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.