

## REPORT OF THE COMMITTEE ON TUBERCULOSIS

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Vice Chair: Michael S. VanderKlok, MI

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The Committee met on November 16, 2010, from 8:00 a.m. to 5:30 p.m. at the Hilton Minneapolis, Minneapolis, Minnesota. There were 138 members and guests in attendance. Dr. Kathleen M. Connell and Dr. James Averill presided. Dr. Averill served as Acting Vice Chair in Dr. Michael S. VanderKlok's absence.

In her opening remarks, Dr. Connell reviewed the day's agenda, welcomed members and guests and made a few housekeeping announcements. The Chair determined that a quorum was present to conduct business.

The Chair established five Subcommittees in 2007 to address specific issues. These Subcommittees included the Diagnostic Test Review Subcommittee, chaired by Dr. Tyler Thacker; the Elephant TB Guidelines Subcommittee, chaired by Dr. Janet Payeur; the TB Test-and-Remove Assessment Subcommittee, chaired by Mr. Phil Durst; the Eventing Cattle Subcommittee, chaired by Dr. Chuck Massengill; and the Education and Communication Subcommittee, chaired by Dr. John Maulsby. Only the Elephant TB Guidelines Subcommittee had a report and a proposed resolution.

After the Chair's opening remarks, Dr. Bob Meyer, Assistant State Veterinarian, Wyoming Livestock Board, gave a tribute to Dr. Mitch Essey for his contributions to the National Bovine Tuberculosis Eradication Program.

*"Thank you for the opportunity today to pay tribute to Dr. Mitchell Essey, a man dedicated to eradicating bovine TB during his career. Even though he is now gone, he remains an inspiration and a good friend to many. Dr. Essey graduated from Michigan State University with a degree in veterinary*

medicine in 1955, and completed further graduate studies at the University of Colorado in Denver where he took a special interest in mycobacterial disease pathogenesis and epidemiology – especially tuberculosis. For much of his professional career with USDA he directed his life pursuing the eradication of bovine tuberculosis and brucellosis from livestock populations in the United States. Dr. Essey was active in both the TB and Brucellosis Committees of USAHA for many years, and played a significant role in reducing the national prevalence of both of these diseases; a benefit that our livestock industries enjoy today.

*Dr. Essey was a mentor to many, myself included. He had a unique and sincere way of instilling the important need to embrace the goal of final eradication of TB and brucellosis. He was scientifically sound, spoke with sincere and honest conviction, and thoroughly understood the epidemiologic and economic consequences of the diseases he worked most closely with. This great understanding allowed him to further develop, support, and champion both the TB and brucellosis programs. In 1991, Dr. Essey was instrumental in developing a successful TB eradication program for the captive cervid industry by working closely with captive cervid producers.*

*Dr. Essey strongly supported efforts in research, and believed that providing new educational opportunities for aspiring epidemiologists was crucial to future program success. Those of us that closely knew Mitch loved him. There was never a moment that Mitch would not take the opportunity to talk with and educate all that he knew. I believe that the many people that had the opportunity to interact with him can truly say it was time so very well spent. Such moments with Mitch were treasured.*

*Dr. Essey poured himself into the philosophy that bovine TB can be eradicated from U.S. livestock if a strong, persistent and collective will exists. And, he believed that commitment to make it become a reality must be continually renewed. If Mitch was here today he would remind us that bovine TB will quickly erase the tremendous gains we've made over the past 100-plus years if it is allowed to regain a foothold. One can only review what has happened in Great Britain over the past 10 years, and this past year alone in several "Accredited Free" U.S. states to better appreciate his beliefs and warnings.*

*Dr. Essey, we thank you and greatly appreciate your contributions. Although you are now gone, your inspiration, friendship, and efforts in TB eradication will not be forgotten."*

Formal presentations began with Dr. Thomas "TJ" Myers, Chief Policy Officer/Associate Deputy Administrator, U.S. Department of Agriculture (USDA) Animal and Plant Health Inspection Service (APHIS) Veterinary Services (VS), who gave a presentation entitled "Progress Update on A New Approach to the National Tuberculosis Program".

Dr. Myers was followed by Dr. Alecia Larew Naugle, National TB Program Manager, National Center for Animal Health Programs, USDA-APHIS-VS, who gave the National Tuberculosis Program Update. The full text of Dr. Naugle's report is included in these proceedings.

A Time Specific Paper was presented, entitled "Identification of Molecular Targets for Diagnosis of Bovine Tuberculosis". The paper was presented by Ms. Ailam Lim, Immunodiagnostics Laboratory, Diagnostic Center for Population and Animal Health, Michigan State University. This paper's abstract is included in its entirety in these proceedings.

The Time Specific Paper was followed by a report of the Elephant Tuberculosis Guidelines Subcommittee given by Dr. Michele Miller, Subcommittee Member. Dr. Miller mentioned that this Subcommittee was formed in October 2007 by the Chair of the USAHA Committee on Tuberculosis, Dr. Kathleen Connell, at the request of the American Association of Zoo Veterinarians Working Group. Since 1996, the National Tuberculosis Working Group for Zoo and Wildlife Species had been responsible for developing and revising the "Guidelines for Control of Tuberculosis in Elephants" in 1997, 2000 and 2003.

The USAHA Elephant TB Guidelines Subcommittee (Subcommittee) was formed and met to review and revise the 2003 guidelines in 2008. The Subcommittee was asked again in 2010 to review and revise the 2008 guidelines in light of new scientific publications, public health concerns by the Centers for Disease Control and Prevention (CDC) at an elephant facility and data collected from official USDA diagnostic testing. The Subcommittee recommends replacing the 2008 Guidelines with the 2010 version of the "Guidelines for Control of Tuberculosis in Elephants". A summary of changes between the versions include:

- Additional clarification and requirements on the classification of treated and exposed elephants within the TB management group options for culture positive or serologically reactive elephants.
- The management groups now include culture results, serological results and *M. tuberculosis* complex exposure history and recommendations for increased frequency of surveillance in some cases.
- Provides for the potential use of a newly developed serological test—Chembio Diagnostic Systems Inc., Dual Path Platform (DPP®) VetTB Assay which was evaluated by Greenwald *et al* in 2009. (Greenwald, R., Lyashchenko, O., Esfandiari, J., Miller, M., Mikota, S., Olsen, J.H., Ball, R., Dumonceaux, G., Schmitt, D., Moller, T., Payeur, J.B., Harris, B., Sofranko, D., and Waters, W.R., Lyashchenko, K. 2009. Highly accurate antibody assays for early and rapid detection of tuberculosis in African and Asian elephants. *Clinical and Vaccine Immunology* 16(5):605-612.)
- Added flowcharts for the TB management groups in the appendices.
- Updated reference information.

The Subcommittee respectfully submitted its report along with the “2010 Guidelines for Control of Tuberculosis in Elephants” to the TB Committee for acceptance. The Guidelines are included in their entirety in this report.

The Subcommittee proposed a resolution on accepting the revised guidelines. After discussion, the resolution was voted upon by the Committee membership and passed.

State perspectives on the National TB Program were provided during a state roundtable moderated by Dr. Connell. States represented included Colorado, Indiana, Kentucky, Michigan, Minnesota, Nebraska, New Mexico, and South Dakota.

Reports began with Keith A. Roehr, DVM, Colorado State Veterinarian. Dr. Roehr reported in March 2010 a Holstein cow found with bovine tuberculosis by USDA, Food Safety and Inspection Service (FSIS) during regular slaughter inspection. The NVSL results were histopathology and polymerase chain reaction (PCR) positive. Culture results on April 29, 2010 were positive for *Mycobacterium bovis* and spoligotyping demonstrated cultures to be genetically similar to a Coronado Feeder strain. The herd of origin was a Colorado dairy herd of approximately 900 adult cows and calves. A whole herd test for bovine tuberculosis on May 2010 consisted of 498 adult cattle skin tested (CFT), 162 skin test suspects (32.5%), comparative cervical tuberculin (CCT) test positives 124 (76.5%), and Gamma interferon test positives 105 (64.8%). Initial test data suggested significant TB infection may exist in the origin herd! Hundreds of trace investigations lead to six facilities found to be TB positive. Five of the six have been depopulated and Cleaned and Disinfected. These herds were non-traditional producers; small feeders/traders that ranged from 6 to 140 head. There was no demonstration of comingling with breeding herds.

Dr. Roehr was followed by Dr. James Hollis, Designated Tuberculosis Epidemiologist, Indiana State Board of animal Health. Dr. Hollis gave a report on the 2010 Ohio TB Slaughter Traces to Indiana. On 07/01/2010, the Designated TB Epidemiologist for Ohio informed the Indiana Veterinary Services Area Epidemiology Officer of possible traces to Indiana for two cattle slaughtered in Pennsylvania and confirmed PCR positive for *Mycobacterium bovis* on 09/21/2010.

The two cattle were feeder animals slaughtered as part of Load #3 on 06/09/2010 and Load #4 on 06/10/2010. They had gone through a market in western Ohio on their way to slaughter. The positive animal from Load #3 was identified as a < 51% black steer with a carcass weight of 844# and no individual identification. The positive animal from Load #4 was initially identified as a > 51% black heifer with a carcass weight of 691.5# and no individual identification. A visit by a Pennsylvania VS VMO on 06/29/2010 confirmed that the animal on Load #3 was a steer and the animal on Load #4 was a heifer.

Preliminary information from the market indicated that four Indiana producers had sold animals that were part of Load #3 and three other producers had sold animals that were part of Load #4. Contact was made with all seven Indiana producers over the next few days. Verbal hold orders were placed on them and a request was made for any documentation they had concerning their sales to the market in the appropriate time frame.

Producer 1 from Load #3 sold one (1) cross-bred steer that had a live weight of 1330.0#. He had ~150 animals on his property. This number includes his feedlot steers, brood cows, heifers, and calves. He said that he very rarely purchases animals out of state. Most of his steers were born on his farm and

he replaces his brood cows with his own heifers. A quarantine was placed on this premise on 07/12/2010. Nineteen head of test eligible cattle TB tested negative with caudal fold test on 09/02/2010. Approximately 30 test eligible are left to be tested. 840 RFID tags were supplied to use on animals going to slaughter.

Producer 2 from Load #3 sold nine (9) cattle. Four animals listed with avg. live weight of 1085# and five animals with avg. live weight of 1168#. He currently has ~35 animals (mostly cows and calves) on pasture. A quarantine was placed on this premise on 07/14/2010. All test eligible cattle are to be TB tested negative and all feedlot animals are to have official IDs in place before going to market.

Producer 3 from Load #3 sold five (5) steers. He buys all of his feeder steers from one source in Kentucky and has done so for the last ten years. He sends all of his feedlot cattle direct to slaughter. Currently on his property, he has 2-3 fat steers and twenty-five (25) 600# feedlot steers. All cattle going to market must be officially identified and any test eligible cattle must be TB tested negative.

Producer 4 from Load #3 sold one (1) old brood cow tagged 722 weighing 1455# live weight. Upon examination of sales documents it was discovered that a steer was also sold and made up part of load #3. This animal had a live weight of 975#. The positive animal on load #3 had a hot weight of 844#. These two animals have been ruled out.

Initially, the producers that contributed to Load #4 were all ruled out as they all sold steers and the positive animal from Load #4 was identified as a heifer. On 08/30/2011, genotype testing at NVSL reported both animals as male. The animal from Load# 3 was typed as predominately Hereford x Angus. They were unable to assign a breed to the animal from Load #4. The breed testing does not include dairy breeds.

Producer 5 from Load #4 sold 11 steers. He runs a feeder operation. He purchases cattle from the market when they are 600 lbs. and up, then fattens them till about 2 years old and sells them back. He currently has approximately 200 cattle on site. He normally sells from 8 to 12 at a time. He thinks he sold all Holsteins the week the trace animals were sold. Originally, he was ruled out because he sold only steers and the positive animal was a heifer. At this time, we are working to identify possible source herds and any connections they may have with other possible sources. He has been supplied with 840 RFID tags to use on animals going to market.

Producer 6 from Load #4 sold 16 steers. He feeds out cattle, getting bucket calves and older, fattening them till they are 18 to 24 months of age and then selling them. As of 09/22/2010, he had only two freezer beef on the premise and is not planning on restocking for some time. The animals he sold of interest originated from two sources, one is an Indiana sale barn. The other is a local cattle dealer. The animals from the sale barn were all tagged. The others were not. We are sending him 840 RFID tags which he will place in all cattle as he purchases them as well as keeping records.

Producer 7 from Load #4 sold two (2) steers. Because he only sold two steers on this shipment and the positive animal was initially identified as a heifer he was initially ruled out as a source.

As of 09/22/10, there are 28 cows present at this time. The animals that went to the market and were part of Load #4 were purchased Holstein steers weighing 1470 # average. They were purchased originally from a local dairy and never had direct contact, including fence line, with their breeding animals or calves. We will be visiting the farm to confirm separation of the breeding herd from the two steers of interest and gather more information.

The plan at this time is to finish testing the two quarantined breeding herds, continue monitoring marketed animals and gather more data on the traces.

The next state report was given by Sue K. Billings, DVM, MSPH, Deputy State Veterinarian, Kentucky Department of Agriculture.

Michigan's report was given by Steven Halstead, DVM, MS State Veterinarian, Michigan Department of Agriculture.

**History of bovine TB in Michigan:**

- TB Free Status in 1979
- Disease re-emerged 1994 in a whitetail deer
- First cattle herd in 1998
- Lost TB Free status in 2000
- Over 38,000 whole herd surveillance tests been conducted
- 1.8 million cattle have been tested

- About 150 cattle been found infected
- 50 infected cattle herds
- 667 of 184,000 deer found positive (96% in MAZ)

**Today Michigan has Split State Status:**

- Upper Peninsula is Accredited Free
- Lower Peninsula has two Federal Zones:
  - Modified Accredited Advanced Zone, and
  - Modified Accredited Zone
- Bovine TB Program focuses on two risks:
  - Cattle to Cattle Transmission
  - Wildlife to Cattle Transmission
- For cattle to cattle transmission Michigan does the following:
  - Whole herd testing
  - Movement testing
  - Movement certificate
  - Traceability program
- For wildlife to cattle transmission Michigan does the following:
  - Active deer surveillance in MAZ
  - Passive deer surveillance rest of state
  - Wildlife Risk Mitigation
    - This is a biosecurity project to reduce cattle and wildlife interaction
    - Focuses of three key principles
      - ✓ Feeding cattle safely
      - ✓ Watering cattle safely
      - ✓ Storing feed safely

**Future of Michigan bovine TB program:**

- Continue to mitigate cattle to cattle transmission through testing
- Continue to address wildlife to cattle transmission through wildlife risk mitigation and deer surveillance
- Submit a new split state status application
  - MAZ: Alcona, Alpena, Montmorency and Oscoda counties (northeast)
  - MAAZ: Antrim, Charlevoix, Cheboygan, Crawford, Otsego, and Presque Isle (northwest)
  - Accredited Free: Remainder of Lower Peninsula

Minnesota followed with its report given by Beth S. Thompson, JD, DVM, Senior Veterinarian, Minnesota Board of Animal Health. Dr. Thompson On October 1, the majority of Minnesota was upgraded to bovine Tuberculosis (TB) Accredited-Free, and the Modified Accredited area in northwest Minnesota was upgraded to Modified Accredited Advanced (MAA). The State of Minnesota has been working with producers since 2005 to eliminate the disease from northwestern Minnesota and regain the state's TB-Free Status. It has been nearly 2 years since any infection has been found in cattle.

Producers raising cattle in the Management Zone will continue the same testing, movement, and fencing requirements. However, this upgrade brings reductions in testing and movement requirements for the rest of the new MAA Zone, including:

- no TB test required for feeder cattle leaving the herd (official ID still required); and
- an Animal Movement Certificate is not required for within-herd movement.

Yearly whole herd tests are still required for all herds remaining in the MAA zone, and all other movement controls remain in place.

The Minnesota Department of Natural Resources (DNR) continues to conduct surveillance in the free-ranging white tail deer population in northwestern Minnesota. The prevalence of TB in deer has continued to decline over the past few years. No infected deer were found during the winter sharpshooting in the core area. Surveillance will continue in the hunter-harvested deer population and sharpshooting will supplement if needed to meet surveillance numbers.

The Nebraska perspective was provided by Dennis Hughes, DVM, State Veterinarian, Nebraska Department of Agriculture. Dr. Hughes gave an overview of *M. bovis* in Nebraska.

Elk—In March of 2009, a captive elk from a TB accredited herd in northeast Nebraska was confirmed by culture to be positive for *M. bovis*. Testing of the herd yielded only 3 SCT responders, but the herd was quarantined and depopulated in June 2009. Incredibly, 60% of the herd had lesions upon necropsy and 70% of the herd cultured positive for *M. bovis*. The facility was cleaned and disinfected, and still remains empty of cervids at this time, awaiting the results of the deer surveillance project. There were no fenceline contacts, and trace-in testing revealed no infection. There were no trace-out sales from the herd. We have completed testing of epidemiologically linked herds and have not found any more positive animals. We do not know the source of this infection.

Beef – Rock County—In May of 2009, an affected cattle herd in north-central Nebraska was detected by means of a slaughter trace of an old cull cow. (The spoligotype of this *M. bovis* organism was different than the cervid herd and labeled a “south west strain”/Mexican strain) Subsequently, a whole herd test revealed one more old cow to be positive for TB. We had hoped that the index herd of approximately 800 cows would be depopulated, but USDA-VS declined. The finding of this herd resulted in the epidemiologic testing of approximately 22,000+ head of cattle from 61 different herds in 20 counties (39 across the fence contacts, 22 involved in trace-in/trace-out)..

Fortunately, no more infected cattle associated with the index herd were found. The index herd was evaluated by a CEAH model, which determined that a test and removal protocol would be implemented to release quarantine. After a total of four whole herd tests (60+ days between each test), plus euthanasia and post mortem of over 100 responders, the quarantine was released in March 2010. Another whole herd assurance test will be conducted in March of 2011.

Beef – South Dakota/Nebraska—In January of 2009, a 20 month old fat heifer that was slaughtered at Cargill in Schuyler, NE, was found to be positive for TB. The only ID was a back tag that showed she was from a feedlot in Yankton County, SD. By process of elimination, SD traced her back to a herd of origin near Irene, SD. Unfortunately they weren't able to test that herd until December of 2009, and we were notified in January of 2010 of possible trace backs to NE. Continued testing in SD revealed four more positive animals, and all five were part of a group of 189 heifers that had entered the SD herd in February of 2008. Many of these animals had been sold through the Bassett Livestock Market and originated from 4 Nebraska herds, with a 5th herd having had summer grazing fence line contact with the infected herd in SD. All five herds were quarantined and all tested negative for TB.

Trace outs from the SD infected herd revealed 5 northeast NE herds that had purchased heifers from the SD herd. Those herds were also quarantined and tested. The cows that had been purchased from the infected herd were euthanized and examined for TB lesions. Unfortunately one of the purchased cows was found to be positive for *M. bovis*. This gave us a second positive NE beef herd. That herd has been depopulated and no more infected animals were found. There were 8 fence line contact herds, and all of them have been tested negative. Testing around the NE herd with the infected SD animal amounted to another 3,700+ head of Nebraska beef cattle that had to be tested.

The spoligotype of the *M. bovis* from the SD herd is the same as our positive cervid herd. In May of 2009, Game and Parks collected and sampled 42 wild white-tailed deer within 2 miles of the location of the cervid herd. Head lymph nodes (parotid, retropharyngeal and mandibular) were collected and examined for TB lesions, but none were found. A much larger sample encompassing Knox and Cedar counties will be collected during the hunting season this fall. The results of this testing, will, to a large extent, dictate how much more surveillance we conduct in cattle herds in the area around where the positive cervid herd was maintained. We have nearly completed surveillance of possible wildlife-exposed herds within a 2 mile radius of the elk herd pasture. Seventeen herds were quarantined and 13 have already been released, with partial testing done on all but one herd. We should be finished with all herds in this group by the end of November. No infected animals have been found.

Dairy—While all this was going on, we received notice of trace outs from a TB infected dairy in Texas. Animals from that dairy were imported to three large NE dairies. Since all of the imports could not be located, it was necessary to do whole herd tests on the dairies. Thanks to a Federal task-force and most of our state people, approximately 16,000 animals were tested in a short time and fortunately all cows tested negative. The animals from Texas that have been located remain under quarantine and will continue to be tested at least 3 times at 8 month intervals.

Staff and expenses—Our field veterinarians and inspectors and their Federal counterparts spent countless long days on the road and in the field. Due to budget constraints over the last 10 years, our

field force has been down-sized to 5 field veterinarians and 5 inspectors (about ½ of the field force we had during the PRV eradication program). They saw very little of their families through most of 2009 and did not get to take vacations, and missed their usual family and children's events. Most worked 60-80 hour weeks while working 6-7 days/week for over 8 months and several weeks required over 100 hours. The Governor issued an "Emergency Declaration" and appropriated \$750,000 to cover employee overtime, hiring cowboys and outside help, and purchasing new hydraulic chutes, hydraulic alleyways, corral panels and other restraint equipment. We have spent nearly \$450,000 of that appropriation. As of this date, 16,000+ dairy cattle have been tested, 31,000+ beef cattle from over 87 beef herds have been tested with the remainder to be completed this fall as described above. When surveillance testing around the cervid herd is completed (anticipated in late November 2010), BAI will have quarantined and tested 95 herds (dairy and beef) and tested approximately 48,000 head of cattle within our borders since June 2009. For the most part, producers have been very cooperative. The stress of not being able to move cattle while on quarantine pushed some to the limit of their patience while waiting in line to get their herd tested. Nebraska's TB Free status has been salvaged, but the cost has taken a tremendous toll on the Department of Agriculture resources and the cattle industry within the state.

New Mexico's report followed given by Dave E. Fly, DVM, State Veterinarian, New Mexico Livestock Board. Dr. Fly reported that the state continues to have a modified accredited advanced zone and is in the process of dealing with a TB trace investigation from Ohio.

The morning's session of state roundtable ended with South Dakota, provided by Dustin Oedekoven, DVM, State Veterinarian, South Dakota Animal Industry Board. Dr. Oedekoven reported that the March 2008 index herd spoligotype was genetically similar to the Nebraska cervid herd. This led to numerous trace investigations, including a herd from Nebraska that was found to be TB positive.

A question and answer and discussion period followed the state roundtable.

This completed the morning session and the Committee broke for lunch.

After lunch, formal presentations continued with the afternoon's first presentation "National Animal Health Monitoring System (NAHMS) Dairy Heifer Raiser Study" presented by Bruce Wagner, PhD, Center Director, National Animal Health Monitoring System (NAHMS), USDA-APHIS-VS Centers for Epidemiology and Animal Health (CEAH). Dr. Wagner's presentation is included in these proceedings.

The next presentation, "Evaluation of Gamma Interferon Testing Under Field Conditions in the United States", was provided by Aaron Scott, DVM, PhD, Diplomate ACVPM, Center Director, National Surveillance Unit, USDA-APHIS-VS-CEAH. Dr. Scott's presentation is included in these proceedings.

The next presentation was provided by Aaron Scott, DVM, PhD, Diplomate ACVPM, Center Director, National Surveillance Unit, USDA-APHIS-VS-CEAH. The presentation was entitled "A Comparison of the Efficacy of the Comparative Cervical Tuberculin Skin Test and the Gamma Interferon Test in a TB-infected Dairy Herd". The presentation is included in these proceedings.

A presentation on "Communicating about Bovine Tuberculosis" was provided by Mr. Phil Durst, Michigan State University, Agriculture Extension Service, and James Averill, DVM, PhD, Bovine Tuberculosis Eradication Program Coordinator, Michigan Department of Agriculture, Animal Industry Division. Their presentation is included in these proceedings.

An update on the National Serum Bank was given by Dr. Alecia Larew Naugle, National Tuberculosis Program Manager, National Center for Animal Health Programs, USDA APHIS VS. The full text of Dr. Naugle's report is included in these proceedings.

Formal presentations and reports concluded with the report of the USAHA Committee on Tuberculosis's TB Scientific Advisory Subcommittee (TB SAS). This report was provided by Mitch Palmer, DVM, PhD, TB SAS Chair. The TB SAS met Monday, November 15, 2010, from 1 pm to 6 pm. The full text of Dr. Palmer's report is included in these proceedings.

## **Committee Business**

At the conclusion of formal presentations, Dr. Connell gave an overview of resolution format, the 2009 Resolutions and proposed 2010 resolutions submitted so far.

There were two 2009 Resolutions:

Resolution 22 National Bovine Tuberculosis Eradication Program

Resolution 23 Expedited Approval of New Bovine Tuberculosis Antibody Tests by the Center for Veterinary Biologics

Dr. Connell read each Resolution, followed by the response from USDA. VS-APHIS-USDA responded promptly in writing to the 2009 Resolutions. Resolutions can be accessed at USAHA's website by selecting "*Committee*", then "*Tuberculosis*".

Three resolutions were approved and forwarded to the Committee on Nominations and Resolutions. Topics included elephant TB guidelines, TB Cervid test, and CFT Test Response Rates.

One recommendation was approved by the Tuberculosis Committee. A letter is being drafted by the chair for signature by the President of USAHA in regards to United States Animal Health Association (USAHA) recommending that the National Tuberculosis Eradication Program provide quarterly status updates for distribution to State Animal Health Officials; with minimum information to include number of affected herds, slaughter surveillance, and trace investigations.

## REPORT OF THE TUBERCULOSIS SCIENTIFIC ADVISORY SUBCOMMITTEE

Mitchell V. Palmer, DVM, PhD, Chair  
Veterinary Medical Officer  
Bacterial Diseases of Livestock Research Unit  
National Animal Disease Center

### USDA Tuberculosis (TB) Serum Bank

Dr. Alecia Naugle, USDA-APHIS-VS

APHIS' goal of obtaining 250 well-characterized samples from TB-infected cattle was exceeded in FY 2010. As a result of successful collaborations with Mexico and the United Kingdom, the TB serum bank received 307 samples from TB-infected cattle in these countries with an additional 111 samples collected from U.S. animals. The serum bank provides well-characterized serum samples with skin test results for samples from uninfected animals and skin test, histopathology, and TB culture results for samples from infected animals. The serum bank samples will be available to researchers and diagnostic companies as they develop and evaluate serologic tests for bovine TB using the criteria recommended by the U.S. Animal Health Association. In addition, large volume samples were also collected from 1,044 uninfected cattle and 486 uninfected white-tailed deer during FY 2009 through FY 2010.

In FY 2011, the serum bank will continue to accept blood and tissue samples from potentially infected cattle and white-tailed deer and blood samples from presumably uninfected cattle and white-tailed deer from AF States.

### Development Update - IDEXX *M. bovis* Antibody ELISA

John C. Lawrence, IDEXX

#### Familiar test format

- Microtiter format based on recombinant *M. bovis* proteins
- ~3-hour test protocol
- Cattle serum or plasma samples (no specialized handling)
- 12-month shelf life
- Supplemental Use

#### Performance on manufacturing-scale kits

- Sensitivity = 65% vs culture positive status (n = 296)
- Detection of positive animals missed by gamma interferon or skin testing
- Specificity = 98% vs regional classification (n = 1473)
- No cross-reactivity with *M. avium* or *M. paratuberculosis*; reactivity w/*M. kansasii*

#### Independent Evaluations

- Sensitivity range of 0% and 80% vs positive status (mean of 66.2%, n = 260)
- Distinct regional differences – collaborations to continue
- Specificity range of 94.6% to 99.5% (mean of 98.4%, n = 1521)
- All sites produced valid assays without supervision
- Independent results similar to internal development efforts

### Field Study on Use of Gamma Interferon as a Screening Test for Bovine Tuberculosis

Dr. James Averill, Michigan Department of Agriculture

Bovine tuberculosis is a rare disease that has major impacts to the cattle industry. Current diagnostic tools do not have the sensitivity and specificity that is desired by many scientist. In March, 2010 Michigan diagnosed 48<sup>th</sup> herd infected with bovine tuberculosis (bTB) since 1998. This herd had a high prevalence of bTB, 33%. Prior to depopulation the Michigan Department of Agriculture wanted to take the opportunity to learn more about bTB. Objective was to determine if gamma interferon could be used as a screening test for bTB. A whole herd test was conducted on all animals 1 day of age and older. On day one 0.1ml of tuberculin was injected into caudal fold of 73 cattle. In addition 10ml of blood was collected from coccygeal vein for gamma interferon test. Seventy-two hours post injection the caudal fold test was read. Tissue samples were collected from each animal upon depopulation. Cultures were performed at National Veterinary Services Laboratory. Of the 73 cattle tested with gamma interferon; 66 negative, 4 positive, and 3 nonviable. The caudal fold test resulted in 60 negative and 13 responders. Twelve

animals were found culture positive, 16% prevalence rate. In this study the sensitivity and specificity for gamma interferon on day of injection was 33% (0.11-0.65) and 1.0 (0.92-1.0) respectively when using culture as gold standard. For caudal fold test sensitivity was 67% (0.35-0.89) and specificity 92% (0.81-0.97). Results of this field study demonstrate that the gamma interferon as a screening test is not a viable option. The caudal fold test sensitivity and specificity is in line with previous literature.

### **Evaluation of the Interferon Gamma Assay (Bovigam) and Comparative Cervical Tuberculin Skin Test Performance in a Colorado TB-infected Dairy**

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Gross lesions compatible with bovine tuberculosis (TB) were found in a Holstein cow upon routine slaughter inspection in March 2010. After post mortem laboratory testing, TB infection was confirmed and traced back to a dairy herd in Colorado with approximately 900 head of cattle. Approximately 500 adult cows were tested with the caudal fold test (CFT) in May 2010. The cattle that tested as TB suspects on the CFT (162) were then tested with both the gamma interferon assay (G-IFN, Bovigam) and the comparative cervical test (CCT).

This herd provided an opportunity to compare the performance of the CCT and the G-IFN in naturally infected cattle. The objectives of this analysis were to: (a) estimate and compare the sensitivity (SE) of the CCT and the G-IFN, (b) compare the SE of the G-IFN test in this herd with the SE of the G-IFN obtained in a study using national TB data, and (c) assess the agreement between G-IFN and CCT results.

Results showed that SEs of the CCT and G-IFN were 89.04% (79.5% - 95.2%) and 81.94% (71.1% - 90.0%), respectively. The agreement expected beyond chance between the CCT and the G-IFN was moderate (Kappa= 45 %, 28%-62%). The SEs of the G-IFN assay as well as the mean OD's for the bovine minus avian purified protein derivative (PPD) responses in infected animals in this analysis were comparable with those of the study based on national TB data. In addition the SE's of the G-IFN and CCT from this study are comparable with previous estimates published both in the US and in other countries. In conclusion, results from this analysis support current guidance to choose either CCT or G-IFN as follow up tests to the CFT.

### **Evaluation of Alternative Antigens for Use in the Bovigam™ Assay with Samples from the Colorado Dairy Herd with TB-infected Cattle**

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Bovine tuberculosis (TB) was detected in a Holstein cow upon routine slaughter inspection in March 2010. Initial ante-mortem testing indicated that the herd of origin (~900 Holsteins, Colorado) had a relatively high within herd prevalence of bovine TB (11 - 25%). The owner agreed to additional TB test evaluation including skin test and collection of blood for blood-based tests. The herd was subsequently depopulated with all animals evaluated for tuberculous lesions upon slaughter. With this herd, three independent studies were undertaken: evaluation of the accuracy of Bovigam™ and emerging serologic assays, comparison of Bovigam™ and comparative cervical test as confirmatory tests, and evaluation of alternative antigens for potential use in the Bovigam™ assay (the topic of this report).

For the study to evaluate use of alternative antigens in the Bovigam™ assay, the objective was to evaluate interferon (IFN)- $\gamma$  responses to ESAT-6/CFP10 peptide cocktail (EC) and purified protein derivatives (PPD) from various manufacturers, as well as to evaluate interactions based upon responses to pokeweed mitogen (PWM, indicative of cell viability) or infection status. The study was performed in collaboration with Prionics Ag, Schlieren, Switzerland (manufacturers of the Bovigam™) and the Texas Animal Health Commission (TAHC) diagnostic laboratory in Austin, Texas. Blood was collected from 126 animals immediately prior to necropsy and 8 hr after transport by truck to the slaughter facility. Whole blood samples were delivered overnight to the TAHC diagnostic laboratory in Austin, Texas for determination of IFN- $\gamma$  responses using the Bovigam™ assay. Whole blood culture treatments included: no stimulation (NS, culture medium only), CSL *M. bovis* origin PPD (PPDb, 20  $\mu$ g/ml), CSL *M. avium* origin PPD (PPDa, 20  $\mu$ g/ml), Lelystad PPDb (300 IU/ml), Lelystad PPDa (250 IU/ml), EC, and PWM (5

□g/ml), each provided by Prionics Ag, Schlieren, Switzerland. Stimulation was in 96 well tissue culture plates at 37°C for 20-24 hrs; plasma was separated via centrifugation and harvested; and plasma IFN-□ concentrations determined by routine ELISA. Animals were considered: infected (n = 54, *M. bovis* cultured from tissues or detected by PCR on histocompatible lesions), exposed (n = 69, no isolation made or no gross lesions), or PCR pending (n = 3).

Without regards to infection status, □IFN-□ responses (i.e., response to antigen minus NS) to Lelystad PPDb exceeded ( $p < 0.001$ , n = 126) corresponding responses to CSL PPDb. Despite differences in magnitude, relative responses to Lelystad and CSL PPDb were highly correlated ( $r^2 = 0.7$ , n = 126). Responses to Lelystad and CSL PPDa were similar in magnitude and highly associated ( $r^2 = 0.8$ , n = 126). PWM responses were lower (mean = 0.77) than expected suggesting potential effects of animal transport and associated stress. The magnitude of responses to PWM did not correlate ( $r^2 = 0.2 - 0.31$ ) with corresponding responses to antigen (i.e., EC and PPDb); however, exclusion of PWM low responders (i.e.,  $< 0.5$  OD) resulted in a greater percentage of antigen responses considered positive (i.e.,  $> 0.1$  □OD). With regards to infection status, responses to EC by infected animals exceeded ( $p = 0.04$ ) that of exposed animals and responses by infected animals to Lelystad PPDb tended to exceed ( $p = 0.1$ ) that of exposed animals. Responses to EC were generally robust (mean = 0.33, n = 126) and comparable to responses Lelystad PPDb ( $r^2 = 0.77$ ). These findings demonstrate the necessity to critically evaluate the choice of antigens for use in the Bovigam™ assay.

### **Report of the USAHA TB SAS Review of Bovigam™**

The USAHA Tuberculosis Scientific Advisory Subcommittee (TB SAS) reviewed summary data provided by Prionics USA, Inc. concerning use of the Bovigam™ assay for the detection of *M. bovis* infection in cattle. A dossier summarizing peer reviewed research on the Bovigam™ and intradermal tuberculin skin testing was supplied by Prionics, Inc. to the TB SAS, with the specific request to evaluate data that may support use of Bovigam™ as a primary TB test in cattle. In that regard, Prionics suggested that Bovigam™ could be used in testing strategies such as import testing, pre-movement testing, test and removal, and herd screening.

#### **Background**

Bovigam™ has been approved by the Office International de Epizooties (OIE) as an ancillary test to confirm or negate the results of an intradermal tuberculin test.

In 2002, Bovigam™ was confirmed as a supplement to the skin test by the European Commission's Standing Committee on the Food Chain and Animal Health.

In 2003, USDA approved Bovigam™ for use as a supplemental test on cattle found suspect on the primary test. The test could be used, at the discretion of the designated TB epidemiologist (DTE) and the regional TB epidemiologist (RTE), in parallel with the comparative cervical test (CCT) or as a replacement to the CCT.

#### **Review**

TB SAS members reviewed the dossier supplied by Prionics, Inc. and conferred via conference call. In ad-hoc fashion, the data was also analyzed by a statistician from APHIS, VS, Center for Veterinary Biologics.

Data submitted involved 15 different field studies conducted or published between 1991 and 2006. Over 12,000 animals were used to evaluate test sensitivity and specificity. Specifically, 10,952 animals were used from 7 different studies to evaluate test specificity, while 1561 animals from 8 different studies were used to evaluate test sensitivity.

Some of these studies were conducted over 15 years ago; the studies were conducted in several countries and test protocols, test reagents, and corrected ELISA optical density cut-off points differed from those in use today in the USDA TB eradication program. Although the data supplied in the dossier remains useful, more recent data evaluating Bovigam™ and tuberculin skin testing, as they are currently used, is most germane for a recommendation from the TB SAS for expanded use of Bovigam™. Recently concluded studies using Bovigam™, various experimental serological assays, and the intradermal tuberculin test in a tuberculous dairy herd in Colorado, suggest Bovigam™, as used, displays lower sensitivity, as a primary test, than that reported in studies cited in the dossier. It is the opinion of the TB SAS that evaluation of the historical performance of the Bovigam™, provided by Prionics, combined with recently reported results (obtained by USDA) from testing within a tuberculous Colorado dairy herd, are not sufficient, at this time, to support use of Bovigam™ as a primary test. The findings do; however,

reinforce the suitability of Bovigam™ as a confirmatory test, which may be used in parallel or in place of the comparative cervical test (CCT).

It is also the opinion of the TB SAS that USDA TB Program staff should work closely with Prionics, Inc. to obtain data necessary for Bovigam™ to move through phases II and III necessary to obtain official status as a primary test, with data collection to allow determination of test parameters that may support such a decision. Specific areas in need of further analysis include, but are not limited to; thorough evaluation of Bovigam™ and CFT in parallel as primary tests without previous sensitization from skin testing and; effect on sensitivity and specificity of same day stimulation compared to stimulation after shipment of blood. To expedite test approval, further evaluations should include assessment of test parameters using reagents, such as PPD, or other antigens, that are currently in use, as well as probable alternatives that may be used in the future.

## IDENTIFICATION OF MOLECULAR TARGETS FOR DIAGNOSIS OF BOVINE TUBERCULOSIS

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Previously, we have conducted a microarray-based transcriptional profiling study using antigen stimulated white blood cells (WBC) from cattle that were either infected or not infected with *Mycobacterium bovis*. The purpose was to identify potential molecular markers that could be exploited for diagnosis of bovine tuberculosis (bTB). In that study, 91 genes were found that likely showed altered levels of expression between infected and non-infected cattle. To date, we have validated altered expression for 14 of the 91 genes, using quantitative real-time PCR. For validation, RNA was extracted from antigen stimulated WBC from 25 cattle that either had bTB (n=5) or that did not have bTB, as determined at post mortem examination. The cattle that did not have bTB were reactors on the primary caudal fold intradermal skin test (CFT) and non-reactors on the secondary comparative cervical skin test or the whole blood gamma interferon assay (single reactors [n=10]), or were reactors on both primary and secondary tests (double reactor [n=10]). The 25 cattle were a mixture of animals used in the previous microarray study and animals sampled after the conclusion of the microarray study. The majority of the 14 genes were involved in various immune response pathways such as cytokine and chemokine signaling, antigen processing and presentation, and arachidonic acid metabolism.

For cluster analysis, quantitative real-time PCR derived expression ratios ( $-\Delta\Delta Ct$ ) of select genes of the study animals relative to comparable genes of pooled healthy control animals were used. The analysis was based on 14 differentially expressed genes; which allowed clustering of bTB infected cattle and non-infected cattle. Two separate clusters were formed, one of which contained all of the post mortem positive cattle and the other contained all of the double reactors, all of the latter were post mortem negative. Some cattle that were only CFT reactors, and were from bTB positive farms, clustered with bTB positive cattle, while other CFT reactors clustered with the double reactors. Linear discriminant analyses were used to select the minimum number of genes required to provide the best separation between groups of infected and non-infected cattle. Various combinations of genes were assessed to determine their merit as classifiers (predictors) of bTB infection. Results of this analysis showed that the expression ratio ( $-\Delta\Delta Ct$ ) of as few as three out of the 14 differentially expressed genes would provide sufficient statistical power to discriminate cattle with bTB from cattle not infected with bTB. The gene targets identified in this study have been shown to be differentially expressed in bTB infected and non-infected cattle; thus, those genes might be used to develop a rapid and sensitive diagnostic assay for bTB.

**Annual Update for the State and Federal Cooperative  
Bovine Tuberculosis (TB) Eradication Program  
Fiscal Year (FY) 2010  
U.S. Department of Agriculture  
Animal and Plant Health Inspection Service (APHIS)  
Veterinary Services**

In October 2009, APHIS published a concept paper entitled "A New Approach for Managing Bovine Tuberculosis" in the *Federal Register* that outlined proposed changes to the TB program. These potential changes represent a new approach to managing bovine TB in the United States that will:

- mitigate the introduction of TB into the U.S. national herd,
- enhance TB surveillance,
- increase options for managing TB-affected animals and herds,
- modernize the regulatory framework, and
- transition the TB program from a State classification system to a science-based zoning approach.

Ultimately, APHIS will amend its TB regulations to align them with the new approach. However, because the rulemaking process can be lengthy, APHIS has implemented several interim measures in FY 2010 to mitigate disease spread while addressing the most urgently needed changes.

#### **New Policy for Management of TB-Affected Herds**

During FY 2010, APHIS continued to alter its approach to the management of TB-affected herds. Historically, Federal funding was used to depopulate entire TB-affected herds and indemnify herd owners as the primary management option. Rather than recommending whole-herd depopulation, we will base our approach on the circumstances surrounding each herd. Whole-herd depopulation will be implemented when the data indicate that other options will not mitigate disease spread, an imminent public or animal health risk exists, or it is financially beneficial to do so. Otherwise, APHIS proposes to manage specific TB-affected herds under a test-and-remove policy in which animals on an affected farm are placed under quarantine and repeatedly tested for TB. The herd will be released from quarantine when there is a high level of confidence that the herd is free of the disease.

To aid in making these decisions and managing TB-affected herds, APHIS developed an epidemiological model. The model estimates the probability of a TB-affected herd being free of infection after implementing a defined herd testing protocol. The model also incorporates specific factors associated with the herd and information about the accuracy of currently approved tests for TB. APHIS is currently developing a memorandum that provides updated guidance for classifying and managing livestock herds affected with TB in light of this new policy.

#### **TB Federal Order**

On April 15, 2010, APHIS issued a Federal Order to initiate other urgent changes. Using this Federal Order, APHIS suspended its enforcement of title 9 of the *Code of Federal Regulations* (9 CFR) 77.7(c) in accredited-free (AF) zones and States and 9 CFR 77.10 for modified accredited advanced (MAA) zones and States. All other existing requirements continue to be enforced. The Federal Order resulted in the following changes to the TB program:

- APHIS will not downgrade an accredited-free State or zone, or any part of that State or zone, where TB-affected herds are confirmed, as long as the State or zone meets the following criteria for controlling the disease:
  - maintaining all affected herds under quarantine,
  - implementing a herd plan for each affected herd to prevent the spread of TB,
  - implementing a program to periodically test the animals under quarantine and remove and destroy those that do not test negative, and
  - conducting surveillance adequate to detect TB if present in other herds or species.
- Cattle and bison that are not known to be infected with or exposed to TB may be moved interstate from MAA States or zones without restriction for TB.

- However, the APHIS Administrator may require increased surveillance within all or part of a State or zone or restrict the interstate movement of cattle and bison from all or part of a State or zone:
  - when necessary to address TB risks from wildlife or
  - under any other circumstances if the Administrator determines it is necessary to prevent the spread of TB.

### **Joint TB and Brucellosis Regulatory Working Group**

The development of the proposed TB regulation is expected to take approximately 2 years. It will require ongoing engagement with a wide group of internal and external stakeholders to obtain input on the proposed strategies, program standards, surveillance plans, and other policy concepts before proposed regulations can be published. Because the bovine brucellosis program is undergoing similar changes, APHIS has formed a joint working group to discuss overarching regulatory concepts for the TB and brucellosis programs. The working group is composed of State, Federal, and Tribal representatives. A kick-off meeting was held on September 21-22, 2010, and the group continues to hold weekly conference calls.

### **Bovine State Status**

At the end of FY 2010, 46 States, two Territories, and three zones were TB accredited-free (AF), including Puerto Rico and the U.S. Virgin Islands. California was MAA and three States had split-State status. New Mexico has AF and MAA status. Michigan has AF, MAA, and modified accredited (MA) status. In December 2009, APHIS published an interim rule that advanced six counties in the western portion of Michigan's current MA zone to MAA status. Minnesota was upgraded from MAA and MA to AF and MAA status on October 1, 2010. Of the AF States and zones, 20 States and the U.S. Virgin Islands have maintained AF status for over 25 years; 20 States have been AF for 15 or more years; 5 States have been AF for 10 or more years; 1 State and Puerto Rico have been AF for 5 or more years; and 1 State and 1 zone have had AF status for less than 5 years.

### **Captive Cervid State Status**

All States and territories have MA status.

### **TB-Affected Herds Identified in FY 2010**

Thirteen affected herds were detected during FY 2010, including 11 beef and 2 dairy herds. These herds are located in Colorado (one dairy, one beef), Kentucky (one beef), Michigan (five beef), Mississippi (one beef), Nebraska (one beef), Ohio (one dairy), and South Dakota (two beef). Seven (54 percent) of the TB-affected herds identified this year (1 dairy and 6 beef herds) were detected as a result of slaughter surveillance and subsequent epidemiologic investigations, demonstrating the integral role of slaughter surveillance in the TB program.

A total of ten cattle herds were depopulated with Federal indemnity. Two Michigan beef herds are under test-and-remove management. An affected Ohio dairy herd undergoing dispersal was identified as affected with TB. At the time of detection, the herd had been mostly dispersed. However, the remaining cattle were sent to slaughter following detection. One Michigan dairy is continuing under a test-and-remove herd plan from 2004; the herd was scheduled for quarantine release in FY 2009 but an infected animal was detected during routine testing. Two California dairies and one Nebraska beef herd under test-and-remove herd plans were released from quarantine during FY 2010. Two captive cervid herds detected in FY 2009 remain under quarantine in the MA (bovine) zone of Michigan.

### **Current TB Strains Resemble TB Cervid Isolates from the 1990s**

In FY 2009-2010, TB strains were isolated from four affected beef herds that match strains isolated from captive cervids during the 1990s. TB infection with a "cervid strain" was confirmed in a South Dakota beef herd in FY 2010. This strain matches the strain found in a TB-affected elk and fallow deer herd identified in FY 2009 in Nebraska. Replacement heifers from the South Dakota beef herd were pastured near the Nebraska herd but there was no direct fence-line contact between the animals.

Subsequently, one beef herd in Nebraska and one beef herd in South Dakota that received replacement heifers from the South Dakota index beef herd were also found to be infected with the same strain of TB. The TB strain isolated from the Kentucky beef herd also matches strains from the 1990's captive cervid TB outbreaks; however, to date an epidemiologic link to captive cervids has not been discovered.

The TB strain from two domestic beef feeder cattle cases identified through routine slaughter inspection in June 2010, match the "cervid strain" isolated from TB-affected captive cervid herds detected in Indiana during FY 2009. Ohio has completed herd testing without detecting infected animals or direct

links to captive cervids. Epidemiological investigations in Indiana are ongoing. At this time, no affected source herd or herds have been identified.

### **National TB Surveillance**

**Granuloma Submissions:** For the period October 1, 2009, through September 30, 2010, 10,914 granulomas were identified during postmortem slaughter inspection and submitted for diagnostic testing. These lesions originated from 157 U.S. establishments that slaughtered 31.4 million cattle, including 6.9 million adult cattle. The minimum standard for slaughter surveillance is five granulomas submitted per 10,000 adult cattle slaughtered annually. This standard is applied to each slaughter establishment. Many establishments substantially exceeded the minimum submission rate in FY 2010. Of the 40 highest volume adult cattle slaughter establishments, 35 (87.5 percent) met or exceeded the submission standard, and 5 (12.5 percent) establishments did not. These 40 highest volume establishments slaughtered 6.7 million cattle, which is 95.5 percent of all adult cattle slaughtered in the United States.

A critical component of the granuloma submission program is diagnostic laboratory support. A total of 8,375 of 10,914 granulomas (76.7 percent) were submitted to the National Veterinary Services Laboratories (NVSL); another 1,165 (10.7 percent) were submitted to the Food Safety Inspection Service (FSIS) Pathology Laboratory in Athens, Georgia; and 1,374 (12.6 percent) were evaluated at the California State Diagnostic Laboratory in Tulare, California. Submissions to the NVSL and California laboratories have increased from 70.9 and 7.9 percent in FY 2008, respectively, with a corresponding decrease in submissions to the FSIS Athens laboratory.

Of the 10,914 granulomas submitted by slaughter establishments in FY 2010, 17 (0.2 percent) had histology consistent with mycobacteriosis. Of these 17 cases, TB was confirmed in 8 cattle. TB is confirmed by a combination of polymerase chain reaction testing of formalin-fixed tissue and culture of fresh tissue.

**Slaughter Cases:** Of the eight TB cases detected in cattle at slaughter during FY 2010, two cases occurred in adult cattle over 2 years of age, and six cases occurred in feeder cattle. The two adult cattle cases include an adult beef cow that led to detection of an affected Kentucky beef herd and an adult Holstein cow that led to detection of an affected Colorado dairy.

Six TB cases were detected in fed cattle at slaughter during FY 2010. These cattle were all beef-type cattle and were from Texas (three cases), Indiana/Ohio (two cases) and Mississippi (one case). Of the three Texas cases, one animal had official Mexican ear tags collected at slaughter indicating the animal had originated from the State of Coahuila, and two cases originated from Mexico but the definitive Mexican State-of-origin could not be determined. The Mississippi case occurred in an aged roping steer, and the subsequent epidemiologic investigation identified TB in an adult beef cow in the herd. The investigation is ongoing for two domestic steer cases that trace back to herds located in Ohio and Indiana.

**Mexican-Origin Slaughter Cases:** As described above, only one Mexican-origin fed cattle case with official Mexican identification was detected through slaughter surveillance in FY 2010, the lowest number ever recorded. This represents a continued decrease compared to FY 2006-09, when there were 26, 17, 11, and 3 Mexican-origin TB cases, respectively. During the 2008-09 import cycles, there were 827,739 and 810,985 animals imported, respectively. This represents approximately a 30 percent decrease from the 1.1-1.4 million imports per year during 2004-07. However, the decrease in imported cattle is substantially less and does not fully explain the decrease in the observed rate of TB cases in Mexican-origin cattle. Other factors may be contributing to the decrease in TB cases.

**Live Animal Testing:** Tuberculin skin testing in live animals is another component of our national TB surveillance. In FY 2010, 1,275,815 caudal fold tuberculin tests of cattle and bison were reported, with 18,217 responders (1.4 percent, 48 States and Puerto Rico/U.S. Virgin Islands reporting). The response fraction by State, for 46 States testing more than 300 animals, ranged from 0.1 to 6.8 percent (median, 1.0 percent). Caudal fold test performance appears to be improving. During FY 2008 through 2010, 13, 24, and 23 States, respectively, had a response fraction of 1 percent or greater. The number of States having a response fraction of less than 0.25 percent was 13, 12, and 5 from FY 2008 through FY 2010, respectively.

Tuberculin testing is the primary means of surveillance for TB in captive cervids as there are no standards for granuloma submissions for establishments that slaughter cervids. During FY 2010, 11,029 single-cervical tests were conducted in captive cervid species with 182 suspects (1.7 percent) reported to APHIS. The number of captive cervids tested annually has ranged from 25,000 in FY 2006 to just over 10,000 in FY 2007.

The gamma interferon test has been available as an official supplemental test in the TB program since 2005. Laboratories in five States (California, Michigan, Nevada, and Texas) and the NVSL are approved to conduct gamma interferon testing. A total of 13,314 tests were conducted in cattle in FY 2010.

### **Collaborations with Mexico**

APHIS continues to work with Mexico to ensure equivalency between the two countries' requirements for controlling TB. To accomplish this, we conducted reviews in Aguascalientes, Chihuahua, Chiapas, Campeche, and Zacatecas during FY 2010. As a result of these reviews, zones in Aguascalientes and Chiapas maintained or were granted their accredited preparatory (AP) status, respectively. Chihuahua will maintain its MA status, but several action items must be addressed before a followup review in FY 2011. Otherwise, APHIS will consider a downgrade of status. The final review reports for Campeche and Zacatecas are pending. Finally, the MA zone of Coahuila was downgraded from MA to AP status effective August 1, 2010. Mexico's efforts to address the recommendations from a 2009 review of Coahuila failed to reduce the risk of TB in imported Mexican cattle as TB continued to be found in imported cattle from Coahuila and exceeded the allowable standard. APHIS appreciates the contributions of the individuals that served on these Mexican review teams.

### **TB Serum Bank**

APHIS' goal of obtaining 250 well-characterized samples from TB-infected cattle was exceeded in FY 2010. As a result of successful collaborations with Mexico and the United Kingdom, the TB serum bank received 307 samples from TB-infected cattle in these countries with an additional 111 samples collected from U.S. animals. The serum bank provides well-characterized serum samples with skin test results for samples from uninfected animals and skin test, histopathology, and TB culture results for samples from infected animals. The serum bank samples will be available to researchers and diagnostic companies as they develop and evaluate serologic tests for bovine TB using the criteria recommended by the U.S. Animal Health Association. In addition, large volume samples were also collected from 1,044 uninfected cattle and 486 uninfected white-tailed deer during FY 2009 through FY 2010.

In FY 2011, the serum bank will continue to accept blood and tissue samples from potentially infected cattle and white-tailed deer and blood samples from presumably uninfected cattle and white-tailed deer from AF States.

### **Selected State Updates**

**Michigan Update:** Five TB-affected beef herds were detected in FY 2010. Three herds were located in northern lower Michigan in the bovine MA zone and two herds were located in a county advanced to MAA status in December 2009. One of the herds located in the MA zone had previously been depopulated in FY 2001 due to TB infection. Four of the five affected herds were identified through surveillance testing and the fifth herd was identified through epidemiological tracing. Three herds have been depopulated with Federal indemnity, and two herds are under a test-and-remove herd plan. One dairy in Michigan's MA region continues under a test-and-remove herd plan. This dairy was identified as affected a second time in 2004, the first infection being found in 2000. During the last herd test for release of quarantine, an *M. bovis*-infected cow was identified. As a result of this finding, the quarantine was not released and the dairy herd is still considered affected. Under the terms of the herd plan, testing will revert to the disease removal phase of the test-and remove protocol and continue until the freedom-from-disease phase is successfully concluded and all requirements for quarantine release have been achieved.

**Minnesota Update:** Following a TB program review in November 2009, Minnesota was upgraded from a split-State status of MAA and MA to AF and MAA on October 1, 2010. No affected herds were detected during FY 2010. To date, all affected cattle herds have been found in a small geographic area in northwest Minnesota. All affected herds in Minnesota identified to date have been depopulated. Surveillance of free-ranging white-tailed deer continues through hunter-harvested and targeted culling sample collection. Twenty-six infected free-ranging white-tailed deer have been identified to date.

**South Dakota Update:** Two beef herds were identified as affected following an epidemiological investigation of a routine slaughter surveillance detection of *M. bovis* in a domestic feeder heifer in FY 2009. The investigation determined that the heifer was from a group of approximately 200 beef heifers that were pastured in close proximity to the Nebraska captive cervid herd identified and depopulated in FY 2009. A total of five infected heifers were detected in this cohort group. The TB strain isolated from these five heifers matches the Nebraska captive cervid strain by genotyping. The first affected herd was identified through epidemiological testing and the second herd was identified through epidemiological

investigations as having purchased heifers from the index herd, one of which was found to be infected. Both herds have been depopulated with Federal indemnity.

**Nebraska Update:** One beef herd was identified as affected as a result of the South Dakota epidemiological investigation. This herd received heifers from the index South Dakota herd and TB was detected in one animal. The herd was depopulated with Federal indemnity.

**Ohio Update:** A dairy herd was identified as TB-affected as a result of movement testing during a dispersal sale. By the time TB was identified, the majority of the herd had been dispersed. Trace investigations of dispersed animals and possible source premises for this herd encompass at least 16 States. To date, no further infection has been identified although epidemiological investigations are continuing.

## **National Animal Health Monitoring System (NAHMS) Dairy Heifer Raiser Study**

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USDA-APHIS-VS

Offsite heifer-raising operations are becoming more common and are now used by about 1 of 10 dairy operations (NAHMS Dairy 2007). Almost one-half of operations with 500 or more cows raised at least some heifers offsite. There are concerns about the commingling of animals on these operations and the potential exposure to Mexican cattle, which could result in the transmission of multiple diseases, including BVD and TB. Although the NAHMS Dairy 2007 study asked producers about offsite heifer raising, NAHMS did not obtain information about the operations themselves.

Many of the dairy operations involved in recent TB outbreaks used offsite heifer raising facilities but it is unknown if the facilities are at high risk for TB transmission. This risk has been known for many years and in 2004, the U.S. Animal Health Association TB strategic planning committee recommended conducting a descriptive analysis of the dairy heifer-raising industry:

*"This information is critical if education efforts regarding risk factors and practices that promote spread of bovine tuberculosis and other disease are to be focused toward this segment of the industry."*

There are three primary objectives of the study addressing critical needs:

1. Provide the first comprehensive information on animal health and management practices for heifer-raising operations,
2. Evaluate the biosecurity risks associated with heifer-raising operations (e.g. commingling cattle from multiple operations, exposing young cattle to Mexican cattle) and.
3. Assist in the development of a biosecurity assessment that can be used to evaluate the risk of disease transmission (e.g. TB, BVD, etc.).

Results of a small survey of 14 Dairy Calf and Heifer Association Members conducted in April and May of 2010 was presented. Although only a small, pilot study, results showed the 209 mean inventory for weaned heifers was 726; pregnant heifers was 508; preweaned heifers was 486; and for lactating / dry cows was 305. The operation average inventory of the dairy heifers and cows was 2,025 with a range of 13 to more than 10,000. The primary source of inventory on these operations was from other dairy operations (83.1 percent of cattle), followed by their own dairy operation (11.4 percent). The operations average 4.1 clients and the cattle arrived at an average age of 88 days and left at 19 months. Five of 14 operations brought in cattle from outside their state and 6/14 sent cattle out of state when they left the operation. Only 1 of the operations tested heifers for TB prior to or at arrival on the operation. Five of 12 operations saw deer in cattle housing areas but no operations reported housing Mexican cattle.

The preliminary results of this small pilot study suggest that there are biosecurity deficiencies on heifer-raising operations. The larger heifer-raising study in 2011 will greatly increase the sample size and our ability to obtain a better description of practices and risk of disease transmission.

## **Interferon Gamma Assay (Bovigam) for Bovine TB: Field Performance Analysis**

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This analysis addresses the field performance of the G-IFN in US herds between 2005 and 2009 and provides feedback on test behavior to those who apply the test and use test results to manage individual animals or herds. The specific objectives of the analysis were (1) to estimate diagnostic sensitivity (SE) and specificity (SP) of the G-IFN under field conditions, (2) to assess the association between G-IFN test results, TB status of cattle and post mortem test results in animals slaughtered due to TB suspicion in the United States, and lastly (3) to explore the variability of the G-IFN optical densities and different cut-off points for classification of test results in adult cattle.

A dataset containing antemortem and postmortem test information from cattle in TB infected herds was used to estimate test SE, to assess the association between tests results and TB status, and to explore different cut off values for the test (dataset#1, n=1001). Another dataset (dataset#2, n=4,123) with ante mortem testing information from herds with low risk of TB infection was used to estimate test SP and to explore G-IFN cut off values.

The SE of the G-IFN computed on 87 confirmed TB infected cattle from dataset #1 was 83.9% (95% CI=76.1%-91.6%) for a cut off value of 0.1. Test SP computed from dataset #2 was 90.7 % (89.8%-91.6 %) for a cut off value of 0.1, 97 % (96.5%-97.5 %) for a cut off value of 0.3, and 98.6 % (98.2%-98.9 %) for a cut off value of 0.5. Likelihood ratios (LRs) were calculated to measure the association between test results and TB status of the animals. Both the LR+ (9.03) and the LR- (0.18), suggest moderate to high test accuracy. In addition, the observed (96%) and expected (94%) agreement computed to explore the association between G-IFN and histopathology results indicate that the G-IFN has the ability to discriminate infected from not infected cattle, which is a desirable characteristic of diagnostic tests. Receiver operating characteristic (ROC) curve analysis was used to explore the effect of different cut off values on test SE and SP and the area under the curve (AUC) was estimated to provide an indicator of test accuracy. The AUC was 0.968, which suggests high test accuracy. ROC analysis concluded that a cut -off value of 0.1 provides a good combination of SE and SP for parallel testing, while cut off values between 0.3 and 0.6 provide high SP desirable in series-testing protocols.

In conclusion, the G-IFN test performs with high accuracy in the field, yielding SE and SP estimates comparable to those reported in previous evaluations. In addition, this study shows that the specificity of the G-IFN test is comparable to the specificity of the comparative cervical skin test (CCT). This result support current guidance to choose either G-IFN or CCT in follow up to a caudal fold test.

## Communicating about Bovine Tuberculosis

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Sufficient and good communication is often a sticking point in relationships and business, but when it involves a disease that can have far-reaching and devastating impacts, it really becomes critical. The basics of good communication don't change, but the need to be reminded of them is probably constant.

In the case of bovine Tuberculosis (bTB), and likewise with other diseases and issues, there are reasons to do communication right; reasons that include the fact that regulations must be accurately conveyed, and that there is the potential for economic impact, fear, controversy and competing agendas. Also we need to get people to "buy-into" what we are saying and we need to hear the thoughts and ideas of others.

Michigan has been communicating about bTB for more than 10 years now and in that time has made some communication errors and we've learned some things in the process that we want to share.

- **Don't** put out inconsistent messages. Credibility is at risk.
- **Do** have very good internal communication so that everyone has the same message.
- **Don't** over-rely on paper communications. Paper is necessary, but we often overestimate the value of it.
- **Do** use face-to-face communication even though it can be costly, more fractious and more demanding of time.
- **Don't** believe you have all the answers.
- **Do** understand that we are talking about people's livelihoods that they have invested years into and that sometimes better solutions will come from them. Be humble, admit mistakes and seek input honestly.
- **Don't** go it alone.
- **Do** develop partnerships with other agencies, organizations or groups that have a stake in the issue. Be a real partner and expect real partnership.
- **Don't** assume your time is more valuable than theirs.
- **Do** go to the producers. Have meetings at locations convenient to them.
- **Don't** think that this is just science.
- **Do** understand that it is beyond science alone; it is personal, it is economic, it is social. Therefore, use personal stories. Be practical. Do demonstrations. Make it real. Empathize.
- **Don't** have people find out through the back door.
- **Do** use a sequence of communication so that people are notified before they find out some other way.
- **Don't** narrow the issue.
- **Do** broaden it. TB is not just an ag issue. Involve more people, not fewer, the whole industry, not just the area affected.

Michigan is currently focused on:

- **MDA Weekly Updates:** E-mail only to internal and key partners
- **Personal visits on each infected farm:** Sit down with producer to go over things and understand operation better.
- **Video Vignettes:** Brief explanations of the program available on internet
- **Bovine TB News:** Monthly e-newsletter communication with a broad audience on various aspects of the battle with bTB

We would like to develop Bovine TB News into a national TB newsletter to communicate with scientists, producers, professionals and regulators working with producers and with various other stakeholders. In order to make it relevant, timely and beneficial we need your involvement. We would like to have an identified contact person in each state on the issue of bTB to share information and updates. Contact: Phil Durst, Michigan State University Extension, [durstp@msu.edu](mailto:durstp@msu.edu), work phone 989-826-1160 or cell phone 989-387-5346.

# GUIDELINES FOR THE CONTROL OF TUBERCULOSIS IN ELEPHANTS 2010

UNITED STATES ANIMAL HEALTH ASSOCIATION (USAHA)  
ELEPHANT TUBERCULOSIS SUBCOMMITTEE  
8 November 2010 DRAFT REVISION

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

1. [http://www.aphis.usda.gov/animal\\_welfare/index.shtml](http://www.aphis.usda.gov/animal_welfare/index.shtml) (available to the public)
2. [www.aazv.org](http://www.aazv.org) (available to AAZV members by password)
3. [www.elephantcare.org](http://www.elephantcare.org) (available to the public)
4. [www.elephanttag.org](http://www.elephanttag.org) (available to the public)

# 1. INTRODUCTION

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

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

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

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

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

## 2. DEFINITIONS

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

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

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

**Atypical mycobacteria:** see non-tuberculous mycobacteria

## Contact transmission:

**Direct contact transmission** may occur during activities such as touching or riding an elephant, being touched by an elephant, examining, medicating, bathing, and handling

**Indirect contact transmission** involves contact with a contaminated intermediate object, such as occurs during cleaning cages and equipment and handling soiled laundry. Injuries from contaminated sharps, such as scalpel blades, needles, and necropsy knives, may result in exposure to pathogens. (NASPHV 2006)

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

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

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

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

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

**Exposure:** Risk of transfer of an infectious agent from a TB infected elephant(s) or contaminated environment through contact (direct, indirect) or airborne modes of transmission.

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

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

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

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

**Infected elephant:** an elephant from which *Mycobacterium tuberculosis* complex has been identified through culture, PCR or other molecular techniques or that is reactive on the ElephantTB STAT-PAK® Assay and the MAPIA™.

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

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

**MultiAntigen Print ImmunoAssay (MAPIA™):** A confirmatory test to the ElephantTB STAT-PAK® Assay for detection of antibodies to *M. tuberculosis* and *M. bovis* in elephant sera or plasma (Lyashchenko 2000, 2006, Greenwald 2009).

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

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

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

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

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

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

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

**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:** Absence of growth of *M. tb* complex organisms from trunk wash, feces, tissue or other samples using standard mycobacterial culture methods. Failure to isolate organisms may be due to the following reasons:

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

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

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

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

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

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

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

**Rapid Test:** see ElephantTB STAT-PAK<sup>®</sup> Assay

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

Report date: **The date the laboratory reports the results.**

Spoligotyping: **A genotyping assay**

Variable number tandem repeat (VNTR): **A genotyping assay**

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

**Test date:** The date the sample is collected.

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

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

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

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

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

**Untested elephant:** An elephant is considered “untested” if it has not had three trunk washes obtained by the method outlined in this protocol within a 12 month period or if fewer than three valid culture results are obtained or if it has not been tested with the ElephantTB STAT-PAK<sup>®</sup> Assay performed by a USDA veterinarian trained and certified to perform the test.

### **3. ANNUAL TESTING**

To adequately address the concerns of TB in the general elephant population, all captive elephants must be tested annually by culture and with the ElephantTB STAT-PAK<sup>®</sup> Assay (a blood test). Samples for cultures and blood must be collected by or under the supervision of a licensed veterinarian according to current USDA requirements. Blood collection for the Guideline-required ElephantTB STAT-PAK<sup>®</sup> Assay must be witnessed by a federal or state veterinarian and performed as licensed by the USDA Center for Veterinary Biologics. See further information below under ElephantTB STAT-PAK<sup>®</sup> Assay. It is required that elephants with a reactive ElephantTB STAT-PAK<sup>®</sup> Assay result be tested using the confirmatory MultiAntigen Print ImmunoAssay (MAPIA™). See item 5 below.

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

Record keeping of TB testing and treatment by the attending veterinarian is of utmost importance. It is recommended that attending veterinarians maintain open communication with the United States Department of Agriculture (USDA) and State Veterinarian, particularly concerning elephants under treatment for TB or in cases of exposure to TB positive elephants. It is recommended that at least a 1 ml aliquot of sera collected at the time of TB testing be sent to the elephant serum bank (See appendix 8).

#### **4. CULTURE COLLECTION PROCEDURE (also see Appendix 3)**

Samples for culture must be collected by or under the supervision of a licensed veterinarian using the “triple sample method.” This method consists of obtaining three samples from the trunk on separate days. If possible, collect samples within a seven-day period. Do not pool samples. Samples should be taken after water has been withheld for at least two hours to reduce sample dilution and contamination. Light exercise prior to collection may facilitate obtaining secretions from lower in the respiratory tract, which is desirable. Of the following methods, the trunk wash with bag seems to provide the most effective way to collect samples at this time. **Samples collected by swab are not acceptable.** As there is a risk of human exposure to sputum produced during this procedure, personal protective measures are recommended for personnel during sample collection. These should include gloves 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. Raise the trunk as high as possible to distribute the fluid deeper into the trunk. Lower the trunk and place a clean, one-gallon plastic bag over the end of the trunk and hold in place until the elephant exhales into the bag. Transfer at least 20 ml of the sample to a sterile leak proof, screw-top container. Sterile 50-ml conical screw-top plastic centrifuge tubes are preferred and are available free of charge from the National Veterinary Services Laboratories (NVSL) – call 515-337-7388.

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

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

#### **Storage**

Do not expose samples to sunlight or heat. Consult receiving laboratory to determine whether samples should be refrigerated or frozen prior to shipment. For those laboratories that recommend freezing (i.e.

NVSL) freeze samples as soon as possible after collection and keep frozen until shipment. Freeze at -20°C (conventional freezer). As standard frost-free freezers undergo cyclic freeze-thaws to limit frost, freezers that do not have this feature are preferred. Freezing at -80°C (ultra-low temperature freezer) is also acceptable. **Frozen samples must be shipped within 2 weeks of sample collection to the testing lab.**

### **Packaging and Shipping**

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

Place samples on ice packs or dry ice and ship overnight via Federal Express, Airborne, or other overnight carrier. **Do not ship by U.S. mail** as samples may be irradiated which will render them unacceptable. Packaging and shipping should be in accordance with the International Civil Aviation Organization Technical Instructions for the Safe Transport of Dangerous Goods by Air 2009-2010 (<http://www.icao.int/icaonet/dcs/9284.html>). Also helpful is the 2007 WHO document “Guidance on Regulations for the Transport of Infectious Substances ([http://www.who.int/csr/resources/publications/biosafety/WHO\\_CDS\\_EPR\\_2007\\_2cc.pdf](http://www.who.int/csr/resources/publications/biosafety/WHO_CDS_EPR_2007_2cc.pdf))

Packaging and shipping samples and cultures should be in accordance with Department of Transportation regulations – 49 CFR Parts 171, 172, 173 and 175- Hazardous Materials: Infectious Substances; Harmonization with the United Nations Recommendations; Final Rule, published June 2, 2006 in the Federal Register. [http://isu1.indstate.edu/terc/infectiousmaterial/pdf/Hazardous%20Materials\\_Infectious%20substances%20harmonization%20with%20the%20UN%20final%20rule.pdf](http://isu1.indstate.edu/terc/infectiousmaterial/pdf/Hazardous%20Materials_Infectious%20substances%20harmonization%20with%20the%20UN%20final%20rule.pdf)  
<http://ecfr.gpoaccess.gov/cgi/t/text/text-idx?c=ecfr&rgn=div5&view=text&node=49:2.1.1.3.8&idno=49>

Send samples to NVSL or other laboratory facility offering comparable procedures for identification of mycobacteria species. When submitting samples to NVSL, use VS Form 10-4, Specimen Submission Form. This form is available online in Word or pdf format: [http://www.aphis.usda.gov/animal\\_health/lab\\_info\\_services/forms\\_publications.shtml](http://www.aphis.usda.gov/animal_health/lab_info_services/forms_publications.shtml).

### **Request mycobacterial culture with species differentiation.**

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

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

## **5. ELEPHANTTB STAT-PAK<sup>®</sup> ASSAY SAMPLE COLLECTION PROCEDURE**

Blood collection for the Guideline-required ElephantTB STAT-PAK<sup>®</sup> Assay must be witnessed by a federal or state veterinarian and performed as licensed. It is advisable to also bank a serum sample. Blood from elephants with reactive ElephantTB STAT-PAK<sup>®</sup> Assay results must be submitted for MAPIA<sup>™</sup> /DPP<sup>®</sup> testing to:

**Chembio Diagnostic Systems, Inc.**

3661 Horseblock Road  
Medford, NY 11763  
Tel: 631-924-1135  
Fax: 631-924-6033  
Email: [customerservice@chembio.com](mailto:customerservice@chembio.com)  
Contact Chembio for shipping instructions.

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

## **6. ANCILLARY SCREENING / DIAGNOSTIC TESTS**

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

### **Intradermal Tuberculin Test**

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

### **Enzyme Linked Immunosorbent Assay (ELISA)**

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

### **Acid Fast Smears**

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

## **7. TB MANAGEMENT GROUPS (1-4)**

**All elephants will fall into one of four management groups (1-4) based on test results or will be untested (group 5). A culture positive elephant is defined as an elephant from which *Mycobacterium tuberculosis* or *Mycobacterium bovis* has been isolated from any body site or specimen. A culture positive elephant is considered positive until it has met the treatment requirements as outlined for Group 4. Exposure history has been incorporated into the Guidelines as ongoing data collection has indicated that it is an important risk factor. Flow charts are included in Appendix 9 to illustrate the management groups.**

**GROUP 1: Culture negative; ElephantTB STAT-PAK® non-reactive; no exposure to culture positive elephant in past 12 months.**

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

- No treatment or travel restrictions.
- No elephant should move into a facility where there is an untested elephant.
- If an elephant has had exposure to other untested elephants in the previous 3 months, then a STAT-PAK® test should be repeated in 3 months time to confirm. If the ElephantTB STAT-PAK® remains non-reactive, the elephant continues in Group 1.

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

Monitor by culture (triple sample method) and ElephantTB STAT-PAK<sup>®</sup> every 3 months for one year post-exposure, then every 6 months for 2 years, then annually thereafter if all cultures remain negative and ElephantTB STAT-PAK<sup>®</sup> remains non-reactive.

- No travel or public contact until 2 additional non-reactive ElephantTB STAT-PAK<sup>®</sup> tests are performed at 3 and 6 months post-exposure (6 month restriction).
  - If non-reactive at 6 months, travel/public contact restrictions removed as long as additional testing can be performed as outlined above.
- If the results during any of the follow-up testing change, the individual elephant will change group. No elephant should move into a facility where there is an untested elephant.

Note: The exact time to sero-conversion is unknown.

### **GROUP 3: Culture negative; ElephantTB STAT-PAK<sup>®</sup> reactive**

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

#### **A. Culture negative; STAT-PAK<sup>®</sup> reactive, MAPIA<sup>™</sup>/DPP<sup>®</sup> non-reactive, no known exposure**

Monitor by culture (triple sample method) every 3 months for the first year after becoming ElephantTB STAT-PAK<sup>®</sup> reactive, then every 6 months for the next 2 years. Repeat MAPIA<sup>™</sup> / DPP<sup>®</sup> every 6 months for the first year if elephant remains STAT-PAK<sup>®</sup> reactive. If all cultures and MAPIA<sup>™</sup>/DPP<sup>®</sup> remain negative/non-reactive during this period, annual testing may resume.

- No treatment or travel restrictions.
- If the culture becomes positive or MAPIA<sup>™</sup>/DPP<sup>®</sup> becomes reactive during any of the follow-up testing the individual elephant will change category.
- No elephant should move into a facility where there is an untested elephant.

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

Monitor by culture (triple sample method) every 3 months for one year post-exposure, then every 6 months for two years then annually thereafter if all cultures remain negative. Repeat MAPIA<sup>™</sup>/DPP<sup>®</sup> every 6 months for the first 3 years if elephant remains STAT-PAK<sup>®</sup> reactive. If all cultures and MAPIA<sup>™</sup>/DPP<sup>®</sup> remain negative/non-reactive during this period, annual testing may resume after 3 years.

- No travel or public contact for first year; if results are unchanged at the first year, restrictions are removed.
- If the culture or MAPIA<sup>™</sup>/DPP<sup>®</sup> results change during any of the follow-up testing and become positive, the individual elephant will change group.
- Culture positive elephants that have completed a course of anti-tuberculosis therapy may remain ElephantTB STAT-PAK<sup>®</sup> reactive and fall into this group. If appropriate treatment has been documented and approved by USDA, these animals will not have travel/public contact restrictions unless there is a change to positive culture and/or reactive MAPIA<sup>™</sup>/DPP<sup>®</sup> results during follow-up testing.

#### **C. Culture negative; STAT-PAK<sup>®</sup> reactive, MAPIA<sup>™</sup>/DPP<sup>®</sup> reactive, no known exposure**

Monitor by culture (triple sample method) every 3 months for one year, then every 6 months for life. Repeat MAPIA<sup>™</sup>/DPP<sup>®</sup> every 3 months for the first year, then every 6 months for an additional 2 years if elephant remains STAT-PAK<sup>®</sup> reactive. If all cultures remain negative after 3 years annual serological testing may resume as described in these guidelines.

- No travel or public contact until the first year of testing has been completed.
- Treatment should be considered. If serological conversions are demonstrated to be recent (within the past 12 months then prophylactic treatment can be used. If serological conversions are longer standing or unknown, then full treatment may be advisable. Individual

cases should be evaluated in conjunction with USDA. If treatment is performed, the elephant may be able to travel and have public contact after 6 months of successful documented USDA approved treatment.

- If the culture or MAPIA™/DPP® results change during any of the follow-up testing the individual elephant will change group.

Note: The STAT-PAK® and MAPIA™ /DPP® tests have been shown to be early indicators of TB infection. Retrospective studies have shown elephants may be serologically reactive months to years in advance of detection by culture (Greenwald 2009).

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

Monitor by culture (triple sample method) every 3 months for one year post-exposure, then every 6 months for life. Repeat MAPIA™/DPP® every 3 months for the first year, then every 6 months for an additional 2 years if elephant remains STAT-PAK® reactive. If all cultures remain negative after 3 years, annual serological testing may resume as described in these Guidelines.

- No travel or public contact until the first year of testing has been completed.
- Treatment should be considered. If serological conversions are demonstrated to be recent (within the past 12 months) then prophylactic treatment can be used. If serological conversions are longer standing or unknown then full treatment may be advisable. Individual cases should be evaluated in conjunction with USDA. If treatment is performed, the elephant may be able to travel and have public contact after 6 months of successful documented USDA approved treatment.
- If the culture or MAPIA™/DPP® results change during any of the follow-up testing the individual elephant will change group.
- Culture positive elephants that have completed a course of anti-tuberculosis therapy may remain ElephantTB STAT-PAK® reactive and fall into this category. If appropriate treatment has been documented and approved by USDA, these animals will not have travel/public contact restrictions unless there is a change in their results during follow-up testing. It has been shown that the MAPIA™/DPP® will decline and may indicate a response to treatment so on-going annual monitoring with MAPIA™/DPP® is required for life as changes in MAPIA™ may detect relapse.

**Considerations for ElephantTB STAT-PAK® reactive elephants.**

Elephants may develop antibodies to mycobacterial antigens months to years prior to detection by culture, however, the time intervals between exposure, seroconversion, and shedding are not precisely known. Numerous variables such as age, genetics, immune status, nutritional condition, other concurrent health problems, and other factors influence the development of disease in an individual animal following exposure to a pathogenic agent. Results of MAPIA™/DPP® testing are useful in helping determine potential risk categories as defined above and determine which animals require more frequent surveillance or should undergo prophylactic treatment (Greenwald 2009).

There may be a possible association with chronic inflammatory conditions, such as arthritis, in elephants that are ElephantTB STAT-PAK® reactive, but non-reactive on MAPIA™/DPP® and with no known TB exposure based on a small number of cases. Review history for possible exposure to a culture positive animal or previous treatment for TB since this may also affect results. Nonetheless, it is important to monitor these elephants for possible development of infection and disease. Retrospective analyses of banked serum samples are strongly encouraged to provide a more complete serological history.

Elephants that are culture negative, ElephantTB STAT-PAK® reactive and MAPIA™/DPP® reactive are at increased risk of either latent or active TB. Factors to consider in the decision to administer treatment vs. increased monitoring include exposure history, age, whether the elephant travels, potential exposure of personnel or public, side effects of treatment, concurrent health problems, etc. Increased monitoring and travel/public contact restrictions is required based on risk. If culture results during any of the follow-up testing become positive, the individual elephant will move to Category 4.

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

Based on a history of exposure to a culture positive animal, or other considerations, the attending veterinarian may elect to administer prophylactic or full treatment after consultation with USDA.

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

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

**Prophylactic Treatment Schedule 1 (preferred):**

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

**Prophylactic Treatment Schedule 2:**

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

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

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

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

**Monitoring of Prophylactically Treated Elephants**

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

**GROUP 4: *M. tuberculosis* complex positive culture**

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

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

### **Positive cultures must be submitted to NVSL for genotyping.**

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

#### **Options:**

**A. Treatment: This is the preferred option for culture positive elephants whenever possible.** 1. If the organism was isolated at a laboratory other than NVSL and they do not perform mycobacterial species differentiation and DNA fingerprinting, the owner must request that the laboratory submit the isolate to NVSL or other qualified laboratory for mycobacterial species differentiation and DNA fingerprinting.

**2. Antimicrobial sensitivity testing should be performed on all positive isolates. Sensitivities should be requested for the following drugs: isoniazid, rifampin, pyrazinamide, ethambutol, ciprofloxacin (or other fluoroquinolone), and amikacin. (Antimicrobial susceptibility testing for *M. tuberculosis* complex organisms is now available at NVSL).**

3. Perform ElephantTB STAT-PAK<sup>®</sup> and MAPIA<sup>™</sup> every 3 months during treatment then every 6 months for 2 years then according to the schedule in the group that the elephant falls into post-treatment. Serological monitoring of treated elephants with MAPIA<sup>™</sup> has shown changes that may indicate successful treatment or recrudescence of infection (Lyashchenko 2006).

4. Beginning with the onset of treatment, cultures should be collected by the triple sample method every 2 months for the first 6 months of treatment, then every 6 months for the remainder of the elephant's life. This intensive screening by culture ensures adequate therapy during the treatment period and 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.

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

**Schedule 1 (preferred):** Administer 3 drugs to which the isolates are susceptible daily for 2 months. The first 60 doses should be administered within a period of 90 days (i.e. no more than 30 days of "refused medication" should occur). Adequate blood levels of all 3 drugs must be demonstrated in two samples collected approximately two weeks apart. Serum samples should be collected as soon as the elephant is

accepting medication reliably. If acceptable levels (see below) are not achieved, the dosage should be adjusted and serum levels tested again (two samples collected approximately two weeks apart). It must be demonstrated that the elephant received the first 60 doses at a dosage level sufficient to achieve adequate drug serum levels. Treatment is then continued daily for an additional 10 months with 2 drugs to which the isolate is susceptible for a total number of doses (with two drugs) of 300. As above, the inclusion of INH is recommended. The total number of doses for the entire treatment is 360. The entire treatment should be completed within 15 months (this allows for “refused medicine” days and periods of interruption that may be needed if side effects are noted).

**Schedule 2:** Administer 3 drugs to which the isolate is susceptible for 2 months. The first 60 doses should be administered within a period of 90 days (i.e. no more than 30 days of “refused medication” should occur). Adequate levels of all drugs must be demonstrated in two samples collected approximately 2 weeks apart. Serum samples should be collected as soon as the elephant is accepting medication reliably. If acceptable levels (see below) are not achieved, the dosage should be adjusted and serum levels tested again (two samples collected approximately two weeks apart). It must be demonstrated that the elephant received the first 60 doses at a dosage level sufficient to achieve adequate drug serum levels. Continue treatment with two drugs at twice the dosage used in the initial period every other day for 10 months (150 doses). It is not necessary to repeat serum drug levels. The total number of doses is 210. The entire treatment should be completed within 15 months (this allows for “refused medicine” days and periods of interruption that may be needed if side effects are noted). Animals that have not completed treatment are considered as non-treated.

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

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

#### **Additional Monitoring of Treated Elephants**

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

**B. Quarantine without treatment:** This option may be considered especially for animals that are already housed alone and not considered a good candidate for treatment (ex. bull elephant). Additional precautions must be taken for human safety (such as the use of N-95 masks, gloves, etc). Quarantined elephants should be kept out of range from non-infected animals and should be monitored for signs of TB disease.

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

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

**Group 5: Untested** If an elephant cannot complete procedures as outlined for official annual testing, it should not be permitted to have public contact that would result in exposure by contact or aerosol

transmission, or contact with other tested elephants (or their enclosures or equipment). Untested elephants should not be moved from their home facilities. A tested elephant should not move into a facility housing an untested elephant unless it can be demonstrated that there will be no direct contact with the untested elephant or with its enclosure or equipment. If a tested elephant(s) is in contact or housed with an untested elephant, the tested elephant cannot travel nor have public contact until the untested elephant is tested unless approved by USDA.

## **8. PRINCIPLES OF ANTI-TUBERCULOSIS THERAPY**

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

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

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

Once susceptibility tests are received, and the sputum has reverted to being smear negative, the number of drugs is decreased to two first line drugs for the remainder of treatment. When the index case is known, and the index isolate is known to be susceptible to all anti-mycobacterial drugs, then initial treatment may be limited to three drugs. However, in the vast majority of cases the index case is not known with certainty and four drugs are given. Moreover, in regions or situations when the frequency of resistance exceeds 10%, empiric initial therapy for humans consists of five drugs.

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

## **9. ANTI-TUBERCULOSIS DRUGS**

Antituberculous agents are divided into first and second line agents. First line agents include isoniazid, rifampin, pyrazinamide, ethambutol, and streptomycin. These are agents with the greatest activity and the best side effect profiles. Second line agents include those with less activity and/or greater side effects. Second line agents include capreomycin, ethionamide, cycloserine, and thiacetazone. The fluoroquinolones (FQ; moxifloxacin, ciprofloxacin, levofloxacin, and enrofloxacin) while not considered as 1<sup>st</sup> line agents have significant bactericidal activity against *M. tuberculosis*. Moreover, published studies report the equivalency of FQ substitution for ethambutol in the treatment of TB in humans and studies are underway to investigate FQ use for the treatment of latent TB infection. Linezolid, a drug active against Gram positive bacteria such as *Staphylococcus aureus*, MRSA, enterococcus, and VRE has also been

shown to have significant activity against *M. tuberculosis* and has been used successfully in salvage regimens. Amikacin, an aminoglycoside (as is streptomycin), is a mainstay in the treatment of non-tuberculous mycobacterial infection and has been used in salvage regimens against MDR-TB. Pharmacokinetic studies of INH, RIF, EMB, and PZA in elephants have been published (Maslow et al. 2005a, Maslow et al. 2005 b, Zhu et al. 2005, and Peloquin et al. 2006).

## FIRST LINE AGENTS

### Isonicotinic acid hydrazide (Isoniazid, INH)

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

**Metabolism and excretion:** INH is acetylated in the liver through the action of *N*-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 those results in more rapid clearance and lower bioavailability. It is not known whether elephants are polymorphic in this enzyme and differ in the speed of acetylation.

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

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

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

### Rifampin (RIF)

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

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

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

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

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

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

## **Pyrazinamide (PZA)**

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

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

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

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

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

## **Ethambutol (EMB)**

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

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

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

**Route of administration:** Ethambutol is administered orally to humans and elephants. Rectal administration 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

### FLUOROQUINOLONES: MOXIFLOXACIN, CIPROFLOXACIN, LEVOFLOXACIN, ENROFLOXACIN

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

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

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

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

## AMIKACIN

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

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

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

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

hours. However, significant toxicity occurred with prolonged use of this drug at this dose (personal communication, Dr. G Dumonceaux ).

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

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

## 10. DOSAGES AND ROUTES OF ADMINISTRATION

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

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

Drug	Dosage (mg/kg)	Route	Formulation	Target conc (µg/ml)	Cmax (hr)
Isoniazid	5	Oral	premixed suspension	3-5	1-2
	4	Oral	Powder	3-5	0.5-1
	4	Rectal	premixed suspension	3-5	0.25-0.5
Rifampin	10	Oral only	Powder	8-24	2-4
Pyrazinamide	30	Oral or rectal	Powder	20-60	1-2
Ethambutol	30	Oral only	Powder	2-5	1-2

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

## 11. BLOOD LEVELS

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

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

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

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

## **12. POSTMORTEM EXAMINATION**

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

[www.elephanttag.org](http://www.elephanttag.org)  
[www.elephantcare.org](http://www.elephantcare.org)

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

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

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

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

### **Protective equipment for tuberculosis cases - Mandatory**

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

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

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

### **Consult these websites for OSHA and CDC guidelines:**

1. OSHA TB standards and rules: <http://www.osha.gov/SLTC/tuberculosis/standards.html>
2. Guidelines for Preventing the Transmission of *Mycobacterium tuberculosis* in Health-Care Settings, 2005:  
[http://www.cdc.gov/nchstp/tb/Federal\\_Register/New\\_Guidelines/TBICGuidelines.pdf](http://www.cdc.gov/nchstp/tb/Federal_Register/New_Guidelines/TBICGuidelines.pdf)

### **Necropsy procedures**

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

## **13. EMPLOYEE HEALTH AND SAFETY**

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

Any employee with a positive intradermal 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 intradermal testing. If inoculation with BCG occurred more than 10 years ago, a positive PPD test should not be considered a reaction due to BCG, but should instead be considered as positive for exposure to TB.

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 or any other route and thus, there are no restrictions placed on animals or humans known to be colonized or infected such organisms.

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 should include the use of respiratory (N95) HEPA filtered masks during all direct or indirect contact with infected animals, such as 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*.

Best practices for the safe conduct of work in biomedical and clinical laboratories and animal facilities in regards to *Mycobacterium tuberculosis* are listed in the 5<sup>th</sup> Edition of Biosafety in Microbiological and Biomedical Laboratories published by the U.S. Department of Health and Human Services in 2007. [http://www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL\\_5th\\_Edition.pdf](http://www.cdc.gov/od/ohs/biosfty/bmbl5/BMBL_5th_Edition.pdf)

## **14. REPORTING**

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

## **15. APPENDICES**

### **APPENDIX 1. REFERENCES CITED AND ADDITIONAL READING**

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

Ramiro Isaza, DVM, MS and Cornelia Ketz, DVM

## 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 °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 through 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.

#### **References**

1. Mikota, S., Sargent, E.L., Ranglack, G.S. Medical Management of Elephants. West Bloomfield, MI, Indira Publishing House. 1994.
2. Mikota, S.K., Peddie, L., Peddie, J., Isaza, R., Dunker, F., West, G., Lindsay, W., Larsen, R.S., Salman, M.D., Chatterjee, D., Payeur, J., Whipple, D., Thoen, C., Davis, D.S., Sedgwick, C., Montali, R.J., Ziccardi, M., and Maslow, J. Epidemiology and diagnosis of *Mycobacterium tuberculosis* in captive Asian elephants (*Elephas maximus*). J Zoo Wildl Med 2001 Mar. 32 (1): 1-16.
3. Michalak, K., Austin, C., Diesel, S., Bacon, J.M., Zimmerman, P., and Maslow, J.N. *Mycobacterium tuberculosis* infection as a zoonotic disease: Transmission between humans and elephants. Emerg Infect Dis. 1998. Apr-Jun 4(2): 283-287.

## APPENDIX 4. TESTING LABORATORIES

### CULTURES, ANTIMICROBIAL SENSITIVITY, GENOTYPING

#### USDA APHIS VS

#### National Veterinary Services Laboratories (NVSL)

1920 Dayton Avenue

Ames, IA 50010

Lab web site: [http://www.aphis.usda.gov/animal\\_health/lab\\_info\\_services/diagnos\\_tests.shtml](http://www.aphis.usda.gov/animal_health/lab_info_services/diagnos_tests.shtml)

#### Dr. Janet Payeur

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#### Dr. Suelee Robbe-Austerman

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Email: [Suelee.Robbe-Austerman@aphis.usda.gov](mailto:Suelee.Robbe-Austerman@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 free of charge from NVSL.

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

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

NVSL Trunk wash cost: \$98 per sample for processing which includes a Gen Probe® DNA probe on any isolate. If the sample is positive for mycobacteria and speciation is requested, the charge is \$122.00 per sample which includes biochemical analysis, 16s rDNA sequencing analysis, spoliotyping and VNTR genotyping. DNA fingerprinting of *M. tuberculosis* or *M. bovis* isolates is also available. Antimicrobial susceptibility testing is available for *M. tuberculosis* complex organisms for \$112.00 per isolate. Please contact NVSL at (515) 337-7388 for test schedule.

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

**(User fees as of October 1, 2010). Call lab before shipping samples for current prices and schedule of testing or check prices at the NVSL web site:**

[http://www.aphis.usda.gov/animal\\_health/lab\\_info\\_services/diagnos\\_tests.shtml](http://www.aphis.usda.gov/animal_health/lab_info_services/diagnos_tests.shtml)

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

Director: [Leonid Heifets, M.D.](#)

1400 Jackson St.

Denver, CO 80206

(303) 398-1384

E-mail: [heifetsl@njc.org](mailto:heifetsl@njc.org)

For price list, shipping instructions, and requisition form:

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

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

**HISTOPATHOLOGY**

**Scott P. Terrell, DVM, Diplomate ACVP**

SSP Pathology Advisor

Disney's Animal Kingdom

1200 N Savannah Circle

Bay Lake, FL 32830

W (407) 938-2746;

H (407) 251-0545;

Cell (321) 229-9363;

Email [Scott.P.Terrell@disney.com](mailto:Scott.P.Terrell@disney.com)

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

**USDA APHIS NVSL Pathobiology Laboratory**

1920 Dayton Avenue

Ames, IA 50010

(515) 337-7521

Fax (515) 337-7527

Lab web site: [http://www.aphis.usda.gov/animal\\_health/lab\\_info\\_services/diagnos\\_tests.shtml](http://www.aphis.usda.gov/animal_health/lab_info_services/diagnos_tests.shtml)

**Dr. Art Davis**

Director of Pathobiology Laboratory

(515) 337-7526

Email: [Arthur.J.Davis@aphis.usda.gov](mailto:Arthur.J.Davis@aphis.usda.gov)

**Dr. Mark Hall**

Head Pathological Investigations

(515) 337-7927

Email: [Mark.Hall@aphis.usda.gov](mailto:Mark.Hall@aphis.usda.gov)

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

**ANTI TB DRUG LEVELS**

**Infectious Diseases Pharmacokinetics Laboratory (IDPL)**

**National Jewish Medical and Research Center**

1400 Jackson St.  
Denver, CO 80206

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

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

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

**Charles Peloquin, Pharm.D.**

Professor and Director  
University of Florida  
1600 SW Archer Rd., Rm P4-33  
PO Box 100486  
Gainesville, FL 32610-0486  
Tel: 352-273-6266  
Fax: 352-273-6804

[peloquin@cop.ufl.edu](mailto:peloquin@cop.ufl.edu)

Call or email for information on sample submission.

**The National Veterinary Services Laboratories**

USDA APHIS NVSL  
1920 Dayton Avenue  
Ames, IA 50010

Web site: [http://www.aphis.usda.gov/animal\\_health/lab\\_info\\_services/diagnos\\_tests.shtml](http://www.aphis.usda.gov/animal_health/lab_info_services/diagnos_tests.shtml)

**Dr. David Kinker**

Head, Serology Section  
515-337-7950

Email: [David.R.Kinker@aphis.usda.gov](mailto:David.R.Kinker@aphis.usda.gov)

Call before shipping samples for current prices.

**Chembio Diagnostic Systems, Inc.**

3661 Horseblock Road  
Medford, NY 11763  
Tel: 631-924-1135  
Fax: 631-924-6033

Email: [customerservice@chembio.com](mailto:customerservice@chembio.com)

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

# APPENDIX 5. USDA Standard Operating Procedure for Processing Elephant Trunk Washes for the Isolation of Mycobacteria

United States Department of Agriculture  
National Veterinary Services Laboratories

## Standard Operating Procedure Processing Elephant Trunk Washes for the Isolation of Mycobacteria

Mention of trademark or proprietary product does not constitute a guarantee or warranty of the product by USDA and does not imply its approval to the exclusion of other products that may be suitable.

### 1. Purpose

The purpose of this Standard Operating Procedure (SOP) is to describe the procedure for processing elephant trunk washes for the isolation of *Mycobacterium tuberculosis* used in the Mycobacteria and Brucella (MB) section.

**Warning: *Mycobacterium bovis*, *M. tuberculosis* and *M. avium* are pathogenic to humans and they are a Class III pathogen. All procedures must be performed in a Class II or Class III biological safety cabinet.**

### 2. Materials

- 2.1 50 ml sterile conical centrifuge tubes
- 2.2 15 ml sterile conical centrifuge tubes
- 2.3 Sorvall RC 3BP refrigerated centrifuge
- 2.4 N-Acetyl-L cysteine (NALC); Sigma catalog number A-7250
- 2.5 NaOH-Sodium citrate solution; (NVSL #10687)
  - 2.5.1.1 Dissolve 29 gm of sodium citrate dehydrate in 1000 ml of Super Q H<sub>2</sub>O. Dissolve 40 gm of sodium hydroxide pellets in 1000 ml of Super Q H<sub>2</sub>O. Combine the 2 solutions and dispense as requested. Autoclave for 15 minutes at 121 °C.
- 2.6 Sterile distilled water
- 2.7 Johne's antibiotic mixture (contains vancomycin, amphotericin B, and nalidixic acid); NVSL #20215
  - 2.7.1 Brain Heart Infusion Broth (NVSL #10009) 18.5 g
    - 2.7.1.1 Combine 37 gm of Difco BHI w/out dextrose (BBL # 250220) with 1000 ml Super Q H<sub>2</sub>O. Bring to a rolling boil. Dispense as requested. Autoclave 20 min. at 121°C for flasks, 15 min. for tubes.
  - 2.7.2 Super QH<sub>2</sub>O 1000 ml
  - 2.7.3 Nalidixic Acid (NVSL #40153) 10 ml
    - 2.7.3.1 Mix 10 g of nalidixic acid with 500 ml of distilled H<sub>2</sub>O. Add 10N NaOH drop by drop until solution clears and QS to 1000 ml. Filter sterilize solution thru a 0.22µm filter into sterile jug with bell end. Dispense (wearing gloves), 20 ml into a 50 ml sterile conical tube. Caution – chemical is carcinogenic.
  - 2.7.4 Vancomycin (NVSL #40151) 10 ml
    - 2.7.4.1 Combine 9.346 gm with 1000 ml of distilled Super Q H<sub>2</sub>O and mix well. Filter sterilize. Dispense into 50 ml sterile tubes in 20.5 ml amounts.
  - 2.7.5 Amphotericin B (NVSL #40154) 5 ml
    - 2.7.5.1 Add 10 ml warm sterile distilled H<sub>2</sub>O to a 100 mg Amphotericin B (Fungizone). Shake gently until dissolved and dispense as requested.
  - 2.7.6 Combine BHI broth and Super QH<sub>2</sub>O. Autoclave for 20 min at 121°C. Cool to 50°C. Remove 25 ml of cooled broth and discard. Add Nalidixic Acid,

Vancomycin, and Amphotericin B. Mix well. Dispense in tubes and cover with foil because the Amphotericin B is light sensitive. Store in -20°C freezer. Media is good for 3 months.

- 2.8 37°C CO<sub>2</sub> incubator preferred
- 2.9 Media set-up (one tube of each per sample):
  - 2.9.1 Middlebrook 7H10 w/glycerol; NVSL #10941 or BBL Middlebrook and Cohn 7H10 Agar tubes (BBL #220959).
  - 2.9.2 Middlebrook 7H11 w/glycerol; NVSL #10942 or BBL Seven H11 Agar tubes (BBL #221392) or BBL Selective Seven H11 Agar tube (BBL #297639).
  - 2.9.3 Stonebrinks; NVSL #10451
  - 2.9.4 Mycobactosel L-J medium (BBL #221414)
  - 2.9.5 Bactec 12B medium vial with Panta and Erythromycin (32 µg/ml)
- 2.10 1 ml tuberculin syringes
- 2.11 5 ml syringes
- 2.12 Slant trays, media tube baskets
- 2.13 Vortex
- 2.14 Sterile swabs

### 3. Procedures

- 3.1 Carefully pour 10 – 12 ml of the trunk wash into a 50 ml conical centrifuge tube. If there is less than 10 ml, use the entire sample.
  - 3.1.1 At this time, also pour 10 - 12 ml of sterile distilled water into a 50 ml conical centrifuge tube; this sample will be labeled “negative control” and will be processed the same as the rest of the samples.
- 3.2 Pour 10 to 12 ml (or whatever is left if < 10 ml) of the remaining trunk wash into a 15 ml conical centrifuge tube for storage.
  - 3.2.1 These 15 ml centrifuge tubes are stored at -20 ± 2° C for a minimum of 8 weeks or until the bacterial isolation procedure is completed.
  - 3.2.2 Samples from cases in which no isolation was made are retained in a -20° ± 2 ° C freezer for at least 6 months.
    - 3.2.2.1 Samples that have no isolation and are older than 6 months can be discarded by the procedure in the current version of MBSOP0008.
  - 3.2.3 Samples from cases in which mycobacteria have been isolated will be retained for one year and stored in a -20° C freezer.
- 3.3 Allow the 10 - 12 ml of the trunk wash in the 50 ml conical tube to stand undisturbed for 15 – 20 minutes to allow sediment to settle to the bottom. Or Alternately: Pulse spin the 10 - 12 ml of trunk wash in the 50 ml centrifuge to spin down excess sediment. This can be accomplished by centrifuging for 1 minute and 40 seconds at 3000RCF, 10°C, using the Sorvall RC 3BP centrifuge.
- 3.4 Slowly pour the supernate, trying not to disturb the sediment, into a sterile 50 ml conical centrifuge tube.
- 3.5 Prepare the N-Acetyl-L-cysteine (NALC)/NaOH- sodium citrate solution according to the following proportions:

Volume (ml)	NaOH- sodium citrate <sup>a</sup> (ml)		NALC (g)
50	50	0.25	
100	100		0.50
150	150		0.75
200	200		1.00
300	300		1.50

- a. 1:1 mixture of 4% NaOH to 2.9% sodium citrate
- b. Allow NALC to dissolve in solution before use
- c. Discard this solution after 24 hours

- 3.6 Add an equal amount of the NALC solution to the trunk wash supernate up to a maximum of 10 ml using a sterile pipette.
- 3.6.1 Be careful to avoid cross contamination of samples when adding the NALC solution.
- 3.7 Vortex or vigorously hand shake for  $20 \pm 5$  seconds
- 3.8 Routinely allow the trunk wash to remain in contact with the NALC for  $15 \pm 5$  minutes.
- 3.8.1 If the sample is extremely cloudy or appears to be contaminated, the NALC solution may need to remain in contact with the sample for up to  $20 \pm 5$  minutes.
- 3.8.2 NALC is a mucolytic agent and this procedure reduces or eliminates contaminating bacteria while releasing Mycobacteria which may be trapped in mucin and cells, allowing them to grow. Observe the tube for clearing before proceeding to the next step.
- 3.9 Add enough sterile distilled water to the NALC/wash solution to fill the centrifuge tube
- 3.10 Centrifuge the water/NALC/wash solution for 20 minutes at 6000g and  $10^{\circ}\text{C}$ .
- 3.11 Carefully pour off and discard the supernatant.
- 3.12 Re-suspend the sediment with 2 mls of sterile distilled water and 1 ml of the John's antibiotic mixture.
- 3.12.1 Vortex the re-suspended mixture for  $20 \pm 5$  seconds.
- 3.13 Incubate overnight at  $37 \pm 2^{\circ}\text{C}$ .
- 3.14 Vortex or vigorously hand shake the incubated sample for  $20 \pm 5$  seconds.
- 3.15 Using a sterile swab per specimen, swab the sample over the entire surface of each of the solid media tubes listed in section 2 of this SOP.
- 3.15.1 The four solid media tubes are Middlebrooks 7H10 w/glycerol, Middlebrooks 7H11 w/ glycerol, Mycobactosel LJ, and Stonebrinks.
- 3.15.2 If one of these media is not available, contact the section head or their designate for which media to be substituted.
- 3.16 Prepare the BACTEC 12B bottles.
- 3.16.1 A 5 ml syringe is used to add 2 ml of Erythromycin to the reconstituting fluid supplied for the PANTA solution for a final concentration of  $32 \mu\text{g/ml}$ .
- 3.16.2 Using a new 5 ml syringe, add 5 ml of the reconstituting fluid to the vial of PANTA.
- 3.16.3 Using a 1 ml tuberculin syringe, each vial of the BACTEC media is inoculated with 0.2ml of the reconstituted supplement.
- 3.17 Inoculate the BACTEC media with your sample.
- 3.17.1 Using a 1 ml tuberculin syringe, inoculate the BACTEC media with  $\leq 0.5$  ml of the sample
- 3.18 Place the inoculated solid media on a slant rack and incubate overnight at  $37 \pm 2^{\circ}\text{C}$  in 10%  $\text{CO}_2$ , if available.
- 3.18.1 Incubation in an atmosphere of  $\text{CO}_2$  will encourage earlier growth.
- 3.18.2 After being incubated overnight on a slant rack, the media tubes can be stored in the  $37^{\circ}\text{C}$  incubator in an upright position.
- 3.19 Place the inoculated BACTEC bottles in a locked  $37^{\circ}\text{C}$  incubator.
- 3.20 Read the media tubes as outlined below and record the results on the appropriate worksheets:
- 3.20.1 Solid Media
- 3.20.1.1 Read tubes weekly for weeks 1-7 after inoculation.
- 3.20.1.2 For tubes in week 8, read and record the results and discard the tubes if there is no suspicious growth for Mycobacteria noted in the tubes.

3.20.1.3 For tubes that contain contamination, discard the media tubes that are overgrown. If the entire set of media is overgrown, the case may need to be retested.

3.20.2 BACTEC Bottles

3.20.2.1 BACTEC bottles are read twice weekly for the first 3 weeks and then once a week until week 6.

3.20.2.2 Results of the growth indicator values are recorded on the BACTEC worksheet assigned to the case on the bottle each time the BACTEC bottle is read by the BACTEC 460 machine.

3.20.2.3 BACTEC bottles that have a growth indicator value of 300 or higher are examined by acid fast staining. See the current version of MBSOP2210 for these procedures. BACTEC bottles that are to be discarded are stored in a locked cabinet in the biological safety level 3 laboratories until they are removed by the personnel from Environmental Health and Safety. See the current version of MBSOP2007 and MBSOP2008 for these procedures.

**4. References**

4.1 Della-Latta, P. and I. Weitzman. 1998. Mycobacteriology. p. 169-204 In H.D. Isenberg (ed.), *Essential Procedures for Clinical Microbiology*. American Society for Microbiology, Washington, D.C.

4.2 Zimbardo, M.J. and D.A. Power. 2003. *Difco & BBL Manual – Manual of Microbiological Culture Media*. Becton, Dickinson and Company, Sparks, MD.

## APPENDIX 6. CONTACTS FOR QUESTIONS

### DIAGNOSIS AND TREATMENT

**Michele A. Miller, DVM, MS, PhD**

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Work: 813-367-4055  
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**Dr. Susan K. Mikota**

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Cell: 931-628-5962  
Email: [smikota@elephantcare.org](mailto:smikota@elephantcare.org)  
Website: [www.elephantcare.org](http://www.elephantcare.org)

**ELEPHANT TB STAT-PAK<sup>®</sup> ASSAY AND MAPIA<sup>™</sup>**

**Konstantin Lyashchenko, Ph.D.**

Research Director, Mycobacterial Immunology  
Chembio Diagnostic Systems, Inc.  
3661 Horseblock Road  
Medford, NY 11763  
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### REGULATORY

**Dr. Denise Sofranko**

USDA-APHIS-Animal Care  
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Voice Mail: 240-461-9142  
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2150 Centre Avenue  
BLDG B Mail Stop #3-W11  
Ft. Collins, CO 80562-8117

### HUMAN HEALTH, ELEPHANT THERAPY AND TREATMENT

**Joel Maslow, MD, PhD**

Division of Infectious Diseases

Philadelphia Veterans Affairs Medical Center and the  
University of Pennsylvania School of Medicine  
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#### **NECROPSY, PATHOLOGY**

**Scott P. Terrell, DVM, Diplomate ACVP**

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Home: 407-251-0545  
Cell: 321-229-9363  
Email: [Scott.P.Terrell@disney.com](mailto:Scott.P.Terrell@disney.com)

#### **INTERNET**

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

1. [http://www.aphis.usda.gov/animal\\_welfare/index.shtml](http://www.aphis.usda.gov/animal_welfare/index.shtml) (available to the public)
2. [www.aazv.org](http://www.aazv.org) (available to AAZV members by password)
3. [www.elephantcare.org](http://www.elephantcare.org) (available to the public)
4. [www.elephanttag.org](http://www.elephanttag.org) (available to the public)

## **APPENDIX 7. SOURCES FOR ANTI-TUBERCULOSIS DRUGS**

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

## APPENDIX 8. Elephant Serum Bank Submission Form

Institution/owner: \_\_\_\_\_

Submitter: \_\_\_\_\_

Address: \_\_\_\_\_

Tel: \_\_\_\_\_ Fax: \_\_\_\_\_ Email: \_\_\_\_\_

### ANIMAL INFORMATION

Asian  African  ISIS# \_\_\_\_\_ Studbook # \_\_\_\_\_

Name \_\_\_\_\_ Age: \_\_\_\_\_  actual  estimate

Sex:  male  female

### SAMPLE COLLECTION INFORMATION

Date of sample collection: \_\_\_\_\_ Time of collection: \_\_\_\_\_

Site of sample collection:  ear vein  leg vein  other: \_\_\_\_\_

Health status of animal:  normal  abnormal

Fasted:  no  yes – how long \_\_\_\_\_

Weight \_\_\_\_\_  actual  estimated

Type of restraint:  manual  anesthetized/sedated  behavioral control

Temperament of animal:  calm  active  excited

Type of blood collection tube:

no anticoagulant (red-top)

EDTA (purple)

heparin (green)

other: \_\_\_\_\_

Sample handling:  separation of plasma/serum by centrifugation

(Check all that apply)  stored as whole blood

frozen plasma

other – describe \_\_\_\_\_

### TB EXPOSURE STATUS

Known infected animal

Known exposure to culture positive source within the past 12 months

Known exposure to a culture positive source within the past 1-5 years

No know exposure to a culture positive source in the last 5 years

### TREATMENT INFORMATION

Is elephant currently receiving any medication or under treatment?  yes  no

If yes, please list drugs and doses: \_\_\_\_\_

\_\_\_\_\_

\_\_\_\_\_

Time between blood collection and last treatment: \_\_\_\_\_

---

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

Send completed form with samples to:

**Michele A. Miller, DVM, MS, PhD**

Chief Veterinary Officer and Director of Conservation Medicine

Palm Beach Zoo, 1301 Summit Blvd., West Palm Beach, FL 33405

Phone: 561-833-7130 ext 224; Cell: 561-727-9630;

Email: [mmiller@palmbeachzoo.org](mailto:mmiller@palmbeachzoo.org)

## Consent Form for Use of Serum by Elephant SSP

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

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

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

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

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

\_\_\_\_\_  
Signature, title

\_\_\_\_\_  
Date

\_\_\_\_\_  
Printed name

\_\_\_\_\_  
Phone number

\_\_\_\_\_  
Institution

\_\_\_\_\_  
Email address

\_\_\_\_\_  
Address

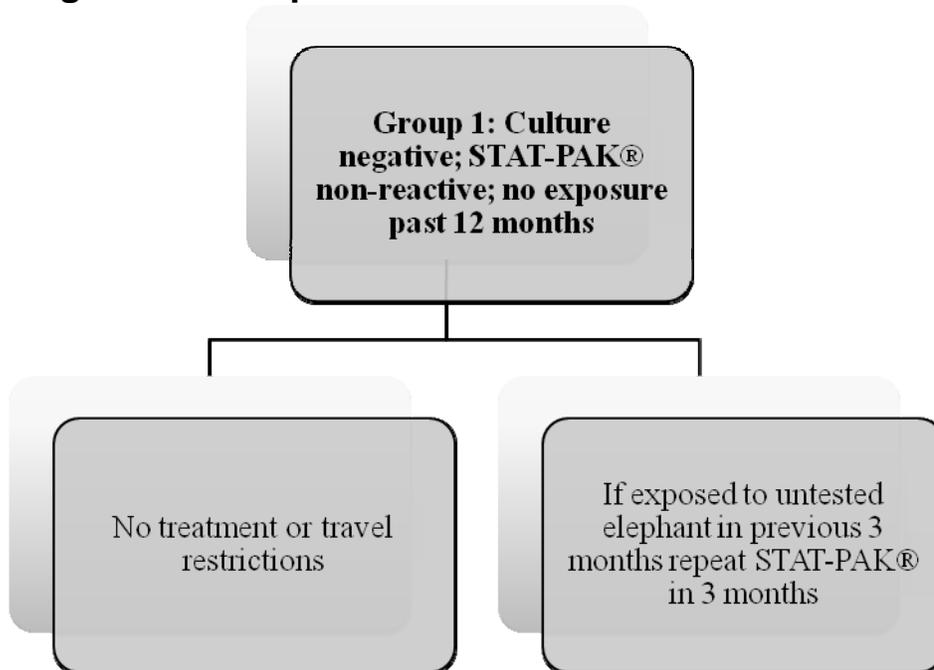
\_\_\_\_\_

\_\_\_\_\_

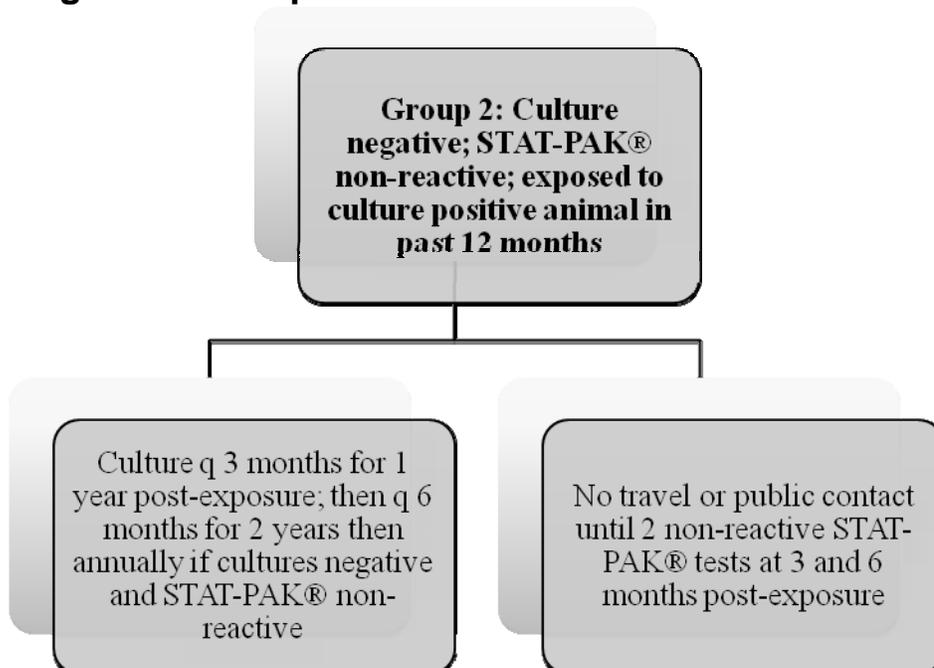
Comments: \_\_\_\_\_

## Appendix 9. TB Management Groups

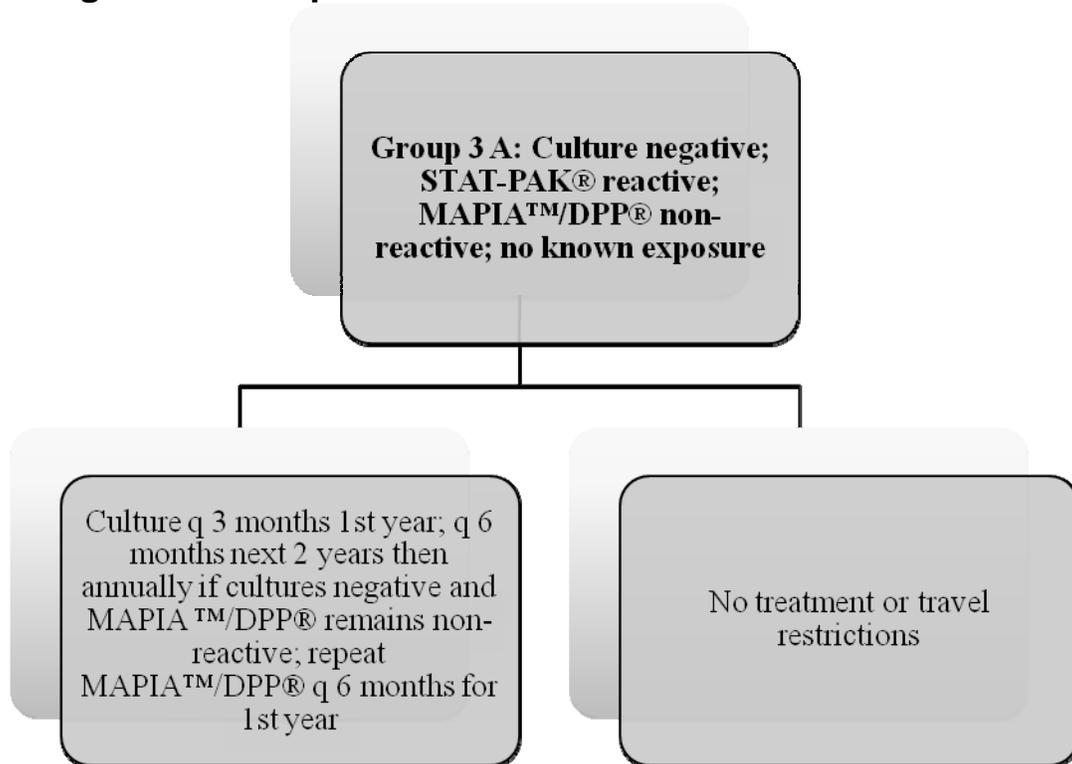
### TB Management Group 1



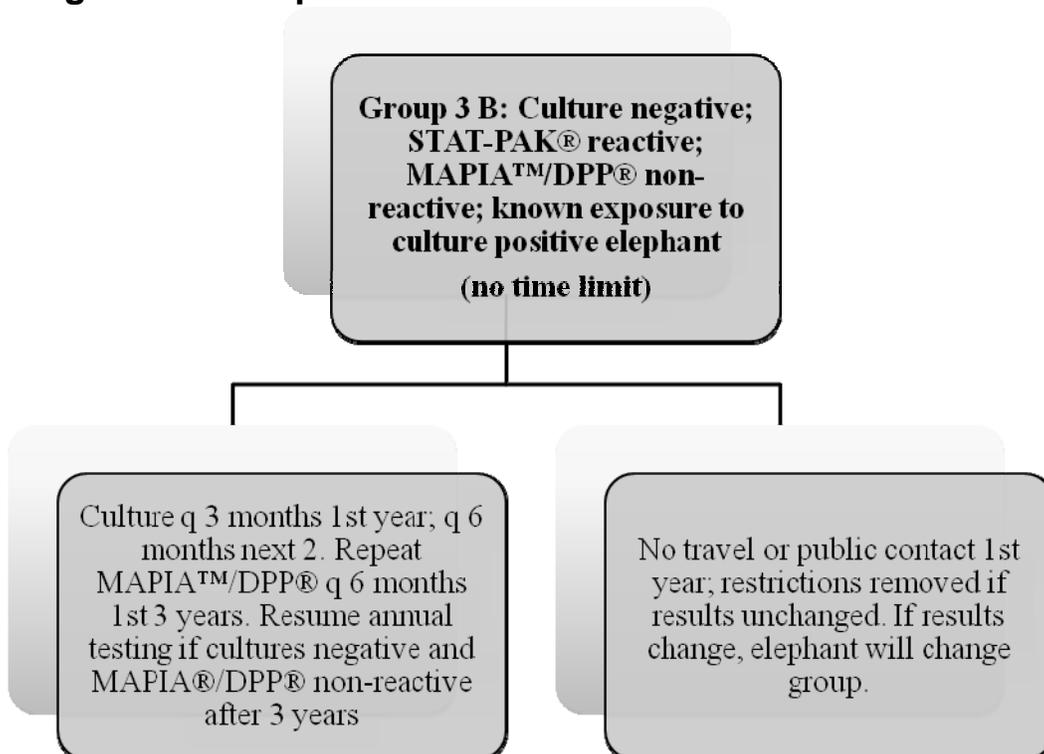
### TB Management Group 2



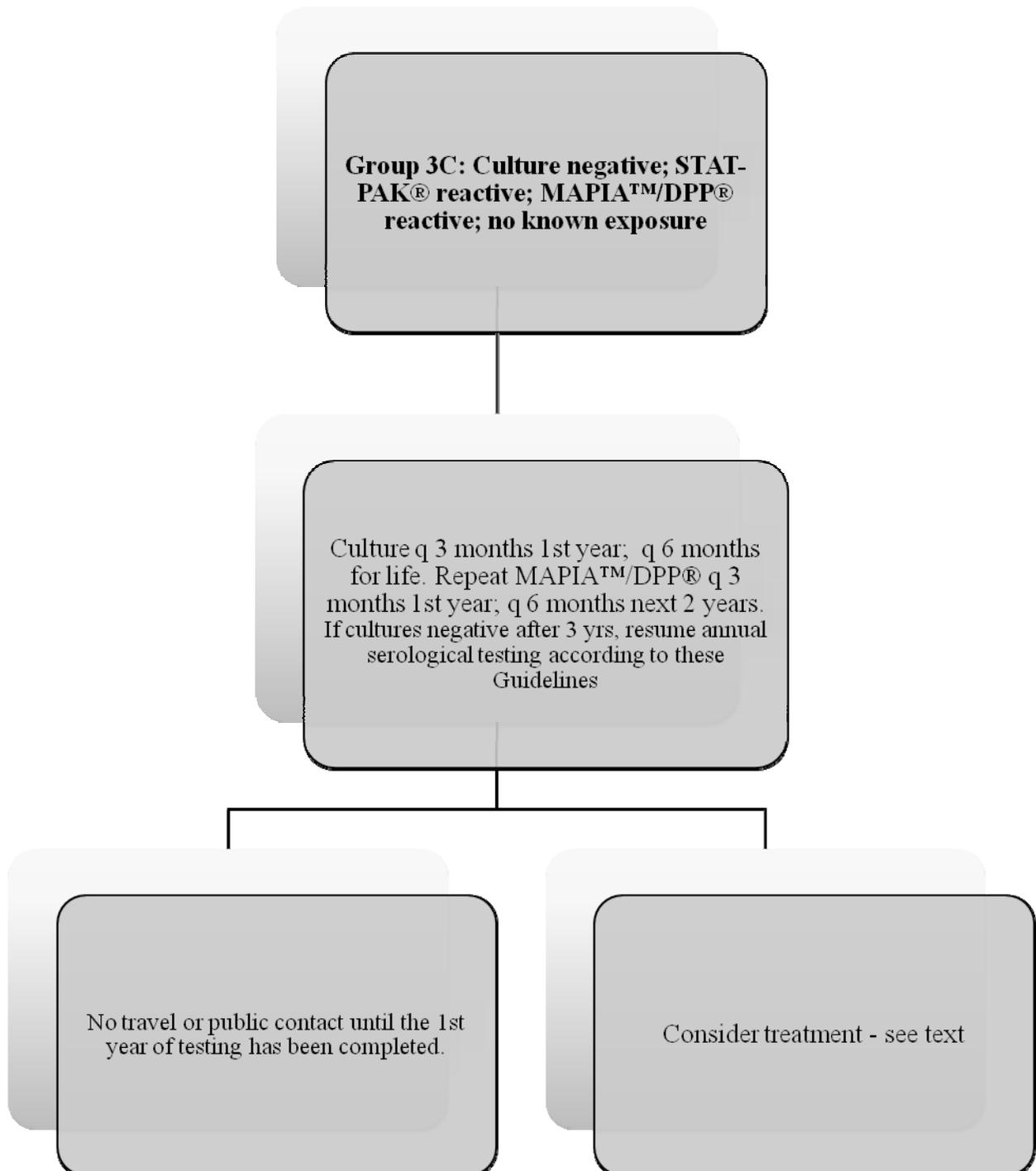
## TB Management Group 3A



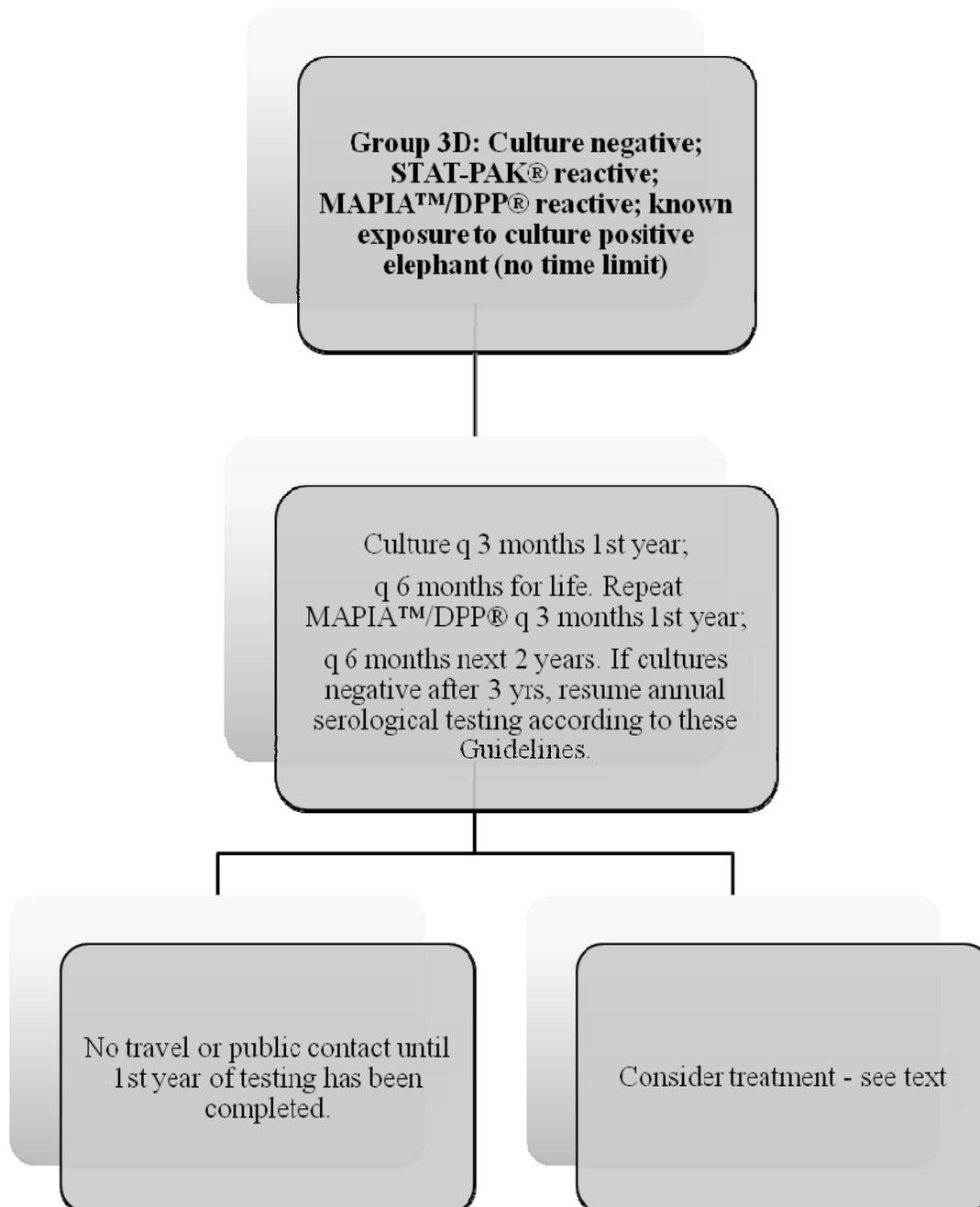
## TB Management Group 3B



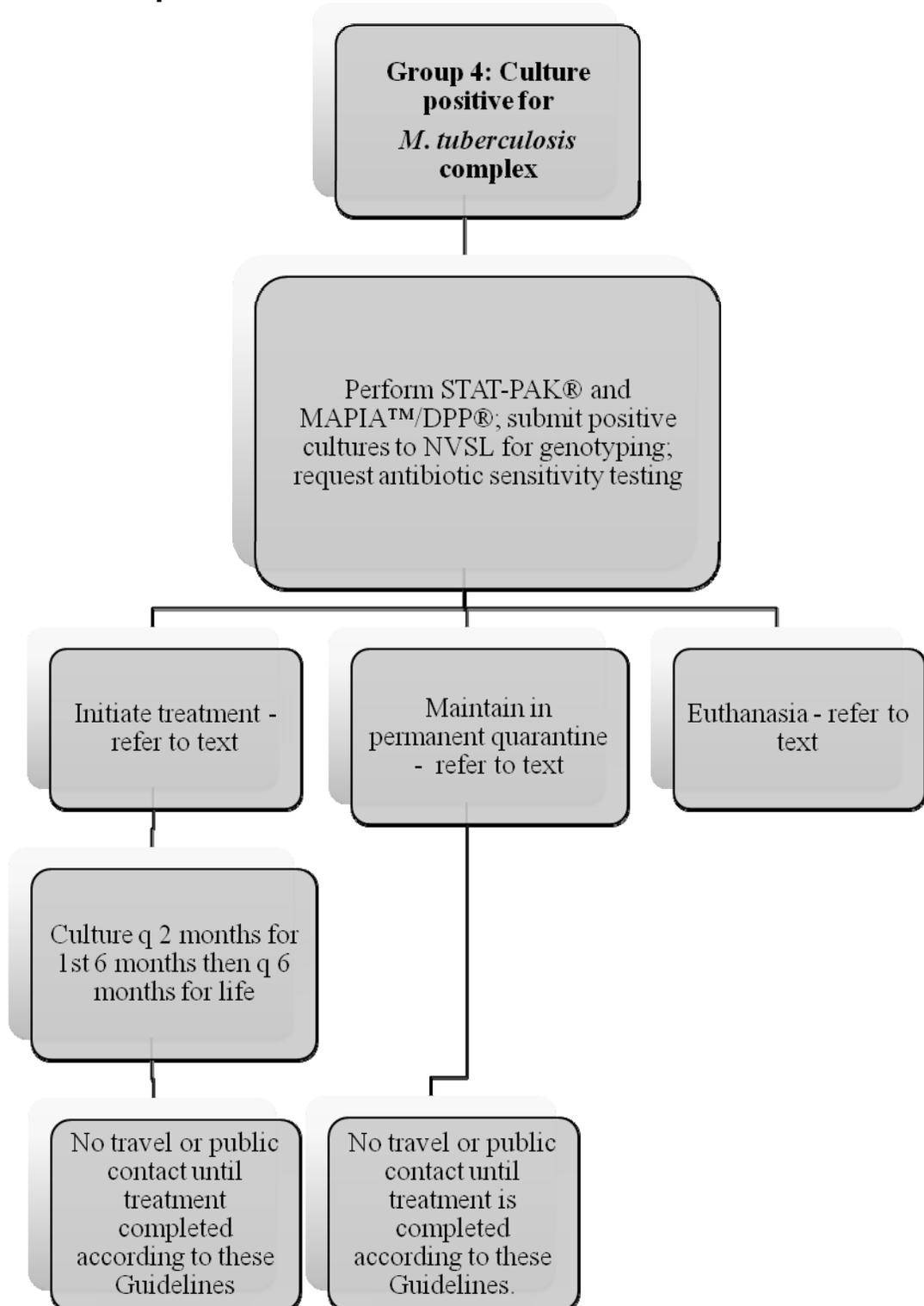
**TB Management Group  
3C**



**Management Group  
3D**



## Management Group 4



\*\*\*\*KIM\*\*\*\*The Bovine TB News September 2010 edition should be inserted here, is enclosed as a pdf document.