MALDI-TOF : 3 YEARS OF EXPERIENCE IN THE UNIVERSITY HOSPITAL OF LIEGE

INTEGRATION IN THE ROUTINE WORKFLOW AND PARTICIPATION TO THE FILAMENTOUS FUNGI PROJECT

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Start with MALDI-TOF

- 1. Validation
- 2. Routine bacterial identifications

1. Validation (1)

May 2009: Microflex [®](Bruker)

Bruker's database evaluation in parallel with classical methods for 2 months

Interpretation criteria:

MS Score ≥ 2.3: **Excellent identification**

MS Score ≥ 2.0 et < 2.3 and the first 3 results are identical: Good identification

MS Score ≥ 1.7 et < 2.0 and the first 3 results are identical: Acceptable identification

1. Validation (2)

418 tested microorganisms

	Number	In agreement with the reference method
Excellent identifications	90	100%
Good identifications	190	100%
Acceptables identifications	42	100%
Inconclusive identifications	36	
Unidentified	60	

- **Total:** 322 identifications on 418 (77%)
- No misidentifications

1. Validation (3)

Evaluation by bacterial groups

Analysis in parallel with classical methods:

- Of the 30 most frequently encountered species
 - 10 to 20 strains by species
- Of the rarer species when encountered (still in progress)

\rightarrow No misidentifications*

2. Routine bacterial identifications

Since July 2009:

First line method for all bacterial identifications: Microflex[®] MALDI-TOF MS

(Bruker Daltonics)

Based on the pairing scores and the defined interpretation criteria:

- Acceptance of the identification
- Second line identification methods when necessary: classical phenotypic methods



2. Routine bacterial identifications

Evaluation: February 2010

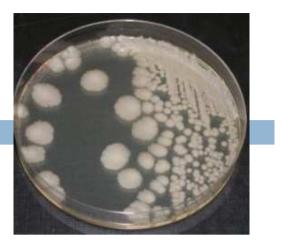
Bacterial group	Number of tested strains	Accepted identifications using the algorithm (%)
Enterobacteriaceae	541	98,7
Non fermentative Gram- negative bacteria	207	95,6
Staphylococcus	83	97,5
Streptococcus	110	89
Haemophilus / Moraxella	42	97,6
Neisseria sp.	4	100
Campylobacter sp.	5	100
Anaerobes	21	95,2
TOTAL	1013	96,7

Other applications in CHULg

- 1. Yeasts
- 2. Filamentous fungi
- 3. Direct identifications from positive blood cultures

1. Yeasts

- Direct identification
 - bad results if using the bacterial algorithm



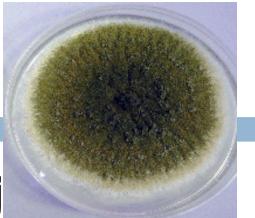
- Formic acid liquid extraction
 - Little contributing and too long for integration in the routine work
- \rightarrow Direct double deposition

→ Extraction by addition of formic acid on the target plate

Modified interpretation criteria:

MS Score \geq 1.4 and the first 3 results are identical and appearance of colonies on agar consistent with

2. Filamentous fungi (1)



Bruker filamentous fungi proj

- Initiated in 2010
- Participation invitation to all interested laboratories involved in fungi diagnosis
- Aim: Contribution of experts to a database creation
- Participating labs from:
 - Europe: Belgium, Netherlands, France, Germany, UK…
 - USA
 - South Africa

2. Filamentous fungi (2)

Project plan part 1

Inter-laboratory reproducibility test:

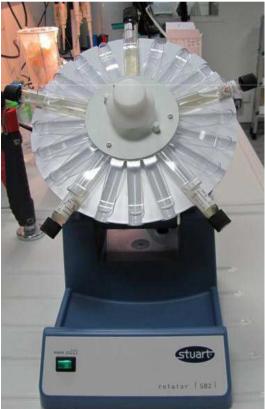
- Capacities of each partner to reproduce:
- Cultivation
- MALDI sample preparation
- MALDI measurement of filamentous fungi according to the recommendations of Bruker
- 6 tests organisms: 4 Aspergillus sp, 1 Microsporum canis and 1 Chrysosporium keratinophylum

2. Filamentous fungi (3)

Cultivation in liquid medium (Sabouraud) To avoid contamination with agar when harvesting To standardize the growth state



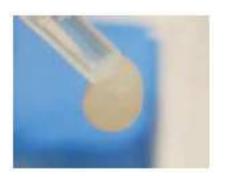
Incubation (30 or 35°) until biological material is observed (1 to 6 days regarding to the species).

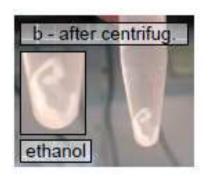


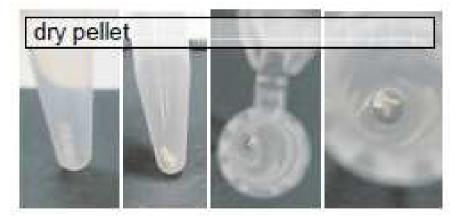
2. Filamentous fungi (4)

Harvesting procedure

- Centrifugation of broth containing the fungi
- Water and ethanol washing of the pellet
- Formic acid extraction on dry pellet







Analysis in the Microflex

2. Filamentous fungi (5)

Project plan part 2

 Sending by the projects participants of well characterized strains to Bruker central lab

 Creation of 2 complementary database with these strains
 In total 244 filamentous fungi spectra
 Now available for participating labs

2. Filamentous fungi (6)

CHULg:

Enrichment of the DB with:

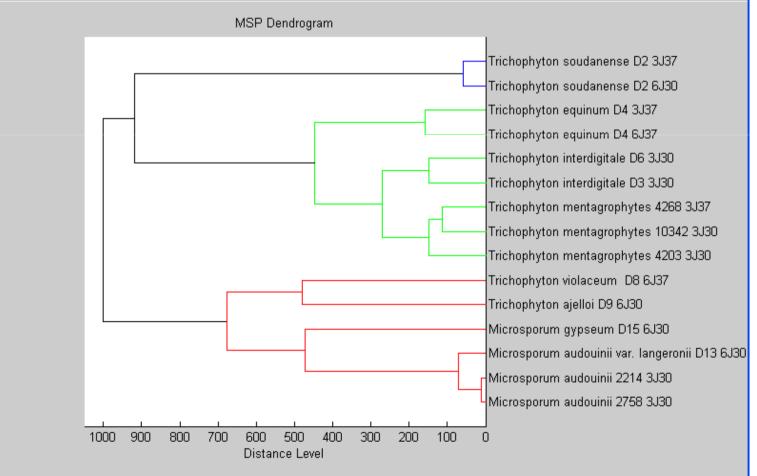
- Reference strains
- Well characterized (sequenced) clinical strains

Necessity of a modified AutoX method

- Slightly highered initial laser energy and Matrix Blast
- Lower « Minimum Intensity Threshold »

2. Filamentous fungi (7)

CHULg: Self introduced spectra (still



2. Filamentous fungi (8)

CHULg:

- From young cultures (3-4 days maximum)
- \rightarrow Direct triple deposition
- → Extraction by addition of formic acid on the target plate
 - Microscopy control still essential
 - Sequencing will be performed to confirm obtained with MALDI and microscopy.



2. Filamentous fungi (9)

CHULg: First results

Good IDs to species with:

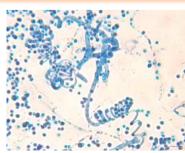
Aspergillus sp., Fusarium sp.,
 Scopulariopsis sp.

Good IDs to gender with:

Trichophyton sp. VS Microsporum sp.









3. Direct IDs from positive blood cultures (1)

March-April 2011

- Evaluation of 2 different bacterial extraction methods
 - MALDI Sepsityper[®] Kit (Bruker)
 - Saponin home made method



113 BacT/Alert[®] (bioMérieux) anaerobic positive blood cultures without charcoal



3. Direct IDs from positive blood cultures (2)

Criteria for acceptation of the species identification

First three results having the best matches with the MALDI Biotyper database are identical, whatever the scores were.

Reference method

Conventional method: MALDI-TOF identification after subculture on agar plate

3. Direct IDs from positive blood cultures (3)

Monomicrobial blood cultures (107)

	Direct identi	fications (%)	
	Sepsityper kit	Saponin lysis	
Gram negative (40)	85%	93%	p = 0.4497
Gram positive (67)	58%	52%	p = 0.4227

Polymicrobial blood cultures (6):

different species never all identified with the direct method but only one of them 3. Direct IDs from positive blood cultures (4)

Integration in the routine work (when sufficient staff)

Clinical investigation: assessing the impact of a faster identification on the antibiotics administration

Untreated patient: Administration of a correct treatment in 86% of cases

Patient already treated: Adaptation of the treatment in only 33% of cases that needed

3. Direct IDs from positive blood cultures (5)

Need to

Inform clinicians on:

Natural resistance of bacteria

The existence and access of guidelines based on our local epidemiology

Target services for which adopt this technique

Integration in the routine workflow

- 1. Organization of the bacteriology lab
- 2. MALDI Docking station
- 3. Connections between devices

1. Organization of the bacteriology lab

LIS : GLIMS

4 « paperless » reading stations

- Urines/stools
- Respiratory samples
- Blood cultures
- Others (liquids, biopsies, genital samples...)

Readings and encoding in the LIS in real time : during the morning

MALDI-TOF



Currently: 1 deposition station ~ 4 series of identifications per

day

- Around 9.00 AM: IDs from blood cultures
- Around 12.00 AM: IDs from the 3 other reading stations
- Around 14.00 AM: IDs from anaerobes, ID checks...
- Around 16.30: IDs from isolations, IDs from blood cultures...

On average

700 MAI DI denneitione ner week

2. MALDI Docking station

bioMérieux software

➡ Link between:



- Samples
- AST cards
- Positions on the target plate

Transfer to Vitek[®] 2 and MALDI-TOF PC

bioMérieux Docking station Work sheet

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« Mass spectrometry » work

« Vitek 2 » work space

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bioMérieux Docking station Application in CHULg

Use only for the creation of MALDI-TOF plates

No use of the VITEK[®] 2 fonction via the Docking station, for the moment.

 AST preparation by conventional methods (VITEK[®] 2 via Smartcarrier or agar diffusion) after identification. 3. Connections between devices (1) Transfer to MALDI Biotyper

An Excel file is generated and transfered to MALDI by computer network

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Connections between devices (2) Export to GLIMS

After analysis: Exportation of all IDs

- Reception of the IDs in GLIMS
- Raw data in the « Internal comment » field

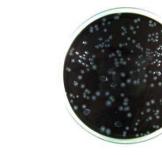
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ID validation

ID confirmation by technicians based on:

- The 10 propositions and pairing scores.
- Colonies morphology checking (consistency)

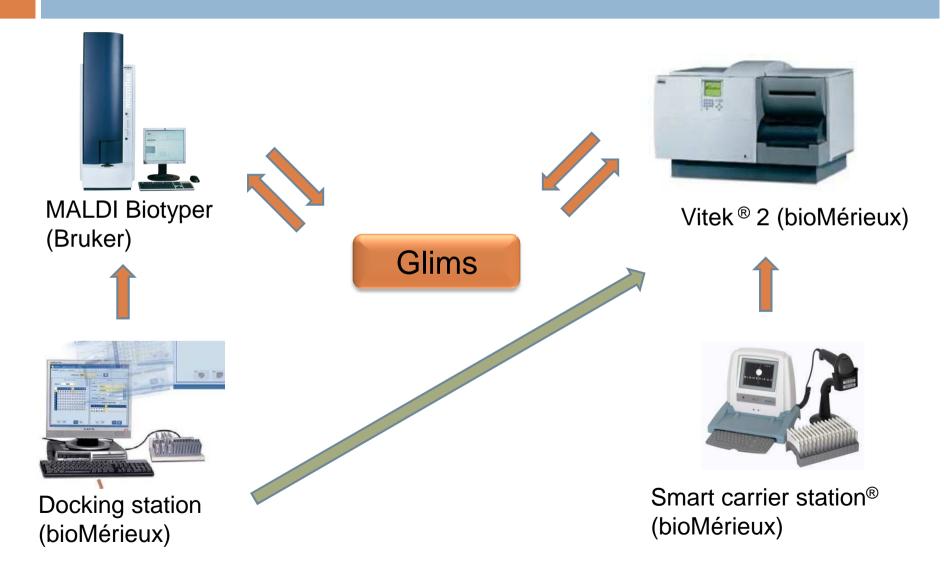
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ID validation by a clinician biologist.

3. Connections between devices (3)



Discussion and conclusions

Discussion (1)

Docking station (bioMérieux)

- MALDI projects creation from the bench
 - Removing the risk of copying errors
- Screen viewing of the MALDI plate
 - Reducing the risk of deposition errors
- Possibility of preparing a Vitek[®] AST simultaneously
- Configuration of the software and PC connections: Invaluable assistance of
 - Bruker
 - Our local IT

Discussion (2)

Developments to consider

Acquisition of more Docking stations

- Budget
- Adaptation of the computer network

Deposition the same time as the reading

- Time saving ? Workflow ?
- Mixing of activities

Deposition the same time as Vitek AST suspension

- Quality (working from the same colony)
- Time saving ? Workflow ?
- Mixing of activities

Conclusions

MALDI Biotyper

Essential for our routine identifications

Filamentous fungi

- Promising database: Improvements and enrichment needed
- Microscopy still essential

bioMérieux Docking station

- MALDI projects creation facilitated
- Future evaluation of simultaneous AST preparation
- Existence of other Docking stations: Bruker, Copan

Computer connections:

- Work simplification
- Auality accurance