New Concepts in Diagnosis for Eumycetoma

Wendy W.J van de Sande, Sarah Abdalla Ahmed, Ahmed Fahal, G Sybren de Hoog

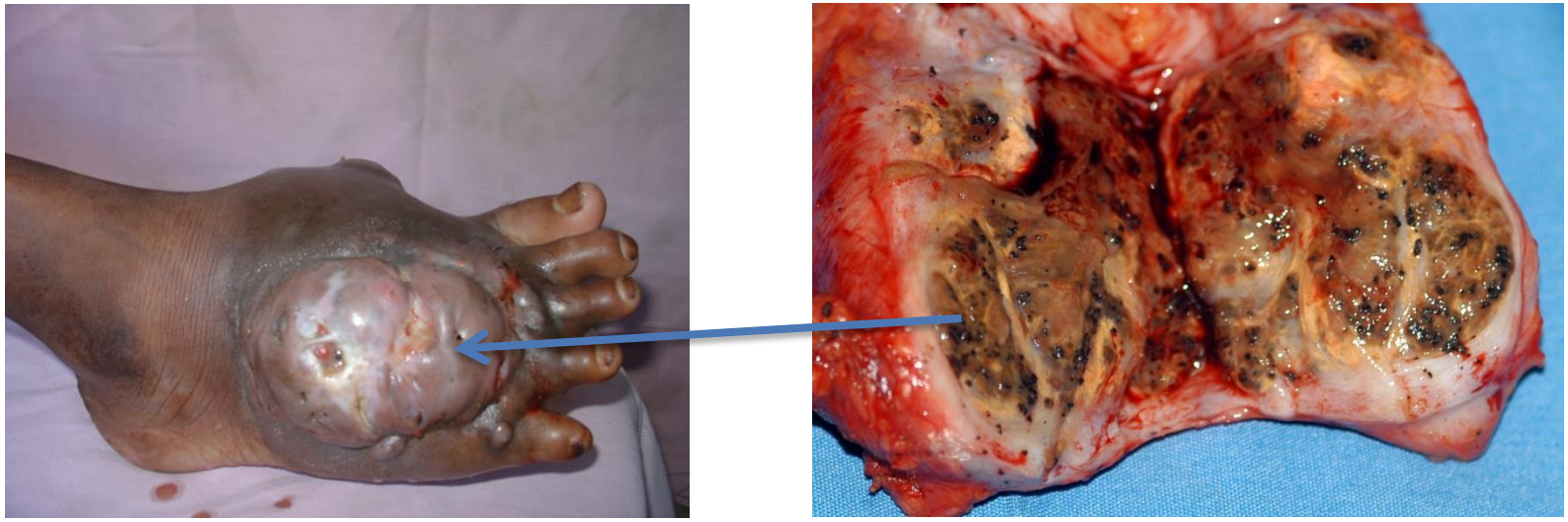
Erasmus MC, Department of Medical Microbiology and Infectious Diseases, Rotterdam, The Netherlands

CBS Fungal Biodiveristy center, Utrecht, The Netherlands

Diagnosing mycetoma

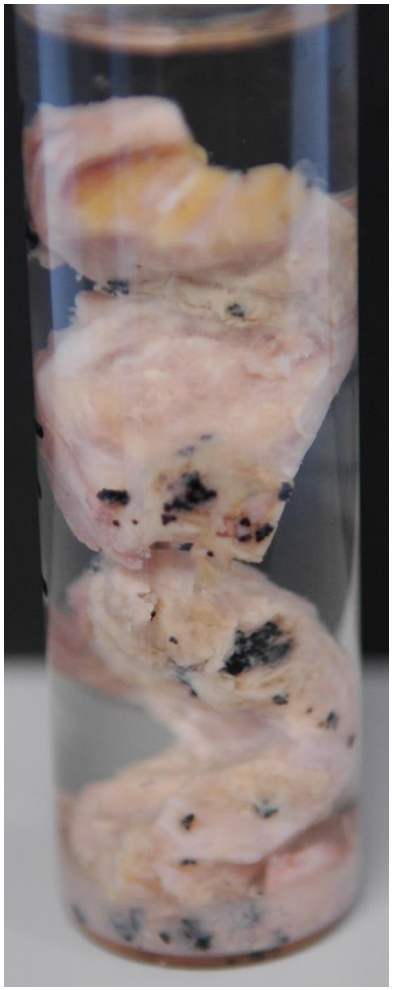
- Eumycetoma can be caused by more than **42 different** fungal species

- Diagnosis is made clinically



Identification of the causative agent

- Colour of the grain



Eumycetoma	
Madurella mycetomatis	●
Scedosporium boydii (synonym: *Scedosporium apiospermum* or *Pseudallescheria boydii*)	W
Falciformispora senegalensis (synonym: *Leptosphaeria senegalensis*)	●
Trematosphaeria grisea (synonym: *Madurella grisea*)	●
Acremonium falciforme (synonym: *Cephalosporium falciforme*)	W
Aspergillus fumigatus	
Exophiala jeanselmei	●
Geotrichum candidum	
Neotestudina rosatii	W
Medicopsis romeroi	●
Medicopsis romeroi or *Biatriospora mackinnonii* (synonym: *Pyrenochaeta* spp)	●
Aspergillus flavus	G
Microsporum audouini	W
Cochliobolus lunatus (synonym: *Curvularia lunata*)	●
Rhinocladiella atrovirens	
Aspergillus nidulans	W
Neoscytalidium dimidiatum	
Fusarium spp	W

Van de Sande, PLoS NTD, 2013

Identification of the causative agent

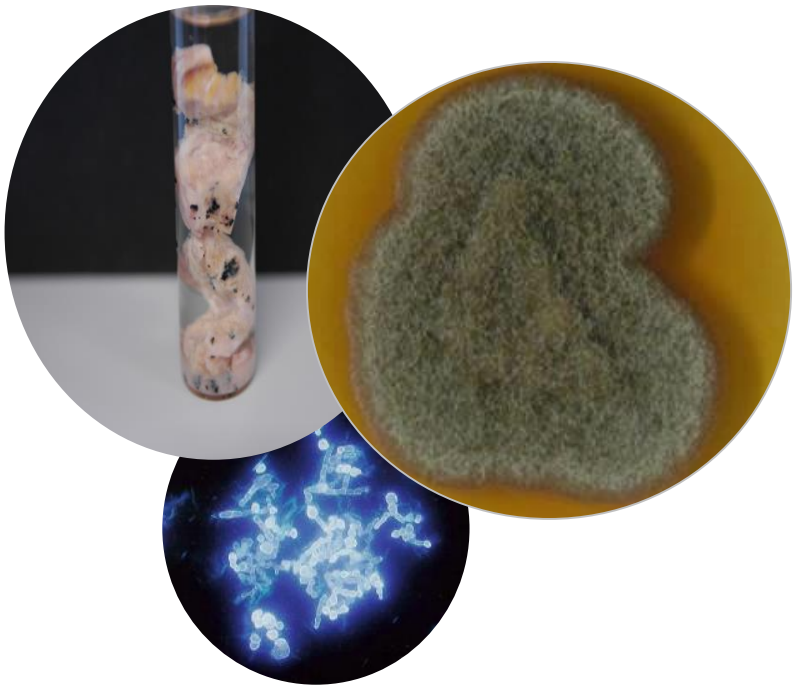
- *In vitro* susceptibility for itraconazole

Causative agent	MIC_{90}
Madurella mycetomatis	0.25
Falciformispora senegalensis	0.125
Trematosphaeria grisea	0.125
Medicopsis romeroi	**>16**
Biatriospora mackinnonii	0.5
Madurella fahalli	**>16**
Madurella tropicana	0.01
Madurella pseudomycetomatis	0.03

De Hoog, JClinMicrob, 2012
Ahmed, MedMycol, 2015

Black grain mycetoma

- Fine needle aspirate to obtain viable grains
  - Direct microscopic smear
  - Cell blocks
  - **Culturing**
    - Gold standard
    - Time consuming
    - Misidentifications
- Species identification only reliable with molecular identification

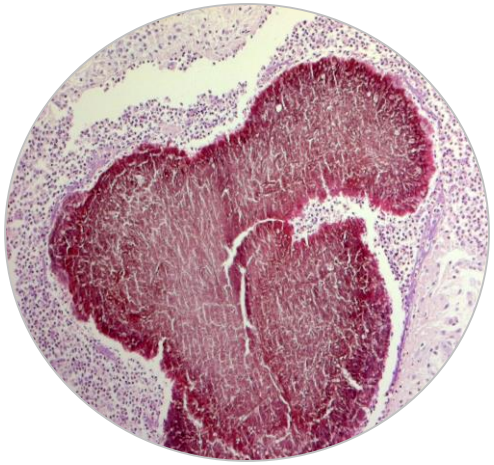


Van de Sande, PLoS NTD, 2013

Molecular identification

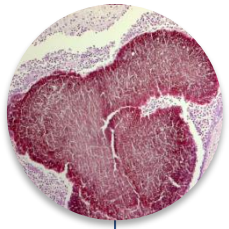
- First requirement: **DNA**

- DNA isolation from cultured isolates



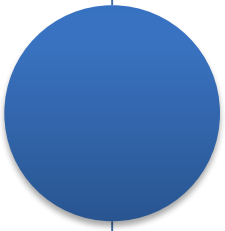
- DNA isolation directly from grains

DNA isolation directly from grains



Grains

Bead-beating with glass beads



CTAB, chloroform and isoamylacohol

No DNA

Bead-beating with Bashing beads

ZR Fungal/Bacterial DNA miniprep

No DNA

Bead-beating with metal beads

Qiagen Dneasy Plant mini kit

DNA

Ahmed, JClinMicrob, accepted

DNA amplification method

- **Standard:** Amplification of ITS region and sequencing
  - Expensive equipment needed:
    - Thermocycler
    - Sequencer
  - Time consuming

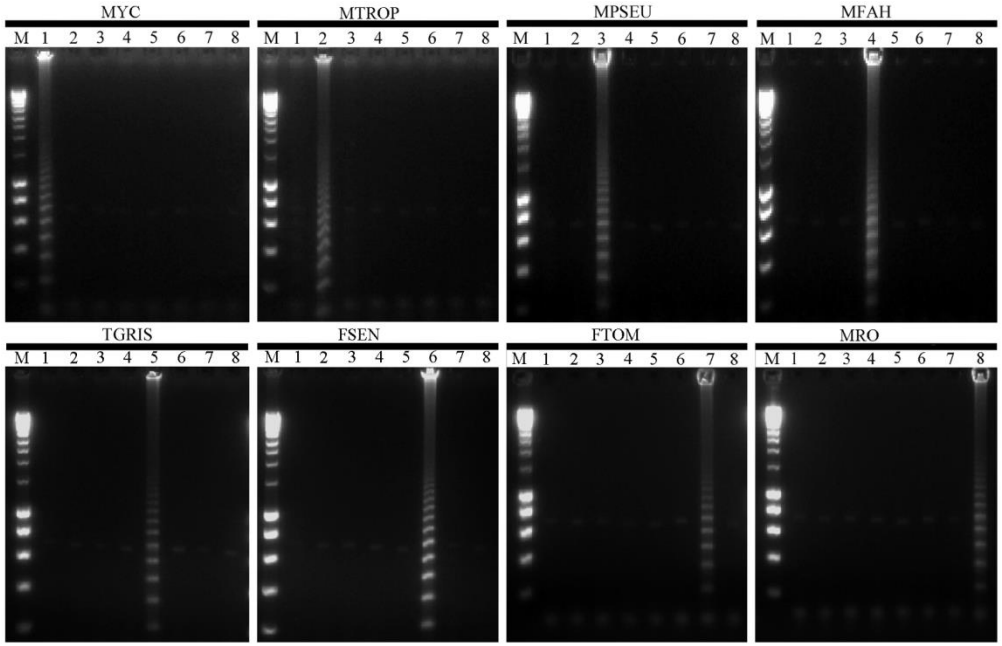
- **Isothermal amplification** to identify black grain causative agents of mycetoma

Rolling circle amplification

- Amplification of ITS region

- Identification of causative agent with **isothermal amplification** with species specific probes to identify

  - *Madurella mycetomatis*
  - *Falciformispora senegalensis*
  - *Trematosphaeria grisea*
  - *Medicopsis romeroi*
  - *Falciformispora tomkinsii*
  - *Madurella fahalii*
  - *Madurela tropicana*
  - *Madurella pseudomycetomatis*



Ahmed, PLoS NTD, 2015

Rolling circle amplification

- Rolling circle amplification is very suitable to identify black grain mycetoma casuative agents to the species level
  - 100% specificity and no cross reactivity in 62 isolates tested
- **BUT** still amplification of the ITS region was needed

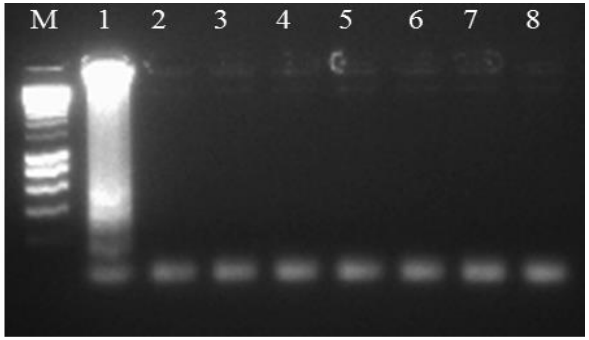
Ahmed, PLoS NTD, 2015

Isothermal DNA amplification

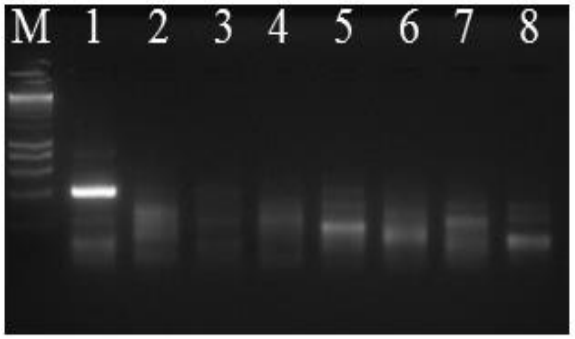
- *Madurella mycetomatis*

- Isothermal DNA amplification methods of ITS region

- Loop-mediated isothermal amplification (LAMP)



- Recombinase polymerase amplification (RPA)



Ahmed, JClinMicrob, accepted

Comparisson of DNA amplification methods

	RCA	LAMP	RPA
Pre-amplification of ITS region with PCR	yes	no	no
Reaction temp	65 ^{o}C	65 ^{o}C	39 ^{o}C
Time to identification	6h	2h	40 min
Detection limit	0.003 ng	0.5 ng	0.2 ng
Specificity	100%	100%	100%

In summary

- A method has been developed to isolate DNA directly from mycetoma grains, reducing the time to identification with 6 weeks

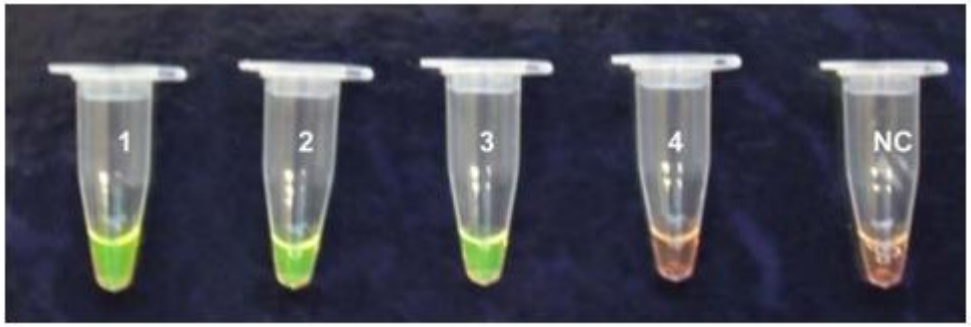
- Three **isothermal DNA amplification** methods have been developed to identify black grain mycetoma causative agents

- **RPA** seems to be the most suited for further development

Further development of RPA

- RPA is only developed to identify *Madurella mycetomatis.* Primers need to be designed and tested for the other causative agents too.

- The electrophoresis step can be omitted when lateral flow detection or fluorescence detection are used.



Further development of RPA

- Set up a clinical trial in which the performance of these novel diagnostic tools is compared to culturing to determine:
  - Sensitivity
  - Specificity
  - Time needed to positive identification

Point of care test

- RPA is well suited for reference labs in the endemic settings
- But field hospitals will not use it
- Need for a point of care test

Ideal point of care test

- Serological assay which detects the most common causative agents of mycetoma

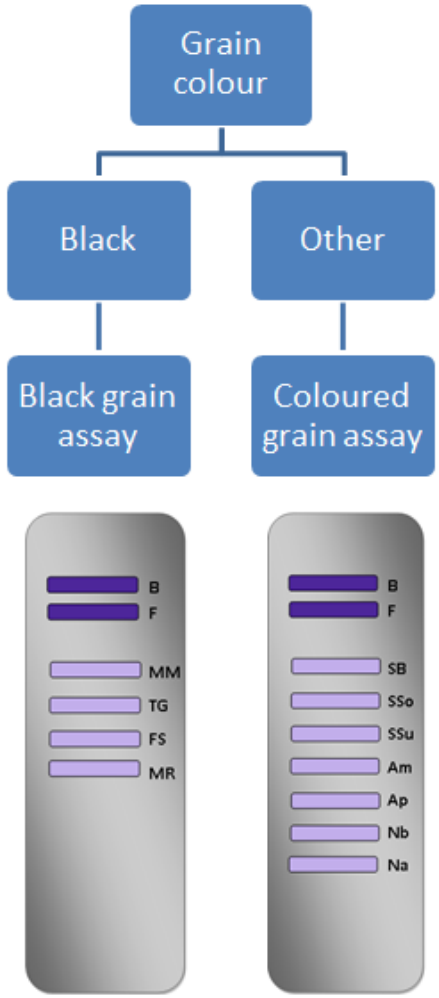
Ideal point of care test

- Serological assay which detects the most common causative agents of mycetoma

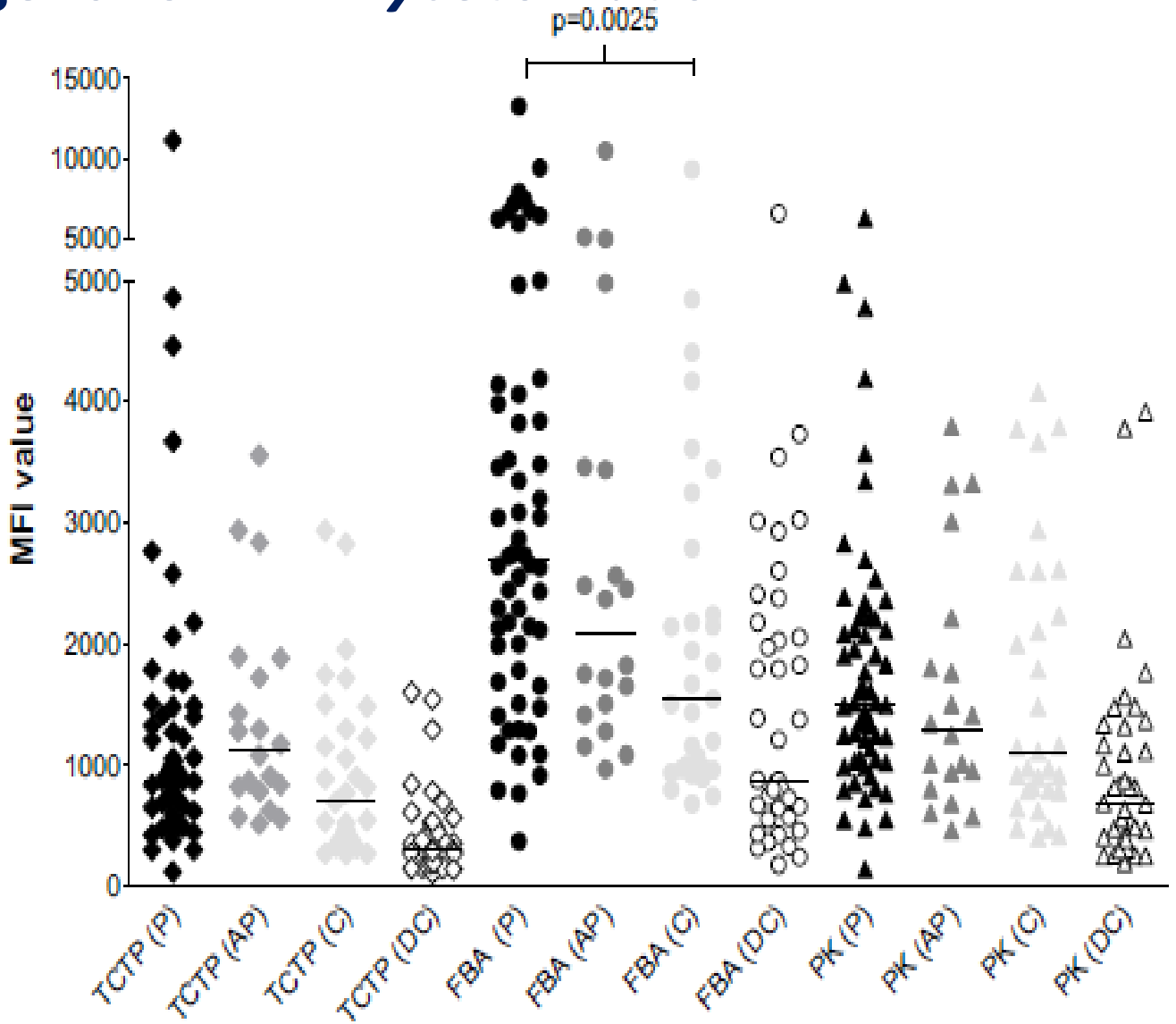
Species	grain colour	Sudan	Mali	Senegal	Madagas-kar	India	Mexico
Madurella mycetomatis	B	1039	20	177	59	442	15
Actinomadura madurae	W/Y/P	6	12	86	1	163	207
Streptomyces somaliensis	Y	337	3	53	3	96	28
Actinomadura pelletieiri	R	16	15	285	61	65	9
Nocardia brasiliensis	W	0		0		75	1694
Nocardia asteroides	W	0		0		63	18
Falciformispora senegalensis	B	0	1	109		20	
Trematosphaeria grisae	B	0		0		52	18
Medicopsis romeroi	B	0		3	7	10	1
Scedosporium boydii	W	0		28	1	18	6

Ideal point of care test

- Serological assay which detects the most common causative agents of mycetoma

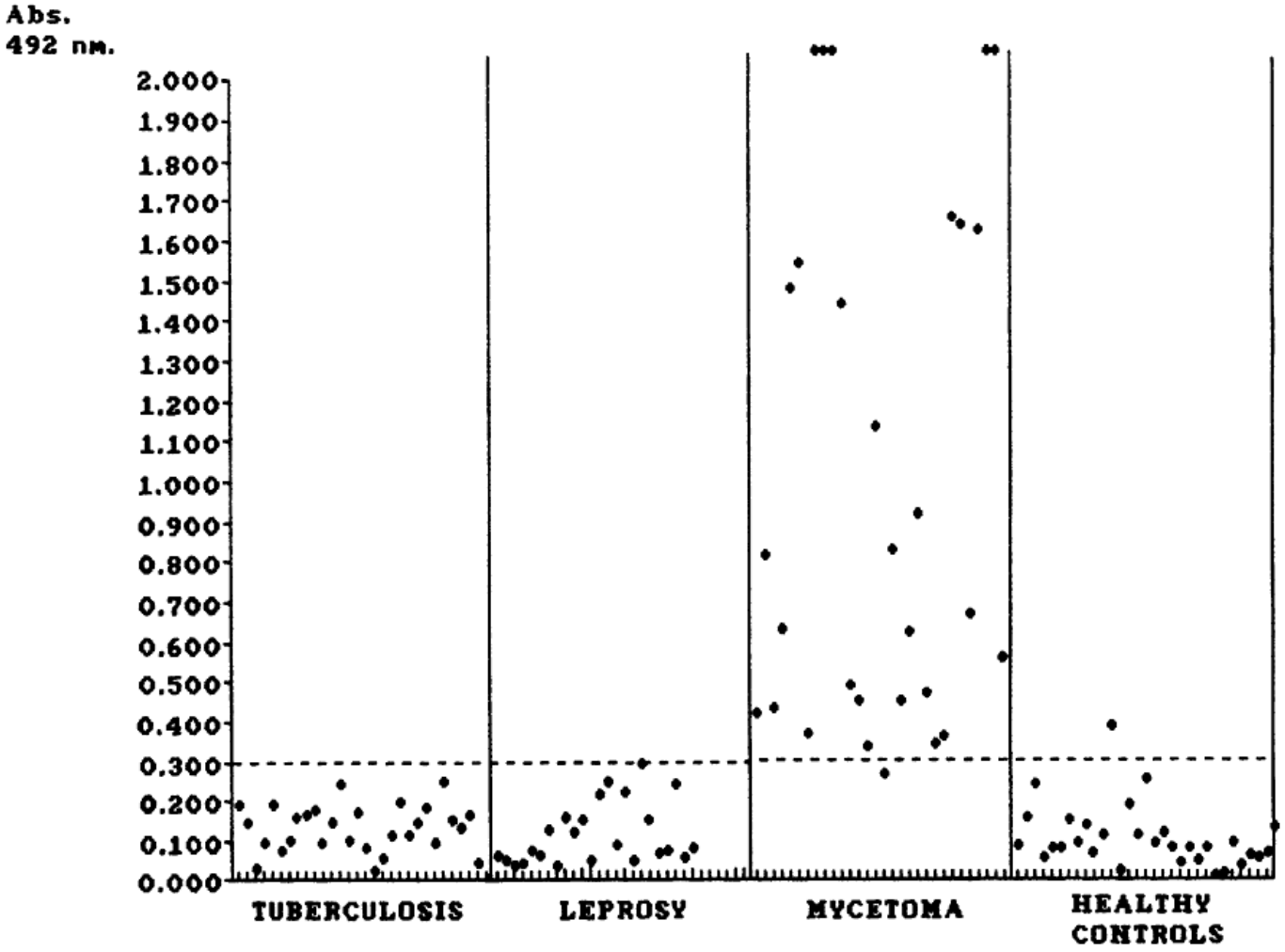


Antigens for *M. mycetomatis*



De Klerk et al, Med Mycol 50 (2012): 143-151; Van de Sande et al, J immunol 177 (2007): 1997-2005

Antigens for *Nocardia brasiliensis*



Salinas-Carmona et al, J Clin Microbiol 31 (1993): 2901-6

Serology

- Only antigens discovered for *M. mycetomatis* and *N. brasiliensis*
- For other causative agents no antigens discovered
- Need to identify suitable antigens for mycetoma causative agents

In conclusion

- DNA based identification tools are developed but need to be further optimised and tested in the endemic reference centers

- Effort should be made to identify antigens for the most common causative agents of mycetoma in order to develop a user friendly, point of care test for field based diagnosis of mycetoma

Contact

w.vandesande@erasmusmc.nl