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

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

Cécile Meex

Medical Microbiology

CHU Liège

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)

→ **No misidentifications***

2. Routine bacterial identifications (1)

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 (2)

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

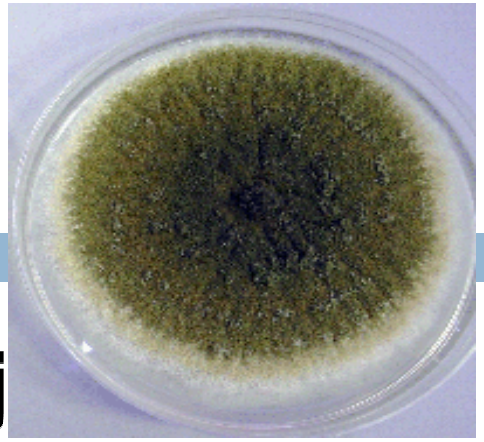
→ **Direct double deposition**

→ **Extraction by addition of formic acid on the target plate**

Modified interpretation criteria:

MS Score ≥ 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

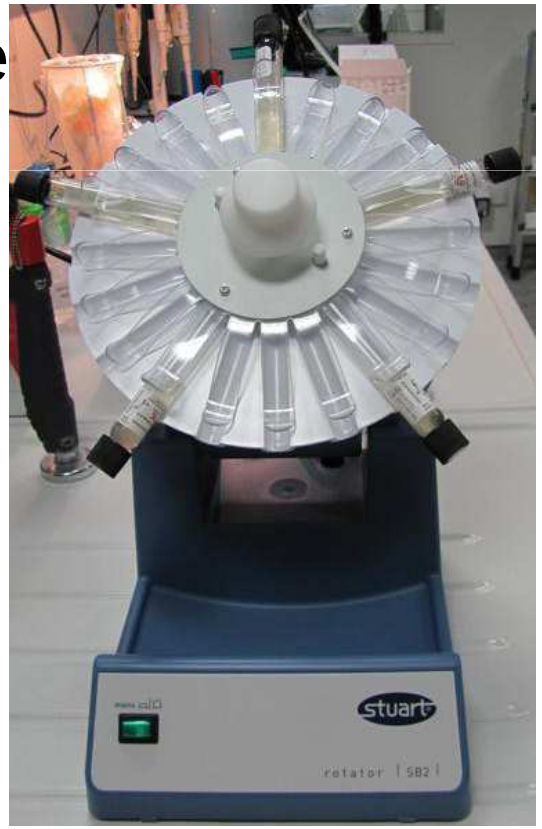
- **Inter-laboratory reproducibility test:**
  Capacities of each partner to reproduce:
  - Cultivation
  - MALDI sample preparation
  - MALDI measurement of filamentous fungi

  according to the recommendations of Bruker

- 6 tests organisms: 4 *Aspergillus sp*, 1 *Microsporum canis* and 1 *Chrysosporium keratinophylum*

2. Filamentous fungi (3)

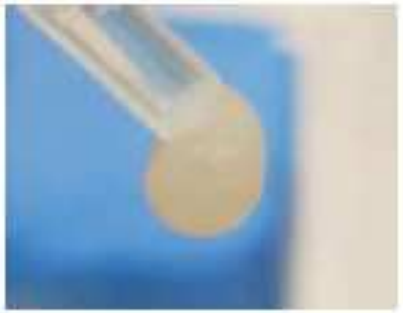
- Cultivation in liquid medium (Sabouraud)
  - To avoid contamination with agar when harvesting
  - To standardize the growth state

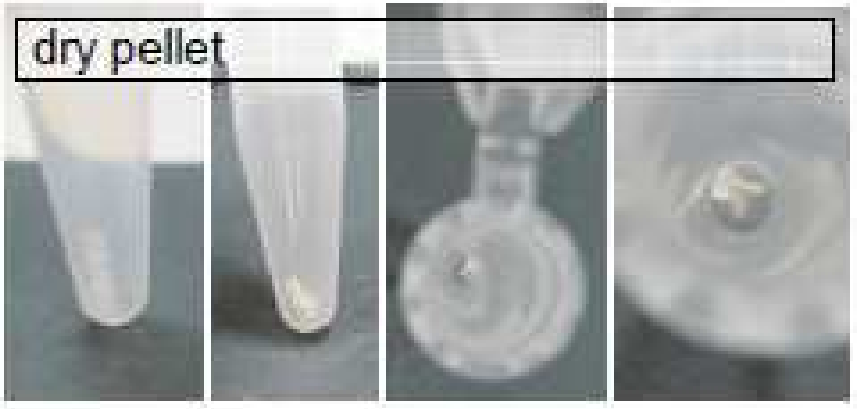


Incubation (30 or 35℃) until biological material is observed (1 to 6 days regarding to the species).

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

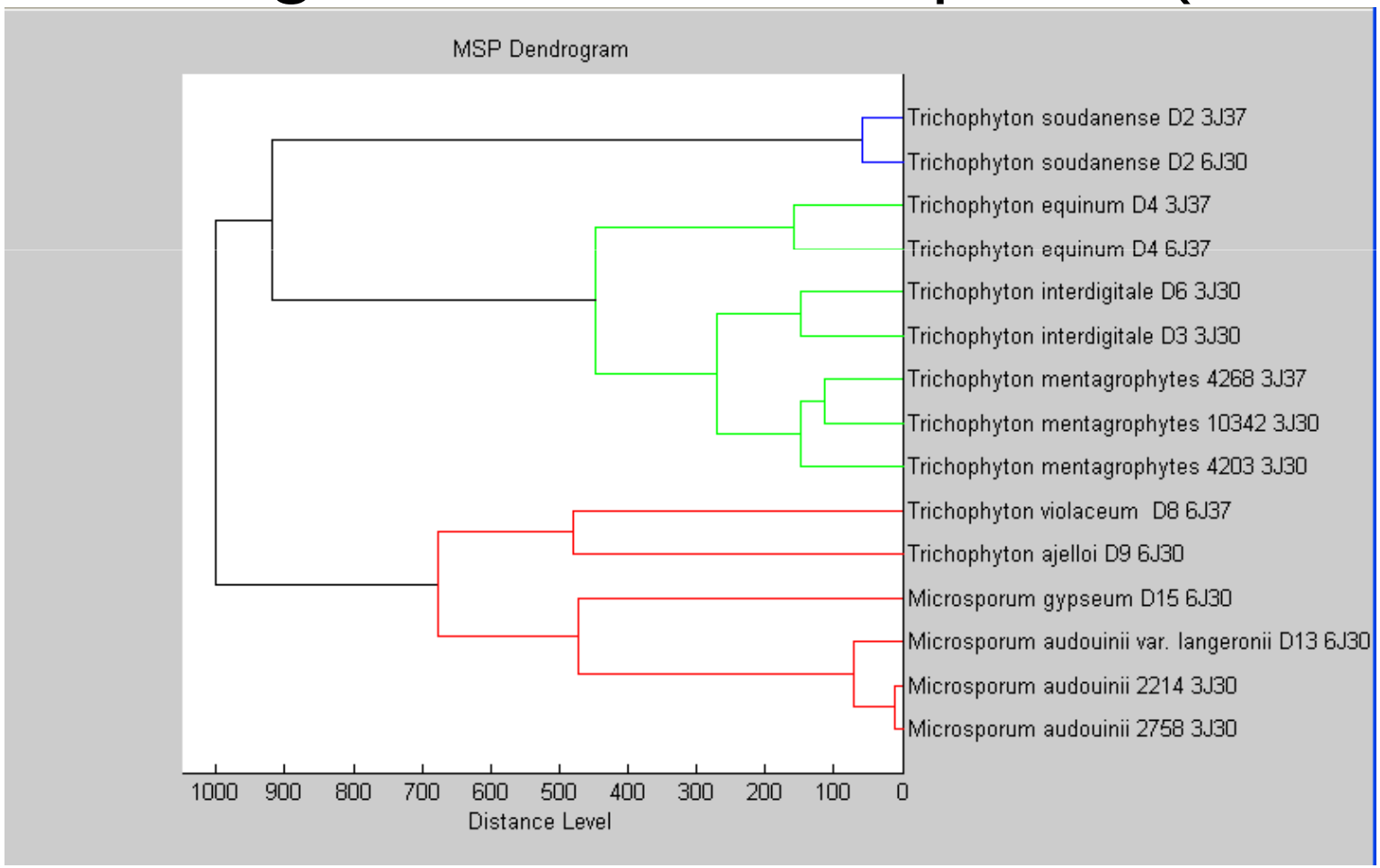
- Sending by the projects participants of well characterized strains to Bruker central lab
- Creation of 2 complementary database with these strains
  - In total 244 filamentous fungi spectra
  - Now available for participating labs

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)

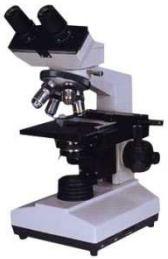
- **CHULg:**

From young cultures (3-4 days maximum)

→ Direct triple deposition

→ Extraction by addition of formic acid on the target plate

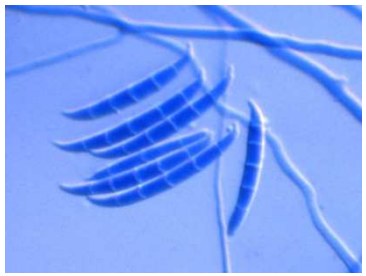
  - Microscopy control still essential
  - Sequencing will be performed to confirm obtained with MALDI and microscopy.

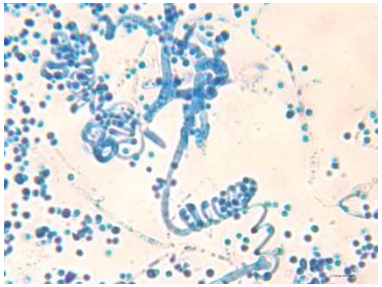


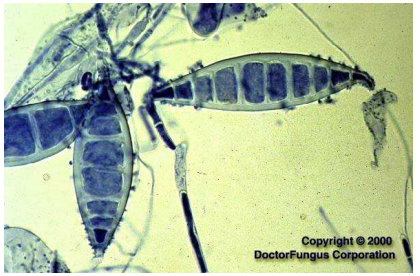
2. Filamentous fungi (9)

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 identifications (%)		
	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

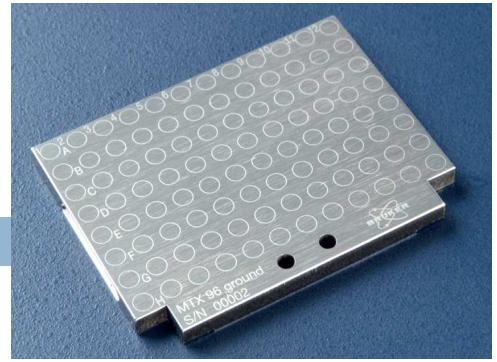
Integration in the routine workflow

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 MALDI depositions per week**

2. MALDI Docking station

bioMérieux software

⟹ **Link between:**

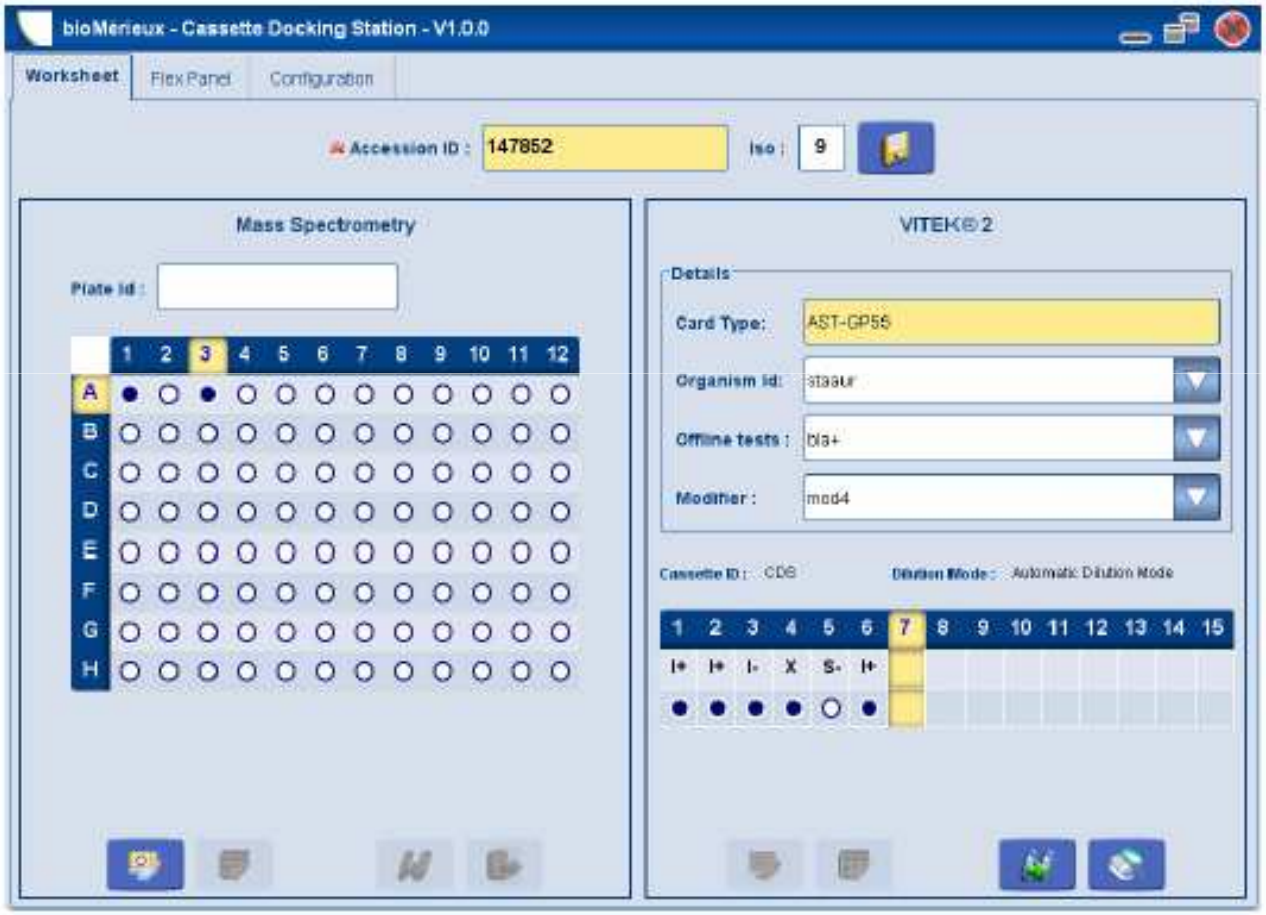
- Samples

- AST cards

- Positions on the target plate

⟹ **Transfer to Vitek® 2 and MALDI-TOF PC**

bioMérieux Docking station
Work sheet



« Mass spectrometry » work space

« Vitek 2 » work space

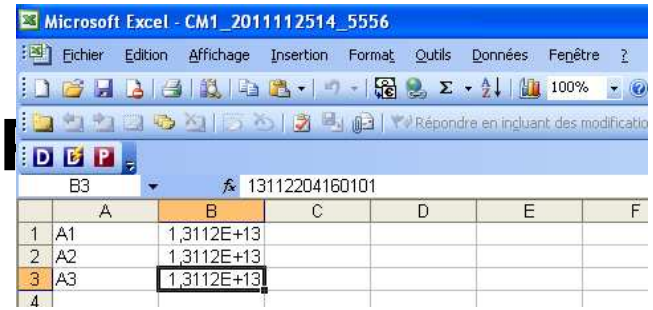
bioMérieux Docking station Application in CHULg

- 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.**

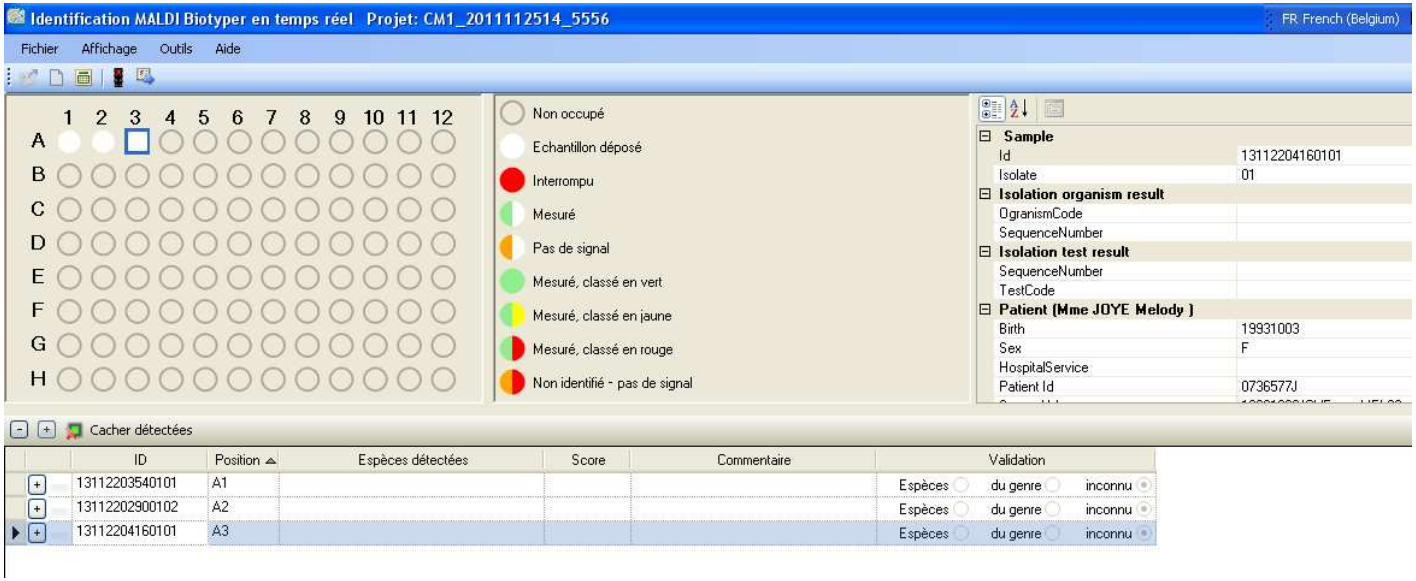
3. Connections between devices

(1) Transfer to MALDI Biotyper

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



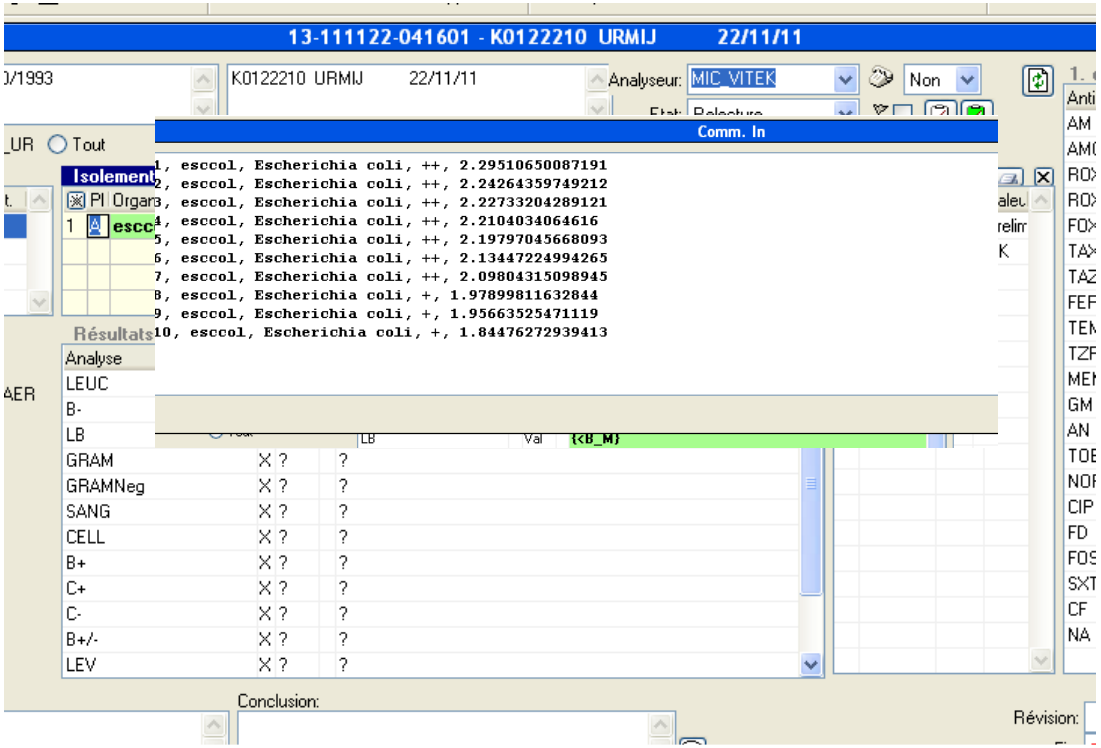
- **Import in the MALDI Biotyper software**



3. 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**



ID validation

- **ID confirmation by technicians based on:**
  - **The 10 propositions and pairing scores.**
  - **Colonies morphology checking (consistency with**

- **ID validation by a clinician biologist.**

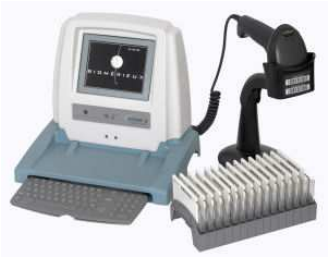
3. Connections between devices (3)

MALDI Biotyper (Bruker)

Vitek ® 2 (bioMérieux)

Glims

Docking station (bioMérieux)



Smart carrier station® (bioMérieux)

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
  - Quality assurance