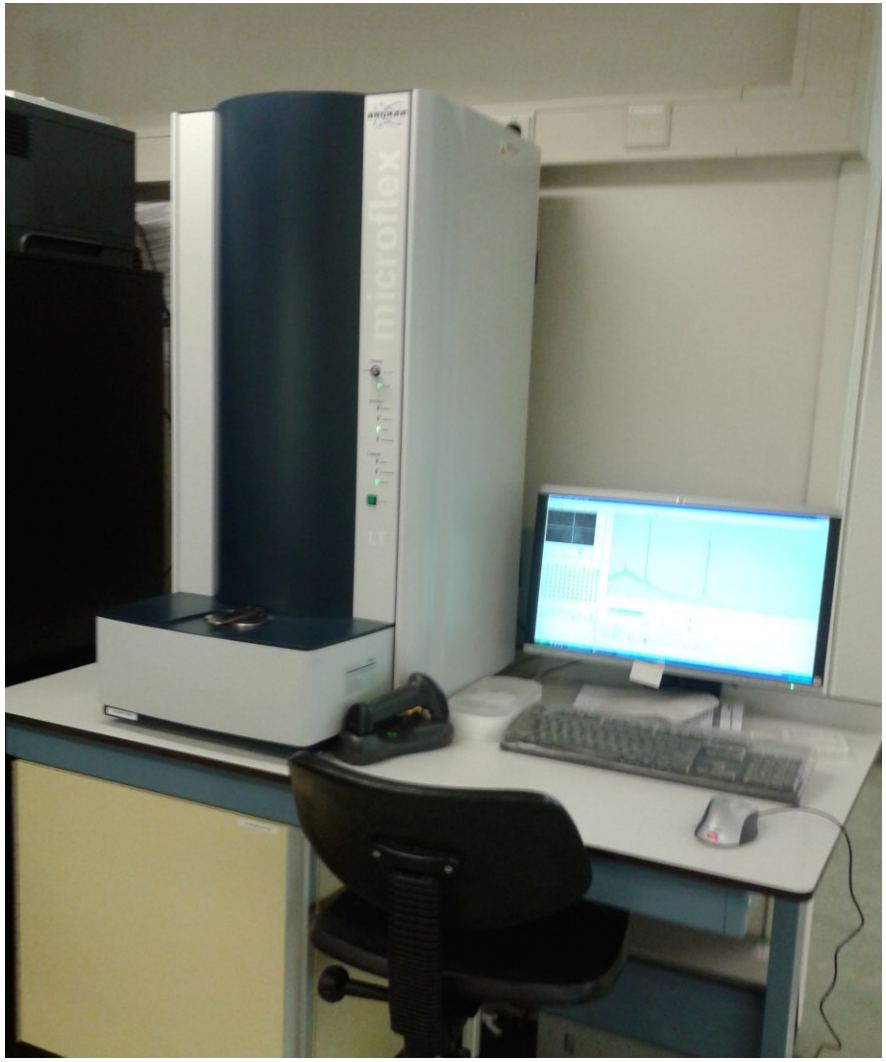
STICHTING PAMM
LABORATORIA VOOR PATHOLOGIE EN MEDISCHE MICROBIOLOGIE

Determinatie van Mycobacteriën met de Maldi TOF

Arjan Jansz 20 juni 2013

Maldi TOF



Impact of MALDI-TOF for Health Care

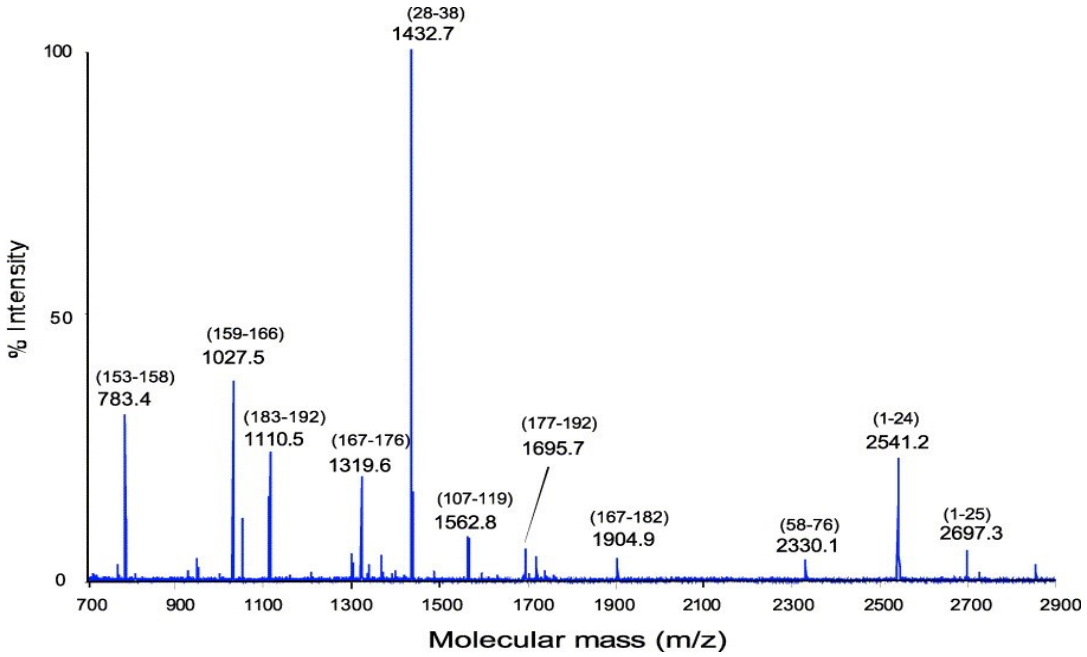
Cleveland Clinic, USA Top 10 Innovations of 2013

#1 Bariatric Surgery for Control of Diabetes:

#2 Neuromodulation Therapy for Cluster and Migraine Headaches:

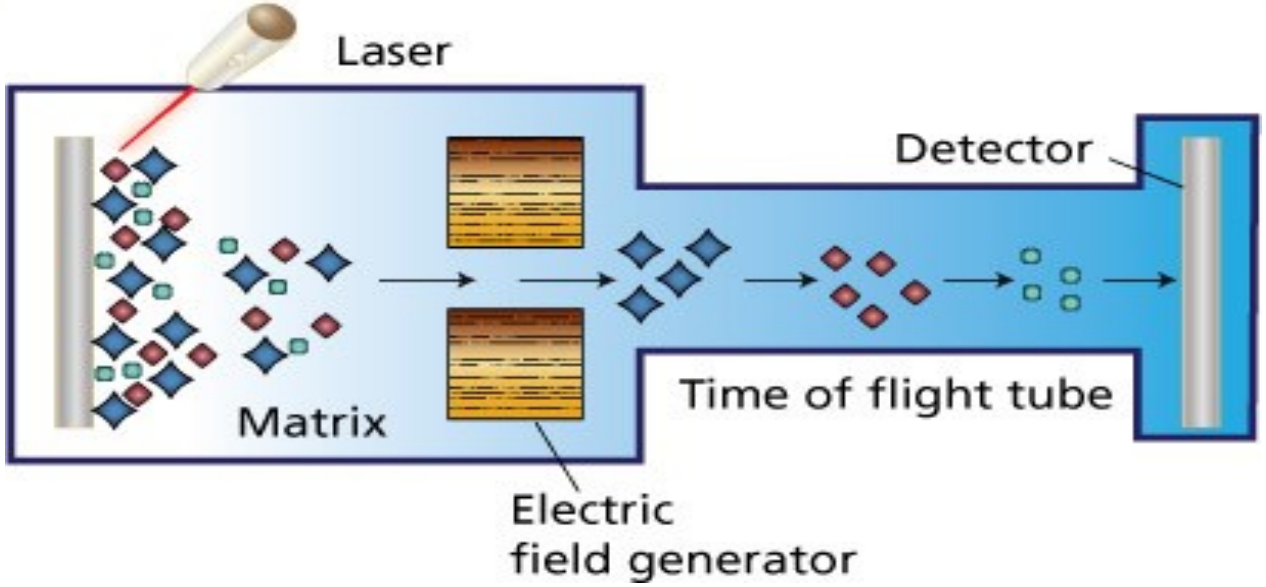
#3 Mass Spectrometry for Bacterial Identification:

http://www.clevelandclinic.org/INNOVATIONS/SUMMIT/topten/2013.html



- Maldi-TOF = matrix- assisted laser desorption/ionization time- of- flight

- Analyse door massa/lading ratio te bepalen



- Pulserende laser wordt afgevuurd op gespot monster
- laserenergie wordt opgenomen in matrix → ionisatie van substraat

Waarom Maldi-TOF

- Moleculair biologisch technieken
  - snellere primaire detectie
  - snelle resistentie detectie
  - snelle determinatie
  - typering

  Maar: sparen

- Huidige determinatie methode kost tijd
- Uniformering determinatie mbv MALDI-TOF op het laboratorium
- Lagere kosten

Vraagstelling

- Kan MALDI-TOF mycobacteriën determineren?

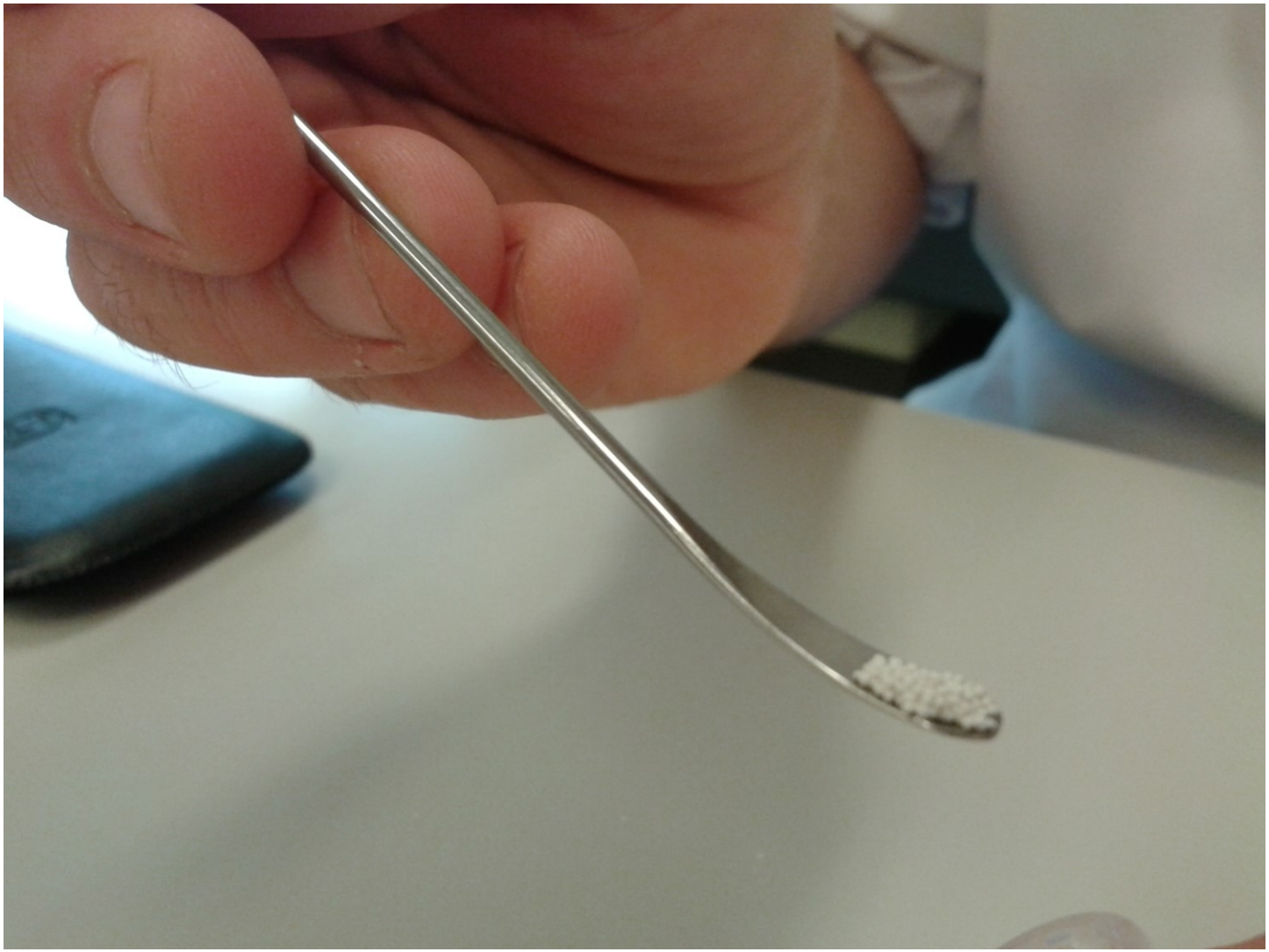
  - routine (reversed line blot)

  vs

  - MALDI-TOF

Methode

- 101 stammen opgekweekt in de BACTEC™ microMGIT™ 960 (7ml buis)
- Validatie Mycobacterie database(V1.0) met behulp van de silica bead methode
- Een keer opgewerkt, twee keer gespot
- Automatisch gerund volgens Bruker protocol



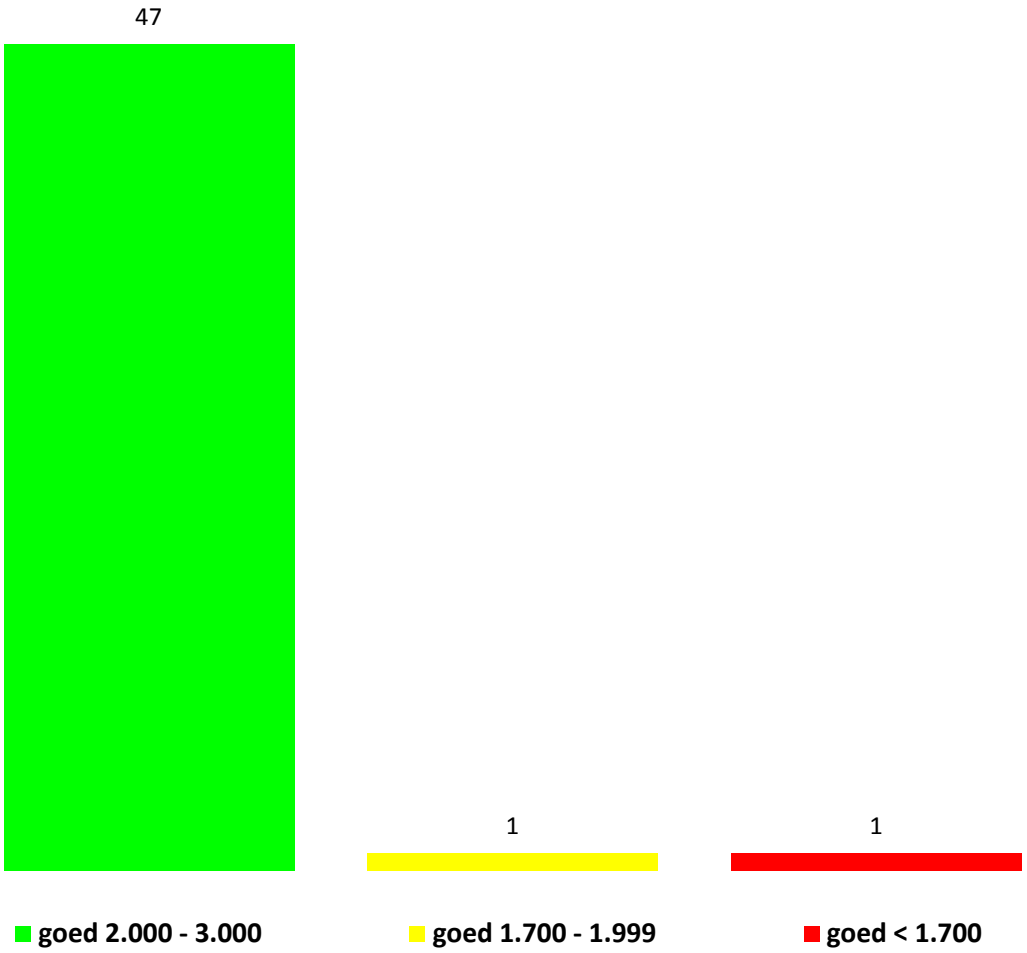
Stammen

Organisme Naam	Aantal
M.tuberculosis	47
M.bovis	2
M.abscessus	3
M.avium	11
M.bohemicum	2
M.chelonae	4
M.fortuitum/peregrinum	6
M.gordonae	5
M.interjectum	3
M.intracellulare	5
M.kansasii	5
M.malmoense	1
M.marinum	2
M.scrofulaceum	1
M.simiae	2
M.xenopi	2
totaal	101

Aandachtspunten

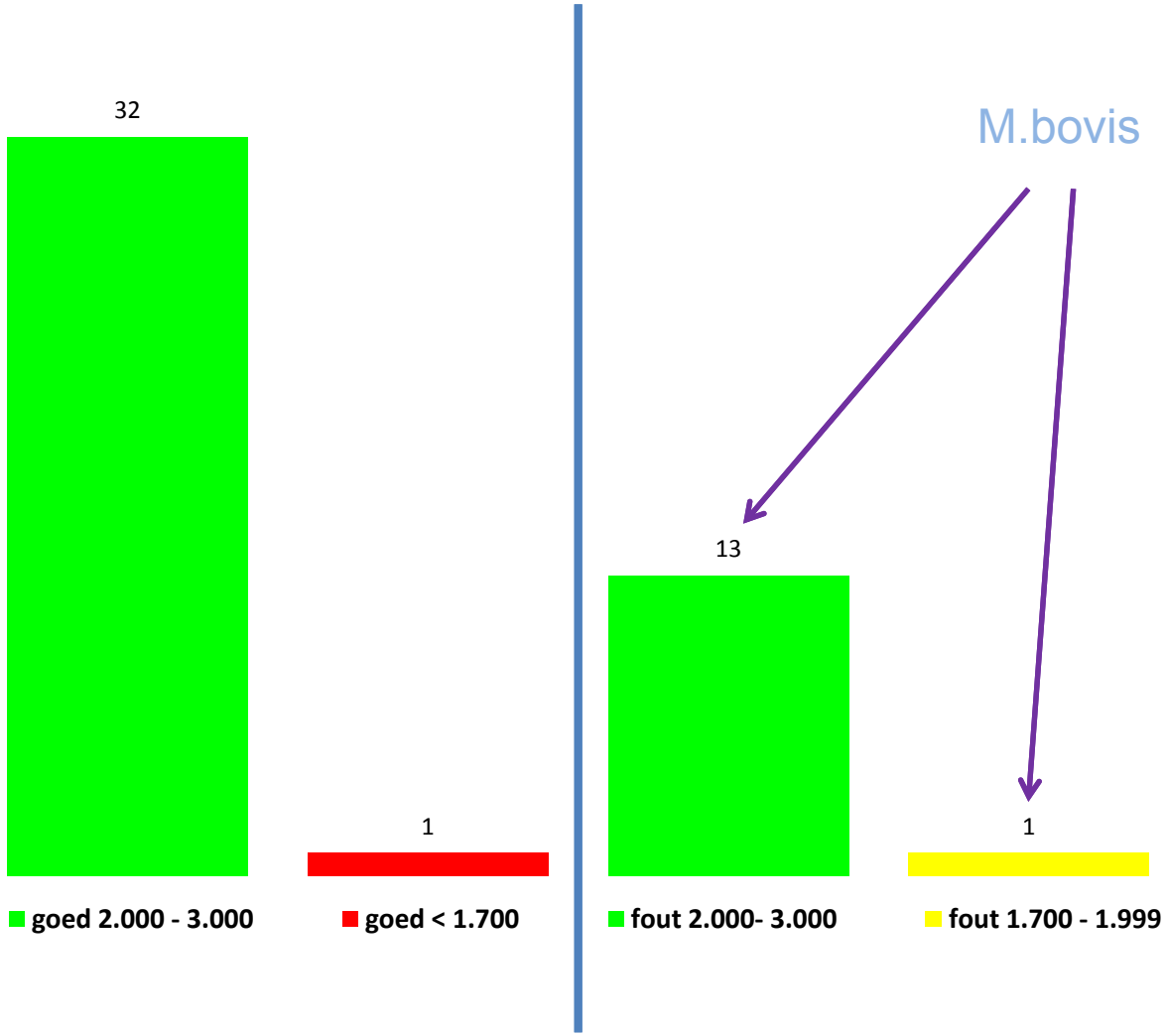
- 80% van de Mycobacterium stammen enkelvoudig in database
  - **Alleen best match beoordelen**
- 97% van duplo spotten komt overeen
  - **bij verwerking van de resultaten een spot beoordeeld**
- Grafieken: kleurcodering van de Malid-tof gebruikt

Resultaat M.tuberculosis-complex(n=49)



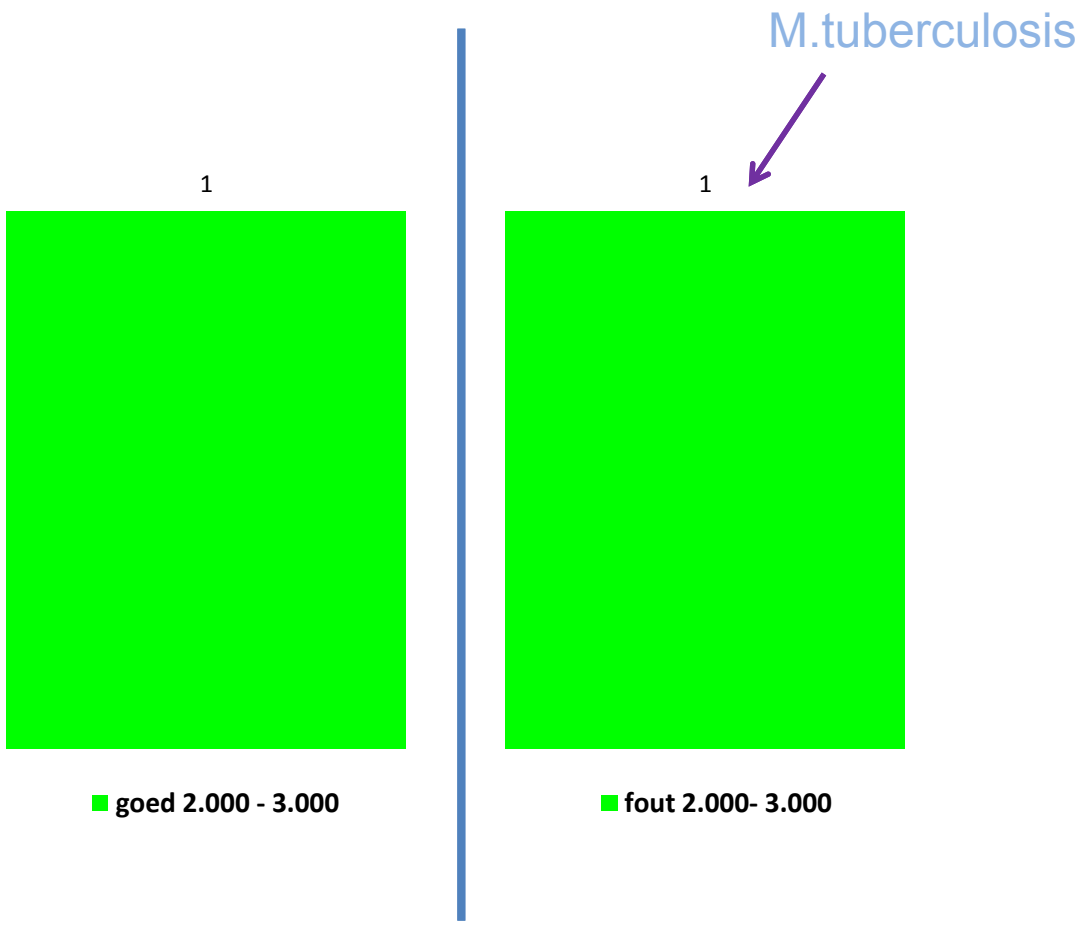
Binnen M.tuberculosis-complex

Resultaat M.tuberculosis (n=47)

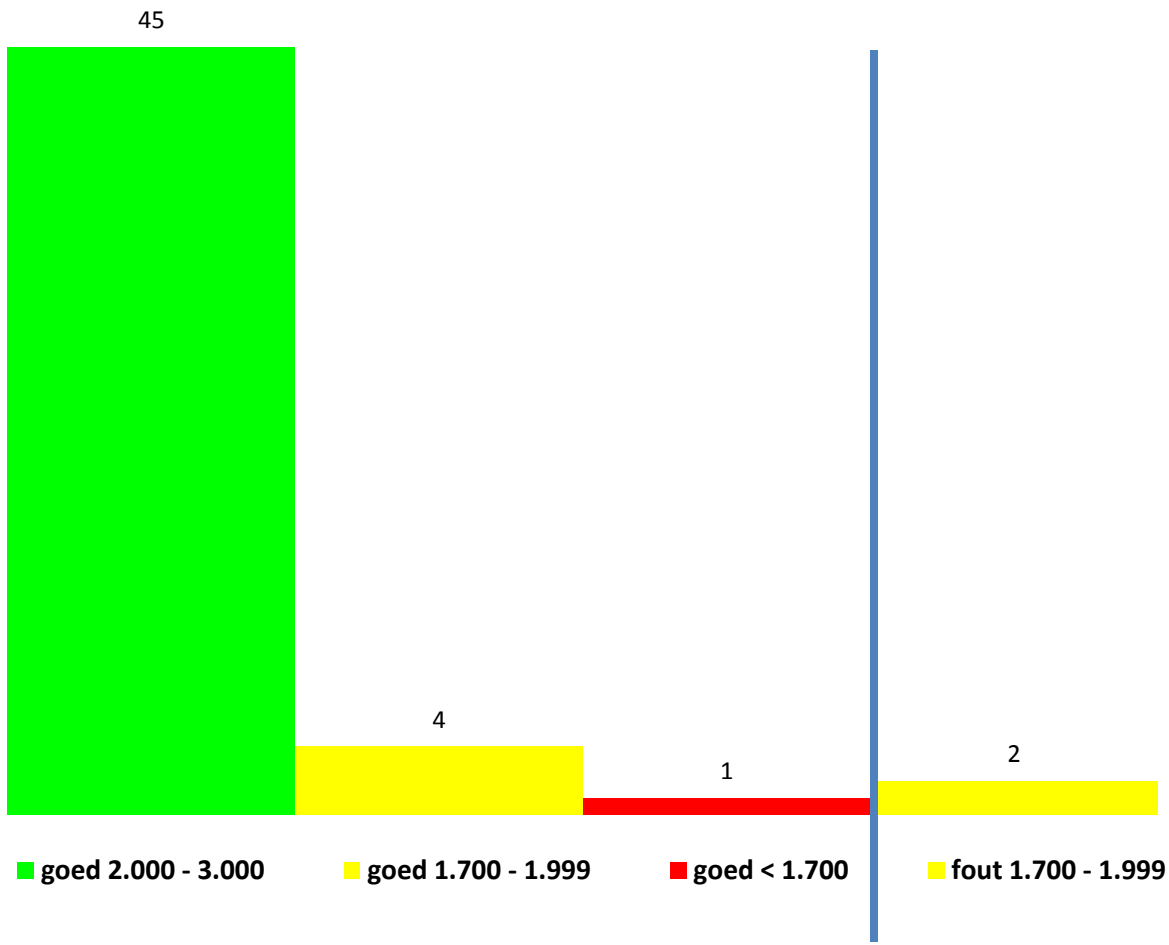


Binnen M.tuberculosis-complex

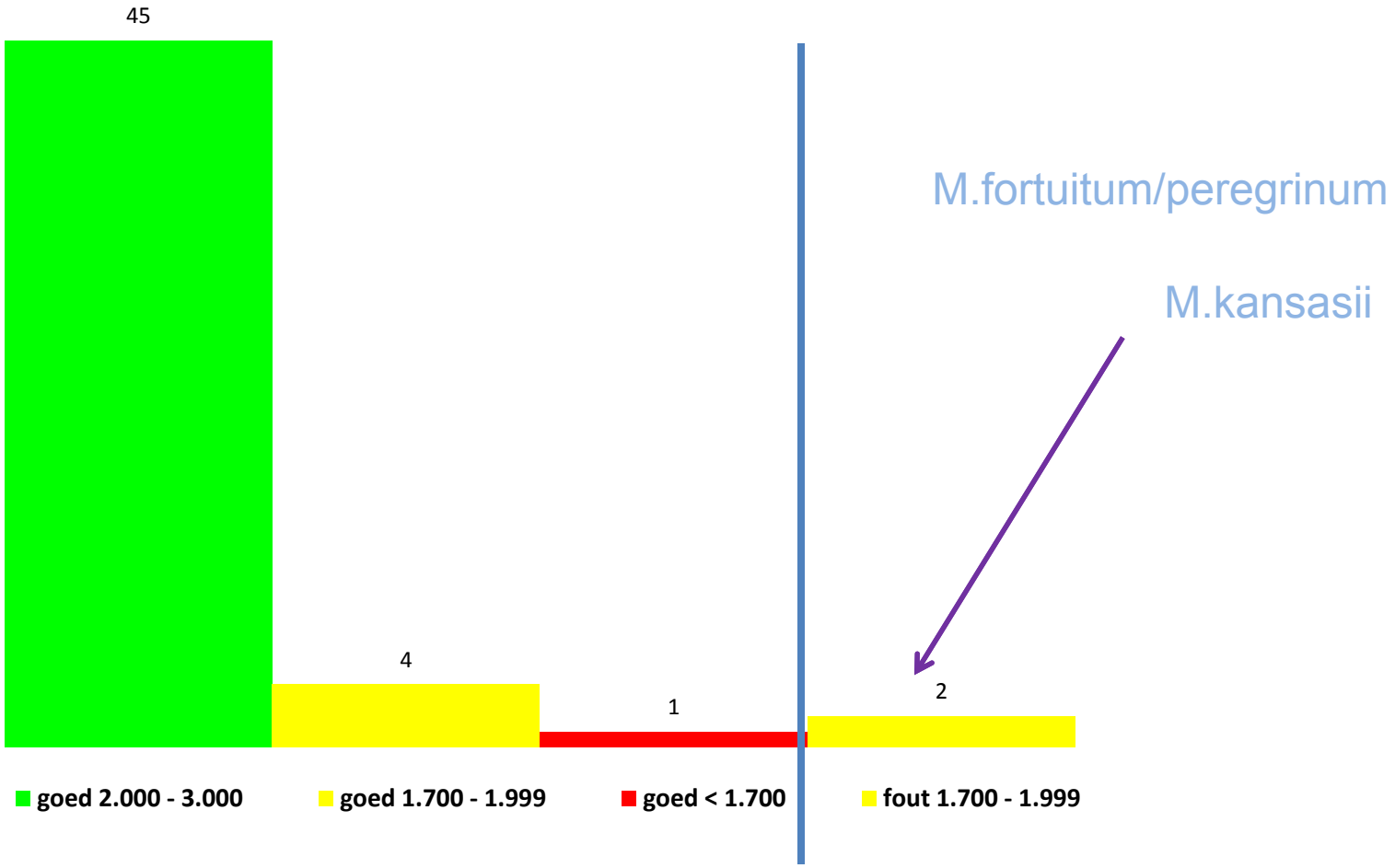
Resultaat M. bovis (n=2)



Resultaat atypische mycobacteriën(n=52)



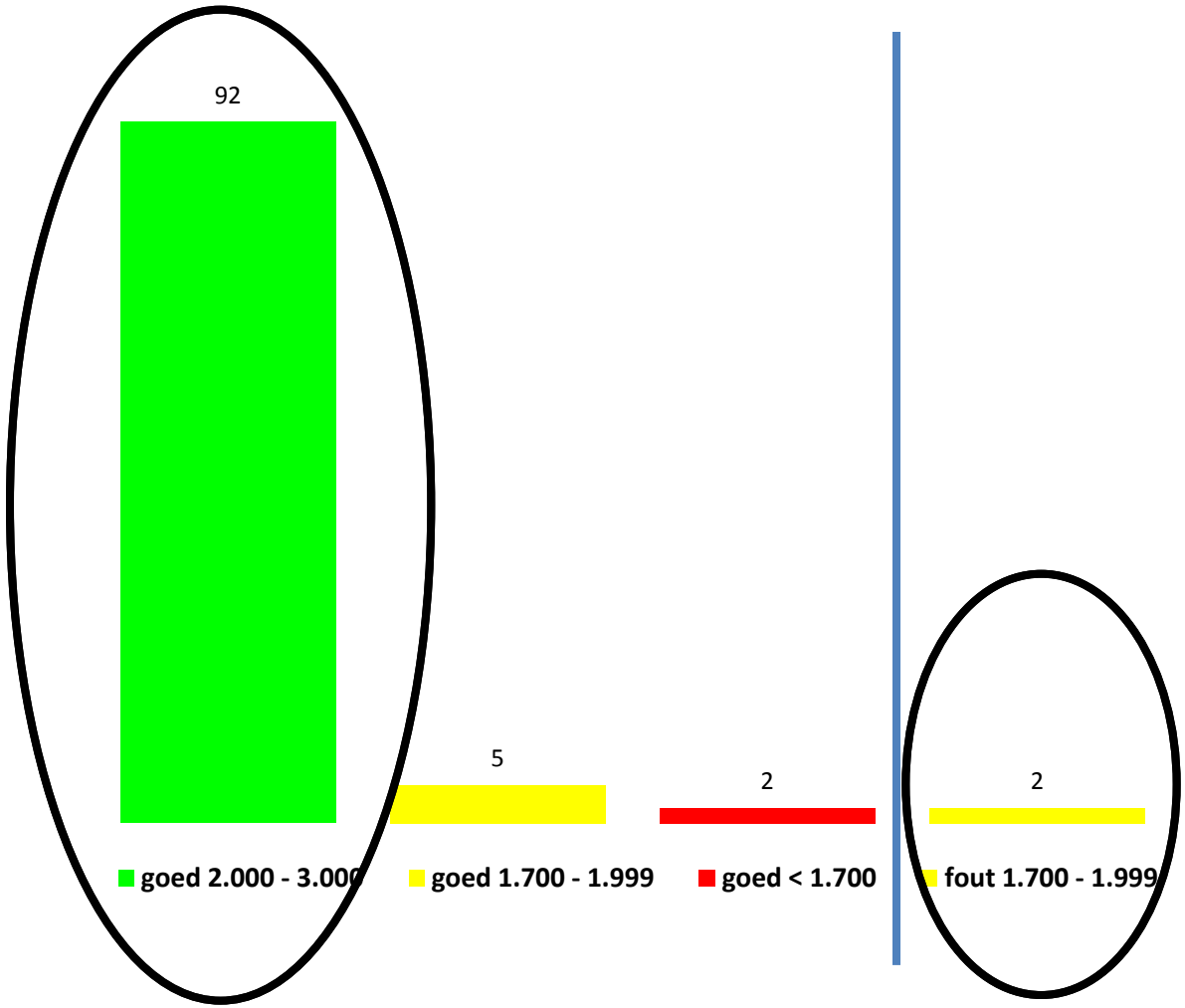
Resultaat atypische mycobacteriën(n=52)



Discrepanties

- Stam 1:
  - Routine: INNO-LiPA blot: M.fortuitum/peregrinum
  - Maldi-TOF: M.septicum (1.822)
  - 16S sequentie analyse: kan geen onderscheid maken tussen:
    - M.porcinum
    - M.fortuitum acetamidolyticum
    - M.fortuitum fortuitum
    - M.peregrinum
    - M.septicum
    - M.boenickei
- Stam 2:
  - Routine: INNO-Lipa blot: M.kansasii type III/IV/V
  - Maldi-TOF: M.bohemicum (1.806)
  - 16S sequentie analyse: niet gelukt

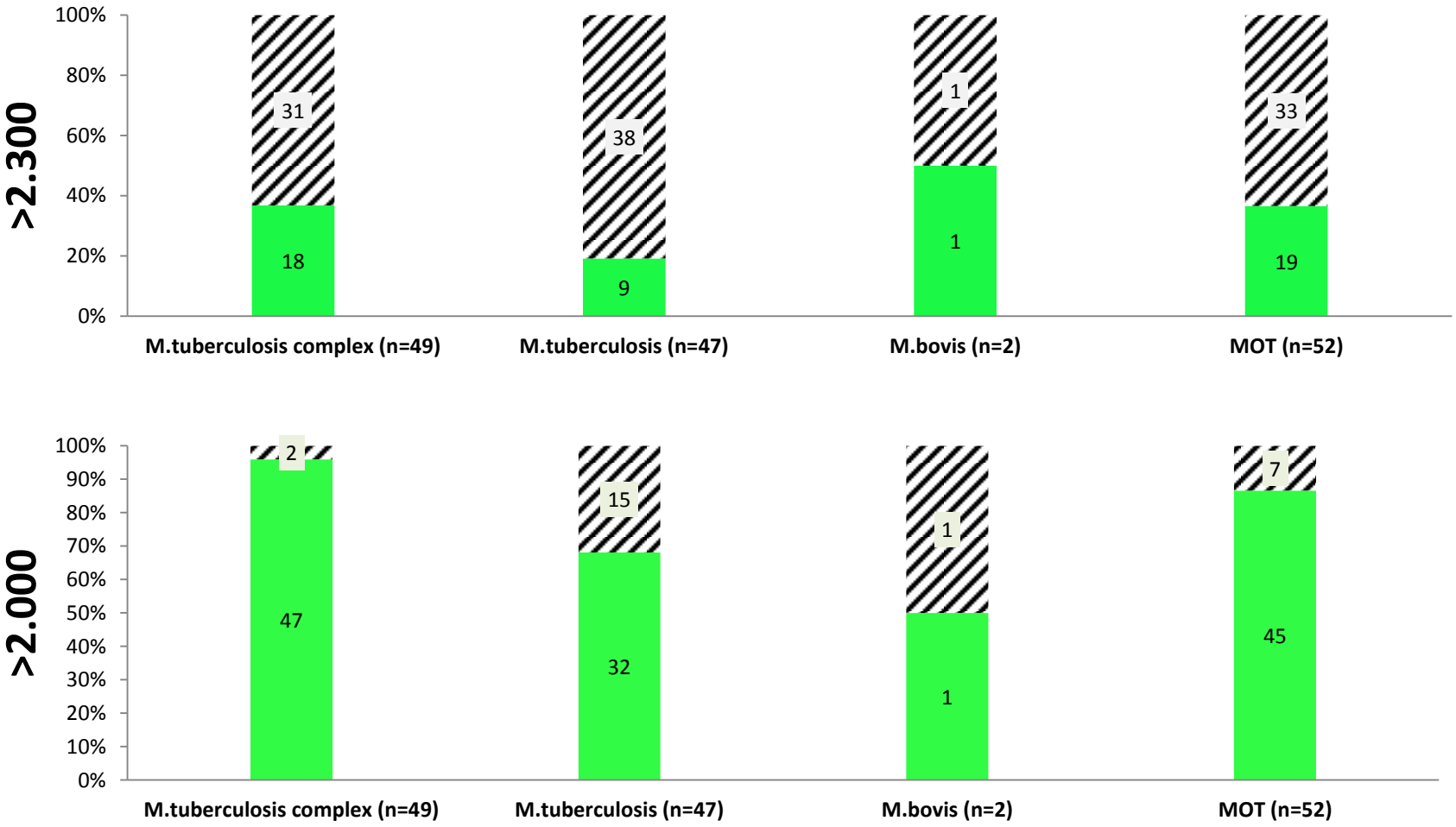
Resultaat totaal (n=101)



Acceptatie criteria Bruker

Range (Bruker)	Description (Bruker)
2.300 … 3.000	highly probable species identification
2.000 … 2.299	secure genus identification, probable species identification
1.700 … 1.999	probable genus identification
0.000 … 1.699	not reliable identification

Impact acceptatie grens



Conclusie

- Determinatie **100%** **correct** bij score >2.000
  - indien men niet tracht te differentiëren binnen het M.tuberculosis complex
- Determinatie <2.000 **niet accepteren**
- Het is niet nodig om bepalingen dubbel te spotten (97% concordantie)
- Uitbreiding database is gewenst

Werkwijze PAMM

Range (PAMM)	Description (PAMM)
2.000 … 3.000	identification accepted
0.000 … 1.999	identification NOT accepted

Resultaten

	>2.000	1.700 - 1.999	0.000 - 1.699
M. tuberculosis_complex	47	1	1
M. abscessus	3		
M. avium	10	1	
M. bohemicum	0	2	
M. chelonae	4		
M. fortuitum/peregrinum	5	1	
M. gordonae	5		
M. interjectum	2		1
M. intracellulare	5		
M. kansasii	4	1	
M. malmoense	1		
M. marinum	2		
M. scrofulaceum	0	1	
M. simiae	2		
M. xenopi	2		
Totaal	92	7	2

incorrecte identificatie

Resultaten over-all

	>2.000	1.700 - 1.999	0.000 - 1.699
M. tuberculosis_complex	47	1	1
M. abscessus	3		
M. avium	10	1	
M. bohemicum	0	2	
M. chelonae	4		
M. fortuitum/peregrinum	5	1	
M. gordonae	5		
M. interjectum	2		1
M. intracellulare	5		
M. kansasii	4	1	
M. malmoense	1		
M. marinum	2		
M. scrofulaceum	0	1	
M. simiae	2		
M. xenopi	2		
Totaal	92	7	2

91%

incorrecte identificatie

Resultaten

	>2.000	1.700 - 1.999	0.000 - 1.699
M. tuberculosis_complex	47	1	1
M. abscessus	3		
M. avium	10	1	
M. bohemicum	0	2	
M. chelonae	4		
M. fortuitum/peregrinum	5	1	
M. gordonae	5		
M. interjectum	2		1
M. intracellulare	5		
M. kansasii	4	1	
M. malmoense	1		
M. marinum	2		
M. scrofulaceum	0	1	
M. simiae	2		
M. xenopi	2		
Totaal	92	7	2

9%

91%

incorrecte identificatie

Very major / major / minor discrepancy

- Very major discrepancy
  - ➢ M.tuberculosis-complex niet onderkend
- Major discrepancy
  - ➢ Verkeerde determinatie score >2.000
    - Wordt op species niveau afgegeven
- Minor discrepancy
  - ➢ Verkeerder determinatie score <2.000
    - Wordt op genus niveau afgegeven
  - ➢ Geen determinatie door score <1.700

Very major / major / minor discrepancy

	Acceptatie **PAMM**	Acceptatie **Bruker**
very major discrepancy		
M.tuberculosis-complex niet onderkend	0	0
major discrepancy		
foute determinatie >2.0	0	0
minor discrepancy		
MOT determinatie niet geaccepteerd	7	-
MOT determinatie op genus niveau geaccepteerd	-	6
MOT determinatie niet geaccepteerd	-	1

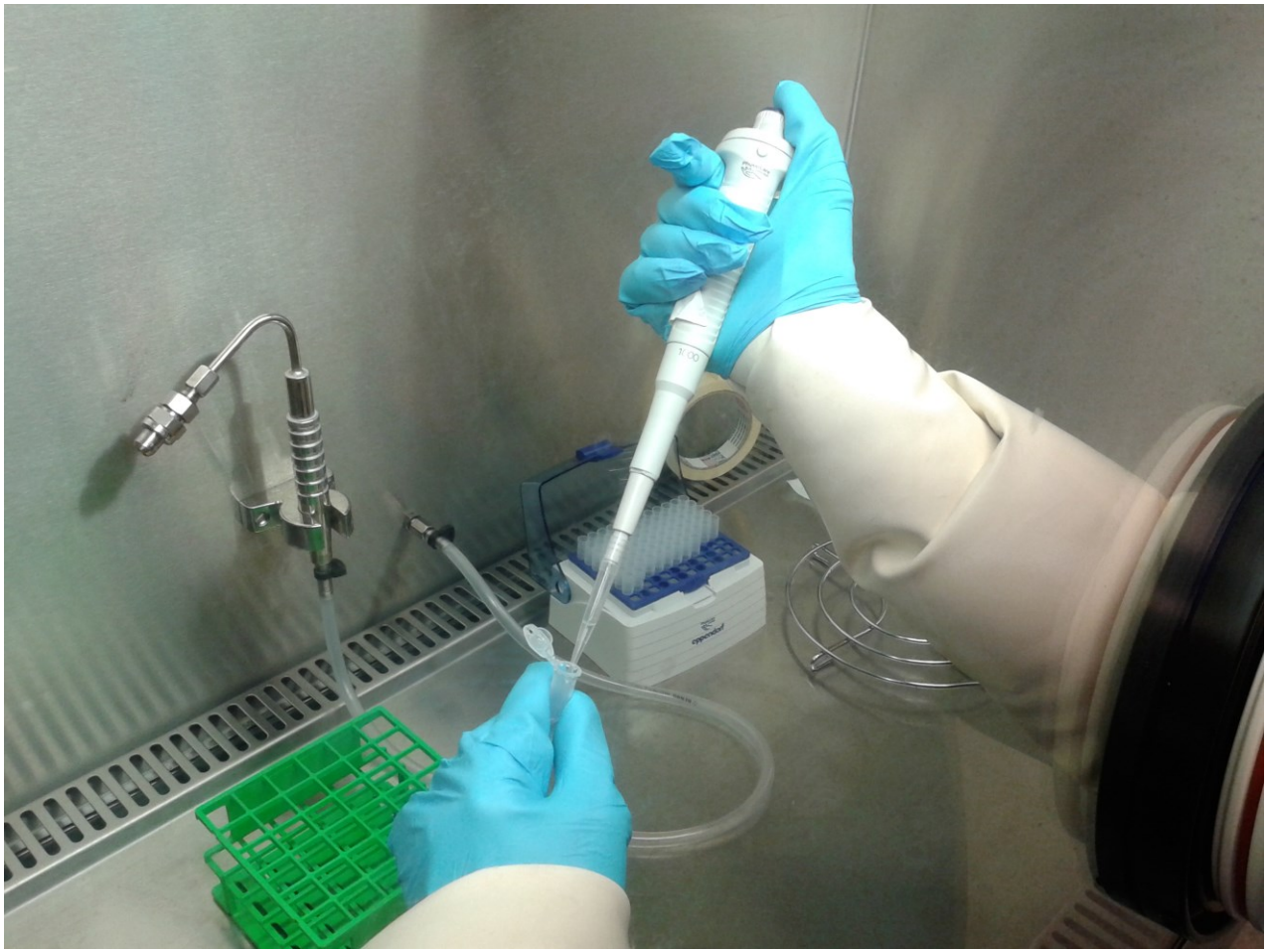
Aanbeveling

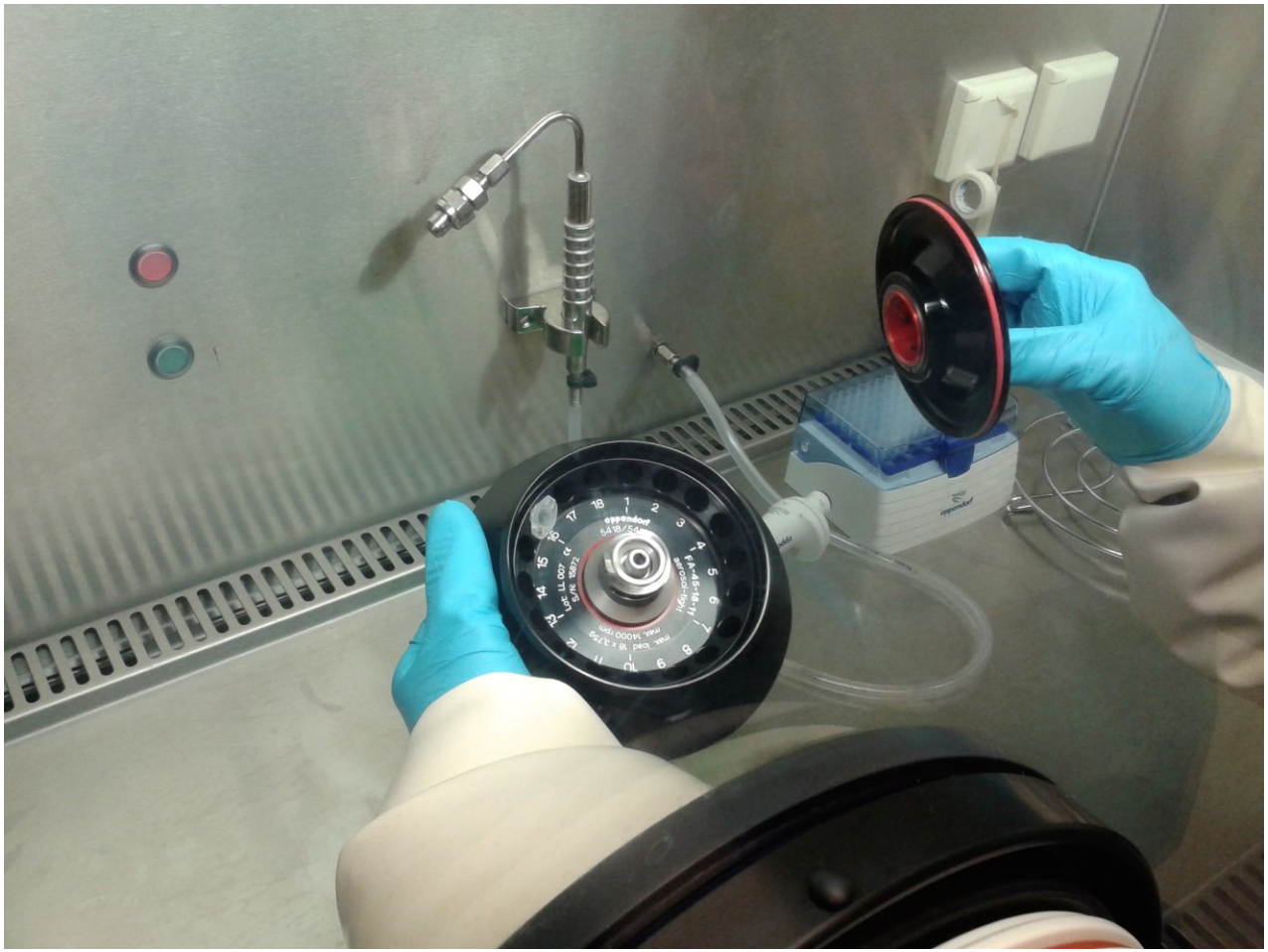
- M.tb-complex >2.000 accepteren, indien gewenst HAIN blot
- M.tb-complex <2.000 niet accepteren, HAIN blot
- MOT >2.000 accepteren
- MOT <2.000 niet accepteren, sequentie analyse

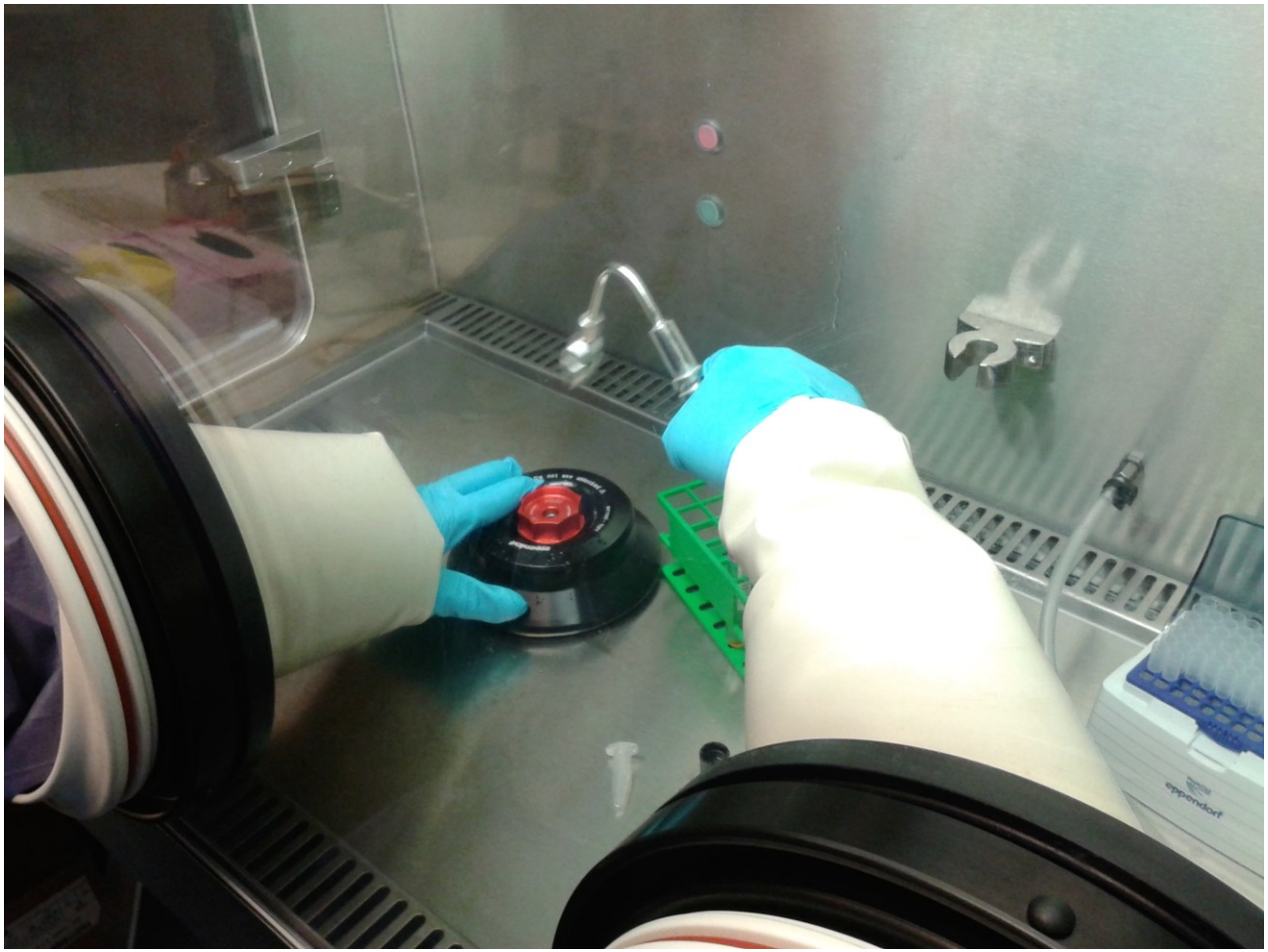

Clean Air
Clean Air

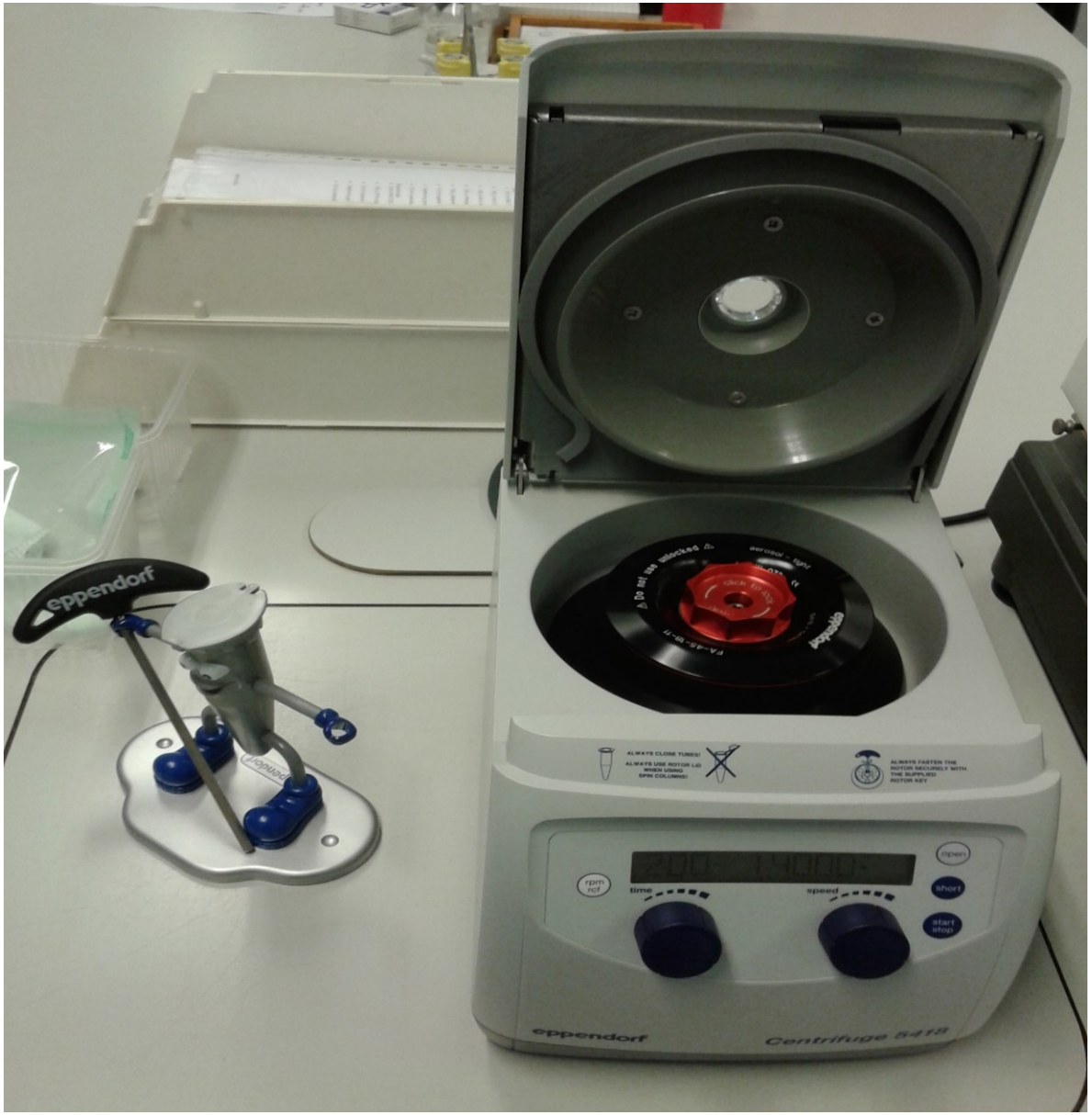
Extractie Bruker I

- Collect 1.2 ml liquid medium from the bottom of the cultivation tube in a 1.5 ml Eppendorf tube.
- Centrifuge at maximum speed (13,000 to 15,000 rpm) 2 min, decant supernatant
- Add 300 µl deionized water
- Add 900 µl $Ethanol_{abs}$, incubate for 10 minutes at room temperature for reducing viable cells
- Centrifuge at maximum speed (13,000 to 15,000 rpm) 2 min, decant supernatant
- Add 500 µl deionized water, suspend pellet
- Centrifuge at maximum speed 2 min, pipette of supernatant
- Add 50 µl deionized water, resuspend pellet
- Heat inactivation by boiling for 30 minutes
- Allow the samples to cool down, add 1200 µl pure ethanol pre-cooled (-18° C)
- Centrifuge at maximum speed 2 min and decant supernatant,
- Centrifuge again and remove residual ethanol carefully by pipetting
- Allow the pellet to air dry
- Add silica beads
- Add pure acetonitrile
- Use a vortex mixer at maximum speed for 1 minute
- Add 70% formic acid (same volume as acetonitrile) and mix by vortexing for approx. 5 seconds
- Centrifuge at maximum speed for 2 minutes
- Place 1 µl of supernatant on a MALDI target and allow to air dry
- Overlay with 1 µl of matrix solution immediately after drying




eppendorf
5418/5424
max. 14000 rpm


eppendorf


eppendorf
eppendorf
Centrifuge 5418
time
speed
rpm
rcf
open
short
start
stop

Extractie Bruker II

- Collect 1.2 ml liquid medium from the bottom of the cultivation tube in a 1.5 ml Eppendorf tube.
- Centrifuge at maximum speed (13,000 to 15,000 rpm) 2 min, decant supernatant
- Add 300 µl deionized water
- Heat inactivation by boiling for 30 minutes
- Add 900 µl $Ethanol_{abs}$, incubate for 10 minutes at room temperature for reducing viable cells
- Centrifuge at maximum speed (13,000 to 15,000 rpm) 2 min, decant supernatant
- Add 500 µl deionized water, suspend pellet
- Centrifuge at maximum speed 2 min, pipette of supernatant
- Add 50 µl deionized water, resuspend pellet
- Allow the samples to cool down, add 1200 µl pure ethanol pre-cooled (-18° C)
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- Add silica beads
- Add pure acetonitrile
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- Add 70% formic acid (same volume as acetonitrile) and mix by vortexing for approx. 5 seconds
- Centrifuge at maximum speed for 2 minutes
- Place 1 µl of supernatant on a MALDI target and allow to air dry
- Overlay with 1 µl of matrix solution immediately after drying

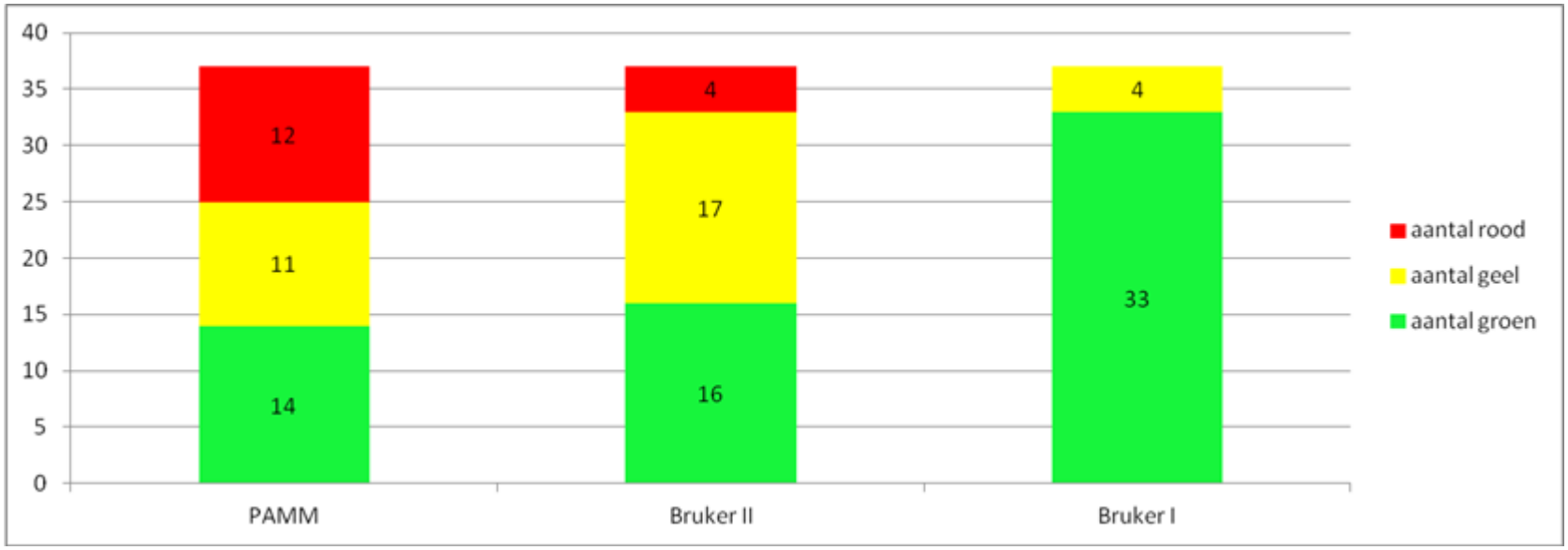
Extractie methode PAMM

- Collect 1.2 ml liquid medium from the bottom of the cultivation tube in a 1.5 ml Eppendorf tube.
- Heat inactivation by boiling for 30 minutes
- Centrifuge at maximum speed (13,000 to 15,000 rpm) 2 min, decant supernatant
- Add 300 µl deionized water
- Add 900 µl $Ethanol_{abs}$, incubate for 10 minutes at room temperature for reducing viable cells
- Centrifuge at maximum speed (13,000 to 15,000 rpm) 2 min, decant supernatant
- Add 500 µl deionized water, suspend pellet
- Centrifuge at maximum speed 2 min, pipette of supernatant
- Add 50 µl deionized water, resuspend pellet
- Allow the samples to cool down, add 1200 µl pure ethanol pre-cooled (-18° C)
- Centrifuge at maximum speed 2 min and decant supernatant,
- Centrifuge again and remove residual ethanol carefully by pipetting
- Allow the pellet to air dry
- Add silica beads
- Add pure acetonitrile
- Use a vortex mixer at maximum speed for 1 minute
- Add 70% formic acid (same volume as acetonitrile) and mix by vortexing for approx. 5 seconds
- Centrifuge at maximum speed for 2 minutes
- Place 1 µl of supernatant on a MALDI target and allow to air dry
- Overlay with 1 µl of matrix solution immediately after drying

Protocol vergelijking

n = 37

7 M.tuberculosis complex, geen Bovis
30 MOT



Workflow in het Laboratorium

Zuurvaste staven gekweekt:
determinatieve pcr/hybridisatie

Positief: Reversed line blot (HAIN Mtb-complex)

Negatief: Maldi buiten de cytolator

Cave: dubbel infecties

Dankbetuiging

Ellen Retera

Saskia van Asten

Ruud Hegge

PAMM

STICHTING PAMM
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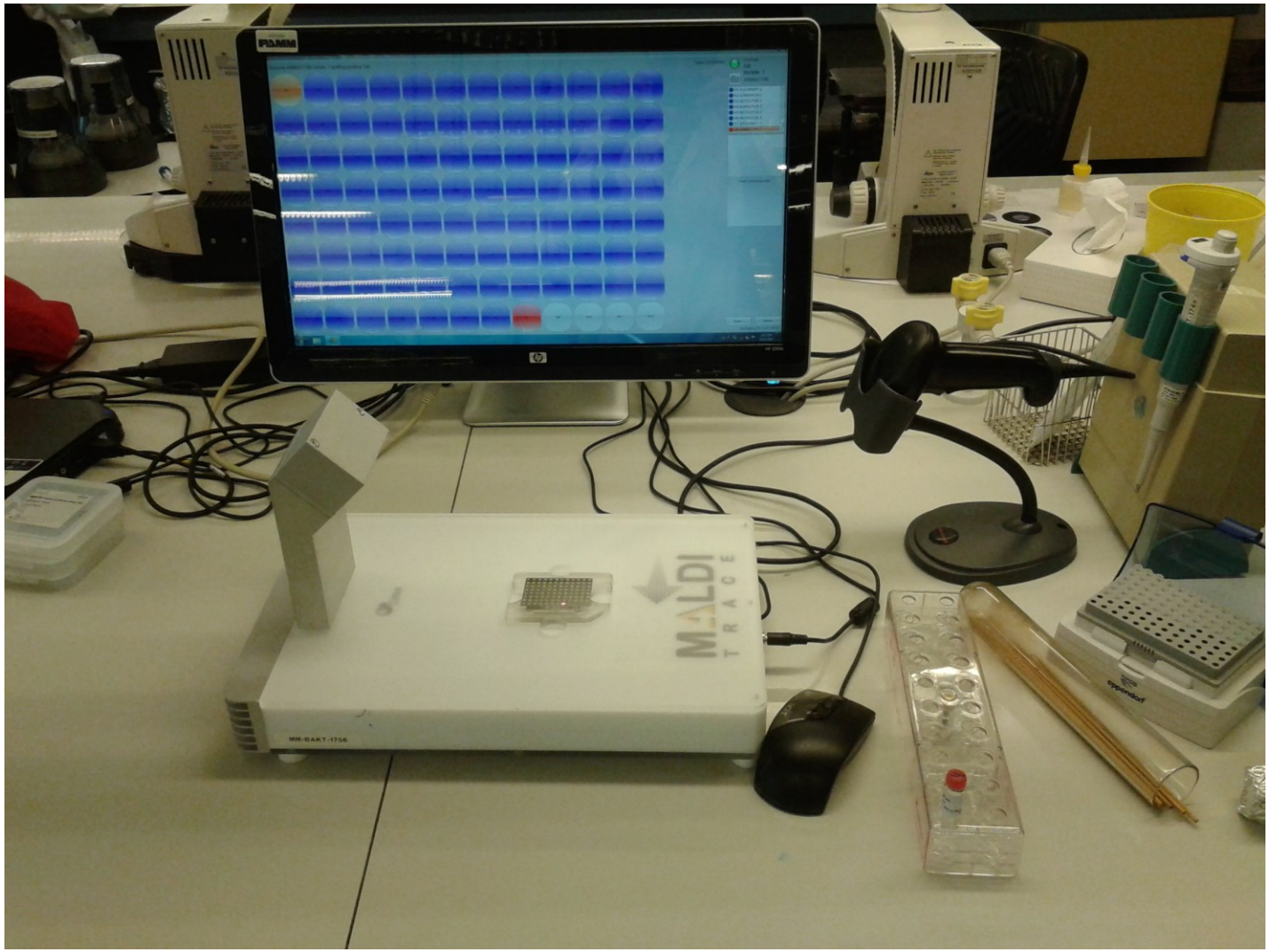
Bedankt voor jullie aandacht!


PAMM
MALDI
TRACE
MM-BAKT-1756