 weeks apart. Serum samples should be collected as soon as the elephant is accepting medication reliably. If acceptable levels (see below) are not achieved, the dosage should be adjusted and serum levels tested again (two samples collected approximately two weeks apart). It must be demonstrated that the elephant received the first 60 doses at a dosage level sufficient to achieve adequate drug serum levels. Continue treatment with two drugs at twice the dosage used in the initial period every other day for 10 months (150

doses). It is not necessary to repeat serum drug levels. The total number of doses is 210. The entire treatment should be completed within 15 months (this allows for “refused medicine” days and periods of interruption that may be needed if side effects are noted). Animals that have not completed treatment are considered as non-treated.

Note: Peripheral neuropathy can sometimes occur in humans receiving INH. Although this side effect has not been reported in elephants, it may be possible. At the discretion of the attending veterinarian, Vitamin B6 (pyridoxine) can be given prophylactically at a dose of 1 mg/kg daily.

Travel: Elephants in Group 4 should not travel or have public contact until treatment is completed according to the guidelines.

Additional Monitoring of Treated Elephants

Elephants should be closely observed for changes in appetite, behavior, and any other signs that may be attributable to adverse drug effects. Monthly blood tests (CBC and serum chemistry profile) are recommended to monitor general health and possible drug effects on the liver. Liver tests (AST, ALT, LDH, bile acids, and bilirubin) should be included in the serum chemistry panel. Isoniazid may cause liver damage and anemia. In addition, leukopenia has occurred in at least one elephant apparently due to INH toxicity).

B. Quarantine without treatment: This option may be considered especially for animals that are already housed alone and not considered a good candidate for treatment (ex. bull elephant). Additional precautions must be taken for human safety (such as the use of N-95 masks, gloves, etc.). Assessment of air movement patterns in buildings housing quarantined elephants is recommended for animal and human health issues. Quarantined elephants should be kept out of range from non-infected animals and should be monitored for signs of TB.

- No travel is permitted.
- No public contact that would result in exposure by contact or aerosol transmission is permitted.
- No exposure to other elephants is permitted.
- Additional testing (trunk wash culture, ElephantTB STAT-PAK[®]/MAPIA[™]/DPP[®]), ancillary tests and nucleic acid amplification are recommended for data collection.

C. Euthanasia: This option may be considered for those animals that are showing clinical signs considered to be poor candidates for treatment, or for other factors based on the clinician’s discretion. A thorough postmortem examination must be performed (see section 11).

Group 5: Untested If an elephant cannot complete procedures as outlined for official annual testing, it should not be permitted to have public contact that would result in exposure by contact or aerosol transmission, or contact with other tested elephants (or their enclosures or equipment). Untested elephants should not be moved from their home facilities. A tested elephant should not move into a facility housing an untested elephant unless it can be demonstrated that there will be no direct or indirect contact with the untested elephant including possible aerosol transmission. If a tested elephant(s) is exposed to an untested elephant, the tested elephant cannot travel nor have public contact until the untested elephant is tested unless approved by USDA.

8. PRINCIPLES OF ANTI-TUBERCULOSIS THERAPY

The American Thoracic Society has published guidelines for the treatment of tuberculosis in humans (see references). In brief, it is necessary to treat active TB with multiple drugs to prevent the emergence of resistant strains of bacteria. For individuals exposed to TB (positive skin test), but no signs of active disease (negative chest radiograph, negative sputum cultures), treatment is typically with a single drug (INH).

The guidelines for the treatment of TB in elephants are based on the assumption that animals with known infection are treated similarly to humans. However, for elephants, the treatment period has been extended. For a category 3 elephant with negative cultures and presumed exposure based on positive serologic response, i.e., positive ElephantTB STAT-PAK[®] (and MAPIA[™]), treatment is a “modified” regime – with two drugs for 9 months. Skin testing is not reliable in elephants. Acid-fast smears are not reliable on elephant trunk washes.

For humans, treatment of primary tuberculosis is to empirically administer 4 first line drugs while waiting for antimicrobial sensitivity testing. This assures that initial treatment includes at least 2 drugs to which the organism is susceptible. And, the additional number of antibiotics results in more rapid clearance of bacteria from the sputum thereby decreasing the public health risk.

Once susceptibility tests are received, and the sputum has reverted to being smear negative, the number of drugs is decreased to two first line drugs for the remainder of treatment. When the index case is known, and the index isolate is known to be susceptible to all anti-mycobacterial drugs, then initial treatment may be limited to three drugs. However, in the vast majority of cases the index case is not known with certainty and four drugs are given.

The length of therapy for humans is currently 6 months for active tuberculosis. This includes the initial period of 3-5 drugs as above and 2-drugs for the remainder of treatment. For individuals with resistance to a single antibiotic, treatment is extended to 12 months with 2 drugs to which the organism is susceptible. For individuals infected with multi-drug resistant tuberculosis (MDR-TB), treatment is for at least 12 months with 2-4 drugs based on the susceptibility pattern (lower numbers of agents are employed if the isolate is susceptible to INH or rifampin). Because the long term outcome and efficacy of treatment for TB of non-human species is currently unknown, treatment of elephants is structured for a 12-month course.

9. ANTI-TUBERCULOSIS DRUGS

Antituberculous agents are divided into first and second line agents. First line agents include isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin. These are agents with the greatest activity and the best side effect profiles. Second line agents include those with less activity and/or greater side effects. Second line agents include capreomycin, ethionamide, cycloserine, and thiacetazone. The fluoroquinolones (FQ; moxifloxacin, ciprofloxacin, levofloxacin, and enrofloxacin) while not considered as 1st line agents have significant bactericidal activity against *M. tuberculosis*. Moreover, published studies report the equivalency of FQ substitution for ethambutol in the treatment of TB in humans and studies are underway to

investigate FQ use for the treatment of latent TB infection. Linezolid, a drug active against Gram positive bacteria such as *Staphylococcus aureus*, MRSA, enterococcus, and VRE has also been shown to have significant activity against *M. tuberculosis* and has been used successfully in salvage regimens. Amikacin, an aminoglycoside (as is streptomycin), is a mainstay in the treatment of non-tuberculous mycobacterial infection and has been used in salvage regimens against MDR-TB. Pharmacokinetic studies of INH, RIF, EMB, and PZA in elephants have been published (Maslow et al. 2005a, Maslow et al. 2005 b, Zhu et al. 2005, and Peloquin et al. 2006).

FIRST LINE AGENTS

Isonicotinic acid hydrazide (Isoniazid, INH)

Mechanism of action: INH acts to inhibit cell wall synthesis through blockage in the mycolic acid pathway. The specific target enzymes are unknown; however, evidence supports a role for the catalase enzyme, *katG*, as modifying INH to an active form. Postulated targets of the activated form of INH include ketoacyl synthetase and *inhA*.

Metabolism and excretion: INH is acetylated in the liver through the action of *N*-acetyltransferase. The acetylated product is then excreted in the urine. Some ethnic groups (Native Americans, Alaskan Natives and Asians as well as others carry a recessive allele encoding for rapid acetylation of INH those results in more rapid clearance and lower bioavailability. It is not known whether elephants are polymorphic in this enzyme and differ in the speed of acetylation.

Toxicity: The major adverse effects documented in humans are hepatitis (principally hepatocellular inflammation with a transaminitis) and peripheral neuropathy. Uncommon adverse reactions include headaches, optic neuritis, seizures, psychosis, encephalopathy, twitching, rashes, and gastrointestinal upset. A histamine like reaction can be observed when products with tyramine (red wine, cheese) are ingested. Risk factors for hepatic toxicity in humans include age greater than 35 yr, concomitant viral hepatitis (Hepatitis B or C), and other hepatic toxins (drugs, alcohol). Vitamin B6 (pyridoxine) is given at a dose of 50 mg daily (~1 mg/kg) to prevent the development of peripheral neuropathy.

Toxicity in elephants: Observed toxicities of INH have included inanition, transaminitis, and anemia. Fermented products (mash or other feeds) should likely be avoided to minimize potential histamine reactions. Liver values (ALT, AST, LDH, bile acids, and bilirubin) should be monitored monthly for 2 months and then bimonthly if no liver toxicity is observed. INH has caused irreversible leukopenia in camels; reversible leukopenia has been observed in one elephant that was considered as possibly / probably related to INH.

Route of administration: In humans INH is administered orally. In elephants, INH is preferentially administered as an oral bolus. However, rectal absorption is efficient, yielding levels similar to oral bolus dosing. In bongo antelope, INH has also been successfully administered via intramuscular injection.

Pharmacokinetics

Food reduces oral absorption so INH is best given on an empty stomach. INH reacts with

reducing sugars so only non-reducing sugars such as sorbitol should be used in compounded preparations for elephants. INH is not stable in blood left at room temperature so samples for serum drug levels should be harvested and frozen quickly (Peloquin 2003).

Rifampin (RIF)

Mechanism of action: Rifampin is a semi synthetic derivative of rifamycin, an antibiotic derived from the fungus *Streptomyces mediterranei*. Rifampin acts to inhibit the DNA-dependent, RNA-polymerase thus blocking formation of messenger RNA (the first step in protein synthesis).

Metabolism and excretion: Rifampin is acetylated in the liver. Both the unaltered and acetylated drug is excreted into the bile. Rifampin is then reabsorbed whereas the acetylated form is not.

Toxicity: The major toxicity of rifampin is hepatitis. Other side effects include gastrointestinal upset, renal failure, hemolysis, acute renal failure, and thrombocytopenia. It is avoided in pregnancy during the first trimester because of possible teratogenicity.

Rifampin is also a strong inducer of the cytochrome P450 hepatic enzymes that may increase the metabolism of concurrently administered drugs. A prime example is exogenously administered steroids used for in vitro fertilization. For animals being treated for other conditions, potential drug-drug interactions should be ruled out.

Toxicity in elephants: The toxicity in elephants is unknown. Similar adverse reactions to humans should be expected. Therefore it is recommended that in addition to liver tests, serum creatinine, electrolytes and CBC be monitored per the schedule listed for INH.

Route of administration: Rifampin is administered to humans orally although intravenous administration is used in patients unable to tolerate oral dosing. In elephants rifampin appears to be absorbed well as an oral bolus although acceptance is low because of the drug's bitterness. Rifampin is not absorbed rectally; there is no known experience with parenteral administration in elephants or other animals. Urine and feces may become orange colored while on this drug.

Pharmacokinetics

Food decreases the C_{max} so rifampin should be given on an empty stomach (Peloquin 2003).

Pyrazinamide (PZA)

Mechanism of action: Pyrazinamide is a synthetic antibiotic derived from nicotinic acid. Its mechanism of action is unknown; however the presence of an intact pyrazinamidase is required. Since *Mycobacterium bovis* lacks this enzyme, it is resistant to PZA.

Toxicity: Toxicities observed in humans include arthralgias and arthritis, hyperuricemia, hepatitis, gastrointestinal upset, and photosensitivity (skin rashes).

Toxicity in elephants: The toxicity for elephants is unknown, however hepatitis may have been observed. Similar adverse effects as documented for humans should be expected.

Route of administration: In humans, pyrazinamide is administered orally. In elephants both oral and rectal dosing have yielded acceptable blood levels. Pyrazinamide has been successfully administered to bongo antelope via subcutaneous injection.

PZA is should not be given if *M. bovis* infection is suspected since this organism is inherently resistant to PZA.

Pharmacokinetics

PZA is reliably absorbed (Peloquin 2003).

Ethambutol (EMB)

Mechanism of action: Ethambutol is a specific inhibitor of the arabinosyl transferase thereby inhibiting formation of arabinogalactose and lipoarabinomannan, which are the dominant lipids in the *M. tuberculosis* cell wall.

Toxicity: The major toxicity of ethambutol is optic neuritis, which may result in decreased visual acuity, a central scotoma, and loss of red-green discrimination. Ethambutol may also cause peripheral neuropathy, headache, rashes, arthralgias, hyperuricemia, and rarely anaphylaxis.

Toxicity in elephants: The toxicity for elephants is currently unknown.

Route of administration: Ethambutol is administered orally to humans and elephants. Rectal administration may be irritating and poorly tolerated resulting in expulsion of the drug although it has been successfully administered in some cases subcutaneous administration has been given successfully to bongo antelope.

Pharmacokinetics

Food does not significantly reduce absorption; however, antacids should be avoided within 2 hours of EMB dosing (Peloquin 2003).

Streptomycin

Mechanism of action: Streptomycin is an aminoglycoside antibiotic derived from the fungus *Streptomyces griseus* that acts on the 30S ribosome to inhibit protein synthesis.

Toxicity: Similar to other aminoglycosides, streptomycin administration may result in auditory-vestibular and renal toxicity. Specific symptoms include ataxia, vertigo, nerve deafness, and renal failure. Most symptoms are reversible if the drug is discontinued immediately after their occurrence.

Toxicity in elephants: The toxicity for elephants is currently unknown but is likely the same as for humans.

Route of administration: Streptomycin is administered via intramuscular injection to humans.

There is no experience in administering streptomycin to elephants.

SECOND LINE AGENTS

Fluoroquinolones: Moxifloxacin, Ciprofloxacin, Levofloxacin, Enrofloxacin

Mechanism of action: Fluoroquinolone antibiotics act to inhibit the topoisomerases DNA gyrase and topoisomerase IV. Both of these enzymes are needed during DNA replication to first unwind supercoiled DNA and then to again achieve a supercoiled structure of DNA. Of the commercially available fluoroquinolones, moxifloxacin has the greatest in vitro activity and in vivo activity in a mouse model of infection followed by ciprofloxacin and levofloxacin (Neurmberger EL et al, Moxifloxacin-containing regimens of reduced duration produce a stable cure in murine tuberculosis, *Am J Respir Crit Care Med* 2004, 170: 1131-4). The anti-tuberculous activity of enrofloxacin, a derivative of ciprofloxacin is unknown. Gatifloxacin also has excellent in vitro activity against strains of TB, although the drug was recently withdrawn due to reports of antibiotic associated diarrhea and QT-prolongation. Studies are underway examining the role of Moxifloxacin in standard treatment and prophylaxis regimens (Burman et al. Moxifloxacin versus ethambutol in the first 2 months of treatment for pulmonary tuberculosis. *Am J Respir Crit Care Med* 2006, 174: 331-8; Pletz MW et al. Early bactericidal activity of moxifloxacin in treatment of pulmonary tuberculosis: a prospective, randomized study, *Antimicrob Agents Chemother* 2004, 48: 780-2).

Toxicity: The quinolone antibiotics may result in arthropathy, cartilage defects in adolescent animals, photosensitivity, antibiotic related diarrhea, and electrocardiographic prolongation of the QT interval.

Toxicity in elephants: The toxicity for elephants is unknown.

Route of administration: These agents are administered either orally or intravenously (levofloxacin only). Oral levofloxacin has been administered to bongo antelope, although poor serum levels were observed. Oral levofloxacin has been used to successfully treat a *Klebsiella spp.* infection of the hock in a horse. (J Maslow, personal communication). Enrofloxacin has been used to treat one elephant with disseminated multi-drug resistant TB as part of a multi-drug regimen. The animal developed photo-induced blepharitis, although this adverse effect had been episodic during infection and was initially detected prior to the institution of enrofloxacin. Thus, the causal association to enrofloxacin is unknown.

Pharmacokinetics

Food may reduce C_{max} slightly; avoid dairy products at the same time. If using antacids, sulcralfate, or multivitamins containing minerals administer 4-6 hours apart (Peloquin 2003).

Amikacin

Mechanism of action: Amikacin is an aminoglycoside antibiotic that acts on the 30S ribosome to inhibit protein synthesis. Isolates that are resistant to streptomycin may be susceptible to amikacin.

Toxicity: Similar to other aminoglycosides amikacin administration may result in auditory-vestibular and renal toxicity. Specific symptoms include ataxia, vertigo, nerve deafness, and renal failure. Most symptoms are reversible if the drug is discontinued immediately after their occurrence.

Toxicity in elephants: The toxicity for elephants is currently unknown but is likely the same as for humans.

Route of administration: Amikacin is administered via intravenous injection to humans. Amikacin has been administered via intramuscular injection to bongo antelope yielding acceptable serum levels (unpublished). A pharmacokinetic study of amikacin in African elephants has been conducted (Lodwick, L.J., Dubach, J.M. and Phillips, L.G., 1994. Pharmacokinetics of amikacin in African elephants. *J Zoo Anim. Med* 25: 367-375). There is no published information regarding amikacin in Asian elephants. Amikacin in one Asian elephant given IM 3 times a week at 14 mg/kg yielded good blood levels (acceptable levels in elephants unknown) and was eliminated almost completely from serum within 72 hours. However, significant toxicity occurred with prolonged use of this drug at this dose (personal communication, Dr. G Dumonceaux).

Other second line agents have not been used for mycobacterial infections in elephants. Clinicians contemplating the use of agents other than those listed should consult with the USDA on an individual basis.

The four first-line drugs used to treat tuberculosis in humans are isoniazid (INH), rifampin (RIF), pyrazinamide (PZA) and ethambutol (ETH). Second-line drugs used in cases of drug intolerance or multi-drug resistant organisms include amikacin and a fluoroquinolone. Both fluoroquinolones and linezolid have been used in cases of multidrug resistance in humans (Vezeris, N. et al. Fluoroquinolone-containing third-line regimen against *Mycobacterium tuberculosis* in vivo. *Antimicrob Agents Chemother* 2003, 47: 3117-22).

10. DOSAGES AND ROUTES OF ADMINISTRATION

Anti TB drugs must be directly administered. Placing drugs over food does not produce reliable blood levels and this is not an acceptable method of treatment. Drugs vary in palatability and acceptance so some experimentation may be required to determine a workable regimen for each individual elephant.

Isoniazid and PZA can be given either orally or rectally. Rifampin and ethambutol should only be administered orally (effective blood levels of rifampin cannot be achieved with rectal administration and ethambutol is quickly expelled when given rectally). Below are suggested starting doses, but actual doses may need to be adjusted in order to achieve adequate blood levels and / or reduce effects of toxicity.

Drug	Dosage (mg/kg)	Route	Formulation	Target conc.	Cmax (hr.)
------	-------------------	-------	-------------	-----------------	---------------

				(µg/ml)	
Isoniazid	5	Oral	premixed suspension	3-5	1-2
	4	Oral	Powder	3-5	0.5-1
	4	Rectal	premixed suspension	3-5	0.25-0.5
Rifampin	10	Oral only	Powder	8-24	2-4
Pyrazinamide	30	Oral or rectal	Powder	20-60	1-2
Ethambutol	30	Oral only	Powder	2-5	1-2

The dosages quoted above are based primarily on the pharmacokinetic studies of drug administration to the first herds of treated elephants as reported (Maslow et al 2005a, Maslow et al 2005b, Zhu et al 2005, Peloquin et al 2006). Recent studies have demonstrated that INH achieves C_{max} much more quickly than previously thought when administered rectally. Dosages are considered as estimates with the goal of achieving target serum concentrations as listed in #10 below without causing significant side effects that interrupt treatment. Serum drug levels or drug side effects may dictate that dosages be adjusted up or down accordingly. Sequential MAPIA™ tests may also be used to monitor response to treatment (Lyashchenko 2006). Second line agents should only be considered and administered following consultation with the facility USDA inspector.

11. BLOOD LEVELS

Target blood levels for elephants treated with each of the anti-tuberculosis drugs are based on the experience in humans. Target serum concentrations are listed in the table above. Blood levels approximating those found in humans have been reported for elephants with each of the four 1st line agents INH, RIF, PZA, and EMB (Maslow et al 2005a, Maslow et al 2005b, Zhu et al 2005, and Peloquin et al 2006).

Blood levels should be determined to measure the maximal concentration of drug (C_{max}). While INH, PZA, and EMB are rapidly absorbed with a C_{max} occurring between 1-2 hrs. drug absorption may vary between elephants and may also vary drug to drug. Recent studies have demonstrated that INH achieves C_{max} much more quickly than previously thought when administered rectally. Importantly, the time to C_{max} (T_{max}) may vary over the course of treatment due to multiple factors such as food intake, drug acceptance, etc. Thus, at the start of treatment and periodically through the course of therapy it is important to measure drug levels at multiple time points until C_{max} for each drug and animal is determined.

For INH, PZA, and EMB it is recommended that drug levels be determined at 1hr, 1.5hr, and 2 hr and for RIF at 2hr, 3hr, and 4hr except if INH is administered rectally and then 15 min and 30 min blood levels are recommended to accurately measure the C_{max}. If the first measured time point represents the greatest level for any drug, then T_{max} may have already passed and earlier time points should be assessed. Conversely, if the last measured time point represents the greatest concentration for any drug, then T_{max} may occur later than the range chosen and later time points should be assessed. During the initial phase of treatment, time ranges should always be assessed to determine the true T_{max}.

NOTE: Target blood levels for anti-TB drugs in elephants have not been rigorously established.

Until further studies can be conducted, target blood levels of anti-TB drugs for elephants must necessarily be based on human data. Although achieving blood levels comparable to humans is the ideal goal, the attending veterinarian should be aware that there is unpublished evidence that some elephants cannot tolerate anti-TB drugs at the doses required to achieve the above levels. Isoniazid, in particular, has caused side effects. It may be necessary to reduce the dose of an anti-TB drug to eliminate side effects, which may result in lower blood levels. The attending veterinarian should carefully document observed side effects, dosage changes and associated anti-TB drug levels in these cases. Variations to these Guidelines require consultation with the facility USDA inspector.

12. POSTMORTEM EXAMINATION

It is essential that a post-mortem examination be performed on all elephants that die. The examination must include a thorough search for lesions of tuberculosis regardless of exposure status. A comprehensive elephant necropsy protocol has been prepared by the Elephant SSP and is available at these websites:

www.elephanttag.org
www.elephantcare.org

Prior to any planned euthanasia of an elephant, trunk washes, blood for serology and any other ancillary tests should be performed regardless of whether or not TB is suspected. In this way, valuable data can be gathered to evaluate the efficacy of the current testing protocol. In the event of a sudden death, collect post-mortem blood and separate serum for other tests.

It is recommended that a trained veterinary pathologist direct the necropsy if possible. In the event of an elephant necropsy (elective or otherwise), contact Dr. Scott Terrell (Elephant SSP Pathology Advisor) for further instructions and possible participation:

Scott P. Terrell, DVM, Diplomate ACVP, SSP Pathology Advisor, Disney's Animal Kingdom, 1200 N Savannah Circle, Bay Lake, FL 32830, W (407) 938-2746; H (407) 251-0545; Cell (321)229-9363; email Scott.P.Terrell@disney.com

The following information is excerpted from the SSP Elephant Necropsy Protocol:

Protective equipment for tuberculosis cases - Mandatory

Respiratory protective equipment should be available during any elephant necropsy procedure regardless of the historical TB testing status of the animal. In animals with an unknown, suspect, or positive TB test history, respiratory protection should be considered **mandatory**. OSHA standards (29CFR1910.134) require that “workers present during the performance of high hazard procedures on individuals (humans) with suspicious or confirmed TB” be given access to protective respirators (at least N-95 level masks).

Similar precautions should be taken during an elephant necropsy. According to the draft CDC guidelines for the prevention of transmission of tuberculosis in health care settings, respiratory protective devices used for protection against *M. tuberculosis* should meet the following criteria:

1. Particulate filter respirators approved include (N-, R-, or P-95, 99, or 100) disposable respirators or positive air pressure respirators (PAPRs) with high efficiency filters)
2. Ability to adequately fit wearers who are included in a formal respiratory protection program with well-fitting respirators such as those with a fit factor of greater than or equal to 100 for disposable or other half-mask respirators
3. Ability to fit the different face sizes and characteristics of wearers. This can usually be met by supplying respirators in at least 3 sizes. PAPRs may work better than half-masks for those persons with facial hair.

Consult these websites for OSHA and CDC guidelines:

1. OSHA TB standards and rules: <http://www.osha.gov/SLTC/tuberculosis/>
2. . Guidelines for Preventing the Transmission of *Mycobacterium tuberculosis* in Health-Care Settings, 2005: <http://www.cdc.gov/tb/publications/guidelines/infectioncontrol.htm>
<http://www.cdc.gov/mmwr/pdf/rr/rr5417.pdf>

Necropsy procedures

All elephants undergoing necropsies should have a careful examination of the tonsillar regions and submandibular lymph nodes for tuberculous appearing lesions. These lymph nodes may be more easily visualized following removal of the tongue and laryngeal structures during the dissection. All lymph nodes should be carefully evaluated for lesions since other sites may also be infected (ex. reproductive or gastrointestinal tract). Collect any nodes that appear caseous or granulomatous for mycobacterial and standard bacterial culture (freeze or ultra-freeze), and fixation (in buffered 10% formalin). In addition, search thoracic organs carefully for early stages of TB as follows: after removal of the lungs and trachea, locate the bronchial nodes at the junction of the bronchi from the trachea. Use clean or sterile instruments to section the nodes. Freeze half of the lymph node and submit for TB culture to NVSL or a laboratory experienced in mycobacterial culture and identification (**even if no lesions are evident**). Submit sections in formalin for histopathology. Carefully palpate the lobes of both lungs from the apices to the caudal borders to detect any firm B-B shot to nodular size lesions. Take **NUMEROUS (5 or more)** sections of any suspicious lesions. Open the trachea and look for nodules or plaques and process as above. Regional thoracic and tracheal lymph nodes should also be examined and processed accordingly. Split the trunk from the tip to its insertion and take samples of any plaques, nodules or suspicious areas for TB diagnosis as above. Look for and collect possible extra-thoracic TB lesions, particularly if there is evidence of advanced pulmonary TB.

13. CONSIDERATIONS FOR PROTECTING EMPLOYEES FROM TUBERCULOSIS INFECTION IN THE WORKPLACE

Any facility housing an elephant with suspected or known infection with *M. tuberculosis* or *M. bovis* should implement an occupational health program to protect employees from tuberculosis infection. The program should include, but is not limited to: pre-placement tuberculosis screening using a two-test approach, annual evaluation for tuberculosis infection in workers, guidelines for the use of personal protective equipment including a robust respiratory protection program, procedures for medical surveillance and reporting of employees with tuberculosis infection or disease, and infection control guidelines to reduce direct and indirect aerosol

transmission. The following resources are provided to aid you in developing a robust program.

The Occupational Safety and Health Agency (OSHA) is a federal regulatory agency to assure safe and healthful working conditions for working men and women by setting and enforcing standards and by providing training, outreach, education and assistance. OSHA has regulations for recording and reporting tuberculosis infection acquired in the workplace. These are outlined at <http://www.osha.gov/SLTC/tuberculosis>.

The Centers for Disease Control and Prevention (CDC) published *Guidelines for Preventing the Transmission of Mycobacterium tuberculosis in Health-Care Settings* in 2005. These Guidelines are available at <http://www.cdc.gov/mmwr/PDF/rr/rr5417.pdf>. Though not specific to elephant care settings, they contain invaluable recommendations and guidance for workers exposed to a source of tuberculosis.

The CDC/National Institute for Occupational Safety and Health Respirator Trusted-Source Information Page is located at http://www.cdc.gov/niosh/npptl/topics/respirators/disp_part/RespSource.html. This website provides information to understand the types of respirators, how to identify approved models and outlets for purchase, a listing of all NIOSH-approved and FDA-cleared surgical N95 respirators, a listing of recently revoked respirator approvals and relevant User Notices. It also contains information on how to implement the use of respirators in the workplace and use them appropriately, and includes commonly asked questions and answers (fact sheets), respirator myths, the science of respirator function and performance, and respiratory protective devices not approved by NIOSH.

Best practices for the safe conduct of work in biomedical and clinical laboratories and animal facilities in regards to *Mycobacterium tuberculosis* are listed in the 5th Edition of *Biosafety in Microbiological and Biomedical Laboratories* published by the U.S. Department of Health and Human Services in 2007. <http://www.cdc.gov/biosafety/publications/bmb15/index.htm>

State Public Health Veterinarians or State Epidemiologists may be willing to provide guidance and consultation. A complete listing of State Public Health Veterinarians and State Epidemiologists can be accessed at the Council of State & Territorial Epidemiologists website www.cste.org.

14. REPORTING

Tuberculosis is a reportable disease. Positive culture results must be reported to the State Veterinarian and appropriate public health agencies.

State Veterinarians: http://www.avma.org/disaster/state_veterinarians.asp

State Public Health Veterinarians:

<http://www.nasphv.org/Documents/StatePublicHealthVeterinariansByState.pdf>

State TB Control Offices: <http://www.cdc.gov/tb/links/tboffices.htm>

15. APPENDICES

APPENDIX 1. REFERENCES CITED AND ADDITIONAL READING

Anon. 2003. Treatment of Tuberculosis. ATS, CDC and Infectious Diseases Society of America, MMWR 52: No.RR-11 (June 20, 2003) 1-88.

Auclair, B., Mikota, S.K., Peloquin, C.A., Aguilar, R., Maslow, J.N. 2002. Population pharmacokinetics of antituberculous drugs and treatment of *Mycobacterium bovis* infection in bongo antelope (*Tragelaphus eurycerus isaaci*). *J Zoo Wildl Med. Sep*; 33(3): 193-203.

Ball, R.L., Dumonceaux, G., Olsen, J.H., Burton, M.S., Lyashchenko, K. Comparison of trunk wash results matched to multiantigen print immunoassay (MAPIA™) in a group of captive Asian elephants (*Elephas maximus*). 2006. Proceedings American Association of Zoo Veterinarians. 303-304.

Centers for Disease Control and Prevention and National Institutes of Health. 2007. Biosafety in Microbiological and Biomedical Laboratories, 5th ed. U.S. Government Printing Office, Washington, D.C. 145-148. <http://www.cdc.gov/biosafety/publications/bmb15/BMBL.pdf>

Davis, M. 2001. *Mycobacterium tuberculosis* risk for elephant handlers and veterinarians. *Appl Occup Environ Hyg.* 16: 350-353.

Dumonceaux, G.A., St. Leger, J., Olsen, J.H., Burton, M.S., Ashkin, D., and Maslow, J.N. 2011. Genitourinary and pulmonary multidrug resistant *Mycobacterium tuberculosis* infection in an Asian elephant (*Elephas maximus*). *J Zoo Wildl Med. Des*; 42(4):709-712.

Greenwald, R., Lyashchenko, O., Esfandiari, J., Miller, M., Mikota, S., Olsen, J.H., Ball, R., Dumonceaux, G., Schmitt, D., Moller, T., Payeur, J.B., Harris, B., Sofranko, D., and Waters, W.R., Lyashchenko, K. 2009. Highly accurate antibody assays for early and rapid detection of tuberculosis in African and Asian elephants. *Clinical and Vaccine Immunology* 16(5): 605-612.

Isaac, R. The elephant trunk wash – An update. Elephant Managers Association Annual Conference. Orlando, Florida. November 9-11, 2001.

Isaza, R, and Ketz, C. A trunk wash technique for the diagnosis of tuberculosis in elephants. Proceedings of the 39th international symposium of the European zoo and wildlife medicine. Vienna, Austria. May 12-16, 1999. 121-124.

Lacasse, C., Terio, K., Kinsel, M.J., Farina, L.L., Travis, D.A., Greenwald, R., Lyashchenko, K.P., Miller, M., and Gamble, K. 2007. Two cases of atypical mycobacteriosis caused by *Mycobacterium szulgai* associated with mortality in captive African elephants (*Loxodonta africana*). *J. Zoo Wildl. Med.* 38 (1): 101-107.

Landolfi, J.A., Mikota, S.K., Chosy, J., Lyashchenko, K.P., Giri, K., Gairhe, K., Terio, K.A. 2010. Comparison of systemic cytokine levels in *Mycobacterium spp.* seropositive and

seronegative Asian elephants (*Elephas maximus*). J Zoo Wildl Med. 41(3): 445-455.

Landolfi, J.A., Schultz, S.A., Mikota, S.K., Terio, K.A. 2009. Development and validation of cytokine quantitative, real-time RT-PCR assays for characterization of Asian elephant immune responses. Vet Immunol Immunopathol. Sep 15; 131(1-2): 73-78.

Larsen, R.S., Salman, M.D., Mikota, S.K., Isaza, R., Montali, R.J. and Triantis, J. 2000. Evaluation of a multiple-antigen enzyme-linked immunosorbent assay (ELISA) for detection of *Mycobacterium tuberculosis* in captive elephants. J Zoo Wildl Med 31: 291-302.

Lewerin, S.S., Olsson, S.L., Eld, K., Roken, B., Ghebremichael, S., Koivula, T., Kallenius, G., and Bolske, G. 2005. Outbreak of *Mycobacterium tuberculosis* infection among captive Asian elephants in a Swedish zoo. Vet Rec. 156(6): 171-175.

Lyashchenko, K., Singh, M., Colangeli, R., and Gennaro, M.L. 2000. A multi-antigen print immunoassay for the development of serological diagnosis of infectious disease. Journal of Immunological Methods 242: 91-100.

Lyashchenko, K., Miller, M., and Waters, W.R. 2005. Application of MAPIA™ (Multiple Antigen Print Immunoassay) and rapid lateral flow technology for tuberculosis testing of elephants. Proc American Association of Zoo Veterinarians, 64-65.

Lyashchenko, K.P., Greenwald, R., Esfandiari, J., Olsen, J.H., Ball, R., Dumonceaux, G., Dunker, F., Buckley, C., Richard, M., Murray, S., Payeur, J.B., Andersen, P., Pollock, J.M., Mikota, S., Miller, M., Sofranko, D., and Waters, W.R. 2006. Tuberculosis in elephants: antibody responses to defined antigens of *Mycobacterium tuberculosis*, potential for early diagnosis, and monitoring of treatment. Clin Vaccine Immunol 13(7): 722-732.

Maslow, J.N., Mikota, S.K., Zhu, M., Isaza, R., Peddie, L.R., Dunker, F., Peddie, J., Riddle, H., and Peloquin, C.A. 2005. Population pharmacokinetics of isoniazid in the treatment of *Mycobacterium tuberculosis* among Asian and African elephants (*Elephas maximus* and *Loxodonta africana*). J Vet Pharmacol Ther. 28(1): 21-27.

Maslow, J.N., Mikota, S.K., Zhu, M., Riddle, H., and Peloquin, C.A. 2005. Pharmacokinetics of ethambutol (EMB) in elephants. J Vet Pharmacol Ther. 28: 321-323.

Maslow, J. Tuberculosis and other mycobacteria as zoonoses. 1997. Proceedings American Association of Zoo Veterinarians. 110-115.

Michalak, K., Austin, C., Diesel, S., Bacon, J. M., Zimmerman, P., and Maslow, J. N. 1998. *Mycobacterium tuberculosis* infection as a zoonotic disease: Transmission between humans and elephants. Emerging Infect. Dis. 4: 283-287.

Mikota, S.K., Dumonceaux, G., Miller, M., Gairhe, K., Giri, K., Cheeran, J.V., Abraham, D., Lyashchenko, K., Larsen, S., Payeur, J., Waters, R., Kaufman, G. 2006. Tuberculosis in elephants: An update on diagnosis and treatment; implications for control in range countries. Proceedings International Elephant Conservation and Research Symposium, 109-118.

- Mikota, S.K., Miller, M., Dumonceaux, G., Giri, K., Gairhe, K., Hamilton, K., Paudel, S., Vincent, B. Elephant tuberculosis diagnosis: implications for elephant management in Asian range countries. 2006. Proceedings American Association of Zoo Veterinarians. 142-143.
- Mikota, S.K., Peddie, L., Peddie, J., Isaza, R., Dunker, F., West, G., Lindsay, W., Larsen, R.S., Salman, M.D., Chatterjee, D., Payeur, J., Whipple, D., Thoen, C., Davis, S., Sedgwick, C., Montali, R.J., Ziccardi, M., and Maslow, J. 2001. Epidemiology and diagnosis of *Mycobacterium tuberculosis* in captive Asian elephants (*Elephas maximus*). J. Zoo Wildl. Med. 32: 1-16.
- Mikota, S.K., Larsen, R.S., and Montali, R.J. 2000. Tuberculosis in elephants in North American. Zoo Biology 19: 393-403.
- Moller, T., Roken, B.O., Lewerin, S.S., Lyashchenko, K., 2006. The elephant Rapid Test (RT) the future diagnostic test for TB (*M. tuberculosis*) in elephants? Call for a validation study in Europe. Proceedings International Elephant Conservation and Research Symposium 119-124.
- Moller, T., Roken, B., Petersson, L., Vitaud, C., Lyashchenko, K., 2005. Preliminary results of a new serological test for detection of TB-infection (*Mycobacterium tuberculosis*) in elephants (*Elephas maximus* and *Loxodonta africanum*) - Swedish Case studies. Verh.ber.Erkrgr.Zootiere. 42, 173-181.
- Montali, R.J., Mikota, S.K., and Cheng, L.I. 2001. *Mycobacterium tuberculosis* in zoo and wildlife species. Revue Scientifique et Technique Office International des Epizooties 20(1): 291-303.
- Montali, R.J., Spelman, L.H., Cambre, R.C., Chatterjee, D. and Mikota, S.K. Factors influencing interpretation of indirect testing methods for tuberculosis in elephants. 1999. Proc. Amer. Assoc. Zoo Vet. 109-112.
- Murphree, R., Dunn, J.R., Warkentin, J.V., Schaffner, W., and Jones, T.F. 2010. *Mycobacterium tuberculosis* infection among employees of an elephant refuge. 2010 National Tuberculosis Conference, 06/22–6/24, 2010, Atlanta, GA.
- National Association of State Public Health Veterinarians (NASPHV), Veterinary Infection Control Committee. 2010. Compendium of veterinary standard precautions for Zoonotic disease prevention in veterinary personnel.
<http://www.nasphv.org/Documents/VeterinaryPrecautions.pdf>
- Oh, P., Granich, R., Scott, J., Sun, B., Joseph, M., Stringfield, C., Thisdell, S., Staley, J., Workman-Malcolm, D., Borenstein, L., Lehnkering, E., Ryan, P., Soukup, J., Nitta, A., Flood, J., 2002. Human exposure following *Mycobacterium tuberculosis* infection of multiple animal species in a Metropolitan Zoo. Emerg Infect Dis 8:1290-1293.
- Payeur, J.B., Jarnagin, J.L., Marquardt, J.G., and Whipple, D.L. 2002. Mycobacterial isolations

in captive elephants in the United States. *Ann N Y Acad Sci* 969: 256-258.

Peloquin, C.A., Maslow, J.N., Mikota, S.K., Forrest, A., Dunker, F., Isaza, R., Peddie, L.R., Peddie, J., and Zhu, M. 2006. Dose selection and pharmacokinetics of rifampin in elephants for the treatment of tuberculosis. *J Vet Pharmacol Ther.* 29: 581-586.

Peloquin, C.A. 2003. Clinical pharmacology of the anti-tuberculosis drugs. In Davies, P.D.O. (Editor). *Clinical Tuberculosis*. London, England. Arnold Publishers, 171-190.

Peloquin, C.A. 2002. Therapeutic drug monitoring in the treatment of tuberculosis. *Drugs* 62(15): 2169-2183.

Peloquin, C.A. 1997. Using therapeutic drug monitoring to dose the antimycobacterial drugs. *Clinics in Chest Medicine* 18: 79-97.

Ryan, C.P. 1997. Tuberculosis in circus elephants. *Pulse Southern California Veterinary Medical Assoc.* 8.

Siegel JD, Rhinehart E, Jackson M, Chiarello L, and the Healthcare Infection Control Practices Advisory Committee, 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings, June 2007.
<http://www.cdc.gov/ncidod/dhqp/pdf/isolation2007.pdf>

Yong , H, Choi GE., G.E., Lee BS., B.S., Whang, J.,, and Shin S.J. 2011. Disseminated infection due to *Mycobacterium avium* subsp. *avium* in an Asian elephant (*Elephas maximus*). *Journal of Zoo and Wildlife Medicine* 42: 743-746.

Zhu, M., Maslow, J.N., Mikota, S.K., Isaza, R., Dunker, F., and Peloquin, C.A. 2005. Population pharmacokinetics of pyrazinamide in elephants. *Journal of Veterinary Pharmacology and Therapeutics* 28: 403-409.

APPENDIX 2. ACKNOWLEDGMENTS

The following individuals have contributed to the historical development of these Guidelines:

Dr. Wilbur Amand, Director Emeritus American Association of Zoo Veterinarians
Dr. Miava Binkley, USDA, Animal Care
Dr. Genevieve Dumonceaux, Florida Aquarium
Dr. Freeland Dunker, Steinhart Aquarium
Dr. Murray Fowler, University of California, Davis
Dr. Werner Heuschele, San Diego Zoo (in memorium)
Dr. Ramiro Isaza, University of Florida – Gainesville
Dr. Barbara Kohn, USDA, APHIS, Animal Care
Dr. Scott Larsen, University of California, Davis
Dr. William A. Lindsey, Feld Inc.
Dr. Konstantin Lyashchenko, Chembio Diagnostic Systems, Inc.
Dr. Joel Maslow, University of Pennsylvania
Dr. Bob Meyer, USDA, APHIS, Veterinary Services
Dr. Susan K. Mikota, Elephant Care International
Dr. Richard Montali,
Dr. C. Douglas Page, Jacksonville Zoo
Dr. Linda Peddie and Dr. James Peddie, America's Teaching Zoo, Moorpark College
Dr. Mo Salman, Colorado State University
Dr. Dennis Schmitt, Feld Inc.
Dr. Scott Terrell, Disney's Animal Programs
Dr. Dominic Travis, Lincoln Park Zoo
Dr. Charles Thoen, Iowa State University
Dr. Gary West, San Antonio Zoo
Ms. Diana Whipple, USDA, ARS, National Animal Disease Center
Dr. Michael Ziccardi, University of California, Davis

The following individuals are members of the U.S .Animal Health Association TB Scientific Subcommittee:

Dr. Chuck Massengill, Missouri Department of Agriculture
Dr. Susan K. Mikota, Elephant Care International
Dr. Michele Miller, Palm Beach Zoo
Dr. Kathy Orloski, USDA, APHIS, Veterinary Services
Dr. Janet B. Payeur, USDA, APHIS, National Veterinary Services Laboratories
Dr. W. Ray Waters, USDA, ARS, National Animal Disease Center

The following individuals have contributed to the 2012 Guidelines:

Dr. Joel Maslow, University of Pennsylvania
Dr. Denise Sofranko, USDA (regulatory advisor only)
Dr. Adam Langer, CDC
Dr. Rendi Murphree, CDC

APPENDIX 3. TRUNK WASH TECHNIQUE FOR THE DIAGNOSIS OF TUBERCULOSIS IN ELEPHANTS

Overview:

- Trunk wash technique collects samples from the distal respiratory tract of the elephant for Mycobacterial diagnostics (culture, PCR)
- A positive results is considered a definitive diagnosis of infection (i.e., identifies an elephant that is shedding mycobacterial organisms).
- A negative result is non-diagnostic.
- An elephant must be trained to allow manipulation of the trunk for successful trunk wash.
- Trunk swabs are not considered acceptable samples.

List of Materials Required

- Sterile saline, 0.9%
- Sterile 60 ml syringe
- 1 gallon plastic zip-lock bag (heavy duty)
- Sterile 50 ml screw top plastic centrifuge tubes (or equivalent)
- Parafilm or plastic seal for caps
- Indelible marker to label tubes
- ± large diameter red rubber feeding tube

Procedure

- Collect 3 separate trunk wash samples, ideally within one-week period
- Collect samples in the morning after water has been withheld; ideal to exercise animal prior to collection of sample
- Manually restrain tip of trunk upward and instill 60 mls sterile saline
- Lift trunk tip to facilitate flow of fluid up trunk
- Place 1 gallon plastic zip-lock bag over trunk tip and low trunk to collect draining fluid; if possible have elephant exhale into bag
- Collect minimum of 20-40 mls fluid containing visible mucus; may contain dirt and food particles
- Transfer sample from plastic bag to labeled centrifuge tube; refrigerate and send to laboratory for processing. If unable to send directly, freeze sample at -20 to -10 °C until it can be sent to the laboratory. Batches of 3 samples can be frozen and submitted to the laboratory together for culture.
- Safety precautions for personnel collecting samples (minimum): gloves, facemask capable of filtering 0.3 micron particles, and working in a well-ventilated area.

APPENDIX 4. TESTING LABORATORIES

CULTURES, ANTIMICROBIAL SENSITIVITY, GENOTYPING

USDA APHIS VS

National Veterinary Services Laboratories (NVSL)

1920 Dayton Avenue

Ames, IA 50010

Lab web site: http://www.aphis.usda.gov/animal_health/lab_info_services/diagnos_tests.shtml

Dr. Janet Payeur

Scientific Outreach Coordinator

Tel: (515) 337-7003 Fax: (515) 337-7397

Email: Janet.B.Payeur@aphis.usda.gov

Dr. Suelee Robbe-Austerman

Head, Mycobacteria and Brucella Section

Tel: (515) 337-7837 Fax: (515) 337-7315

Email: Suelee.Robbe-Austerman@aphis.usda.gov

*Laboratory SOP for mycobacterial cultures available upon request

GENOTYPING of TB Isolates

Dr. James Higgins

Microbiologist

Tel: 515-337-7034

Email: james.a.higgins@aphis.usda.gov

Send trunk washes to NVSL on icepacks by overnight express (Federal Express handles diagnostic samples). Containers should be leak proof and double-bagged (50 ml conical screw-top centrifuge tubes are preferred) and are available free of charge from NVSL.

If lesions are submitted for culture, tissues should be frozen and sent on ice packs overnight. Lesioned tissues should be split and ½ should be sent to the histopathology lab so PCR can be run to see if the tissue is compatible for tuberculosis. There is no charge for histopathology on lesioned tissue.

Use the VS Form 10-4 for submission, not the VS 6-35 form found in the TB kit. If the formalized tissue is sent separately from the frozen tissue, please indicate on the submission forms that there are 2 separate packages coming from the same animal so that the reports can be combined and accession numbers coordinated when they reach NVSL. It is also helpful to call or email NVSL contacts when sending TB suspects to schedule testing and relay any relevant history of the case.

NVSL Trunk wash cost: \$98 per sample for processing which includes a Gen Probe® DNA probe on any isolate. If the sample is positive for mycobacteria and speciation is requested, the charge is \$122.00 per sample which includes biochemical analysis, 16s rDNA sequencing

analysis, Spoligotyping and VNTR genotyping. DNA fingerprinting of *M. tuberculosis* or *M. bovis* isolates is also available. Antimicrobial susceptibility testing is available for *M. tuberculosis* complex organisms for \$112.00 per isolate. Please contact NVSL at (515) 337-7388 for test schedule.

To establish an account at NVSL for billing, contact **Connie Osmundson** (515) 337-7571 or Email: Connie.J.Osmundson@aphis.usda.gov .

(User fees as of October 1, 2011). Call lab before shipping samples for current prices and schedule of testing or check prices at the NVSL web site:

http://www.aphis.usda.gov/animal_health/lab_info_services/diagnos_tests.shtml

**Mycobacteriology Laboratory at National Jewish Medical and Research Center
National Jewish Medical and Research Center**

Director: [Leonid Heifets, M.D.](mailto:Leonid.Heifets@njc.org)

1400 Jackson St.

Denver, CO 80206

Tel: (303) 398-1384

E-mail: heifetsl@njc.org

For price list, shipping instructions, and requisition form:

<http://www.nationaljewish.org/research/clinical-labs/about/learn/mycobac/index.aspx>

Serum sample submission: it is important to protect the samples from light by wrapping the tubes in tinfoil and to separate the serum and freeze it without delay, transferring the serum to a tube or cryovial that is also wrapped in tin foil. Samples should be sent on dry ice as well.

HISTOPATHOLOGY

Scott P. Terrell, DVM, Diplomate ACVP

SSP Pathology Advisor

Disney's Animal Kingdom

1200 N Savannah Circle

Bay Lake, FL 32830

Tel: (407) 938-2746 W;

Tel: (407) 251-0545 H;

Cell (321) 229-9363;

Email Scott.P.Terrell@disney.com

Send sections in formalin of any gross lesion and complete set of tissues including lung, liver, spleen, mesenteric lymph nodes, bronchial lymph nodes and other major organs. Use leak proof container.

USDA APHIS NVSL Pathobiology Laboratory

1920 Dayton Avenue

Ames, IA 50010

Tel: (515) 337-7521

Fax (515) 337-7527

Lab web site: http://www.aphis.usda.gov/animal_health/lab_info_services/diagnos_tests.shtml

Dr. Art Davis

Director of Pathobiology Laboratory

Tel: (515) 337-7526

Email: Arthur.J.Davis@aphis.usda.gov

Dr. Mark Hall

Head Pathological Investigations

Tel: (515) 337-7927

Email: Mark.Hall@aphis.usda.gov

Send formalin sections of any gross lesion and target tissues (lung, liver, mesenteric and bronchial lymph nodes). Use leak proof container. **Please indicate on submission form if a sample was submitted for culture so that the testing can be coordinated and results combined on one form.**

ANTI TB DRUG LEVELS

Infectious Diseases Pharmacokinetics Laboratory (IDPL)

National Jewish Medical and Research Center

1400 Jackson St.

Denver, CO 80206

Web site: <http://www.nationaljewish.org/research/clinical-labs/about/learn/infectious/idpl.aspx>

Refer to the above website for specimen handling instructions and to download Requisition forms.

Infectious Disease Pharmacokinetics Lab, College of Pharmacy, and Emerging Pathogens Institute

Charles Peloquin, Pharm.D.

Professor and Director

University of Florida

1600 SW Archer Rd., Rm P4-33

PO Box 100486

Gainesville, FL 32610-0486

Tel: 352-273-6266

Fax: 352-273-6804

peloquin@cop.ufl.edu

Call or email for information on sample submission.

SEROLOGY -

The National Veterinary Services Laboratories

USDA APHIS NVSL

1920 Dayton Avenue

Ames, IA 50010

Web site: http://www.aphis.usda.gov/animal_health/lab_info_services/diagnos_tests.shtml

Dr. David Kinker

Head, Serology Section

Tel: 515-337-7950

Email: David.R.Kinker@aphis.usda.gov

Call before shipping samples for current prices for ElephantTB STAT-PAK®.

Dr. Jeffrey Nelson

Veterinary Medical Officer

Tel: 515-337-7966

Email: Jeffrey.t.nelson@aphis.usda.gov

Chembio Diagnostic Systems, Inc.

3661 Horseblock Road

Medford, NY 11763

Tel: 631-924-1135

Fax: 631-924-6033

Email: customerservice@chembio.com

Call Chembio before shipping samples for current prices on veterinary products such as ElephantTB STAT-PAK®, MAPIA™ or DPP®.

APPENDIX 5. CONTACTS FOR QUESTIONS

DIAGNOSIS AND TREATMENT

Michele A. Miller, DVM, MS, PhD

Chief Veterinary Officer and Director of Conservation Medicine
Palm Beach Zoo
1301 Summit Blvd.
West Palm Beach, FL 33405
Phone: 561-833-7130 ext. 224
Cell: 561-727-9630
Email: mmiller@palmbeachzoo.org

Dr. Genevieve Dumonceaux

Staff Veterinarian
Palm Beach Zoo
1301 Summit Blvd.
West Palm Beach, FL 33405
Work: 561-833-7130 ext. 252
Email: gdumonceaux@palmbeachzoo.org

Dr. Susan K. Mikota

Director of Veterinary Programs and Research
Elephant Care International
166 Limo View Lane
Hohenwald, TN 38462
Tel: 931-796-7102
Cell: 931-628-5962
Email: smikota@elephantcare.org
Website: www.elephantcare.org

ELEPHANT TB STAT-PAK[®] ASSAY AND MAPIA[™]

Konstantin Lyashchenko, Ph.D.

Research Director, Mycobacterial Immunology
Chembio Diagnostic Systems, Inc.
3661 Horseblock Road
Medford, NY 11763
Tel: 631-924-1135, ext.111
Fax: 631-924-6033
Email: klyashchenko@chembio.com

REGULATORY

Dr. Denise Sofranko

USDA-APHIS-Animal Care
Field Specialist for Elephants
Voice Mail: 240-461-9142

Email: Denise.M.Sofranko@aphis.usda.gov
2150 Centre Avenue
BLDG B Mail Stop #3-W11
Ft. Collins, CO 80562-8117

HUMAN HEALTH, ELEPHANT THERAPY AND TREATMENT

Joel Maslow, MD, PhD

Division of Infectious Diseases
Philadelphia Veterans Affairs Medical Center and the
University of Pennsylvania School of Medicine
University & Woodland Avenues
Philadelphia, PA 19104
Tel: 215-823-4021
Fax: 215-823-5171
Email: joel.maslow@med.va.gov

NECROPSY, PATHOLOGY

Scott P. Terrell, DVM, Diplomate ACVP

SSP Pathology Advisor
Disney's Animal Kingdom
1200 N Savannah Circle
Bay Lake, FL 32830
Tel: 407-938-2746
Home: 407-251-0545
Cell: 321-229-9363
Email: Scott.P.Terrell@disney.com

INTERNET

These guidelines are available on the Internet at the following sites:

1. http://www.aphis.usda.gov/animal_welfare/index.shtml
http://www.aphis.usda.gov/animal_welfare/downloads/elephant/elephant_tb.pdf (2008 guidelines available to the public)
2. www.aazv.org (available to AAZV members by password)
3. www.elephantcare.org (2008 guidelines available to the public)
4. www.usaha.org (Look under Tuberculosis committee for 2010 guidelines)

APPENDIX 6. SOURCES FOR ANTI-TUBERCULOSIS DRUGS

There are various veterinary compounding pharmacies that have experience with formulations for elephants. Please contact one of the consultants in Appendix 6 for information. Select veterinary compounding pharmacies are also listed on www.elephantcare.org.

APPENDIX 7. Elephant Serum Bank Submission Form

Institution/owner: _____
 Submitter: _____
 Address: _____

 Tel: _____ Fax: _____ Email: _____

Animal Information

Asian African ISIS# _____ Studbook # _____
 Name _____ Age: _____ actual estimate
 Sex: male female

SAMPLE COLLECTION INFORMATION

Date of sample collection: _____ **Time of collection:** _____
Site of sample collection: ear vein leg vein other: _____
Health status of animal: normal abnormal
Fasted: no yes – how long _____
Weight _____ actual estimated
Type of restraint: manual anesthetized/sedated behavioral control
Temperament of animal: calm active excited
Type of blood collection tube:
 no anticoagulant (red-top)
 EDTA (purple)
 heparin (green)
 other: _____
Sample handling: separation of plasma/serum by centrifugation
 (Check all that apply) stored as whole blood
 frozen plasma
 other – describe _____

TB EXPOSURE STATUS

Known infected animal
 Known exposure to culture positive source within the past 12 months
 Known exposure to a culture positive source within the past 1-5 years
 No know exposure to a culture positive source in the last 5 years

TREATMENT INFORMATION

Is elephant currently receiving any medication or under treatment? yes no
 If yes, please list drugs and doses: _____

Time between blood collection and last treatment: _____

Ship samples overnight frozen with shipping box marked “PLACE IN FREEZER UPON ARRIVAL”

Send completed form with samples to:

Michele A. Miller, DVM, MS, PhD
 Director of Conservation Medicine
 Palm Beach Zoo, 1301 Summit Blvd., West Palm Beach, FL 33405
 Phone: 561-833-7130 ext. 224; Cell: 561-727-9630;
 Email: mmiller@palmbeachzoo.org

Consent Form for Use of Serum by Elephant SSP

I give consent for the serum submitted to the Elephant Species Survival Plan (SSP) serum bank to be used for research on any elephant related issues based on recommendations by the veterinary advisor and/or steering committee.

The results could be reviewed and used by the SSP veterinary advisor in providing health-related recommendations and publications.

I understand that all results and recommendations regarding the individual elephant will be kept confidential.

_____ Yes, I agree to allow the SSP to use our sample for designated research and testing results.

_____ No, I do not consent to the use of our sample and test results unless specified.

Signature, title

Date

Printed name

Phone number

Institution

Email address

Address

Comments: