
MARTI-test demonstrates that anti-mycolic acid antibodies are surrogate markers for active TB

Jan Verschoor

Department of Biochemistry

Tuberculosis

- A cough or sneeze from a person with active TB can leave droplets that contain mycobacteria in the air for 5 hours.
- Inhalation of those droplets may cause TB, especially in the HIV compromised challenged person.



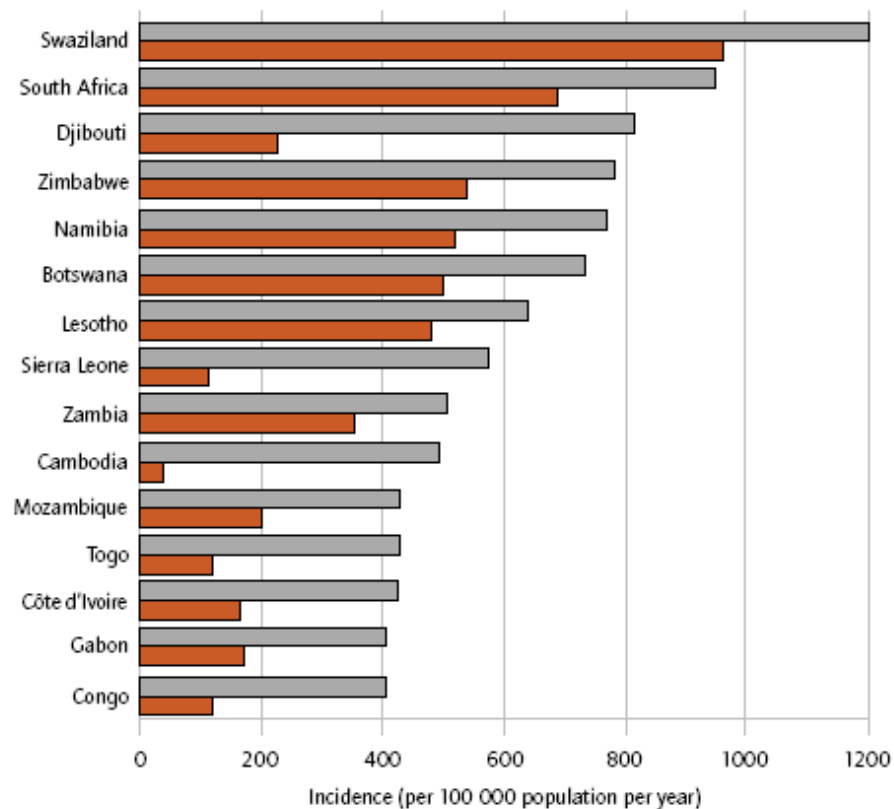
Prognosis of TB

- Curable disease, requiring nine months of combination therapy
- Compromised by:
 - Co-infection with HIV
 - Reduces life expectancy to weeks if left untreated
 - Complicates diagnosis:
 - Standard methods underestimate by 30%
 - *M avium* disease
 - Drug resistance
 - Consumes most of the budget to control TB in SA

WHO report: Global TB control 2009

FIGURE 1.4

Fifteen countries with the highest estimated TB incidence rates per capita (all forms; grey bars) and corresponding incidence rates of HIV-positive TB cases (red bars), 2007



TB diagnosis: State of the art

- Exposure: Skin test – once only

replaced with

Interferon- γ release assays (IGRA) – frequent repeats possible to monitor clinical staff at risk.



- Active disease: Sputum culture (6 wks)

replaced with

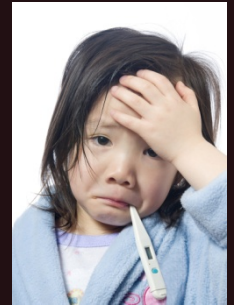
Nucleic Acid Amplification Tests (NAAT) – sputum based (3 days)



TB diagnosis: TB **disease** challenges

■ Paediatric TB

- Hyper-susceptibility in the 1 – 5 year olds: no sputum samples, often extrapulmonary
- High natural resistance to TB in 5-12 year olds



■ HIV patients

- Skin test and chest x-ray fail due to immune anergy
- Extrapulmonary TB manifestation
- Disease due to non-TB mycobacteria

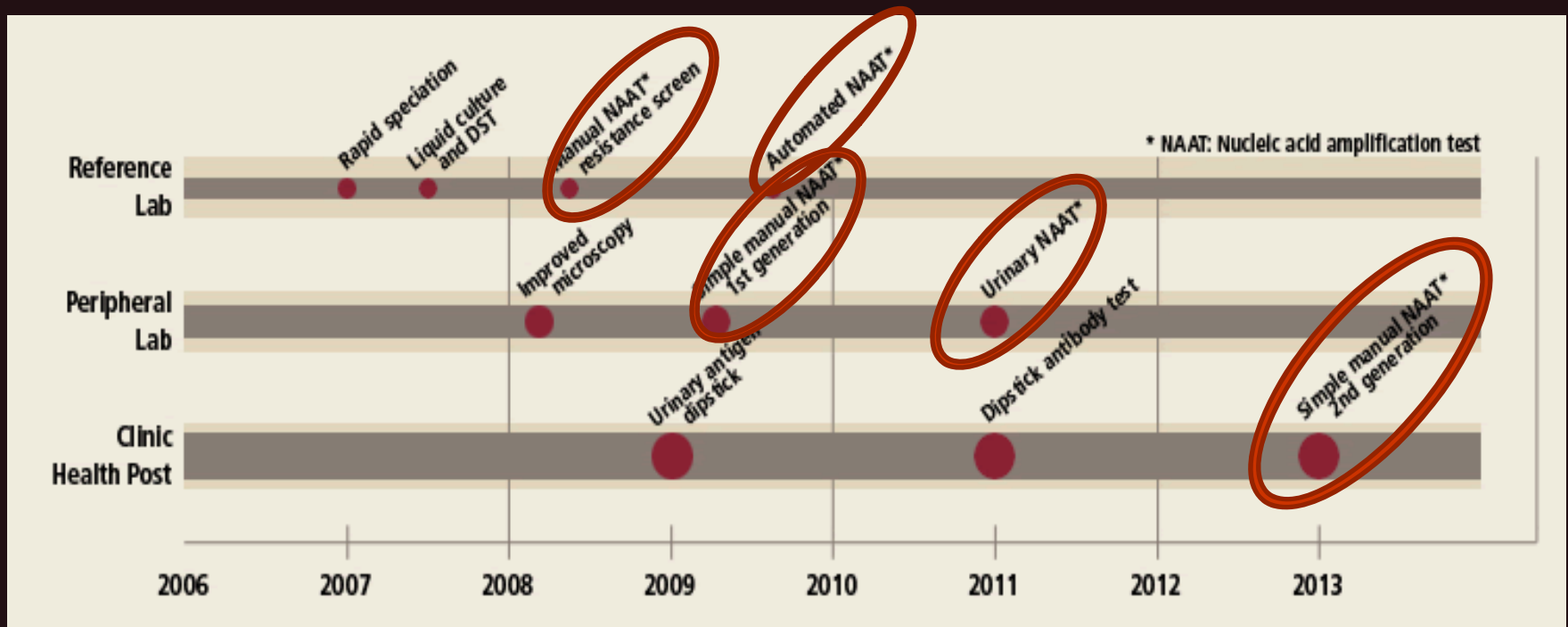


■ Nosocomial TB in TB/HIV burdened environment



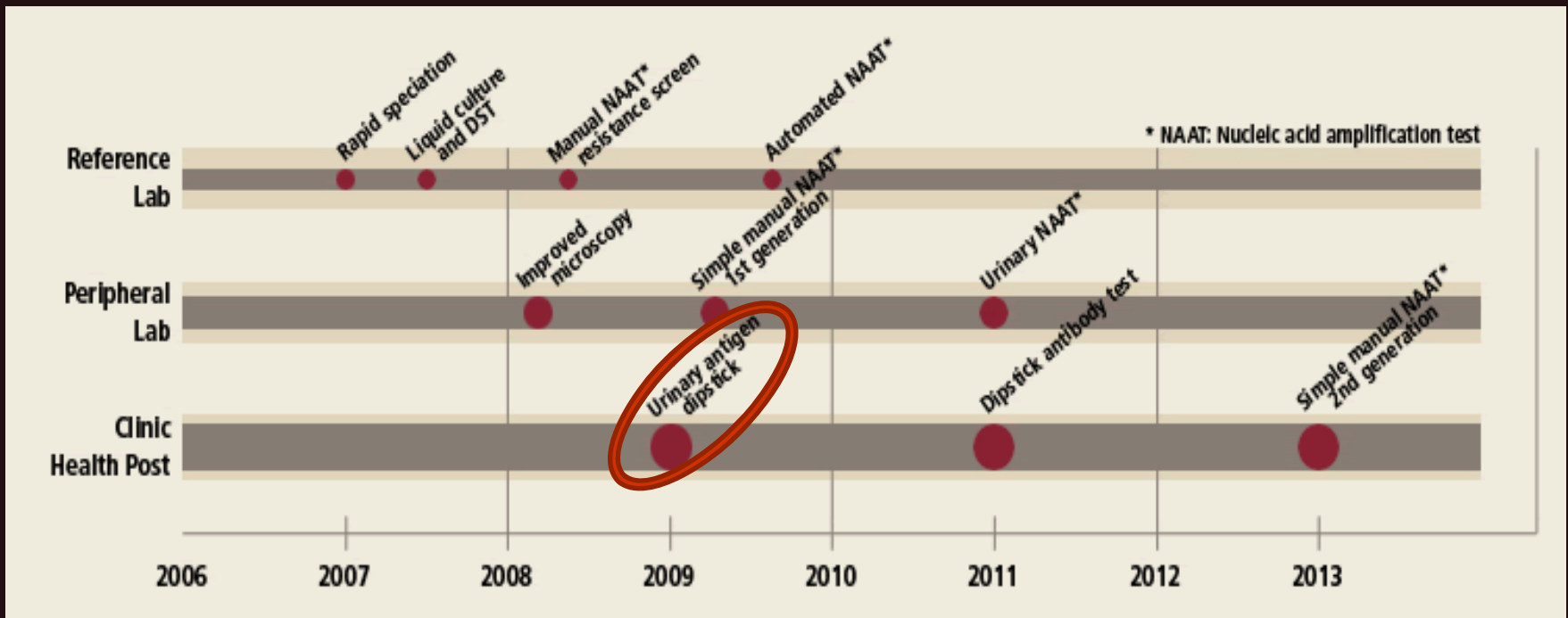
New diagnostics: targets (1)...

- Mycobacterial components: Proteins, lipids and nucleic acids – FIND focus on nucleic acids



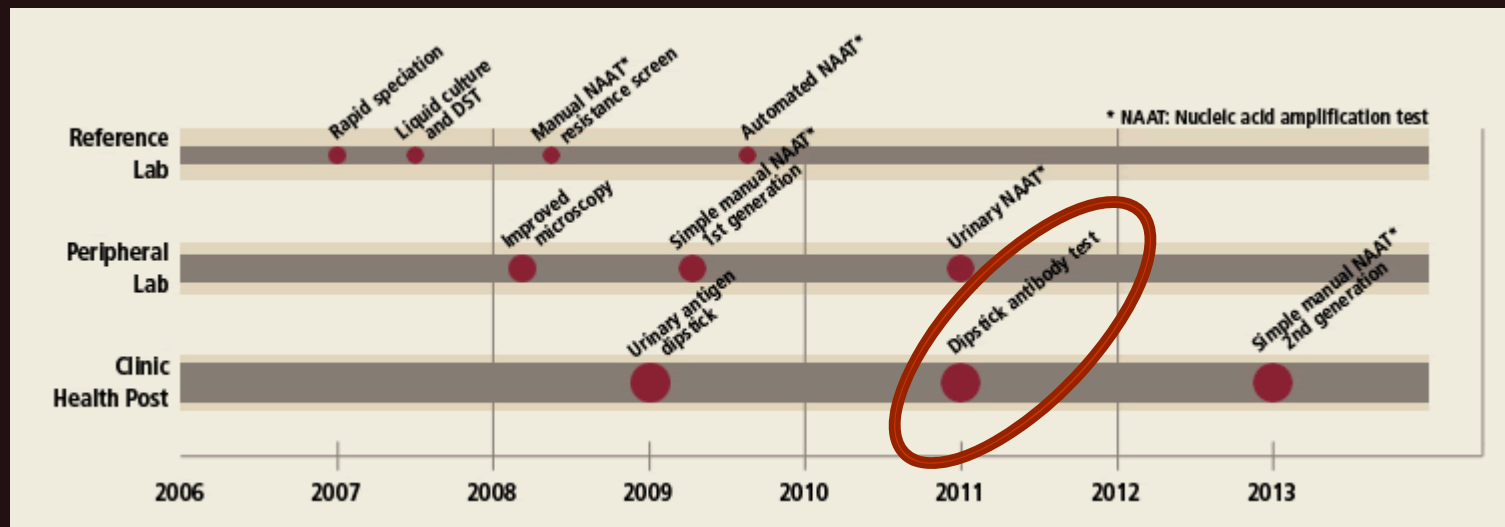
New diagnostics: targets (2)...

- Mycobacterial components: FIND secondary focus on lipids: LAM



New diagnostics: targets (3)...

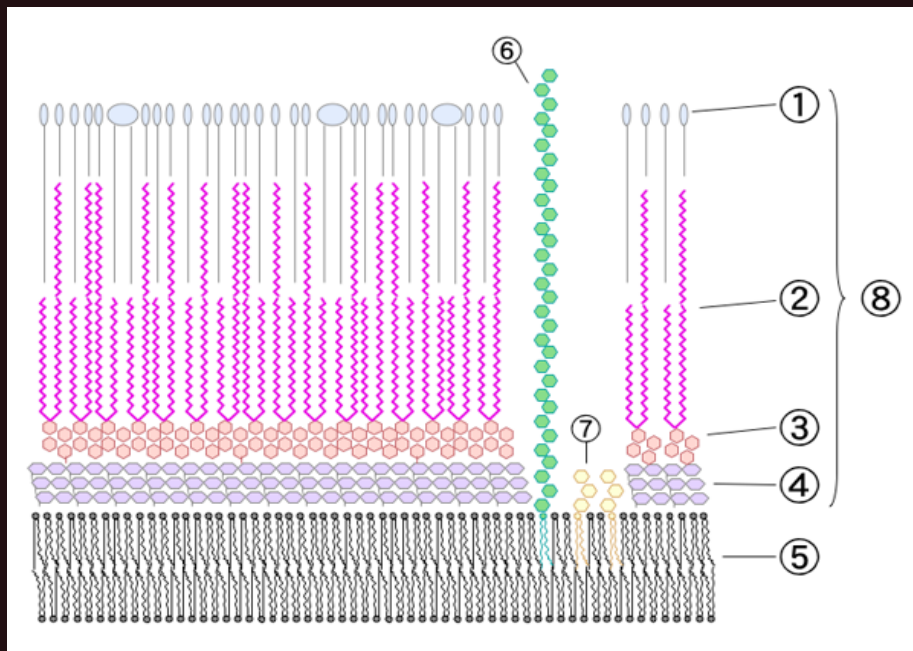
- Surrogate markers for TB
 - Antibodies ... but to which antigen?



Requirements for TB **disease** serodiagnostics

- Antibody activity to remain unaffected by HIV
- Blind towards previous vaccinations and skin tests - should have short memory
- Blind towards Non-TB mycobacteria infection
- Sensitive, specific and reproducible
- Affordable, amenable to field screening, easy to automate and operate

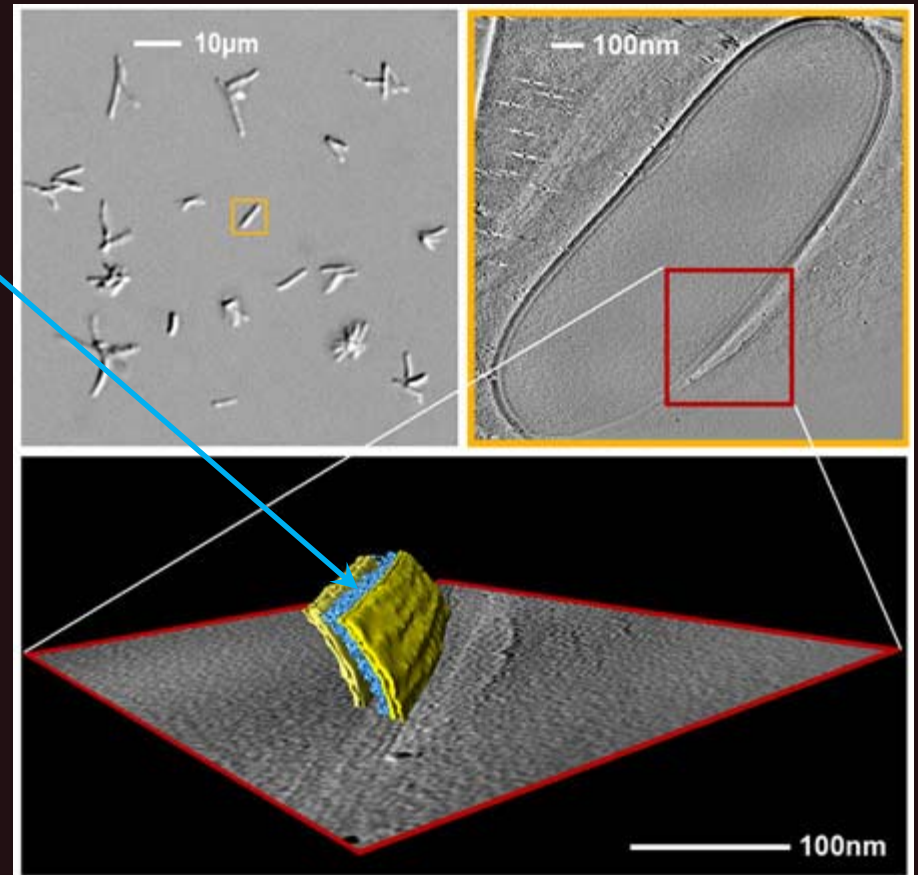
The opportunity provided by mycolic acids from the Mtb cell wall



1. outer lipids
2. mycolic acid
3. polysaccharides (arabinogalactan)
4. peptideglycan
5. plasma membrane
6. lipoarabinomannan (LAM)
7. phosphatidylinositol mannoside
8. cell wall skeleton

Mycolic acids: Unique cell wall wax

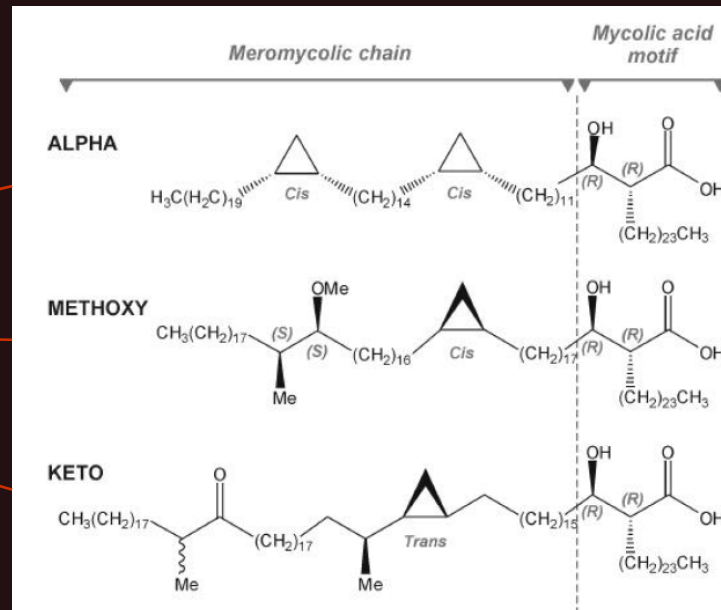
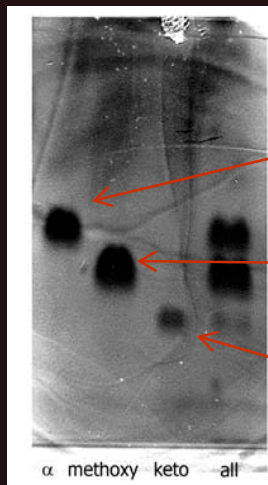
- Most abundant cell wall lipid
- Pumped out as biofilm matrix*
- Distributed in the body by lipoproteins



*Ohja *et al.* (2008) *Mol. Microbiol.* 69: 164

Mycolic acids (MA) = Mtb identikit

- Unique MA composition each for 60 different species of *Mycobacterium*!
- Mycobacterial MA molecules larger than from other species, eg *Nocardia* and *Corynebacterium*
- Three main classes of MA for *M. tuberculosis*:



Mycolic acids and immunity: 1994

- Breakthrough, Harvard, USA: A lipid (MA) presenting role described for the CD1 protein on antigen presenting cells:
 - Stimulation of T cell immunity (and antibodies?) without needing the CD4 T helper cell¹.
 - A possibility to bolster immunity in HIV infected patients with MA².
 - Diagnosis: Anti-MA antibodies as surrogate markers?

1. Beckman *et al.* (1994) *Nature* 372: 691

2. Verschoor & Onyebujoh (1999) *Bioessays* 21: 365-366

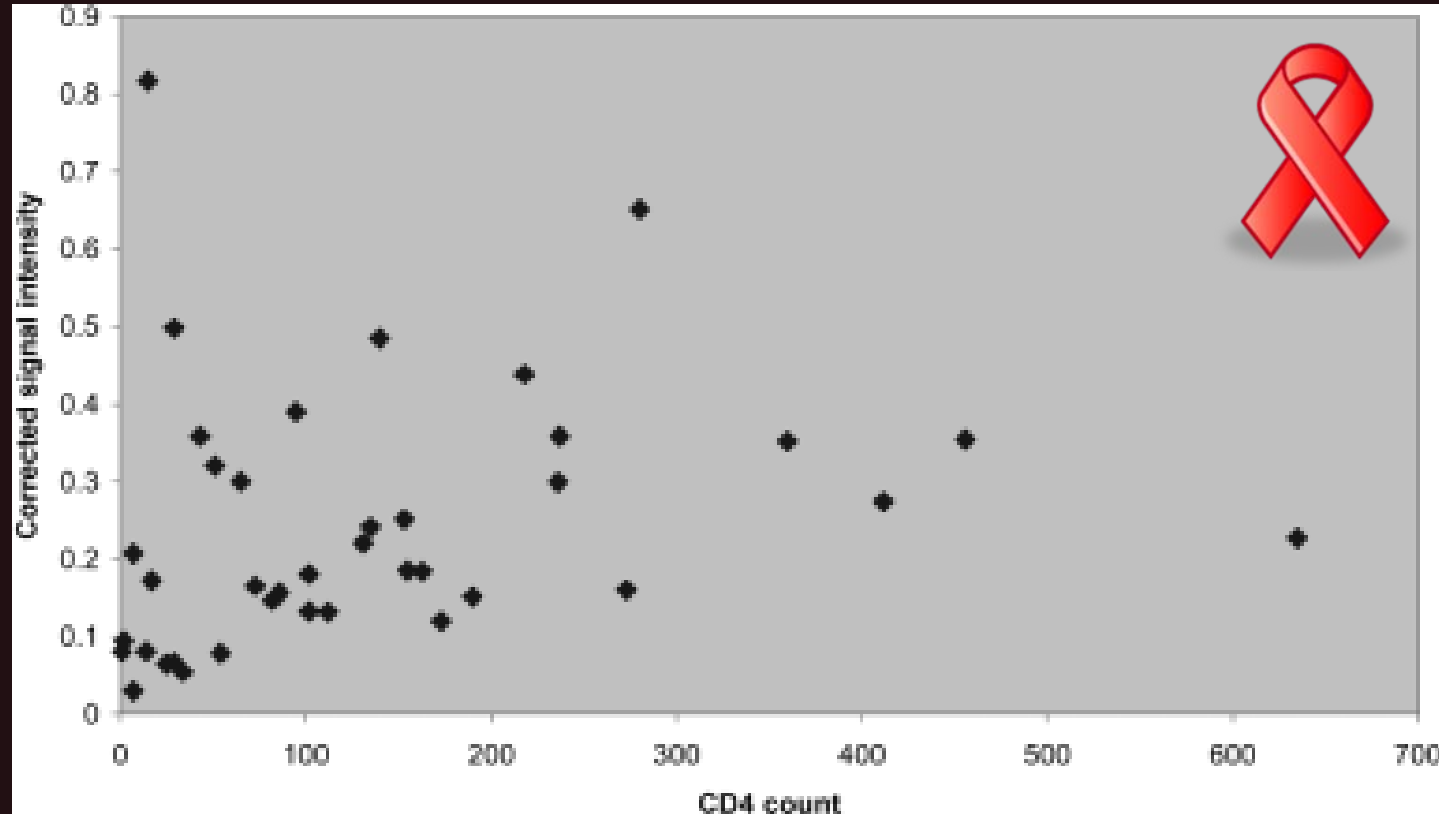
Mycolic acids antibody assays

- Serodiagnosis to detect anti-TB antibodies is impaired by HIV-infection¹
Antibodies to MA not affected by HIV/AIDS²
- Popular antibody tests not to desired standard
Biosensor technology can overcome this
- Biosensor technology is laboratory bound, complex
Biosensors are being automated and simplified, similar to what is currently being achieved with NAAT

1. Clin. Inf. Dis. (2004) 39: e1-e7 ,

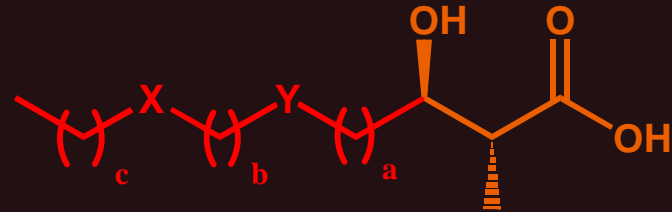
2. Clin. Chem. Lab. Med. (2002) 40: 882

Anti-MA antibodies in HIV patients

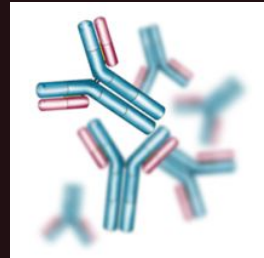


Introducing the MARTI-test

M - Mycolic acid

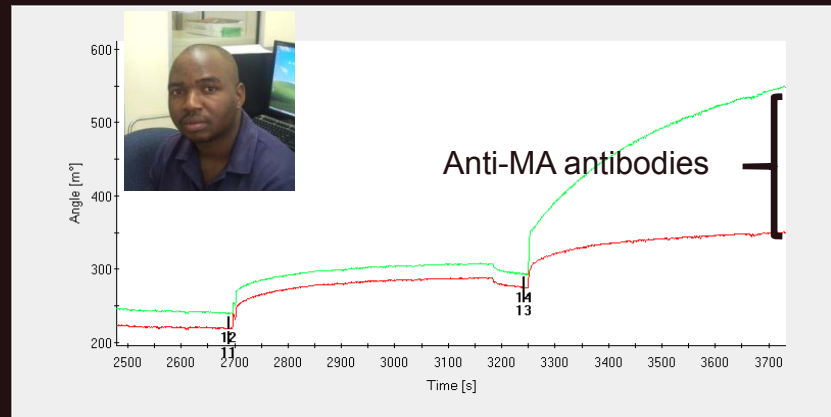


A - Antibody

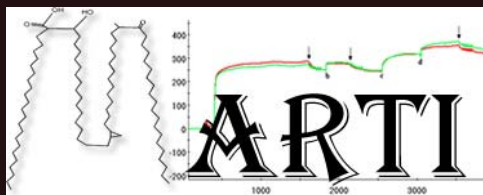
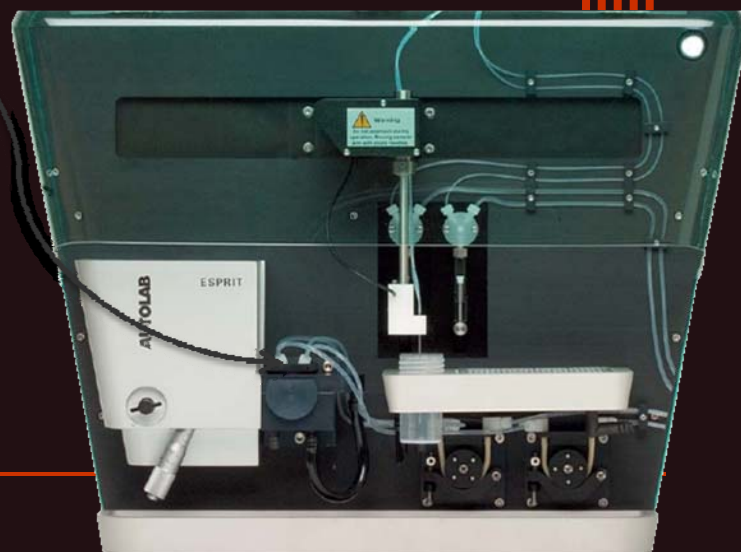
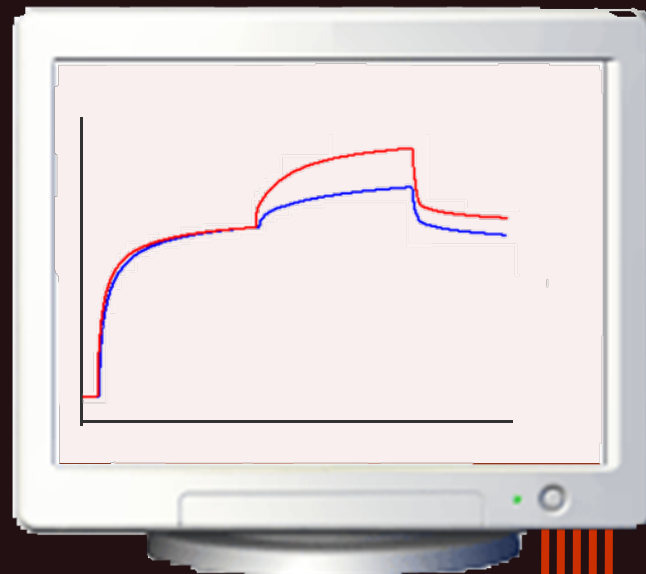
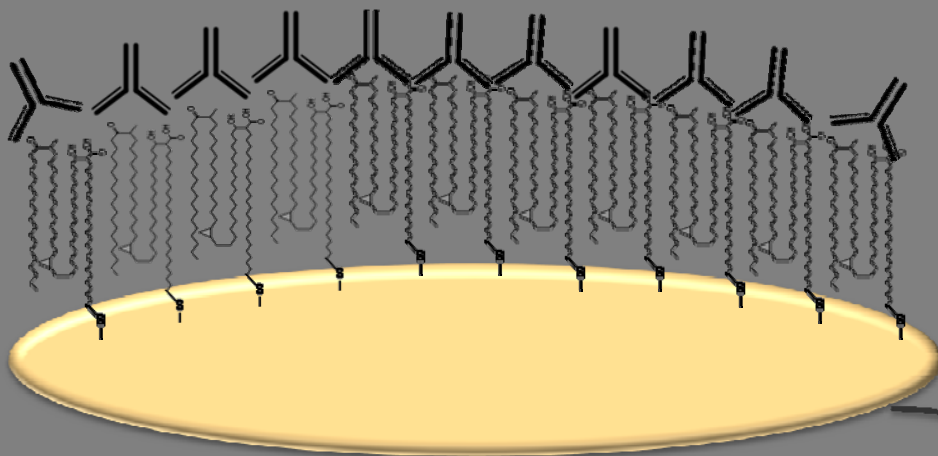


RT - Real-time

I - inhibition

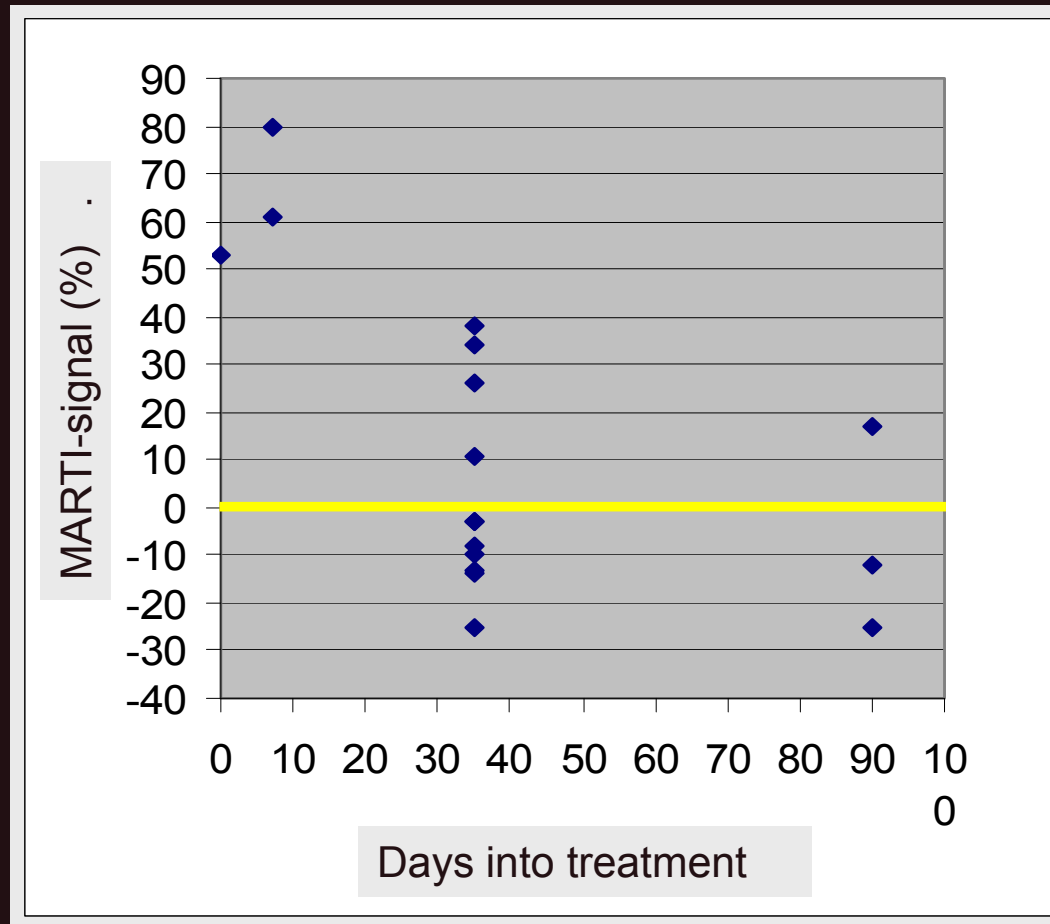


How MARTI Works

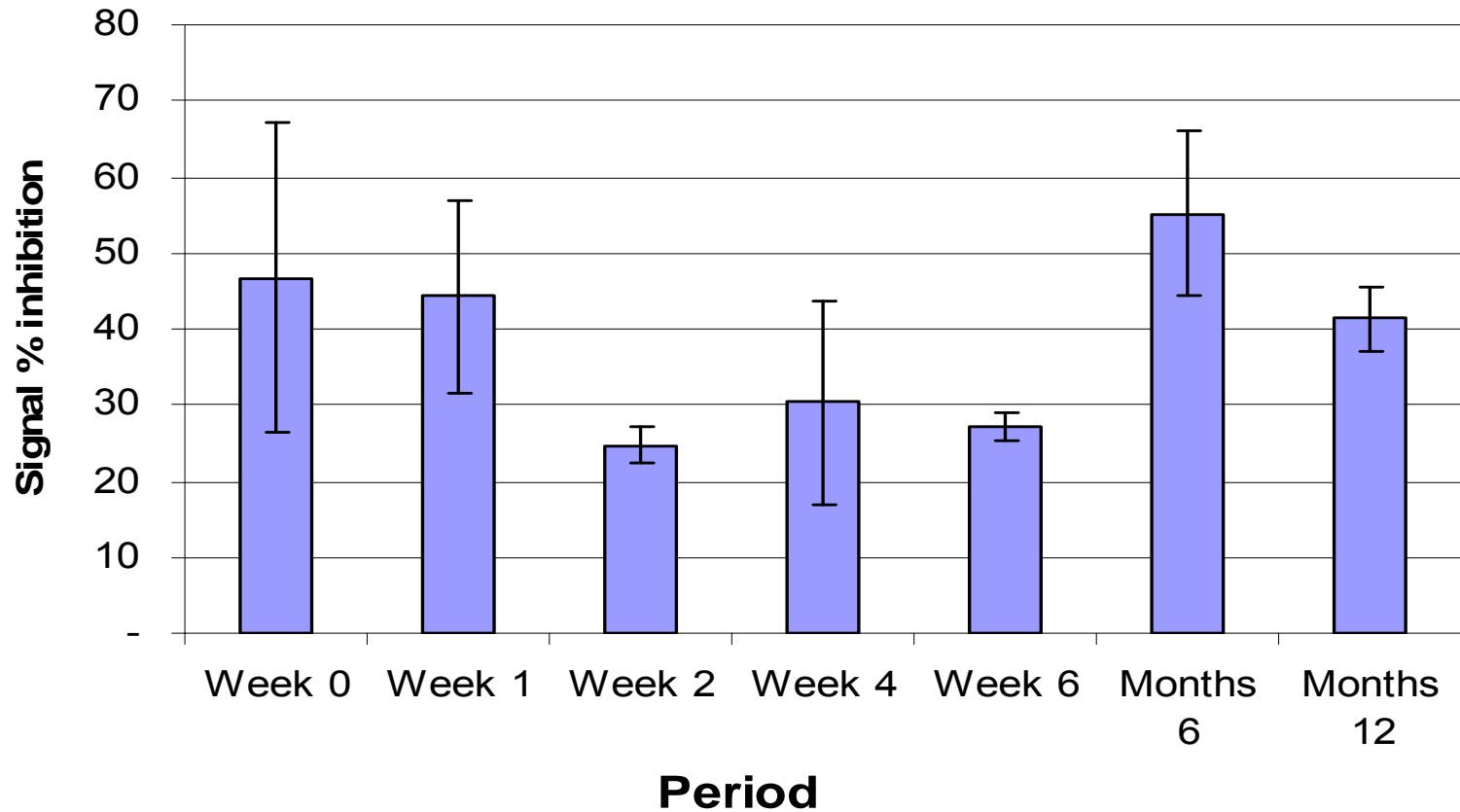


Short memory of antibodies:

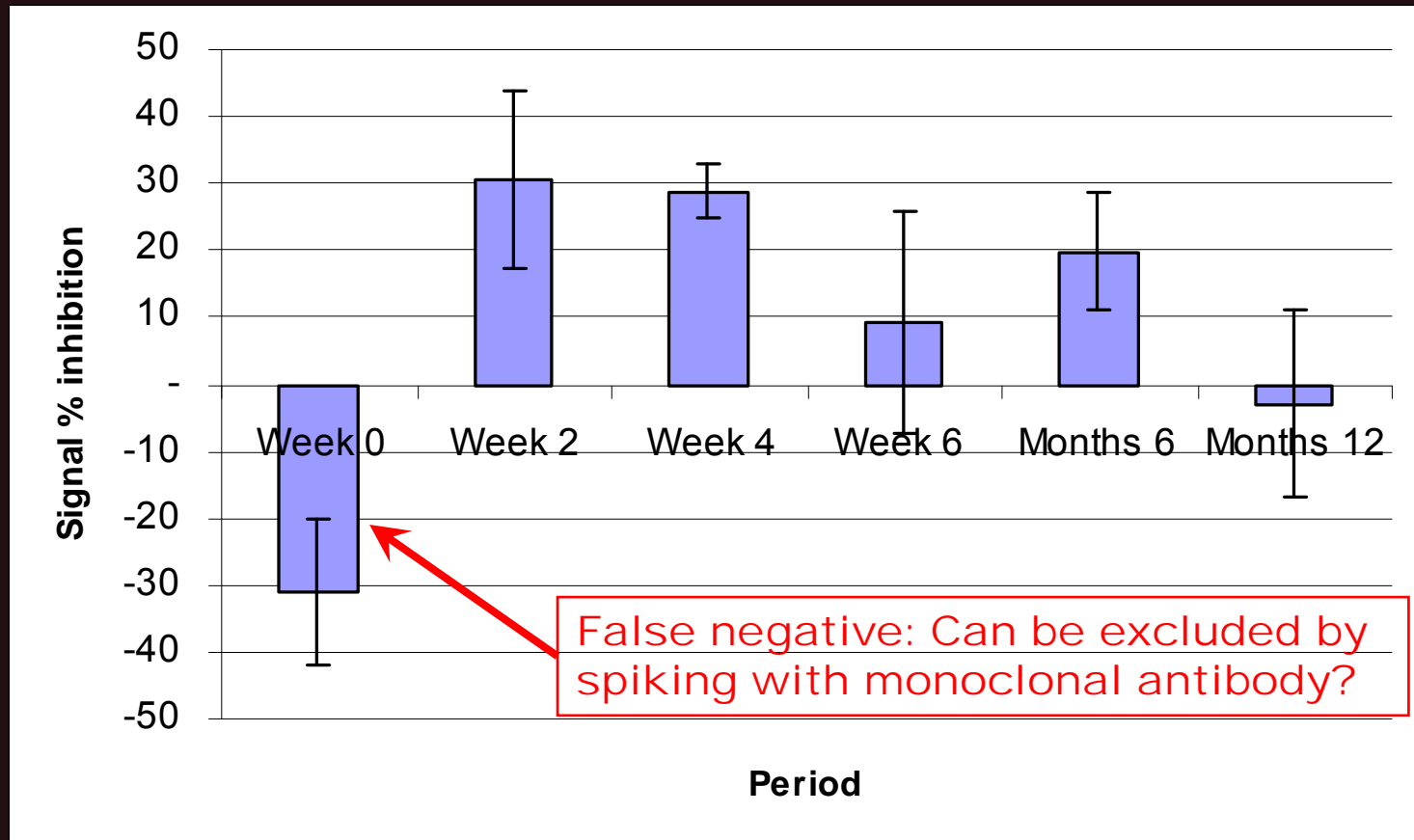
21 blind samples (EDCTP – Univ Stellenbosch)



MARTI in MDR patient (EDCTP P5121)

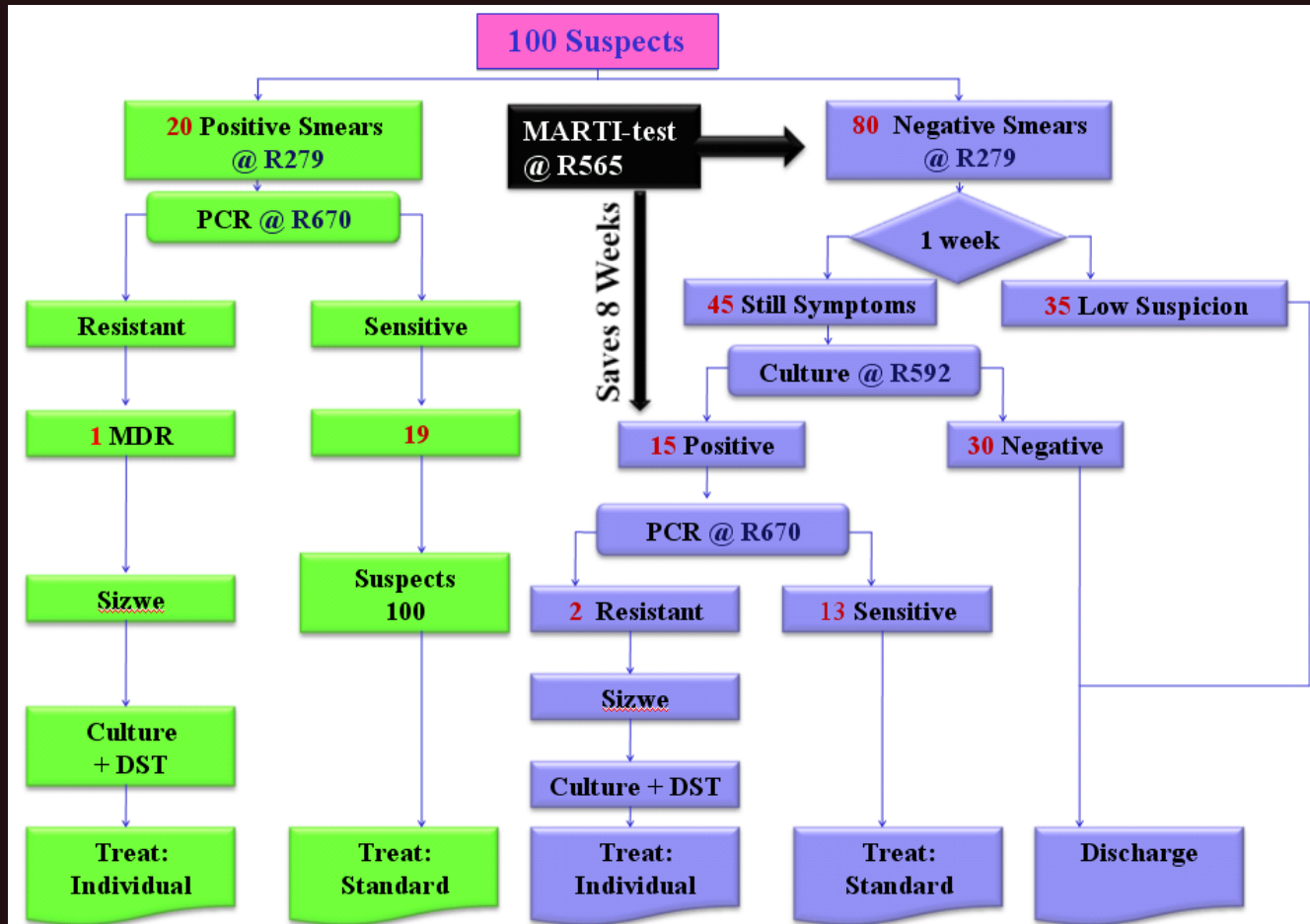


MARTI in cured TB patient (EDCTP P3897)



MARTI-test niche – overall costs

Gauteng:
proposed
flowchart for
PCR
diagnosis, Dr
Gerrit
Coetzee,
Head of the
NHLS
National TB
Reference
Laboratory



Current available diagnostics: **R18 980** **R59 010** Total **R77 990**

MARTI-tests for all suspects **R56 500**

Downstream savings (clinic fees, hospitalization, decreased transmissions) **R100,000s**

MARTI-test now

- A South African invention
PCT patent (2005), granted in 2008, tested at >80% accuracy
- False negatives avoided: >90% accuracy
- Proof of principle in wave-guide biosensor:
Thanyani *et al.* (2008) J Immunol Methods 332: 61-72
- Methodology in SPR biosensor (EDCTP)
Lemmer *et al.* (2009) Methods in Enzymology (In press)
- Methodology in electro-impedance (EDCTP)
Mathebula *et al.* (2009) Chem Comm 2009:3345-3347

MARTI-test future

- Cape Biotech:

Validation in HIV / avoidance of *M avium* disease

- India - South Africa bilateral agreement

MARTI application in child TB and extrapulmonary TB

- MRC:

Three year validation for sensitivity and specificity

- To be coordinated and applied for:

Biosensor hardware engineering for high sample throughput

Acknowledgements: international

- Jan Castrop Eco Chemie NL
 - Biosensor hardware development
- Mark Baird Bangor University UK
 - Chemical synthesis of mycolic acids
- EDCTP EU
 - Funding to initiate translational research

Acknowledgements: national

- P van Helden and team Univ. Stellenbosch
- Hulda Swai and team CSIR
- NRF, MRC, Cape Biotech/Lifelab

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