

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

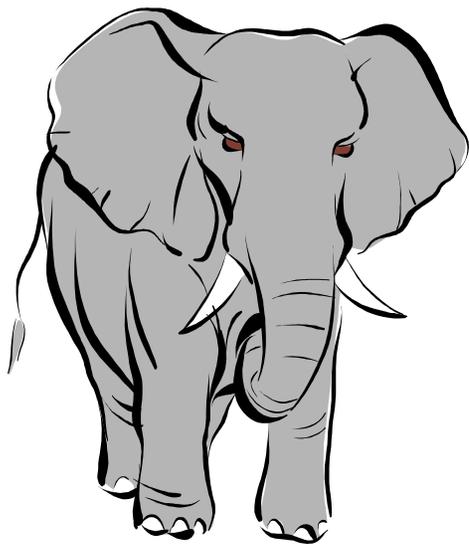


Elephant TB Seminar
Kansas City, MO
April 5-6, 2011

Konstantin Lyashchenko

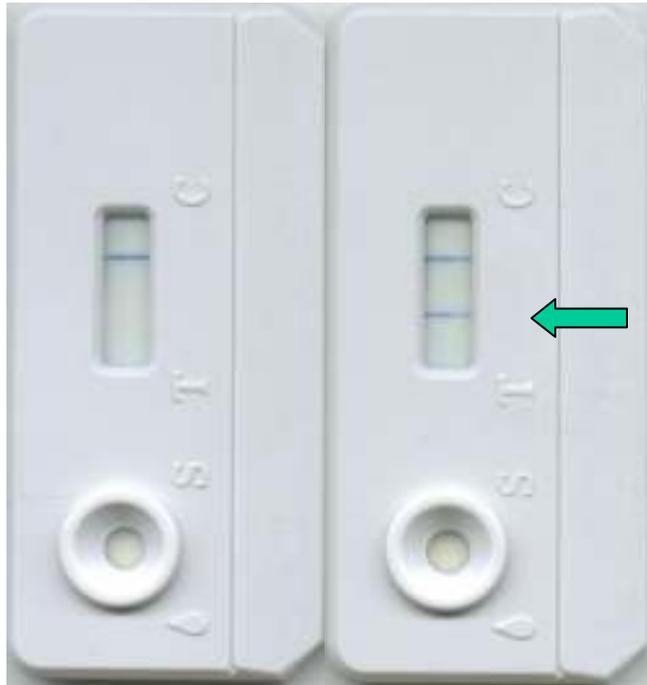


Chembio Immunoassay Technologies for Elephant TB Testing



- Lateral-Flow
ImmunoChromatography
(Rapid Test)
- MultiAntigen Print
ImmunoAssay (MAPIA)
- Dual Path Platform (DPP)

Chembio lateral-flow tests for TB



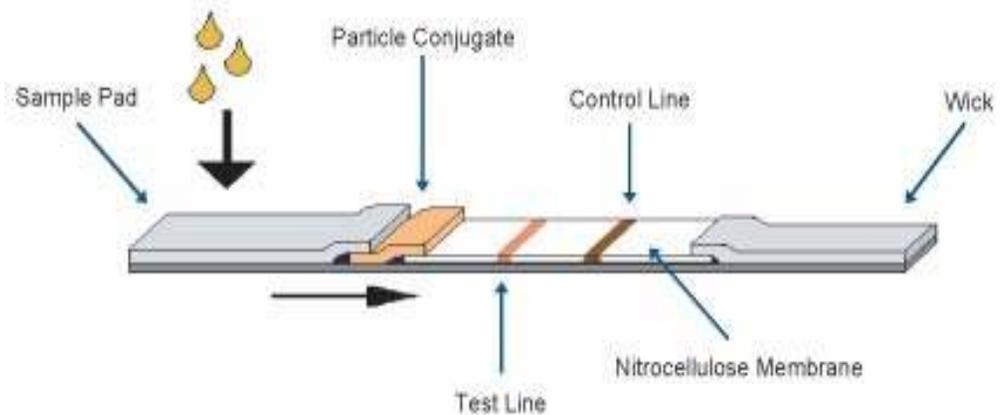
Neg

Pos

PrimaTB STAT-PAK Assay

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

Typical Lateral-Flow Assay Format

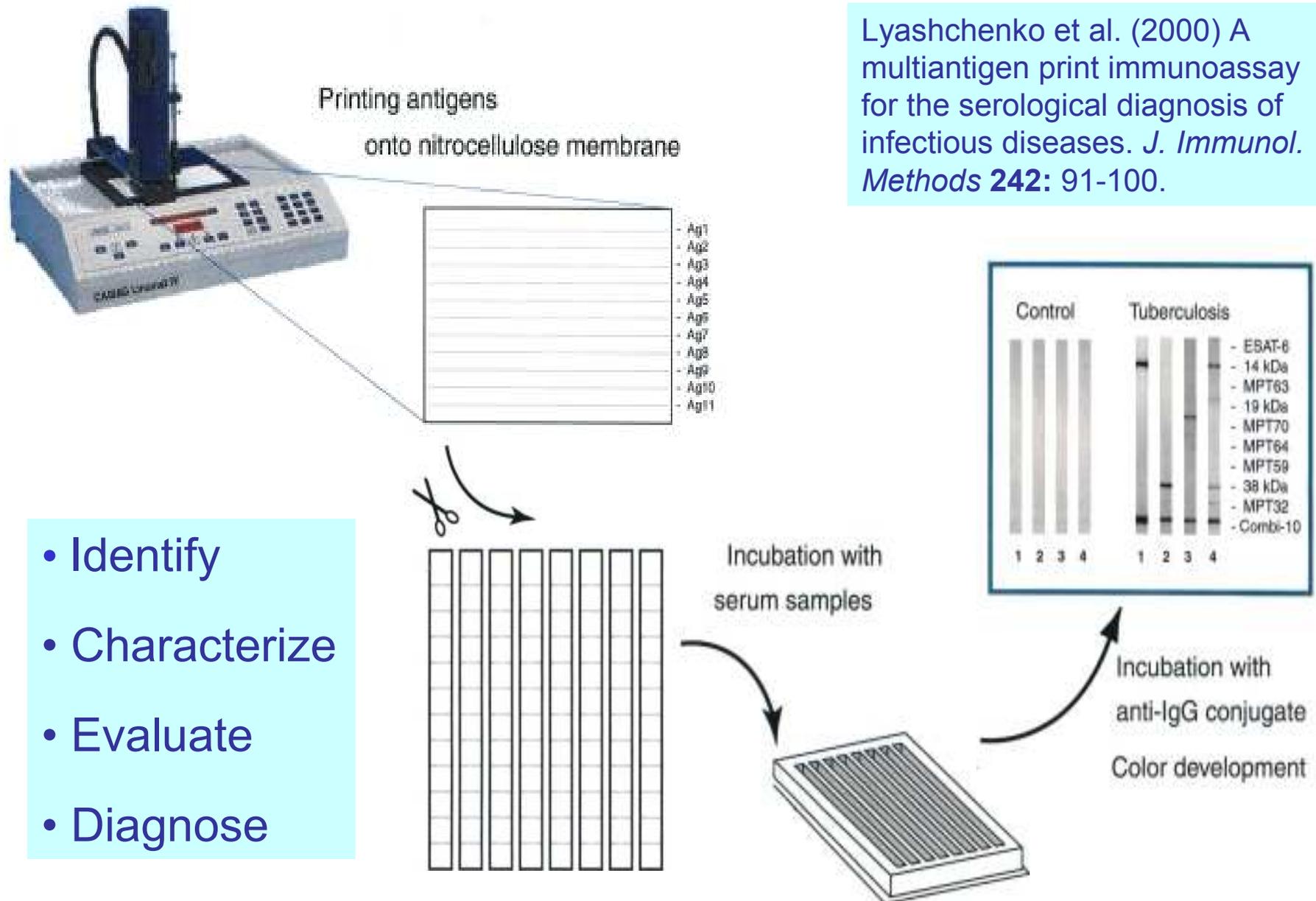


ElephantTB STAT-PAK Assay

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)

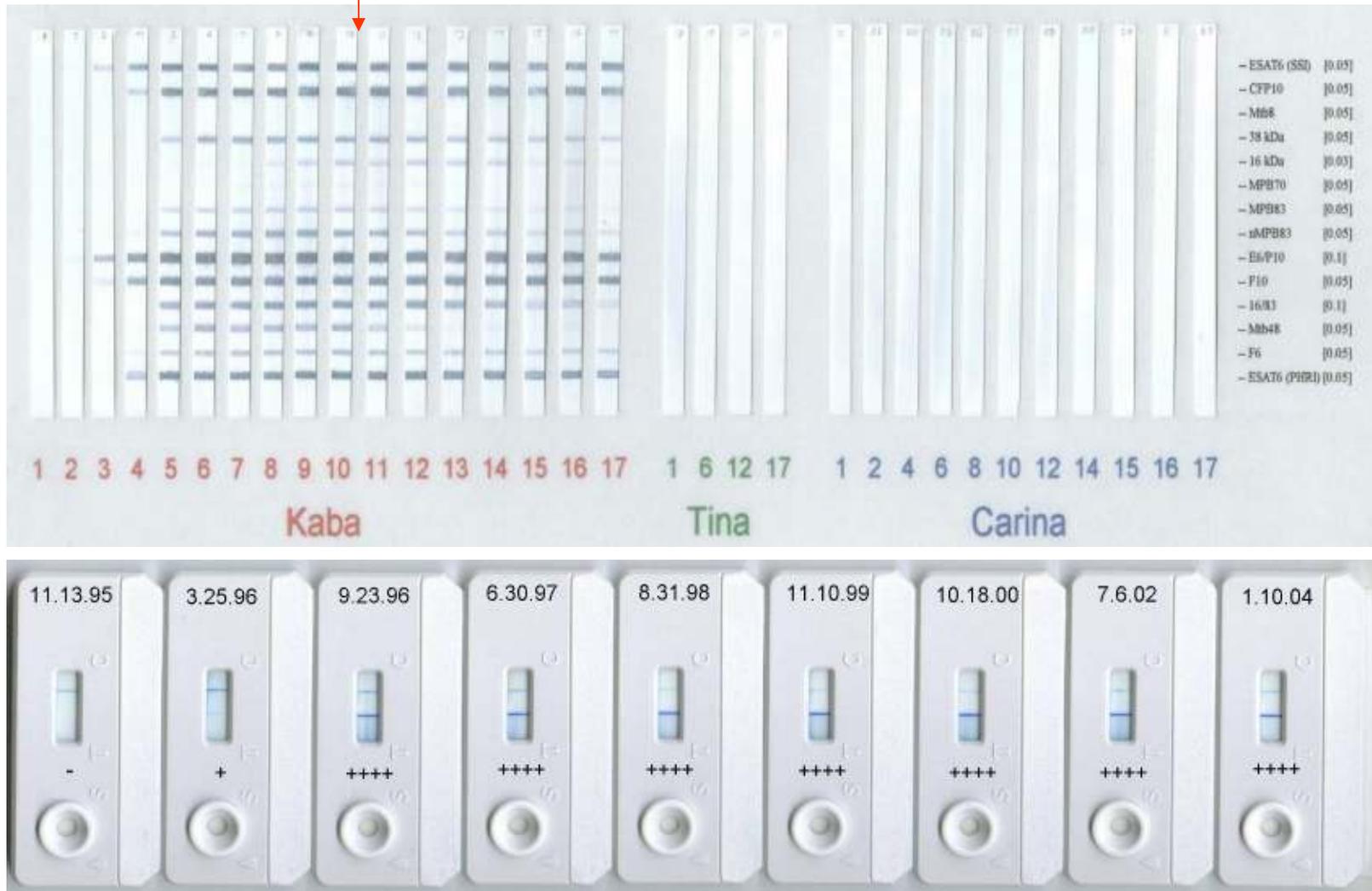
Lyashchenko et al. (2000) A multiantigen print immunoassay for the serological diagnosis of infectious diseases. *J. Immunol. Methods* **242**: 91-100.



- Identify
- Characterize
- Evaluate
- Diagnose

Elephant TB detection: MAPIA and Rapid Test

M. tuberculosis (+) May 2000
treatment initiated

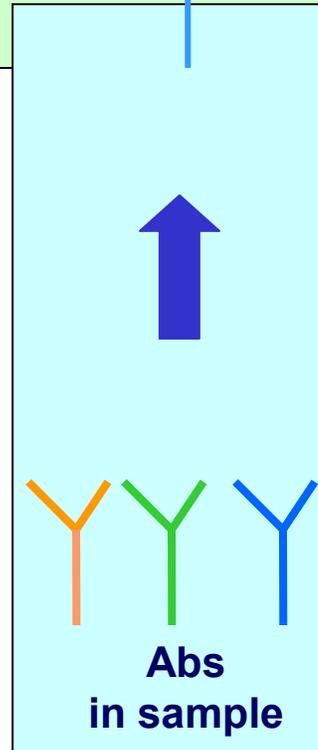
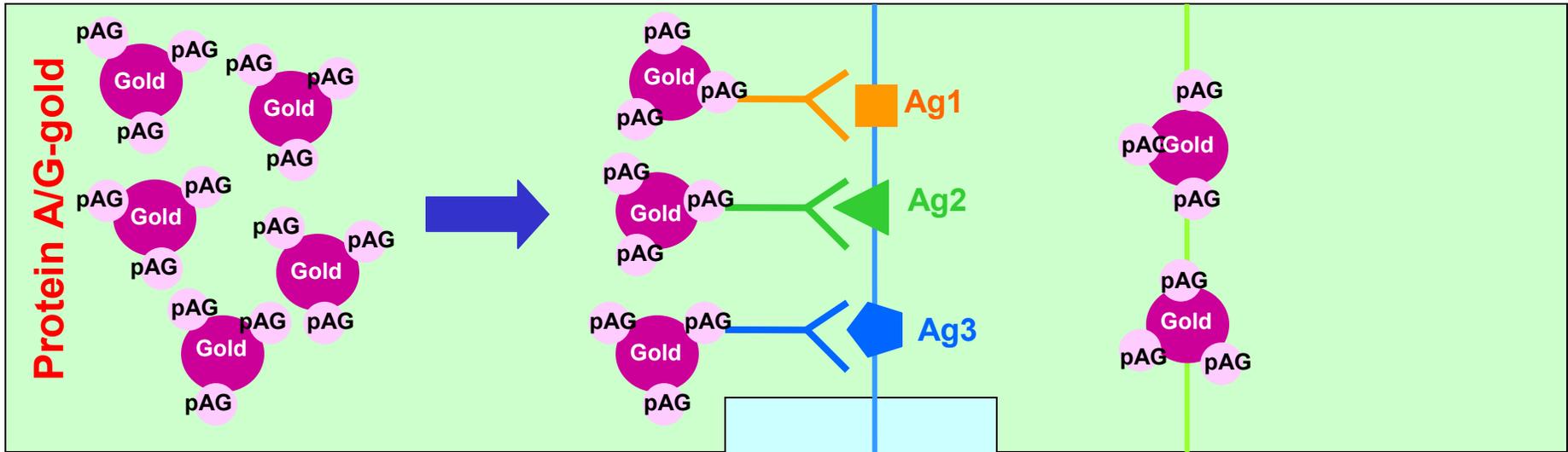


M. tuberculosis (+)

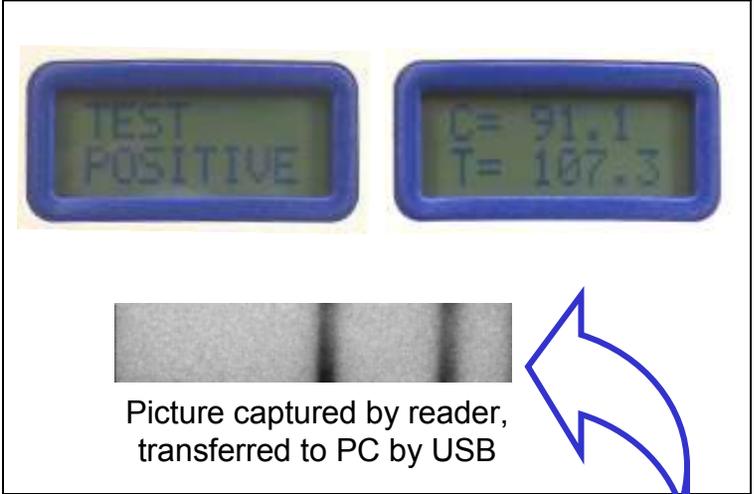
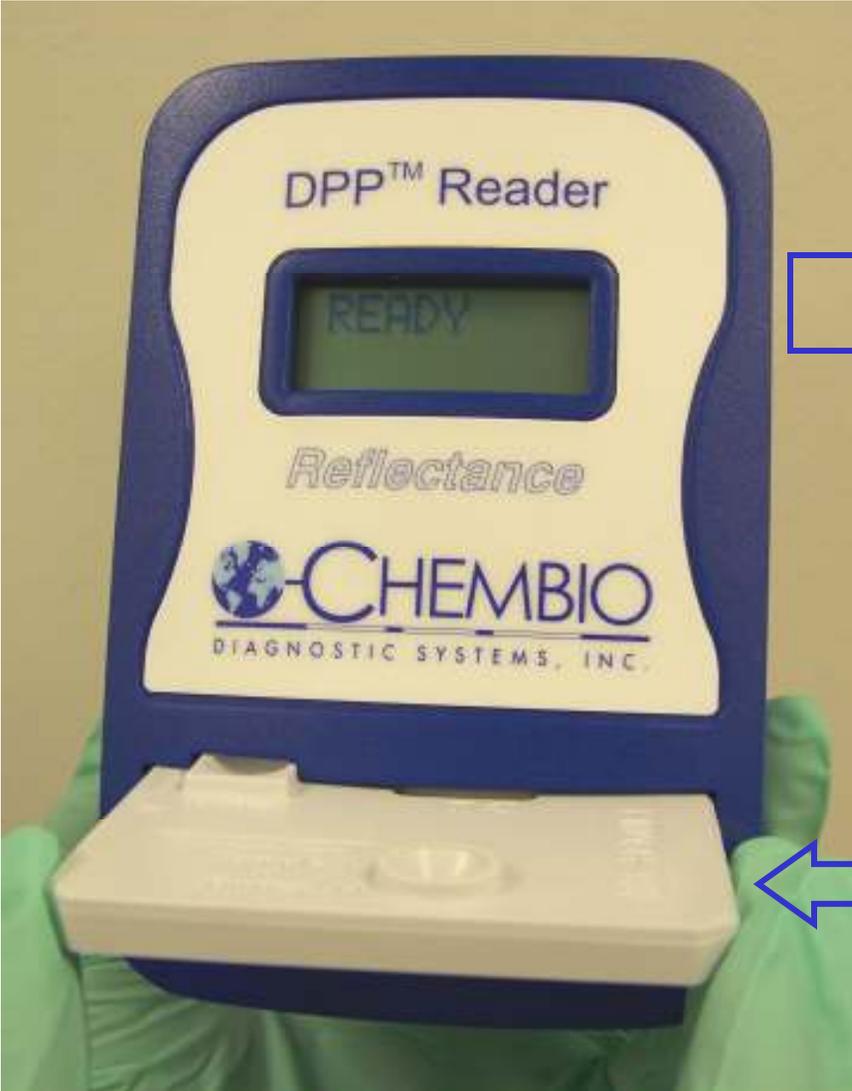
Dual Path Platform (DPP[®]) Assay



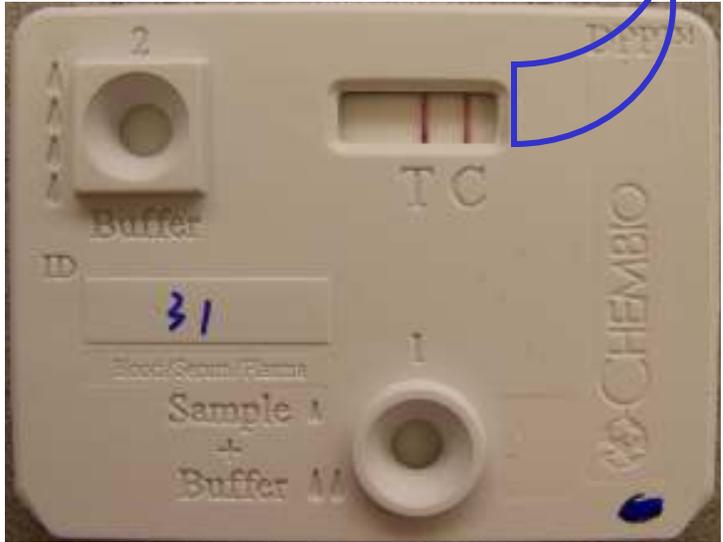
Chembio DPP Assay



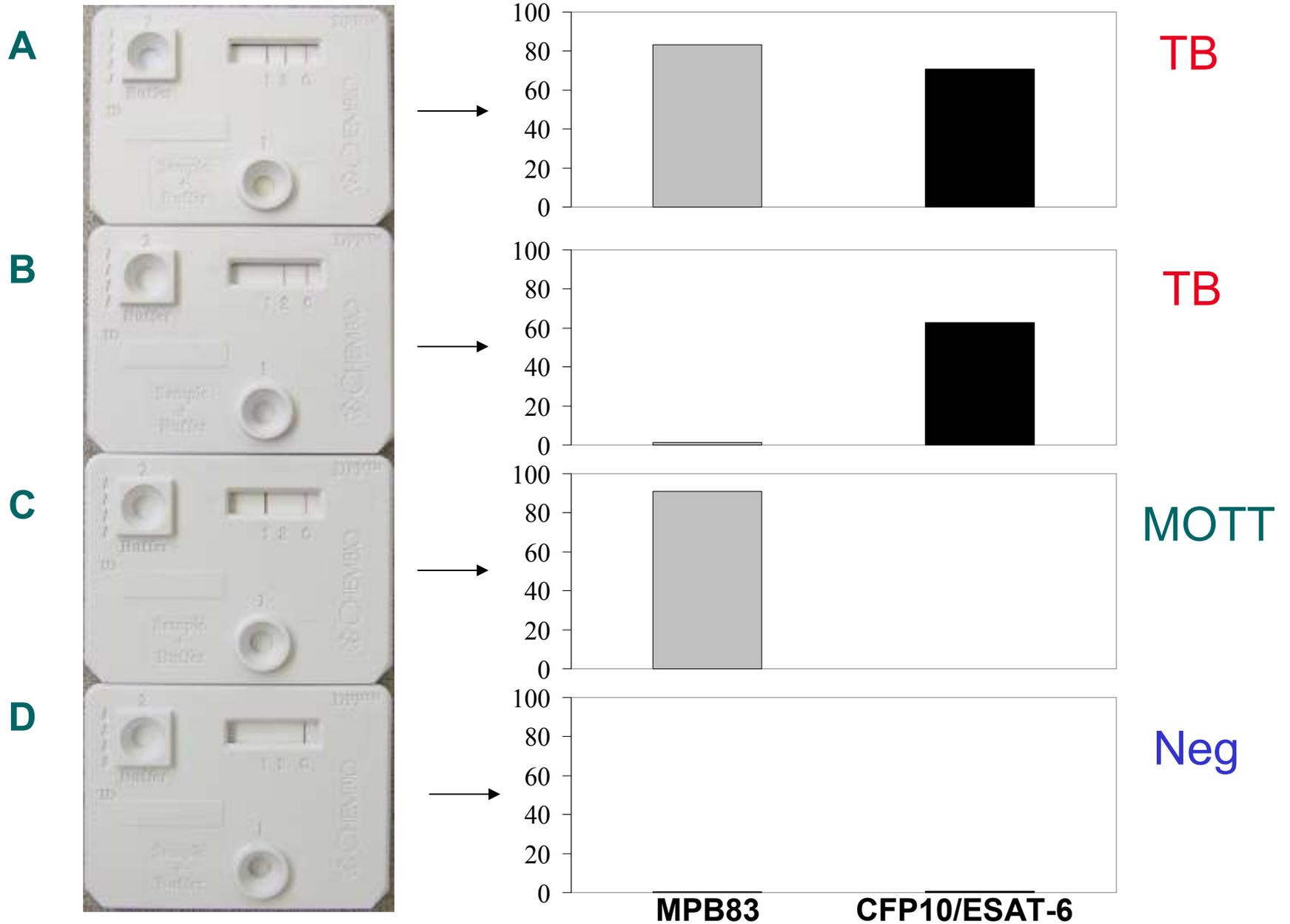
DPP Assay Reader



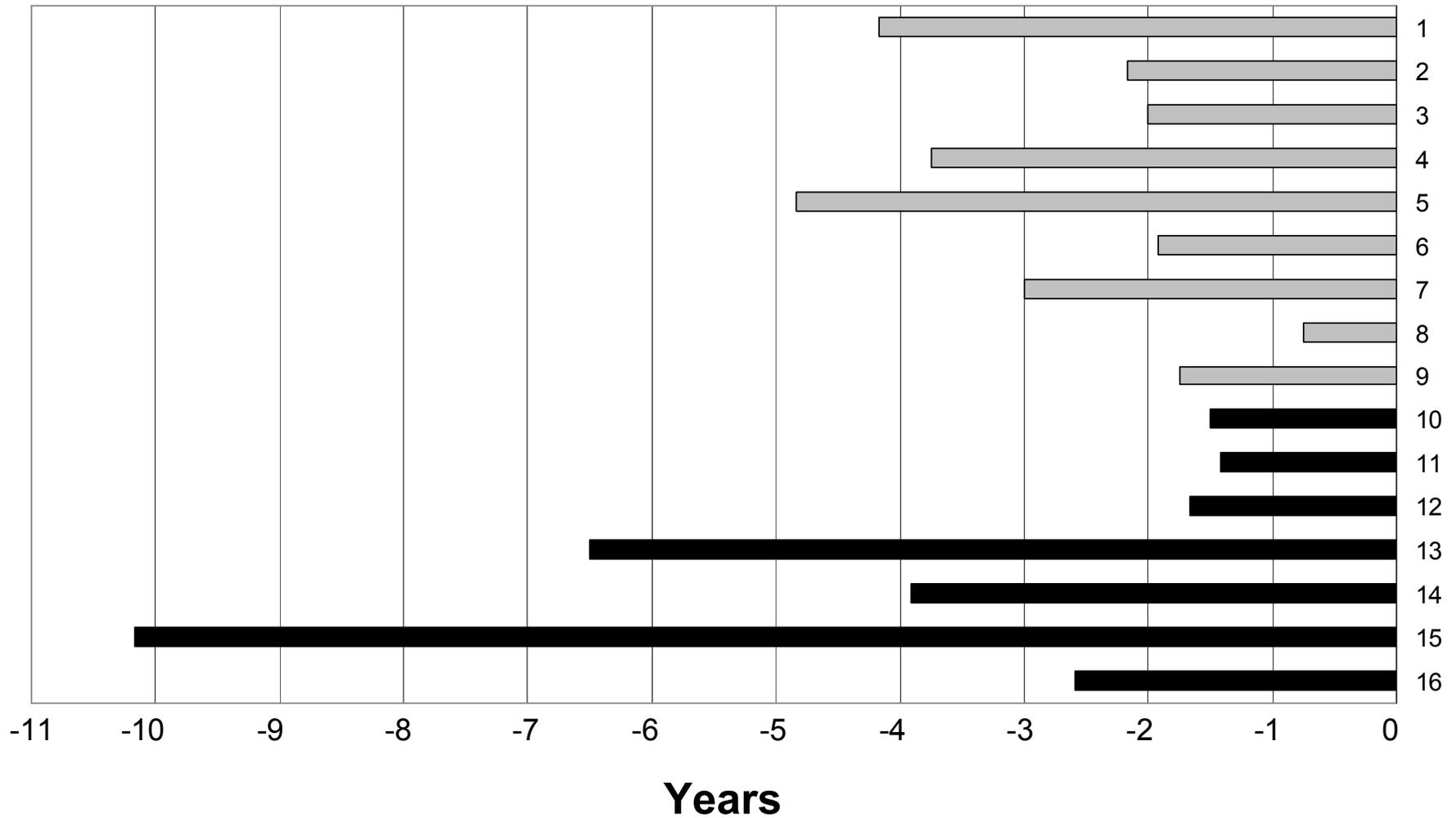
Picture captured by reader, transferred to PC by USB



DPP VetTB assay interpretation (for elephants only)



Seroconversion time to first positive culture from trunk wash samples (gray) or from tissues (black) post-mortem



Serodiagnosis of TB in elephants

Status	No. of elephants	ElephantTB STAT-PAK	MAPIA	DPP
Healthy/other disease	144	4	0	0
Tuberculosis	27	27	27	27
MOTT/ <i>M. szulgai</i>	3	3	3*	3*

* 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:

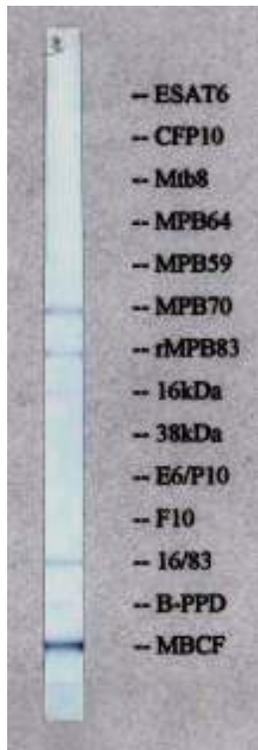
- Se. 100% (95% CI, 84-100%)
- Spe. 100% (95% CI, 97-100%)

Prospective Studies

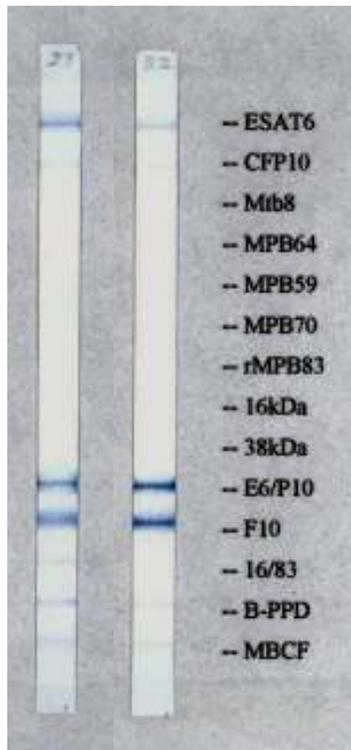
- In the published set of data (*Lyashchenko et al. 2006*, *Greenwald et al. 2009*), 3 live TWC- elephants tested Ab+ to predict disease prior to post-mortem TB diagnosis
- In the US, among 398/451 (88%) elephants tested during 02/2010-03/2011, 95 were ElephantTB STAT-PAK reactive (~24%) and 37 of those 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

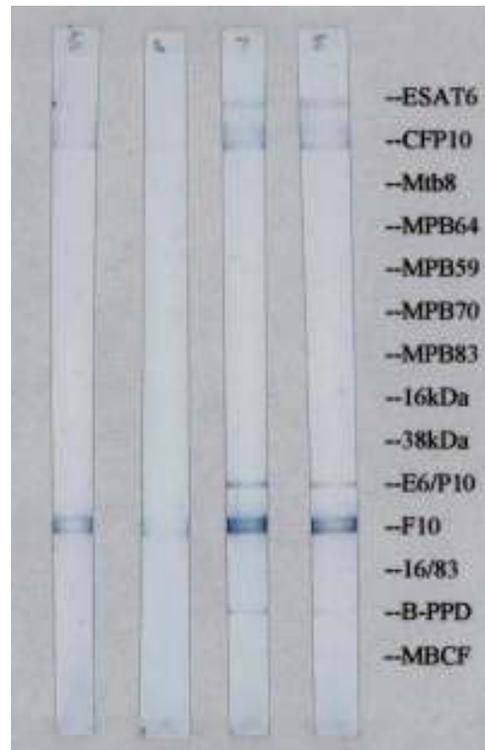
PC PT S



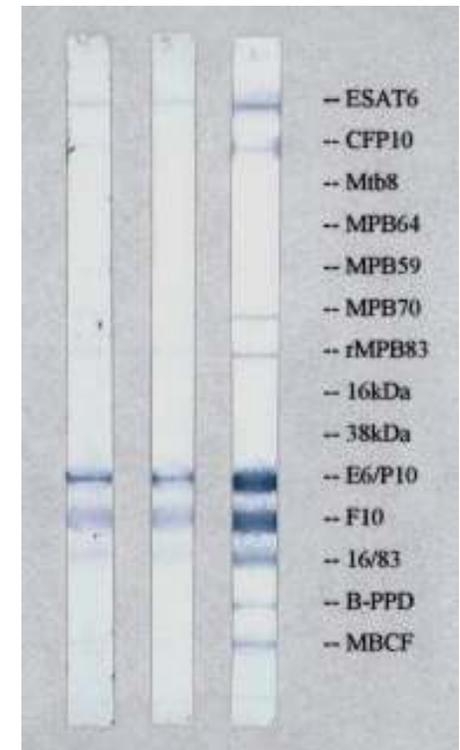
B



I A



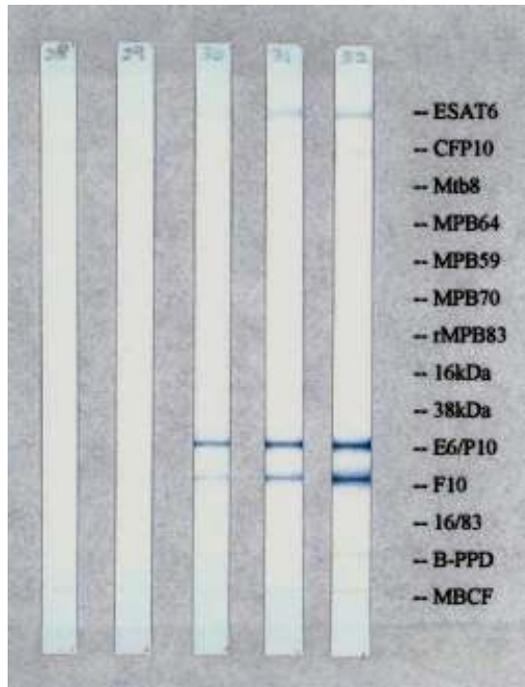
R (2003-2010)



L

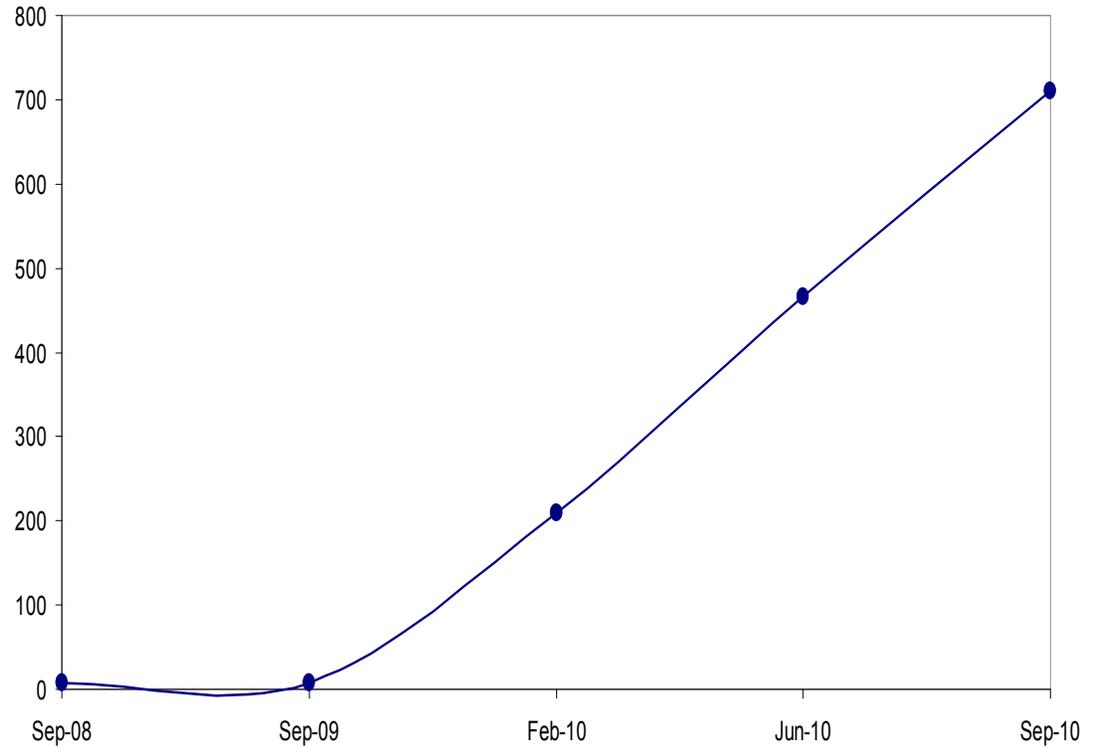
Recent Seroconversion in Elephant A

MAPIA



Sep 2008
 Sep 2009
 Feb 2010
 Jun 2010
 Sep 2010

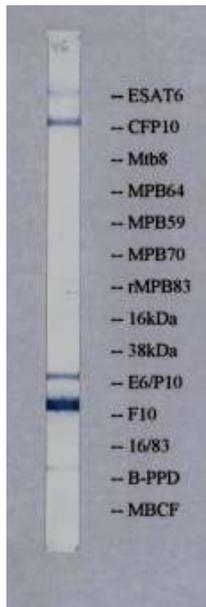
DPP VetTB



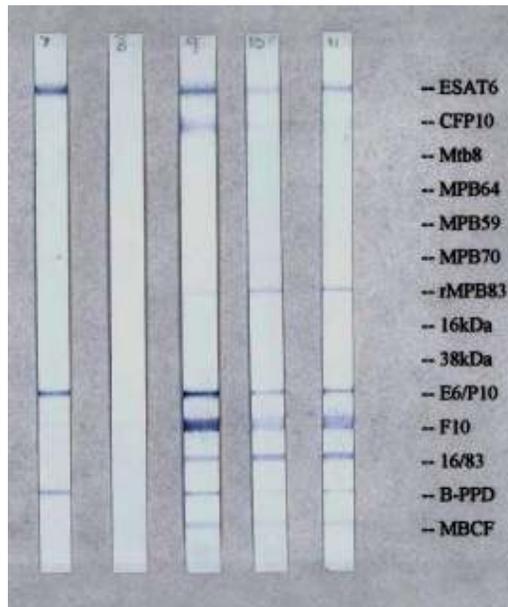
ElephantTB STAT-PAK: - ++ +++ +++

Trunk wash positive for *M. tb*: Feb 2011 (in the News)

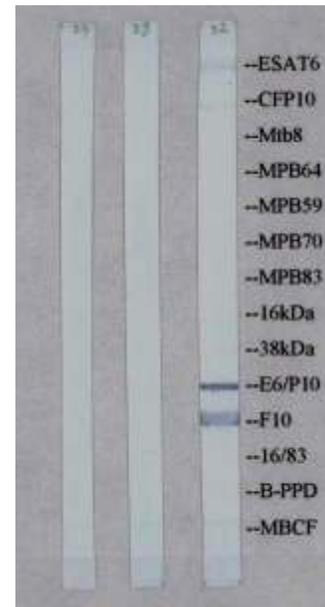
Examples of MAPIA antibody positive results in trunk wash culture negative elephants



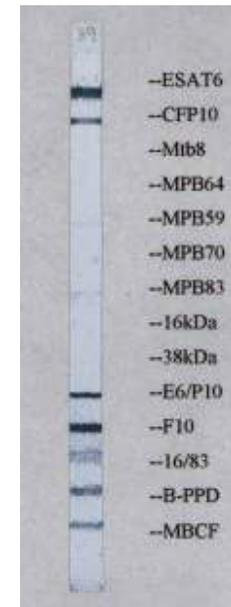
P



R P L S1 S2



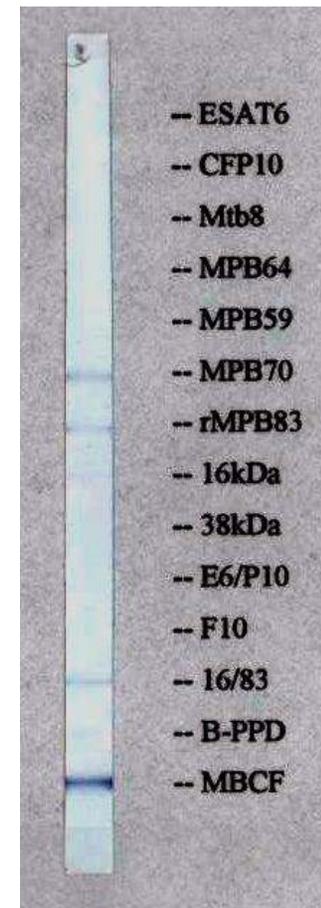
P E D



S

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. tb*/*M. szulgai* coinfection?)

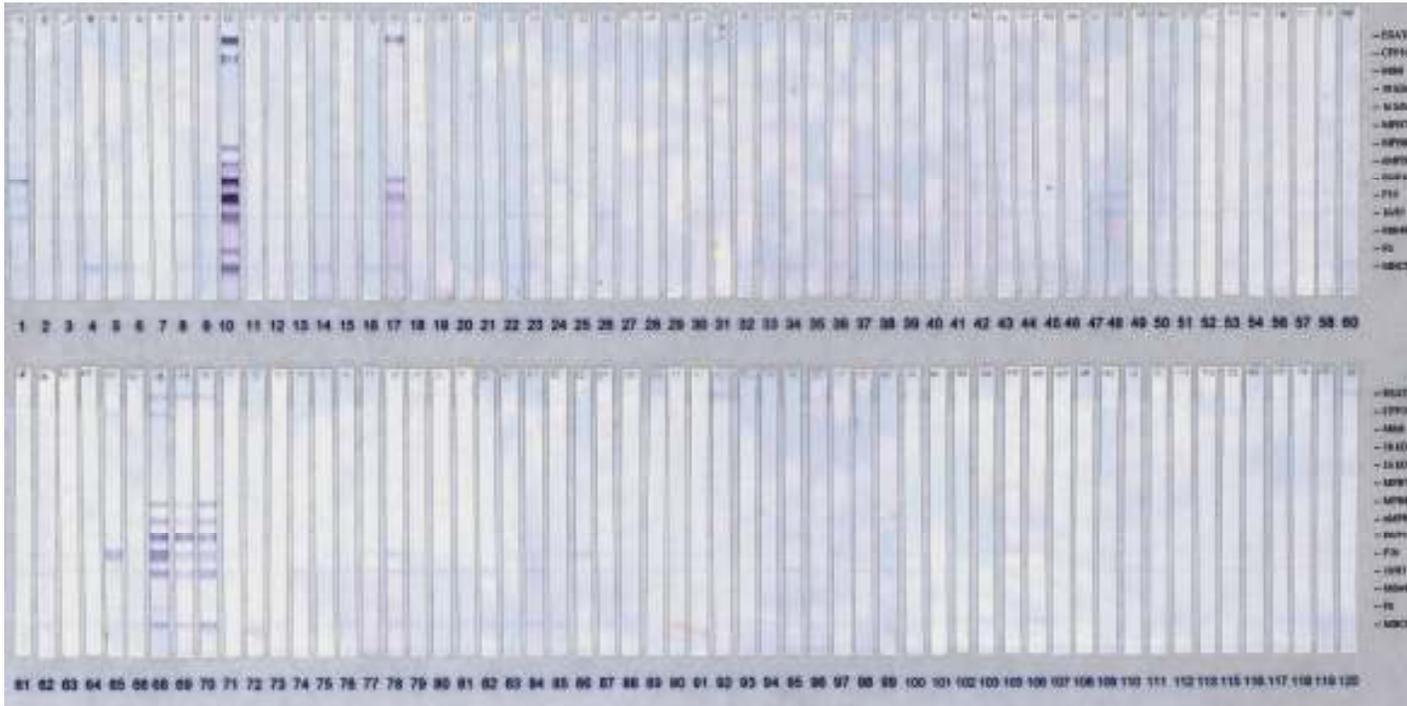


Elephant TB Study in Nepal

(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

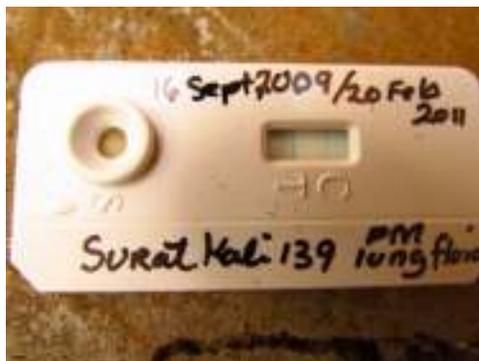


- #68: died 2007, TB-like VL

- #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
- Trunk wash negative: Oct 2004 (Thailand), Dec 2006, Dec 2007, Dec 2008, May-Aug-Nov 2009, Feb-May-Aug 2010
- 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. 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

ABC News, Feb 5, 2011: Taronga Zoo Elephant Diagnosed with TB

Top News United States, Feb 5, 2011: Elephant Suffering from TB

- 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



19-year old mother and her 3-month old calf

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?



2004



2011

