GUIDELINES FOR THE CONTROL OF TUBERCULOSIS IN ELEPHANTS 2003

THE NATIONAL TUBERCULOSIS WORKING GROUP FOR ZOO & WILDLIFE SPECIES

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

1. www.aphis.usda.gov/ac/ElephTBGuidelines2003.html (available to the public)
2. www.aazv.org (available to AAZV members by password)
3. www.elephantcare.org (available to the public)

1. INTRODUCTION

Tuberculosis (TB) is a bacterial disease affecting numerous species. 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. The 2003 Guidelines reflect knowledge that has been gained over the past six years. The Guidelines are for the testing, treatment, and surveillance of *M. tuberculosis* complex (*M. tuberculosis, M.bovis, M. africanum, M. microti*) in elephants. These guidelines should be used with the understanding that neither ancillary diagnostic tests nor treatment regimens have been completely validated in elephants. These Guidelines will be updated periodically as new information becomes available. Ancillary tests may be incorporated in future diagnostic requirements if reliable correlation to actual infection with pathogenic strains of mycobacteria can be documented.

Record keeping of tuberculosis testing and treatment by the attending veterinarian is of utmost importance. Variations to these guidelines require prior USDA approval. It is recommended that attending veterinarians maintain open communication with USDA, particularly concerning elephants under treatment for tuberculosis or in cases of exposure to TB positive elephants.

2. DEFINITIONS

Ancillary diagnostic tests: Diagnostic tests for TB other than culture.

At-risk person: Any person who has had direct contact with an infected animal(s) or who handles waste or other biological materials collected from an enclosure in which such animal(s) resides.

Culture positive: Isolation and identification of *M. tuberculosis* or *M. bovis* from any site using standard mycobacterial methods. Specimens that yield mycobacteria other than those in the *M. tuberculosis complex* are not considered as having TB. Examples of the non-tuberculous mycobacteria that have been commonly isolated from elephants include *Mycobacterium fortuitum* and *Mycobacterium avium*.

Culture positive elephant: An elephant from which *Mycobacterium tuberculosis* or *Mycobacterium bovis* has been isolated from any body site or specimen. A culture positive elephant is considered positive until 1) it has completed six months of treatment with documentation that adequate TB drug serum levels have been achieved on two separate testing dates and 2) it can be demonstrated that sputum cultures (obtained according to the procedures outlined in this protocol) on at least two consecutive months are negative.

Direct public contact: any activity which involves touching or riding an elephant and includes "meet and greet" activities or any other activities in which the public is in the immediate area of an elephant and is able to be touched by an elephant.

Effective therapy: the administration of a specified number of doses of anti-TB drugs within a specified time.

ELISA: Enzyme-linked immunosorbent assay; a test used to detect and measure either antigen or antibody.
Fomite: An inanimate object or material on which disease-producing agents may be conveyed.

Gamma-interferon test: A whole blood *in vitro* assay currently being evaluated as a diagnostic test for TB.

Herd: A group or groups of animals, maintained on common ground or two or more groups of animals under common ownership or supervision that are geographically separated, but that 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 the disease is first diagnosed.

Intradermal tuberculin test (skin test): The injection of 0.1 ml of purified protein derivative (PPD) tuberculin beneath the skin for the purpose of detecting exposure to tuberculosis. In cattle, the test site is the caudal fold 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 arm and the test is read at 48-72 hours. The intradermal tuberculin test is not considered a reliable test in elephants.

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

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

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

***Mycobacterium tuberculosis* complex (MTB Complex):** A group of mycobacteria which includes *M. tuberculosis, M. bovis, M. bovis BCG, M. Africanum, and M. microti.*

Mycobacterium Tuberculosis Direct Test (MTD): A nucleic acid amplification test used in the diagnosis of TB. The MTD utilizes a technique that replicates RNA from bacteria of the *M. tuberculosis* complex.

No isolation: No growth of *M. tuberculosis* or *M. bovis* from trunk wash, feces, tissue or other sample using approved mycobacterial culture methods. Does not rule out the possibility of *M. tuberculosis* or *M.bovis* infection. Possible explanations are:

1. Non-infected animal
2. Infected animal but not shedding organisms at the time of testing (may indicate early infection or latent disease)
3. Sampling error (culture overgrowth by contaminating organisms, inadequate sample, or laboratory error)
4. Effectively treated animal
5. Improperly handled or shipped sample

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 DNA are replicated, allowing for detection of target sequences that otherwise would not be present in high enough numbers to be detected.

Premises: A parcel of land containing animals, administered by a person, government entity (city, county, state, region) or organization (zoological society, corporation). A group of animals (one or more) maintained on common ground or two or more groups of animals under common ownership or supervision that are geographically separated, but may have an interchange or movement of animals without regard to health status.

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

Report date: the date the laboratory reports the results

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 guidelines established in this protocol

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

Trunk wash: A procedure used in elephants to obtain a sputum sample. Using a catheter tip syringe, 60 ml of sterile saline is instilled into the trunk. A clean, one-gallon plastic bag is immediately placed over the end of the trunk and held in place until the elephant exhales into the bag. The sample (at least 20 ml) is transferred to a sterile leak proof, screw-top container. Alternatively, a 14 French feeding tube is used to introduce the sterile saline into the trunk and the sample is obtained by aspiration.

Sensitivity: A measure of the ability of a test to identify infected animals

Specificity: A measure of the ability of a test to identify non-infected animals

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 culture results are obtained.

3. ANNUAL TESTING BY CULTURE

To adequately address the concerns of TB in the general elephant population, all captive elephants should be tested annually by culture under the direct supervision of a licensed veterinarian. If possible, elephants should be tested the same month each year so that annual test dates are 12 months apart. 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."

4. ANCILLARY SCREENING / DIAGNOSTIC TESTS

A number of other ante mortem tests are under investigation to diagnose TB in elephants. Research done to date has not demonstrated a correlation between the intradermal tuberculin test (skin test) and culture results. Therefore, intradermal skin testing cannot be deemed reliable for screening or diagnosis and is not recommended.

Preliminary investigations suggest ELISA testing of serum for the presence of antibodies against TB and Nucleic Acid Amplification tests to detect the presence of bacteria in sputum or other samples may be useful, however, additional data is needed to validate these tests. To collect the data necessary to determine the sensitivity and specificity of these and other diagnostic tests, it is strongly recommended that all elephants be tested using the ancillary diagnostic tests described in these guidelines. Results will be used to develop improved strategies for diagnosis and management of tuberculosis in elephants in the future. In addition, serum should be banked for use in ancillary tests that may be developed in the future.

5. CULTURE COLLECTION PROCEDURE

Samples for culture should be collected under the direct visual 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, protective measures are recommended for personnel during sample collection. These should include gloves and Hepa-filter masks certified by the National Institute for Occupational Safety and Health (NIOSH) to protect against TB (see Employee Health and Safety).

A. Trunk wash with bag (or other suitable container) - Using a catheter tip syringe, instill 60 ml sterile saline into the trunk. Immediately 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. (50- ml conical screw-top centrifuge tubes are preferred and are available, free of charge from NVSL).

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.

Do not expose samples to sunlight or heat. Freeze samples as soon as possible after collection and keep frozen until shipment. Freeze at –20ºC (conventional freezer). As standard frost-free freezers undergo cyclic freeze-thaws to limit frost, freezers which do not have this feature are preferred. Freezing at -80ºC (ultra-low temperature freezer) is also acceptable. All three samples can be submitted together, frozen and shipped overnight on ice packs. Label containers with animal ID and date of collection and put the same information on the submission form. Place screw-top containers in double zip-lock baggies). **Do not send samples in glass containers or packaged only in plastic bags.** Sterile 50 ml conical centrifuge tubes are preferred.

For shipment, place samples on ice packs 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. 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 (included in this protocol). Request mycobacterial culture with species differentiation. Positive cultures from laboratories that do not have the capability to differentiate *M. tuberculosis* complex organisms should be forwarded to NVSL for speciation. Culture of mycobacteria requires a minimum of eight weeks.

Note: Other Mycobacteria species such as *M. avium*, *M. kansasii*, and *M. fortuitum* have been isolated from elephants. At this time, there is no substantive evidence that these organisms are pathogenic for elephants.

6. TB MANAGEMENT GROUPS (A,B,C,D,E)

Based upon the culture results (for *M. tuberculosis complex*) and status of exposure to known culture positive animals, all elephants should fall into one of the following five management groups (A, B, C, D, or E). A culture positive elephant is defined as an elephant from which *Mycobacterium tuberculosis* or *Mycobacterium bovis* has been isolated from any body site or specimen. A culture positive elephant is considered positive until 1) it has completed six months of treatment with documentation that adequate TB drug serum levels have been achieved on two separate testing dates and 2) at least two consecutive months of negative cultures obtained according to the procedures outlined in this protocol can be demonstrated. These groups are intended for initial classification only. Follow-up procedures for future years are outlined in each group.

Group A **(Negative culture; no exposure to culture positive animal in the last five years).** Monitor annually by triple sample method on separate days. Ancillary tests for data collection

encouraged but not required. No treatment or travel restrictions. No elephant should move into a facility where there is an untested elephant.

Group B **(Negative culture; exposure to culture positive animal between one to five years ago).** Monitor quarterly by the triple sample method for one year and then annually thereafter if all cultures remain negative. No treatment or travel restrictions. Ancillary tests are encouraged but not required. No elephant should move into a facility where there is an untested elephant.

Group C **(Negative culture; exposure to culture positive animal within last 12 months).** This group should be tested and then handled according to either *Option 1* (Prophylactic Treatment) or *Option 2* (Monitor by Culture).

Testing

1. As soon as initial culture results are known, re-culture by triple sample method.
2. Request antimicrobial susceptibility results from the culture positive elephant (index case).
3. Perform ancillary diagnostic tests: (strongly recommended but not required)

A. ELISA test
B. Nucleic acid amplification test
C. Gamma interferon test

4. If a lab other than NVSL is used, please submit *M. tuberculosis* culture to NVSL for DNA fingerprinting

Option 1: Prophylactic Treatment

Following the initial negative culture result, animals in this group should be re-cultured by the triple sample method immediately. (Results of these second cultures should be available in eight weeks which may coincide with the completion of the first two months of treatment.) After the second set of samples is taken, begin 9 months of effective therapy. Effective therapy for Group C is defined as the administration of a specific number of doses of two anti-TB drugs within a specified time. The isolate from the index case must be sensitive to the two anti-TB drugs used. 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), and ethambutol (ETH). Isoniazid is recommended as one of the two drugs if the index case isolate is INH sensitive. Treatment can be administered using either of the following schedules:

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). 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 demonstrated that the elephant received 270 total doses at a dosage level sufficient to achieve adequate drug serum levels.

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 demonstrated 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: 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.

Concomitant use of anti-TB medications with other hepatotoxic drugs should be done with caution.

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

Travel: No Option 1 elephant should travel or have any direct contact with the public or previously non-exposed elephants until it has received 60 anti-TB drugs doses within 90 days and adequate drug blood levels have been documented. If animals are cultured at the end of the 60-dose regimen, and if the culture is negative for mycobacteria, the animal may travel and resume public contact provided it receives the next seven months of treatment. No elephant should move into a facility that has an untested elephant.

Monitoring of Option 1 Elephants

1. During the 9 months of treatment, monthly blood tests (CBC and serum chemistry profile) are recommended to monitor general health and possible drug effects on the liver. Liver functions tests (AST, ALT, LDH, and bilirubin) should be included in the serum chemistry panel. (INH may cause liver damage and anemia. In addition, leukopenia has occurred in at least one elephant apparently due to INH toxicity).
2. Beginning with the onset of treatment, cultures should be collected by the triple sample method every other month for 18 months. If all cultures are negative, collect three culture samples annually thereafter.
3. If treatment is discontinued before completion, the full conditions of Option 2 must be met.

Option 2: Monitor by Culture

If treatment is not elected, Group C elephants are restricted from traveling for 12 months. During this time, cultures should be collected by the triple sample method every other month for 18 months. If all cultures are negative, culture annually thereafter by triple sample method.

Group D (Current M. tuberculosis positive culture)

Animals that have had *Mycobacterium tuberculosis* complex isolated from any sample (sputum, stool, tissue, etc.) are considered as culture positive for *M. tuberculosis*. A culture positive

elephant is defined as an elephant from which *Mycobacterium tuberculosis* or *Mycobacterium bovis* has been isolated from any body site or specimen. A culture positive elephant is considered positive until 1) it has completed six months of treatment with documentation that adequate TB drug serum levels have been achieved on two separate testing dates and 2) at least two consecutive months of negative cultures obtained according to the procedures outlined in this protocol can be demonstrated. These elephants should be separated from the public for the duration of the treatment period. Separation from previously non-exposed elephants is also recommended. It is recommended that precautions to safeguard personnel health and safety 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. Group D should be tested and treated as follows:

Testing

1. If the organism was isolated at a laboratory other than NVSL, request that the laboratory submit the isolate to NVSL 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, and amikacin. (Antimicrobial susceptibility testing for *M. tuberculosis* complex organisms is now available at NVSL).
3. Perform ancillary diagnostic tests (recommended but not required):

- ELISA test
- Nucleic acid amplification
- Gamma interferon test
- Bank serum at start and end of treatment period.

Treatment

Pending antimicrobial susceptibility results, initiate empiric therapy with three or four of the following drugs: isoniazid, rifampin, pyrazinamide, and ethambutol. 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 three drugs to which the isolates are susceptible daily for two 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 three 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 two 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 three drugs to which the isolate is susceptible for two 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 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. 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 D should not travel or have public contact during the treatment period (12-15 months). Following completion of treatment, the attending veterinarian must verify that the elephant has received treatment as outlined in this protocol, and that adequate drug levels have been achieved. If treatment requirements are not met, travel and public contact is restricted until these are met.

Monitoring

1. During the 12 months of treatment, monthly blood tests (CBC and serum chemistry profile) are recommended to monitor general health and possible drug effects on the liver. Liver functions tests (AST, ALT, LDH, and bilirubin) should be included in the serum chemistry panel. (INH may cause liver damage and anemia. In addition, leukopenia has occurred in at least one elephant apparently due to INH toxicity).

2. Beginning with the onset of treatment, cultures should be collected by the triple sample method every other month during the treatment period and every other month during the 6-month post-treatment period. This intensive screening by culture ensures adequate therapy during the treatment period and is continued for 6 months after treatment has ended to ensure that the animal does not revert to a positive culture which would again pose a risk to animals or humans. If all cultures are negative, collect three culture samples annually thereafter.

GROUP E (Untested) If an elephant cannot be tested, it should not be permitted to have direct public contact 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 contact with the untested elephant or with its enclosure or equipment.

7. PRINCIPLES OF ANTI-TUBERCULOSIS THERAPY

The American Thoracic Society has published guidelines for the treatment of tuberculosis in humans (Am. J. Respir. Crit. Care Med. 1994; 149: 1359-1374). 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 active disease are treated similarly to humans. However, for elephants, the treatment period has been extended. For a Group C elephant (that has negative sputum cultures but has had recent exposure to a known positive elephant), treatment is a "modified" regime – with two drugs for 9 months. Skin testing is not reliable in elephants.

For humans, treatment of primary tuberculosis is to empirically administer four 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. Moreover, in regions or situations when the frequency of resistance exceeds 10%, initial therapy consists of five drugs. Acid-fast smears are not reliable on elephant trunk washes.

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 a single resistance, treatment is extended to 12 months with 2 drugs to which the organism is susceptible. For individuals infected with multi-resistant bacteria, 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.

8. 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, thiacetazone, and the quinolones (ciprofloxacin, levofloxacin).

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*-acetyl-transferase. The acetylated product is then excreted in the urine. Some ethnic groups (Native Americans, Eskimos, and Orientals) as well as others carry a recessive allele encoding for rapid acetylation of INH that results 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 possibly rocking behavior. Fermented products (mash or other feeds) should likely be avoided to minimize potential histamine reactions. Liver enzymes (SGOT, SGPT, bilirubin) should be monitored monthly for 2 months and then bimonthly if no liver toxicity is observed. INH has caused irreversible bone marrow failure in camels; a similar reaction in elephants has not been documented.

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.

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 which 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 function 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.

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.

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. Ethambutal 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 is irritating and poorly tolerated resulting in expulsion of the drug. Subcutaneous administration has been given successfully to bongo antelope.

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

Amikacin

Mechanism of action: Amikacin is an aminoglycoside antibiotic that acts on the 30S ribosome to inhibit protein synthesis.

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.

Ciprofloxacin, Levofloxacin

Mechanism of action: These two drugs are quinolone antibiotics which act to inhibit the DNA gyrase, The DNA gyrase is used to attain a supercoiled structure of DNA during DNA synthesis.

Toxicity: The quinolone antibiotics may result in antibiotic related diarrhea, photosensitivity, 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). There is no known experience in elephants.

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 ciprofloxacin.

9. DOSES 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.

Isoniazid	5 mg/kg (Oral or rectal)
Rifampin	10 mg/kg (Oral only)
Pyrazinamide	30 mg/kg (Oral or rectal)
Ethambutol	30 mg/kg (Oral only)

10. BLOOD LEVELS

Below are target human serum levels (ug/ml) 2 hours post-administration for the primary anti-TB drugs:

Drug	Target serum level (ug/ml)
Isoniazid	3-5
Rifampin	8-24
Pyrazinamide	20-60
Ethambutol	2-6

NOTE: We have found (Maslow et al, in preparation) that compared to humans, oral Rifampin absorption is slower in elephants. Peak RIF levels in elephants occur at about 6 hours post-administration. PZA and INH peak between 1-3 hours in elephants independent of route (PO, rectal).

Target blood levels for anti-TB drugs in elephants have not been 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 prior USDA approval.

11. NECROPSY

A post-mortem examination should be performed on all elephants that die. The examination should include a thorough search for lesions of tuberculosis regardless of exposure status. It is recommended that a trained veterinary pathologist direct the necropsy. Send representative sections of any gross lesions as well as sections of lung, liver, mesenteric lymph nodes, and bronchial lymph nodes (fixed in formalin) to NVSL in Ames, IA. Representative sections of the same tissues should be frozen and sent to NVSL for culture. Tissues intended for culture should be packed in individual whirl-paks or zip-lock baggies, labeled with a sharpie, and sent frozen. Freeze at -20ºC or on dry ice and send overnight on ice packs. Send a complete set of all tissues, as well as sections of any gross lesions to Dr. Montali at the National Zoo. See attached elephant necropsy protocol for further details.

Prior to any planned euthanasia of an elephant, trunk washes and 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 ELISA and other tests. **Note:** Dr. Richard Montali from the National Zoo should be contacted prior to any euthanasia and may be available

to assist in the necropsy.

12. EMPLOYEE HEALTH AND SAFETY

All employees that are in contact with elephants should be skin-tested for TB annually following established human testing guidelines. New employees should be tested prior to contact with elephants.

Any employee with a positive skin test (i.e. a positive intradermal reaction to purified protein derivative (PPD) of M. tuberculosis), should be evaluated for the possibility of active TB. It is recommended that health care providers who manifest a positive PPD receive INH prophylaxis unless there is a contraindication to treatment. Conversely, those declining treatment are followed yearly with a chest radiograph and clinical evaluation to determine whether they have developed active disease.

A positive skin test may result from either exposure to M tuberculosis, M. bovis, BCG injection, or exposure to non-tuberculous strains of mycobacteria. The American Thoracic Society has published guidelines for the interpretation of skin testing. If inoculation with BCG occurred more than 10 years ago, a positive PPD test should not be considered a reaction due to BCG, but should instead be considered as positive for exposure to TB.

Employees with acid-fast positive sputum smears should be removed from animal contact until it is determined whether this represents infection with an organism of the M. tuberculosis complex (*M. tuberculosis* or *M. bovis*). Treatment guidelines and recommendations for contact with animals and humans are available through state public health departments. At the present time there is no known transfer of non-tuberculous strains of mycobacteria between humans and animals (or human to human) via aerosolization.

Any facility housing a known culture-positive (*M. tuberculosis* complex*)* animal should develop a program to protect employees from TB exposure, to include the use of appropriate face masks (N95 HEPA filtered masks, certified by the National Institute for Occupational Safety and Health to protect against TB), disinfection procedures, and the use of separate implements for infected animals. The local public health department should be contacted for further guidelines.

Measures to protect staff from infected animals include the use of respiratory (N95) HEPA filtered masks during all direct or indirect contact with infected animals, including cage cleaning, medication administration, feeding, watering, etc. The facility should contact local health agencies and should provide additional other protective gear such as gowns, gloves, etc.

No specific precautions are necessary for animals that are culture positive for mycobacteria other than *M. tuberculosis* and *M. bovis*.

13. REPORTING

Ancillary test results will be compared with culture and necropsy results over the coming years to determine their validity. Please forward all test results to AAZV for compilation using the attached reporting form. Banking of frozen serum prior to treatment is recommended to facilitate future research needs.

APPENDIX 1. REFERENCES

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Montali, R. J., L. H. Spelman, R. C. Cambre, D. Chatterjee, and S. K. Mikota. Factors influencing interpretation of indirect testing methods for tuberculosis in elephants. 1999. Proc. Amer. Assoc. Zoo Vet. 1999: 109-112.

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APPENDIX 2. ACKNOWLEDGMENTS

The following individuals contributed to the development of these guidelines.

Dr. Wilbur Amand, American Association of Zoo Veterinarians
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Dr. Genevieve Dumonceaux, Busch Gardens
Dr. Freeland Dunker, San Francisco Zoo
Dr. Murray Fowler, University of California, Davis
Dr. Werner Heuschele, San Diego Zoo (in memorium)
Dr. Thomas Hoyt, USDA
Dr. Ramiro Isaza, Kansas State University
Dr. Barbara Kohn, USDA, APHIS, Animal Care
Dr. Scott Larsen, Colorado State University
Dr. William A. Lindsey, Feld Inc.
Dr. Harold McCoy, USDA
Dr. Joel Maslow, University of Pennsylvania
Dr. Bob Meyer, USDA, APHIS, Veterinary Services
Dr. Susan K. Mikota, Elephant Care International
Dr. Michele Miller, Disney's Animal Kingdom
Dr. Richard Montali, National Zoological Park
Dr. C. Douglas Page, Jacksonville Zoo
Dr. Janet Payeur, USDA APHIS National Veterinary Services Laboratories
Dr. Linda Peddie, Drs. Peddie
Dr. James Peddie, America's Teaching Zoo, Moorpark College
Mr. James Rogers, USDA
Dr. Mo Salman, Colorado State University
Dr. Dennis Schmitt, Southwest Missouri University
Dr. Denise Sofranko, USDA, APHIS, Animal Care
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

APPENDIX 3.

A TRUNK WASH TECHNIQUE FOR THE DIAGNOSIS OF TUBERCULOSIS IN ELEPHANTS

Ramiro Isaza, DVM, MS and Cornelia Ketz, DVM

Address
From the Department of Clinical Sciences, College of Veterinary Medicine, Kansas State University, 1800 Denison Avenue, Manhattan, Kansas, 66506, USA.

Summary
A trunk wash is a practical method of collecting a sample from an elephant's distal respiratory tract for Mycobacterium culture and is the technique recommended in the "Guidelines for the Control of Tuberculosis in Elephants" by the National Tuberculosis Working Group for Zoo and Wildlife Species. The procedure, however, is potentially dangerous to the handlers and requires cooperation of the elephant. Because of the limitations of using culture results as a screening test, the trunk wash results should be interpreted with care. A positive culture result identifies an elephant that is shedding tuberculosis organisms whereas a negative result is non-diagnostic.

Introduction
Tuberculosis in Asian elephants (Elephas maximus) has been sporadically reported in the literature for many years (1, 2). The isolation of Mycobacterium tuberculosis from elephants in the United States has resulted in the development of the "Guidelines for the Control of Tuberculosis in Elephants" by the National Tuberculosis Working Group for Zoo and Wildlife Species (http://www.aphis.usda.gov/ac/ElephTBGuidelines2000.html). Compliance with this policy requires that all elephants have annual mycobacterial cultures. In these guidelines, the trunk wash is recommended as the most practical method of obtaining a culture sample from an elephant. This paper describes the trunk wash technique as the authors are currently using it.

Materials and methods
The trunk wash technique requires that the elephant allow the handlers to restrain and manipulate the tip of trunk. This is difficult in an untrained elephant in that most elephants resent this manipulation, and the trunk is many times stronger than the combined force of several handlers. It is therefore important that the animals be trained to present the trunk, allow gentle manual restraint, and manipulation of the trunk tip during the collection of the sample. The training period varies with the individual elephant, the prior behavioral conditioning of the animal, and the skill of the handlers. In our experience, most animals can be adequately trained for the procedure in 2-4 weeks.

The materials needed for a trunk wash include: Sterile 0.9% saline solution, sterile 60 ml syringe, 1 gallon plastic zip lock type bags (heavy duty), and sterile, 50 ml, screw top, plastic jar or centrifuge tube. As long as attention is given to collecting a clean sample from the distal nasal passages, the materials and techniques for the sample collection can be modified. For example, some clinicians prefer to use a 14-gauge red rubber tube feeding tube inserted into the trunk tip instead of simply flushing the sterile saline into the trunk tip. Another common variation is to

use a sterile plastic container to catch the trunk wash fluid instead of a plastic bag.

Procedure
A routine screening of an elephant should consist of a series of three trunk wash samples collected on separate days within a one-week period. Trunk washings should be collected in the morning and prior to water being offered to the animal. These recommendations are made in an attempt to obtain a representative sample of the nasal flora from the previous night, and to avoid the dilution effect caused by elephants drinking water with their trunks.

The elephant's trunk is manually restrained by the handlers so that the tip is held up. The 60 ml syringe filled with sterile saline is then inserted into one of the nostrils and the saline quickly flushed into the trunk. The handler then lifts the trunk tip as high as possible to help the fluid flow as far into the trunk as possible. The 1 gallon plastic bag is then slipped over the trunk tip and the tip of the trunk is lowered to allow the fluid to drain. If possible, the elephant is allowed to exhale into the bag during this collection phase of the procedure. A good sample should retrieve a significant portion of the saline that was placed into the trunk (about 40 ml). The sample should contain visible mucus from the inside of the trunk and often contains dirt and food particles that are normally found inside the trunk. The collection of moderate amounts of foreign material does not invalidate the sample. If, however, the collector feels the contamination is excessive, a second flush may be attempted.

Once the sample is collected in the plastic bag, it is carefully transferred into a labeled container. Ideally, the sample is refrigerated and sent directly to a laboratory for processing and mycobacterial culture. If the sample cannot be sent directly for culturing, it may be frozen in a regular freezer (-20 to -10 ^{O}C) until it can be sent to the laboratory. Often the recommended three daily cultures samples are collected and frozen until all samples are collected and the batch of samples can be sent to the laboratory together.

Discussion
Identification of a M. tuberculosis infected animal has significant management implications to both the animal and the collection. Management of the infected animal may require isolation of the exposed herd, potential removal of the animal from exhibit or shows, and if elected, treatment of the animals and exposed herd which can be very expensive. In the worst case, a positive diagnosis may lead to euthanasia of the infected animals. For these reasons, the screening test selected needs to be definitive and have as few false positives as possible. A positive culture of M. tuberculosis is, therefore, the only diagnostic test result used as a basis for making decisions in the guidelines .

The trunk wash as a method of collecting a culture sample from elephants was selected by the National Tuberculosis Working Group for Zoo and Wildlife Species because it is a practical method of obtaining a culture sample from a large proportion of the elephant population. The procedure requires no sedation or undue stress to the animal. Additionally, the procedure requires no specialized or expensive equipment.

An important consideration of this procedure is that it can potentially be very dangerous to the handlers. This is particularly true when attempted on an uncooperative elephant, because any

attempts to manually restrain the trunk in an uncooperative elephant can lead to injury. The time spent training the elephant to accept this method will greatly increase the efficiency and safety of the procedure. In some cases, with potentially dangerous or unpredictable animals, an increased level of handler safety can be obtained by having the animal lie in sternal or lateral recumbency prior to sample collection. This technique does not guarantee safety or successful sample collection, as it still requires cooperation of the animal and does not replace adequate training. In the case of elephants managed under protective contact, the animal's trunk can be handled though a set of bars. This method still requires that the animal is fully cooperative and, therefore, usually requires extensive training prior to the collection.

A second safety issue is the potential for zoonotic infection. Recently there has been documentation of a zoonotic transmission of tuberculosis between humans and elephants (3). During the collection of the trunk wash sample, there is exposure to aerosolized mucus from the elephant's respiratory tract. The authors, therefore, suggest that the collectors and handlers wear protective gear during the collection process. Minimal precautions would include a well fitted respirator or face mask capable of filtering 0.3 micron particles, disposable gloves, and working in a well-ventilated, sunlit, area.

Mycobacterial culture as the primary method of detecting infected animals has several limitations that are best illustrated by examination of the underlying biological assumptions. The first assumption is that most infected elephants have respiratory infections. Although the literature suggests that most infected elephants have respiratory infection, there have been no comprehensive necropsy studies to confirm these observations. The second assumption is that most infected animals shed mycobacterial organisms into the respiratory tract. There is little data that determines if and when an infected animal will begin shedding organisms. It is unknown what proportion of elephants can carry latent or "walled off" infections that would be missed with culturing techniques. A third assumption is that animals that are shedding will pass mycobacteria organisms at least once in the three-day testing period. Currently it is unknown if shedding animals pass organisms periodically or continuously. Finally, the samples collected from the distal trunk are often contaminated with normal bacterial flora and foreign material. It is assumed that these contaminants do not routinely overgrow or mask the growth of pathogenic mycobacteria, although no studies have tested this assumption. The interpretations of the culture results should, therefore, be limited. A positive culture is strong evidence that the animal is shedding mycobacteria and is infected; negative culture results provide little information as to whether the elephant is infected or not.

Culturing the distal trunks of all the animals in a population will only detect animals shedding tuberculosis through the trunk, and not detect all animals that are infected. However, with time and repeated cultures of all animals in the population, it may be possible to detect and treat most of the elephants shedding infectious organisms. If these animals are then treated properly and shedding of organisms stops, the spread of tuberculosis from elephant to elephant should decrease in the population.

APPENDIX 4. TESTING LABORATORIES

CULTURES

USDA APHIS VS
National Veterinary Services Laboratories (NVSL)
Dr. Janet Payeur , Head, Mycobacteria and Brucella Section
1800 Dayton Avenue / PO Box 844
Ames, IA 50010
(515) 663-7676 Fax: (515) 663-7315
Email: Janet.B.Payeur@aphis.usda.gov

Send trunk washes to NVSL either frozen or 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 from NVSL. If lesions are submitted for culture, tissues should be frozen. Lesioned tissues should be split and ½ should be sent to the histopathology lab so PCR can be run if the tissue is compatible for tuberculosis. Use the VS Form 10-4 for submission. Cost: $27 per sample for processing plus minimum of $54 for speciation of Mycobacterial positive cultures. To establish an account for billing, contact Connie Osmundson (515) 663-7571. Antimicrobial susceptibility testing for *M. tuberculosis* complex organisms is now available at NVSL. DNA fingerprinting of *M. tuberculosis* isolates is also available. Please call to arrange testing.

HISTOPATHOLOGY

Dr. Richard Montali
National Zoo - Department of Pathology
3001 Connecticut Ave.
Washington, DC 20008
(202) 673-4869 Fax: (202) 673-4660
Email: rmontali@nzp.si.edu

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.

****

Dr. Art Davis (Chief of Pathology) 515-6637526
Arthur.J. Davis@aphis.usda.gov
or Dr. Mark Hall (Head Pathological Investigations) 515-663-7927
Mark.Hall@aphis.usda.gov

USDA APHIS NVSL Pathobiology Laboratory
1800 Dayton Avenue
Ames, IA 50010
(515) 663-7521
Fax 515-663-7527

Send formalin sections of any gross lesion and target tissues (lung, liver, mesenteric and bronchial lymph nodes). Use leak proof container.

ELISA (CSU-APHI)

This test has been evaluated in many elephants and results-to-date suggest that it may be very useful in rapidly diagnosing Asian elephants with active *M. tuberculosis* infection. Validation studies using a small number of confirmed infected elephants and a limited number of probable negative elephants have shown the test to have extremely good predictive value in determining infection status. More testing needs to be completed in order for this test to be fully validated. The validity for this ELISA for African elephants and/or elephants infected with *M. bovis* is unknown. Until the validity of the ELISA test in elephants is further evaluated, culture should still be used as the definitive diagnostic test.

Dr. Mo Salman
Mycobacterium bovis Testing Laboratory
Animal Population Health Institute
College of Veterinary Medicine and Biomedical Sciences
Environmental Health Building, Room 107
Colorado State University
Ft. Collins, CO 80523-1676
(970) 491-7950; Fax: (970) 491-1889

Send 4 ml of frozen serum for each test; send overnight on ice packs. Currently no charge. See submission form Appendix 7. For questions regarding sample submission, test results, or test interpretation, please contact: Dr. Scott Larsen; (919) 661-3565; Email: rslarsen@unity.ncsu.edu

NUCLEIC ACID AMPLIFICATION TEST (NAAT)

The most commonly used NAAT is the Gen Probe® *Mycobacterium tuberculosis* Direct Test (MTD). The MTD utilizes transcription-mediated amplification to specifically replicate ribosomal RNA from bacteria of the *M. tuberculosis complex* (*M. tuberculosis, M. bovis, M. bovis BCG, M. africanum*, and *M. microti*). A positive MTD test result means that RNA from *M. tuberculosis* complex organisms may be present in the sample. Both live and dead organisms are detected. The MTD test has the advantage of quick turn around time. Results are usually available within one week of submission compared to eight weeks that is generally required for culture. NOTE: Several elephants are MTD-positive but culture-negative. Until further evaluation of the validity of the MTD test in elephants, culture should still be used as the definitive diagnostic test.

Send samples to NVSL (see above address) or other suitable laboratory. Call first. Collect trunk wash samples in 20 ml sterile tubes; ship overnight on ice packs. Cost: ~$30.

ANTI TB DRUG LEVELS

Dr. Charles Peloquin
National Jewish Medical and Research Center
1400 Jackson St.
Denver, CO 80206
(303) 398-1925, 398-1448, 398-1427 Fax: (303) 270-2229
E-mail: Peloquin@NJC.org Web site: www.njc.org

Collect blood in 8-10 ml red top tubes. A minimum of 2 ml of serum is needed for each drug. Centrifuge, harvest serum and freeze (-70C preferred, otherwise -20C immediately). Ship on dry ice overnight Monday-Thursday. Contact lab by e-mail or fax for submission form. Cost is approximately $50/time point/ drug.

GAMMA INTERFERON TEST

Diana Whipple, Research Leader
Bacterial Diseases of Livestock Research Unit
USDA, ARS
National Animal Disease Center
2300 Dayton Avenue, PO Box 70
Ames, IA 50010
(515) 663-7325 Fax: (515) 663-7458
E-mail: dwhipple@nadc.ars.usda.gov

This test is still in the developmental stage. It is essential to contact the lab prior to shipment. Sample must arrive within 16 hours. Results may not be available for some time. Send one 10 ml heparin tube of whole blood at room temperature by overnight delivery. Pack in styrofoam container to protect from temperature extremes during shipment. Do not use cold packs or ice. No charge at this time.

APPENDIX 5. CONTACTS FOR QUESTIONS

DIAGNOSIS AND TREATMENT

Dr. Michele Miller
Disney's Animal Kingdom
Department of Veterinary Services
PO Box 10,000
Lake Buena Vista Florida 32830-1000
Tel: (407)-939-7316; Fax: (407)-938-1909
Email: Michele.Miller@disney.com

Dr. Genevieve Dumonceaux
Busch Gardens
PO Box 9158
Tampa FL 33674
Tel: (813) 987-5561; Fax: (813) 987-5548
E-mail: genevieve.dumonceaux@anheuser-busch.com

Dr. Susan K. Mikota
Elephant Care International
3520 Mimosa Ct.
New Orleans LA 70131
Email: smikota@yahoo.com; www.elephantcare.org

REGULATORY

Dr. Denise Sofranko
USDA, APHIS, AC
1629 Blue Spruce Drive, Suite 204
Ft. Collins, CO 80524-2013
Voice Mail : (703)-812-6682; Fax: (505)-293-7466
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HUMAN HEALTH, ELEPHANT THERAPY AND TREATMENT

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NECROPSY, PATHOLOGY

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National Zoo
Department of Pathology
3001 Connecticut Ave., NW
Washington, DC 20008
Tel: (202) 673-4869; Fax: (202) 673-4660
E-mail: rmontali@nzp.si.edu

ELISA TEST

Dr. R. Scott Larsen
Medicine and Epidemiology Dept.
School of Veterinary Medicine
University of California
Tupper Hall
Davis, CA 956161
Tel: (530) 752-1393; Fax: (530) 752-0414
E-mail: slarsen@ucdavis.edu

REPORTING TEST RESULTS (USE ATTACHED FORM)

Dr. Wilbur Amand
American Association of Zoo Veterinarians
6 North Pennell Rd.
Media, PA 19063
Tel: (610) 892-4812 Fax: (610) 892-4813
E-mail: aazv@aol.com

INTERNET

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

1. www.aphis.usda.gov/ac/ElephTBGuidelines2000.html (available to the public)
2. www.aazv.org (available to AAZV members by password)
3. www.elephantcare.org (available to the public)

APPENDIX 6. SUPPLIERS OF ANTI-TUBERCULOSIS DRUGS

Congaree Veterinary Pharmacy
1309-B State Street
Cayce, S.C. 29033
Tel: (877)-939-1335 (toll free); Fax (803)-939-0073
Contact: Terry Fiffick, R.Ph.
Email: congaree@ix.netcom.com

Premier Pharmacy
8269 Commercial Way
Weekiwachee, Florida 34613
Contact: Vern Allen
Tel: (800)-752-7139

Spectrum Chemical Mfg. Corp.
14422 S. San Pedro St.
Gardena, CA 90248
310-516-8000
800-342-6615 for bulk chemicals; must order 25 kg minimum

APPENDIX 7. VS FORM 10-04

Instructions for Completing VS Form 10-4

ITEM 12 - Definitions of Diagnostic Case Categories

General Diagnostic Case - A case in which the tests conducted are for the purpose of diagnosing or confirming a domestic disease, and/or the analysis of environmental products that may be contributing to an existing disease condition.

FAD/EP Diagnostic Case - A case in which the tests conducted are for the purpose of diagnosing or confirming a foreign disease, or for the eradication of a foreign disease that has gained entrance into the United States.

NVSL Intralab Diagnostic Case - A case in which the tests conducted are for the purpose of diagnosing or confirming a disease condition, analyzing environmental products that may be contributing to a disease condition or for analyzing chemical products for another laboratory of NVSL.

Surveillance/Monitor Case - A case in which the tests conducted are for the purpose of monitoring for a specific disease, for a specific insect or insect vector, or for analyzing specific products that are used in treating animals or poultry or for decontamination of animal poultry facilities.

Developmental/Research Case - A case in which the tests conducted are for the purpose of supporting a developmental or research project conducted by another laboratory of NVSL, by staff or field personnel of VS or by other laboratories, institutions, or agencies.

Reagent Evaluation Case - A case in which the tests conducted are the purpose of evaluating a reagent produced by another laboratory of NVSL or by other laboratories, institutions, or agencies.

Import Case - A case in which the tests conducted are for the purpose of qualifying animals or poultry, including wild animals and birds, or animal or poultry products for importation into the United States.

Export Case - A case in which the tests conducted are for the purpose of qualifying animals or poultry, including wild animals and birds, or animal or poultry products for exportation to a foreign country.

TB - A case with a specific request for diagnostic of TB.

ITEM 20 - Identification

Identify Samples with Consecutive Numbers - Record animal identification (number or name) adjacent to appropriate sample number. Laboratory results will be reported by sample identification number. Indicate approximate age in years (y), months (m), weeks (w), or days (d), and indicate sex of each animal. See sample below. When more than 10 samples, use VS Form 10-4A.

IDENTIFICATION		AGE	SEX	IDENTIFICATION		AGE	SEX
Sample	Animal			Sample	Animal		
1	12ABC0000	3y	F	6	12ABC0005	10d	F
2	12ABC0001	2y	M	7	12ABC0006	10m	F
3	12ABC0002	1y	F	8	12ABD0007	8m	M
4	12ABC0003	6m	F	9	12ABC0008	2 1/2y	F
5	12ABC0004	3w	M	10	12ABC0009	15m	M

Send a copy of the VS 10-4 to the Veterinarian-in-Charge (in submitter's state).
Retain a copy for your records.

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
NATIONAL VETERINARY SERVICES LABORATORIES
P.O. BOX 844, 1800 DAYTON AVENUE
AMES, IOWA 50010
(515) 663-7212

SPECIMEN SUBMISSION

INSTRUCTIONS: Use a separate form for each species and each owner/broker. See instructions for completing VS FORM 10-4 for definitions (Item 12) and instructions for identification (Item 20).

PAGE ___ OF ___

1. NAME OF SUBMITTER

2. NAME OF OWNER

MAILING ADDRESS (Street, City, State, and Zip Code)

CITY

STATE

3. LOCATION OF ANIMALS

COUNTY

STATE

Phone No.

FAX No.

4. PAYMENT METHOD ("X" applicable item and provide information)

☐ USER FEE ACCOUNT NO.: _____

☐ MC/VISA NO.: _____

EXP. DATE:

☐ CHECK/MONEY ORDER ENCLOSED (Made payable to "USDA" in U.S. Dollars)

5. HERD/FLOCK SIZE

6. NO. IN HERD/FLOCK AFFECTED

7. NO. IN HERD/FLOCK DEAD

8. EXAMINATIONS REQUESTED

9. COLLECTED BY

10. DATE COLLECTED

11. AUTHORIZED BY

12. PURPOSE OF SUBMISSION ("X" one) (See instructions for definitions)

☐ General Diagnostic ☐ Surveillance ☐ Import ☐ Interstate Movement

☐ FAD/EP Diagnostic ☐ Developmental Research ☐ Export

☐ NVSL Intralab Diagnostic ☐ Reagent Evaluation ☐ TB

13. COUNTRY OF ORIGIN/DESTINATION

14. REFERRAL NUMBER

15. PRESERVATION ("X" applicable item(s))

☐ None ☐ Ice Pack ☐ Dry Ice ☐ Formalin ☐ Borax ☐ Alcohol ☐ Other (specify)

16. SPECIMENS SUBMITTED ("X" applicable item(s))

☐ Blood ☐ Feces ☐ Parasite ☐ Serum ☐ Tissue ☐ Whole Bird ☐ Other (specify)

☐ Culture ☐ Feed ☐ Plant ☐ Soil ☐ Urine ☐ Fetus

☐ Extract ☐ Milk ☐ Semen ☐ Swab ☐ Water

17. TOTAL NUMBER OF SPECIMENS SUBMITTED

18. SPECIES OR SOURCE ("X" one)

☐ Cattle ☐ Goat ☐ Environment ☐ Chicken ☐ Bison ☐ Deer ☐ Other (specify)

☐ Swine ☐ Horse ☐ Reagent ☐ Turkey ☐ Dog ☐ Elk

☐ Sheep ☐ Donkey ☐ Pet Bird ☐ Cat ☐ Fish

19. NUMBER OF ANIMALS SAMPLED

20. IDENTIFICATION (See instructions)

Sample ID	Animal ID/Breed	Age	Sex

IDENTIFICATION (See instructions)

Sample ID	Animal ID/Breed	Age	Sex

21. ADDITIONAL DATA (History, clinical signs, post mortem findings, remarks, tentative diagnosis, etc. Use additional sheets if necessary.)

22. SIGNATURE OF SUBMITTER AND DATE

NVSL USE ONLY

CONDITION	PRIORITY	DISTRIBUTION	RECEIVED BY

NVSL ACCESSION NO

VS FORM 10-4 (JULY 97)

reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden, to Department of Agriculture, Clearance Officer, OIRM, Room 404-W, Washington, D.C. 20250, and to the Office of Information and Regulatory Affairs, Office of Management and Budget, Washington, D.C. 20503.

FORM APPROVED
OMB NO. 0579-0090

U.S. DEPARTMENT OF AGRICULTURE
ANIMAL AND PLANT HEALTH INSPECTION SERVICE
NATIONAL VETERINARY SERVICES LABORATORIES
AMES, IOWA 50010

CONTINUATION SHEET FOR SPECIMEN SUBMISSION

INSTRUCTIONS: Use this form only as a continuation of information on VS FORM 10-4.

PAGE

OF

2. NAME OF OWNER/BROKER (Last, First, MI)

13. REFERRAL NUMBER

CITY

STATE

21. IDENTIFICATION				IDENTIFICATION			
Sample ID	Animal ID/Breed	AGE	SEX	Sample ID	Animal ID/Breed	AGE	SEX

VS FORM 10-4A
(JUN 92)

VS Form 10-04 is also available through the APHIS website:

Submission form 10-4 (NVSL sample submission) at :
http://www.aphis.usda.gov/vs/nvsl/Forms/vsform10-4.pdf

Continuation form 10-4a:
http://www.aphis.usda.gov/vs/nvsl/Forms/vsform10-4a.pdf

APPENDIX 8.

TB ELISA SAMPLE SUBMISSION FORM FOR ELEPHANT SAMPLES

PAGE___
OF ___

Mycobacterium bovis Testing Laboratory
Center of Veterinary Epidemiology and
Animal Disease Surveillance Systems
Colorado State University
Environmental Health Bldg. RM 107
Fort Collins, CO 80523-1676

Attn: Joni
Phone: (970) 491-2379 Fax: (970) 491-2940

For Questions Regarding Sample Submission,
Test Results, or Interpretation Please Contact:

Dr. R Scott Larsen
Phone: (919) 661-3565
rslarsen@unity.ncsu.edu

LAB USE ONLY

JTPID:

Date Rec'd:

Location:

SUBMITTED BY:	PHONE:	NUMBER OF SAMPLES:
CLINIC/INSTITUTION:	FAX:	
ADDRESS:	E-MAIL:	
CITY: STATE: ZIP:	OWNER OF ANIMALS:	

Date Serum Taken	Eleph Name	Stud-book or ISIS #	Species	Sex	Age	Birth Date	Time at Current Facility	Trunk Wash Date	Trunk Wash Results	Skin Test Date	Skin Test Results	PCR Date	PCR Results

APPENDIX 9.

NATIONAL JEWISH

1400 Jackson Street
Denver, Colorado 80206
Client Services (303)-398-1974

Infectious Disease Pharmacokinetics Laboratory Requisition

Most tests require 3-7 days to complete.

Patient Last, First Name, M.I. (**Required**)		Date of Birth	☐Male ☐Female
Collection Date:	Collection Time:	Patient ID:	Diagnosis (ICD-9) Code:
Referring Physician:		Physician UPIN #	Physician Phone #
Fax: (☐ Fax Results)		Facility Phone #	
Mail Results to: (**Required**) P.O. #			

COMPLETE SECTION BELOW ONLY IF YOU DO NOT WANT YOUR ACCOUNT BILLED

Name of Responsible Party (Last, First, M.I.):		Medicare (HIC) #
Street Address		Medicaid ID #
City State Zip Code		Patient Bills must be prepaid or accompany specimen: ☐Visa ☐Mastercard ☐ Other_____ CC#_____ Exp.Date_____
Telephone #	Relation to Patient:	
Please Bill: ☐**Medicare** ☐**Medicaid Colorado**		☐**Patient (Pre-Pay)** ☐ Check ☐ Credit Card

(Please submit a separate requisition for each sample collection time) Specimen source (circle one): serum cerebrospinal fluid other:

REQUIRED	Drug 1	Drug 2	Drug 3	Drug4
Drug name to be Assayed				
ICD-9 Code				
Drug Dose (mg) (Specify: PO, IV, IM)				
# Doses per week				
Date of last dose				
Time of last dose (For IV: Start/End)				
Date blood drawn				
Time blood drawn				

The number of hours after the dose to collect "peak" concentrations is shown in parentheses after each drug name below. To test for delayed drug absorption, a second sample may be collected 4 hours after the "peak". Trough concentrations (prior to next dose) may be helpful for Amprenavir, indinavir, nelfinavir, ritonavir, saquinavir and Efavirenz.

Drugs(s) to be assayed *(provide 2 ml serum per test)*

AMPL	Amprenavir	(2-3 & trough)	**FLUCZ**	Fluconazole	(2 h)	**PASH**	p-Aminosalicylic acid	(6 h)
AZL	Azithroumycin	(2-3 h)	**INDL**	Indinavir	(1-2 h & trough)	**PZAH**	Pyrazinamide	(2 h)
CIPH	Ciprofloxacin	(2 h)	**INH**	Isoniazid	(1-2 h)	**RBN**	Rifabutin	(3 h)
CLART	Clarithromycin	(2-3 h)	**ITRL**	Itraconozole	(3-4 h)	**RIFN**	Rifampin	(2 h)
CFH	Clofazimine	(2-3 h)	**KMH**	Kanamycin**	(2 h)	**RFPTN**	Rifapentine	(5 h)
CSH	Cycloserine	(2-3 h)	**LOPL**	Lopinavir	(4-6 h & trough)	**RTVL**	Ritonavir	(2-3 h & trough)
EFVL	Efavirenz	(5 h-trough)	**NLFL**	Nelfinavir	(2-3 h & trough)	**SAQL**	Saquinavir	(2-3 h & trough)
EMBH	Ethambutal	(2-3 h)	**NEVL**	Vevirapine	(2 h & trough)	**SMH**	Streptomycin	(2 h)
ETAH	Ethionaminde	(2 h)	**OFLHL**	Ofloxacin/Levofloxacin	(2 h)			

** Kanamycin is determined using a bioassay. All other antibiotics must be stopped at least 24 hours prior to sample collection for these drugs. These tests take an additional week to complete. Please call for more information.

Sample preparation and shipment:

- Collect in a plain, 8-10 ml red top tube, which typically provides enough serum for 2 drugs to be assayed.
- Centrifuge, harvest all serum (minimum 2 ml per test).
- Freeze at -70°C if possible (otherwise -20°C) immediately in labeled polypropylene or similar tube. Allow room for expansion of sample inside tube.
- Ship on $\geq$ 3 lbs. Dry ice via overnight mail Monday through Thursday to: National Jewish Medical and Research Center, 1400 Jackson Street, Denver, CO 80206, Attn: IDPL, Rm. D106.

List other medications patient is currently taking:

_____

_____

_____

For NJC Use Only
Date Received:_____
Time Received:_____

Condition: **(circle one)**

frozen partially frozen thawed

One copy for the LAB --- One copy for CLIENT

APPENDIX 10.

Annual Elephant Tuberculosis Surveillance Report Form for 200___

Institution:_____
Submitter: _____
Address:_____
_____
Tel: _____Fax: _____ email: _____

Asian [ ] African [ ] ISIS # _____Studbook # _____Name _____
Age: _____ [] actual [ ] estimate Sex: [ ] male [ ] female

EXPOSURE STATUS:
[ ] Known infected animal
[ ] Known exposure to culture positive source within the past 12 months
[ ] Known exposure to a culture positive within the past 1-5 years
[ ] No known exposure to a culture positive source in the last 5 years

CULTURE RESULTS
Source (trachea; trunk; oropharynx; other): _____
Method: (swab; trunk wash; other);_____
Test date:_____
Laboratory:_____
Results: (negative; M.tb complex**;** M.tb; M. bovis; M. avium; other (list): _____

(Note: Results reported as M.tb complex do not differentiate between M.tb and M.bovis.)

ELISA RESULTS
Date of last tuberculin test: _____ Site: _____ Tuberculin used:_____
Test date: _____
Lab: _____ Result: _____

NUCLEIC ACID AMPLIFICATION TESTS
Test: _____
Sample source: _____
Test date: _____
Lab: _____ Result:_____

Send completed form to:
Dr. Wilbur Amand
American Association of Zoo Veterinarians
6 North Pennell Rd
Media PA 19063
T: 610-892-4812; F:610-891-4813; email:aazv@aol.com

APPENDIX 11.

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/serum
[ ] 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:
Dr. Michele Miller
Disney's Animal Kingdom-Dept. of Vet. Services
1300 N. Savannah Circle West
Bay Lake, FL 32830
(407) 939-7316; email: Michele.Miller@disney.com