Serological Testing: Elephant TB
STAT-PAK, MAPIA, and DPP VetTB

Elephant TB Seminar
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Chembio Immunoassay Technologies for Elephant TB Testing

- Lateral-Flow Immunochromatography (Rapid Test)
- MultiAntigen Print ImmunoAssay (MAPIA)
- Dual Path Platform (DPP)
Chembio lateral-flow tests for TB

**PrimaTB STAT-PAK Assay**
for antibody detection in NHP infected with *M. tuberculosis* or *M. bovis* (USDA-approved 2007)

**ElephantTB STAT-PAK Assay**
for antibody detection in elephants and other exotic wildlife species (rhinoceros, tapir, llama, sea lion, etc.) infected with *M. tuberculosis*, *M. bovis*, *M. microti*, or *M. pinnipedii* (USDA-approved 2007)
MultiAntigen Print ImmunoAssay (MAPIA)


- Identify
- Characterize
- Evaluate
- Diagnose
Elephant TB detection: MAPIA and Rapid Test

*M. tuberculosis (+)* May 2000 treatment initiated

*M. tuberculosis (+)*
Dual Path Platform (DPP®) Assay
Chembio DPP Assay

Protein A/G-gold

Abs in sample

Abs in sample
DPP Assay Reader

Picture captured by reader, transferred to PC by USB
DPP VetTB assay interpretation (for elephants only)

A

B

C

D

TB

TB

MOTT

Neg
Seroconversion time to first positive culture from trunk wash samples (gray) or from tissues (black) post-mortem
### Serodiagnosis of TB in elephants

<table>
<thead>
<tr>
<th>Status</th>
<th>No. of elephants</th>
<th>ElephantTB STAT-PAK</th>
<th>MAPIA</th>
<th>DPP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy/other disease</td>
<td>144</td>
<td>4</td>
<td>0</td>
<td>0</td>
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<tr>
<td><strong>Tuberculosis</strong></td>
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<tr>
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<td>27</td>
<td>27</td>
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</tr>
<tr>
<td><strong>MOTT/M. szulgai</strong></td>
<td>3</td>
<td>3</td>
<td>3*</td>
<td>3*</td>
</tr>
</tbody>
</table>

* MPB83 Ab only, no ESAT-6 and/or CFP10 Ab

**ElephantTB STAT-PAK:**
- Se. 100% (95% CI, 84-100%)
- Spe. 95% (95% CI, 90-98%)

**MAPIA and DPP VetTB:**
- Se. 100% (95% CI, 84-100%)
- Spe. 100% (95% CI, 97-100%)
Prospective Studies

• In the published set of data (Lyashchenko et al. 2006, Greenwald et al. 2009), 3 live TWC- elephants tested Ab+ to predict disease prior to post-mortem TB diagnosis.

• In the US, among 398/451 (88%) elephants tested during 02/2010-03/2011, 95 were ElephantTB STAT-PAK reactive (~24%) and 37 of those were MAPIA positive (9%):
  • 2 became TWC+
  • 4 died and showed evidence of TB at necropsy

• In Nepal, 3/6 MAPIA positive elephants tested in 2006 died of TB (confirmed at necropsy) during 2007-2009.

• In Australia, 1/7 Asian elephant in a zoo tested positive by ElephantTB STAT-PAK and DPP VetTB consistently since 2004 and that elephant converted into trunk wash culture positive in November 2010.
Examples of MAPIA antibody positive results in culture positive elephants

PC   PT   S

B     I     A

R (2003-2010)

L
Recent Seroconversion in Elephant A

MAPIA

DPP VetTB

ElephantTB STAT-PAK:   - ++ +++ +++

Trunk wash positive for \textit{M. tb}: Feb 2011 (in the News)
Examples of MAPIA antibody positive results in trunk wash culture negative elephants
Examples of difficult interpretation of ElephantTB STAT-PAK/MAPIA results

- **Elephants M, B, J:** TWC+ and treated or died of TB confirmed PM; Ab- (effect of treatment)

- **Elephant L:** TWC+ (12/2008) before seroconversion (08/2009) (effect of treatment)

- **Elephant M:** Ab+, died during treatment, no evidence of TB at necropsy (cause of death? effect of treatment?)

- **Elephant A:** Ab seroconversion with unusual MAPIA pattern (recent GnRH vaccination)

- **Elephant B:** Ab+, MPB83 recognized, not ESAT-6/CFP10 (\(M. \text{tb}/M. \text{szulgai}\) coinfection?)
Elephant TB Study in Nepal  
(by Elephant Care International)

- In 2006, 6/115 elephants tested reactive by ElephantTB STAT-PAK/MAPIA
- 3/6 Ab+ suspects died 2007-2009 with all showing evidence of TB at necropsy
- One antibody negative in 2006 died recently without any evidence of disease at PM examination
- Another seropositive suspect became TWC+ but then escaped to India
- Remaining MAPIA positives and newly identified DPP reactors under treatment
- Postmortem TB-diagnosed elephant (2009) without serum sample available was Ab+ by retrospective testing lung fluid obtained from frozen tissue (in agreement with a similar finding made in the US previously)
Nepal Study

- #68: died 2007, TB-like VL
- #70: died 2009, TB-like VL, M.tb+
- #47 (2006 RT+/MAPIA-): died 2007, TB-like VL
- #10: on anti-TB treatment

#139: not tested by serology in 2006, died of TB in 2009, no serum sample available, lung fluid from frozen tissue used for antibody testing
2010 Elephant TB Case in an Australian Zoo

• ~14-yr old female Asian elephant moved from Thailand to Australia in Aug 2006


• Apr 2009: ElephantTB STAT-PAK reactive

• Feb 2010: ElephantTB STAT-PAK and DPP VetTB reactive

• Nov 2010: gave birth to a female calf

• Nov 2010: trunk wash culture positive for \( M. \text{tb} \) (pan-sensitive strain of Beijing) from Nov 24 sample; samples of Nov 23 and 26 culture neg; all three samples direct PCR and AFB neg

• No clinical symptoms suggestive of TB

• Treatment initiated in Dec 2010

• Retrospective serology: reactive by ElephantTB STAT-PAK and DPP VetTB in Aug 2006 (move to Australia); reactive by ElephantTB STAT-PAK in 2004 (Thailand), DPP VetTB pending

• Quarterly testing of all 8 elephants by trunk wash culture and serology is in progress
Elephant diagnosed recently with TB tested negative when she came to Australia from Thailand four years ago.

The zoo keepers believe that the recent pregnancy almost certainly brought on the illness.

The baby has tested negative for TB, as well as the seven other elephants at the zoo.

The elephant with TB is still on display and has not been quarantined, with the zoo experts saying she does not pose any risk to the public.
Senior Veterinarian,
Dec 16, 2010:

“Unfortunately, one of our elephants has returned a positive trunk wash result (culture PCR positive for Mycobacterium tuberculosis complex)… She is also STAT-PAK and DPP VetTB test positive. We have been doing trunk washes every 3 months for 2 years now. My worst nightmare!! “

Lost opportunity for earlier TB diagnosis?