



EUROPEAN COMPLIANCE  
ACADEMY

# Rapid identification of environmental bacteria by MALDI-TOF Mass Spectrometry

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# Rapid identification of environmental bacteria by MALDI-TOF Mass Spectrometry VITEK<sup>®</sup> MS system (bioMerieux)

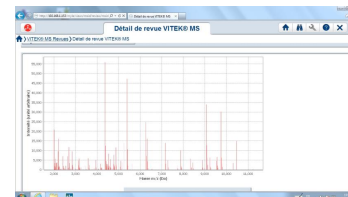
- MALDI-TOF MS
  - Definition – Principle
  - MALDI-TOF VITEK<sup>®</sup> MS System
  - Overview and workflow of analysis
- Evaluation
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    - ⇒ Reference identification
  - Databases
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    - ⇒ No ID
    - ⇒ Bad ID
    - ⇒ Robustness
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      - Duplicates
      - Cultures
      - Operators

# MALDI-TOF MS: Definition-Principle

## Matrix Assisted Laser Desorption Ionisation - Time of Flight Mass Spectrometry

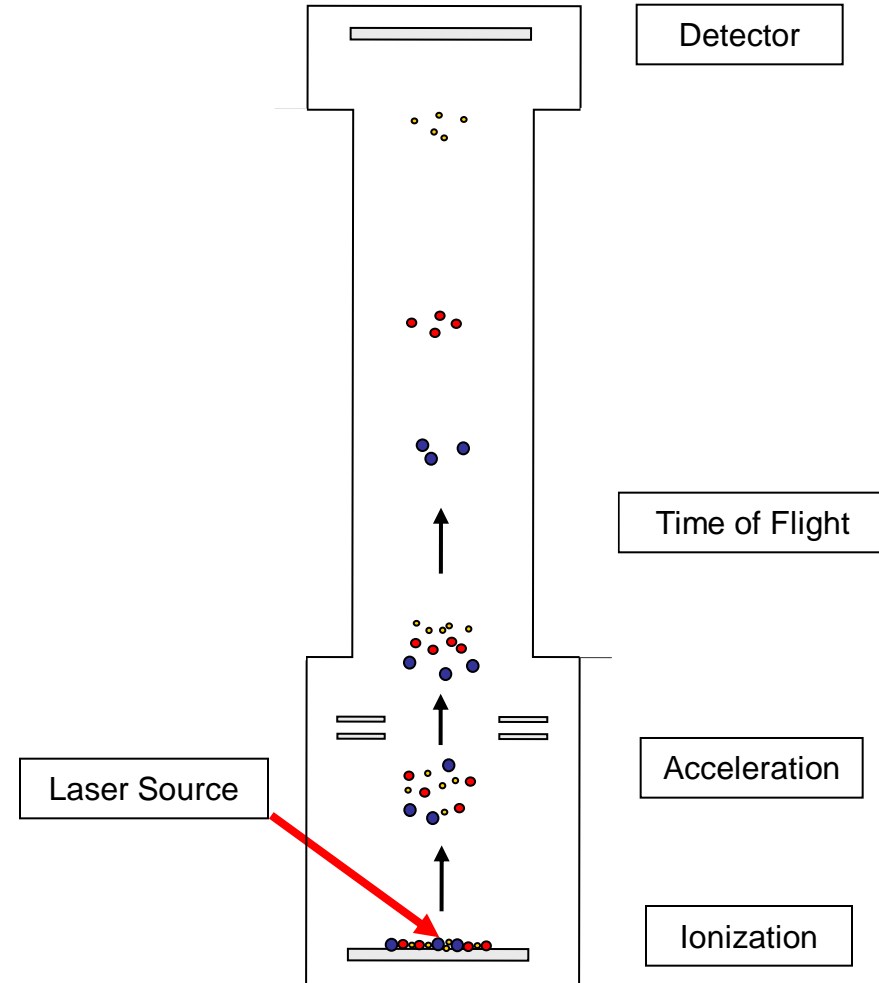
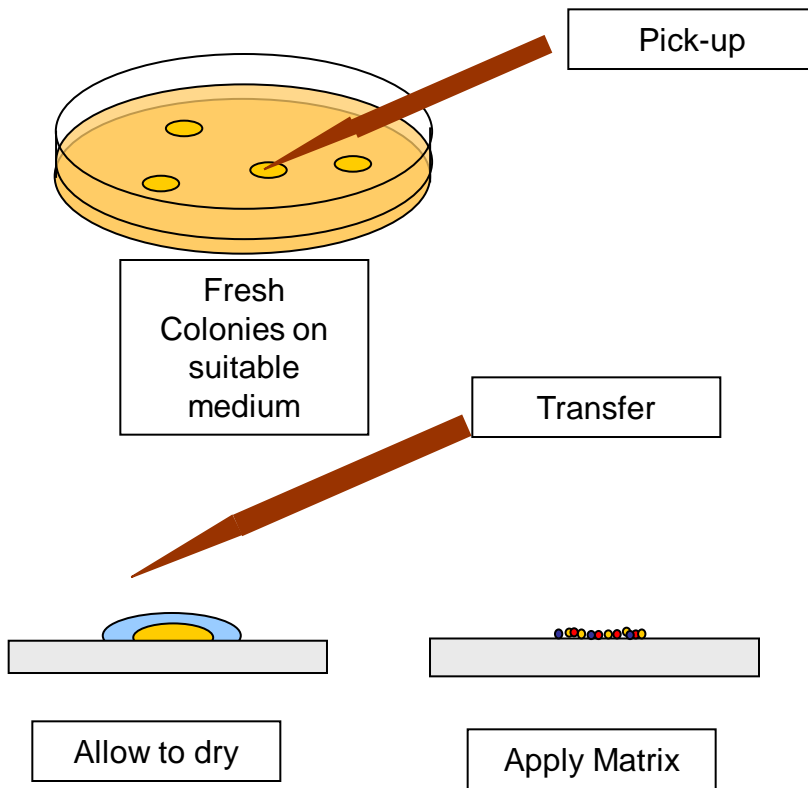
Time of flight analysis of ionised molecules by using a matrix and laser for ionisation and desorption of molecules

- micro-organisms spotted on slide
- laser beam targetting spot on slide : matrix drives energy to molecules
- desorption and ionisation of molecules of different MW
- separation of charged molecules according to their sizes in a vacuum tube based on effect of electric field
- analysis of molecules sizes : spectra

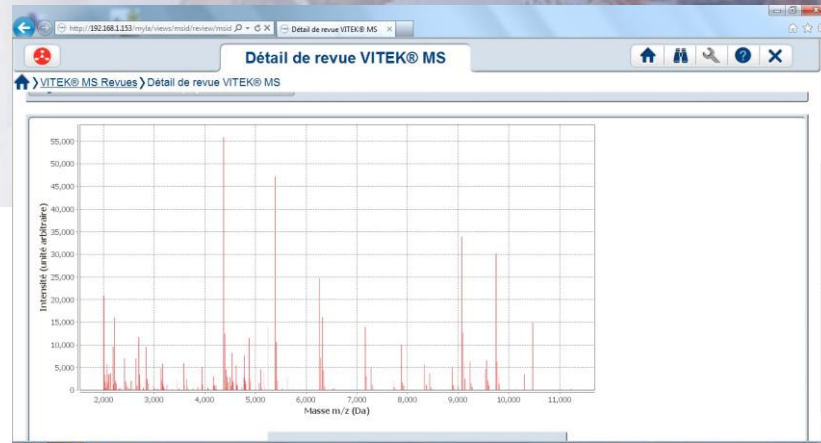




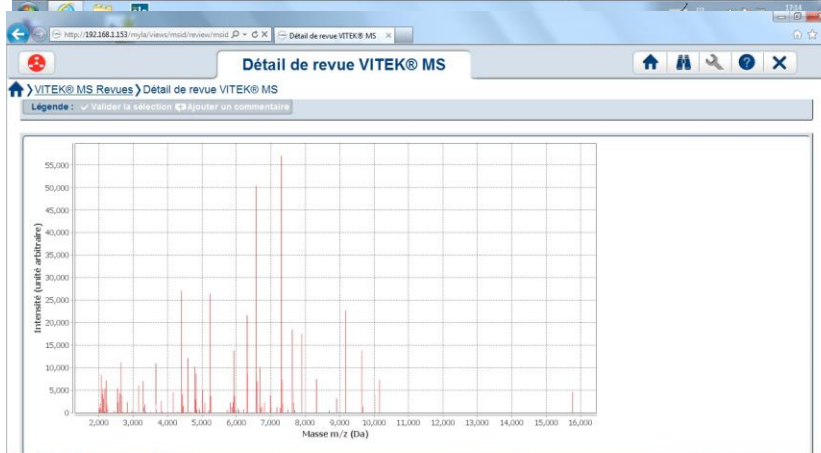
# Identification by MALDI-TOF



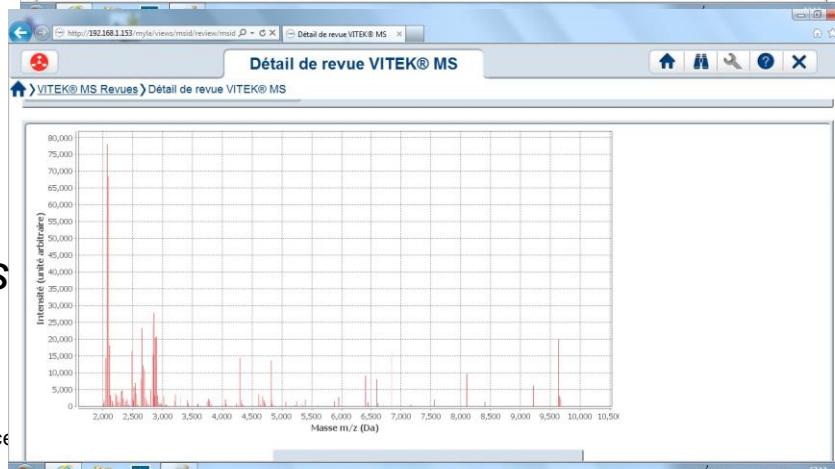
*E. coli*



*R. Insidiosa*



*S. epidermidis*



Comparison of data  
(bins, weights, matrix)  
to database of data to get  
the identification

## MALDI-TOF VITEK® MS System (bioMerieux)

### MASS Spectrometer :

Schimadzu's Mass Spectrometer (Tanaka Nobel Prize 2002)

World leader in MALDI-TOF MS technology

Tanaka received the prize for the development of soft desorption ionization methods for mass spectrometric analyses of biological macromolecules to identify and reveal the structures of such molecules.

### Databases :

IVD 3.0 (bioMerieux): (closed)

- 38,000 spectra of 15,572 strains representing 1,050 species
- More than 78 % of the claimed species are represented by more than 5 strains
- Intra-species variability is taken into account
- FDA de novo Clearance (2013) for Gram- and Gram+ clinical bacteria

identification

RUO (bioMerieux): (open)

- 5600 strains representing 2,447 species

### Interpretation :

Identification at the species level

Confidence level



# MALDI-TOF : VITEK MS system

## Work Flow Overview (part. 1)

Colony  
on plate

Spot  
on slide

Prep  
Station

VitekMS  
Mass Spectra generation



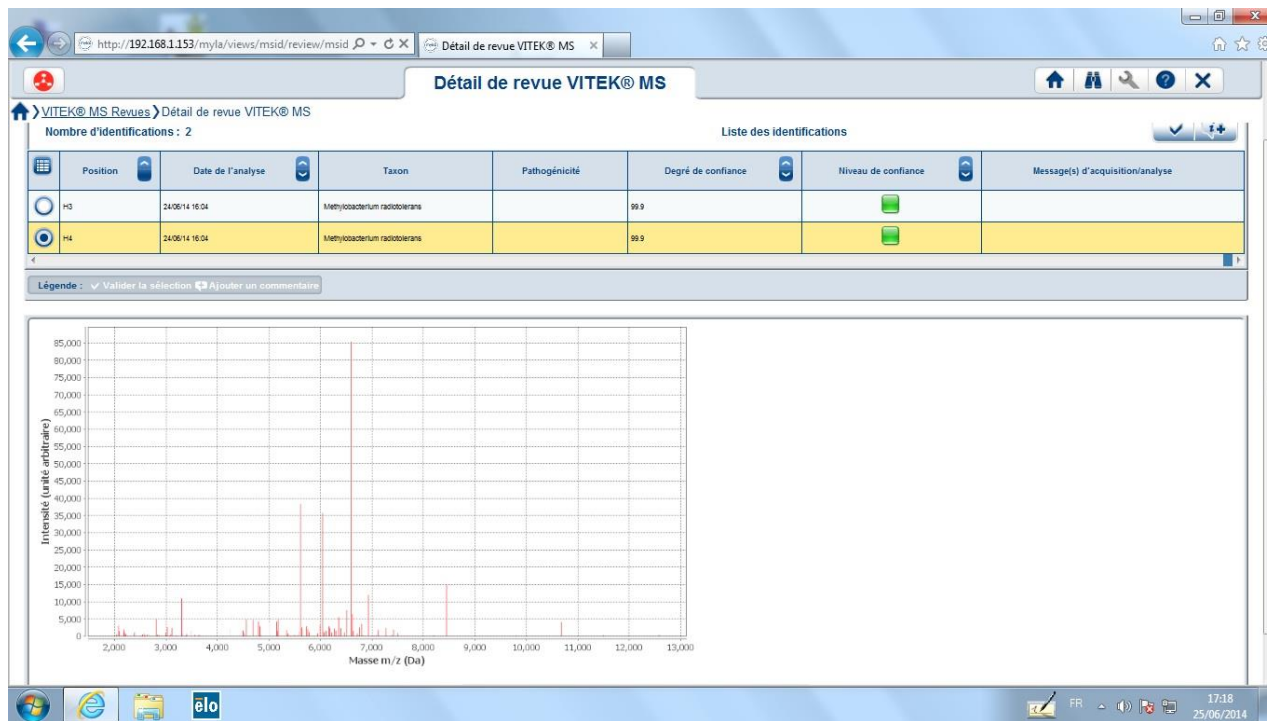
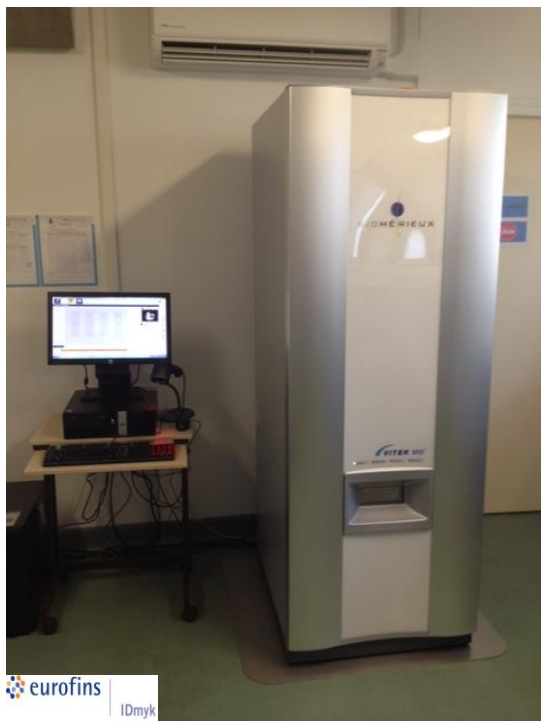
Slides Bar Code Read

# MALDI-TOF : VITEK MS system

## Work Flow Overview (part. 2)

VitekMS  
Raw Mass Spectra generation

Analysis vs. Database  
Comparison of data



Two databases

- IVD (closed)
- RUO (open)



# MALDI-TOF : VITEK<sup>®</sup> MS system

## Work Flow Overview (part. 3)

### TAT :

- Sample preparation : 10-20 minutes/48 samples
- Spotting of colony part, matrix deposit on spot, dry
- 48 samples per slide and up to 4 slides (192 samples  $\leq$  60 min.)



# MALDI-TOF : VITEK® MS system

## Work Flow Overview (part. 4)

**TAT :**

- Sample MS analysis : 5-10 minutes/**16** samples
- ≠ 60-120 minutes / **192** samples

The screenshot shows a web browser window with the URL <http://192.168.1.153/myla/views/msid/review/msid> and a tab titled "VITEK® MS Revues". The interface displays a table with the following data:

Sample ID	Organism	Confidence (%)	Status	Action	Notes
CA79063-1	Escherichia coli	99.9	Green	A revoir	Hors délai
CA72010-1			Yellow	À sélectionner	Hors délai
CA70033-1	Moraxella osloensis	99.9	Green	A revoir	Hors délai
CA67054-1	Enterococcus casseliflavus	99.9	Green	A revoir	Hors délai
CA62028-1			Yellow	À sélectionner	Hors délai
CA60045-1	Ralstonia insidiosa	99.9	Green	A revoir	Hors délai
CA60032-1	Methylobacterium radiotolerans	99.9	Green	A revoir	Hors délai
CA59006-1			Red	A revoir	Hors délai
CA52032-1	Pseudomonas stutzeri	99.9	Green	A revoir	Hors délai
CA10006-1			Red	A revoir	Hors délai
BM0614322-1			Yellow	À sélectionner	Hors délai
BM06143211-1			Yellow	À sélectionner	Hors délai
BM0614319-1	Burkholderia cepacia	99.9	Green	A revoir	Hors délai

# MALDI-TOF VITEK® MS : Evaluation

## Samples : Environmental bacteria tested

Taxa of the samples to include in the present evaluation were selected according to their frequency of identification (by comparative sequencing), in our laboratory, based on data of the last quarter of year 2013. The isolates were from environment of pharma industries.

The references and characteristics of the isolates studied, with their genera, species, and results have been recorded. They were subcultured from our collection, checked for purity, and fresh cultures were tested with the VITEK® MS system, using IVD V3.0 database.

Some of them were further checked by comparative sequencing, in case of discordant results between initial ID and VITEK® MS system ID.

Molecular identification (by 16S rRNA gene almost complete (1,350 bp) sequencing [taxonomic] and comparison to Eurofins IDmyk Eubacteria V11 database (8,470 species type strains), and/or MultiLocus comparative sequencing) was retained as the reference.

# MALDI-TOF VITEK® MS : Evaluation

## Samples : bacterial isolates

We have made a survey of **3,000 molecular identifications** of bacterial isolates from pharmaceutical industries (environmental isolates) done in our lab in the last quarter of 2013.

We have selected :

- **300 isolates (tested in duplicate)**
- representing **163 of the most frequently encountered taxa**
- from **55 genera**

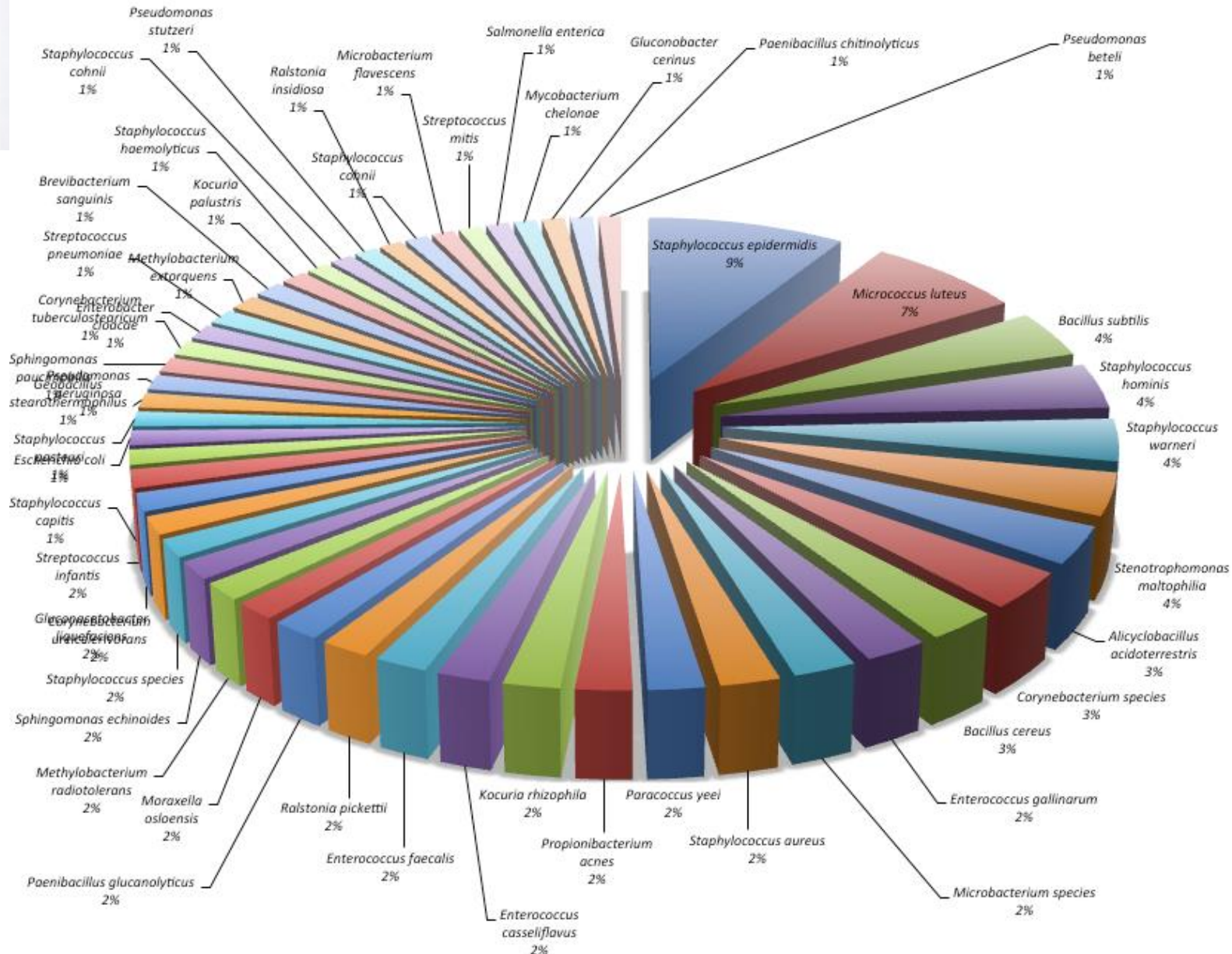
This represented more than 60 % of the total isolates of the different taxa identified at our lab during the period.

Multiple strains (2 to 9) were investigated for the most important taxa (n=68). Isolates of a same species have been typed by molecular methods to ensure that they corresponded to different strains.

Isolates of taxa unclaimed in the IVD database V3.0 were included (25 taxa, 27 isolates) according to their representativity



# Top 50 of the species selected for Vitek MS testing



A. Carlotti (2014)  
(personal communication)



## Global Results

- Good ID at the genus level  
270 isolates out of 300 (90 %)
- Good ID at the species level  
263 isolates out of 300 (88%)
- No ID  
30 isolates out of 300 (10 %)
- Bad ID at the genus level  
0 isolate out of 300 (0%)
- Bad ID at the species level  
7 isolates out of 300 (2%)

## Good ID results : details

- Good ID at the genus level

270 isolates out of 300 (90 %)

- **52 genera out of 55**

- (3 No ID belong to *Asaia* (1), *Globicatella* (1), *Leifsonia* (1))

- Good ID at the species level

263 isolates out of 300 (88%)

**131 taxa out of 163**

[claimed (n=138) and unclaimed (n=25) species in IVD V3.0]

131 of the most frequently encountered species in environmental samples of pharma industries have been well identified at the species level (including multiple isolates) using MALDI-TOF VITEK® MS, as compared to molecular identifications

## Good ID results : details (part.1/5)

EIDmyk reference identification		Isolates				GID species
EIDmyk reference		Nb tested	good ID	%GID	No ID	
<i>Acinetobacter</i>	<i>johnsonii</i>	2	2	100%		
<i>Acinetobacter</i>	<i>lwoffii</i>	1	1	100%		
<i>Alcaligenes</i>	<i>faecalis</i>	1	1	100%		
<i>Alicyclobacillus</i>	<i>acidoterrestris</i>	3	3	100%		
<i>Bacillus</i>	<i>amyloliquefaciens</i>	1	1	100%		
<i>Bacillus</i>	<i>assamensis</i>	1	0	0%	1	-
<i>Bacillus</i>	<i>atrophaeus</i>	3	3	100%		
<i>Bacillus</i>	<i>cereus group</i>	7	7	100%		
<i>Bacillus</i>	<i>circulans</i>	2	1	50%	1	-
<i>Bacillus</i>	<i>firmus</i>	2	0	0%	1	-
<i>Bacillus</i>	<i>gracilicentis</i>	1	0	0%	1	-
<i>Bacillus</i>	<i>idriensis</i>	1	1	100%		
<i>Bacillus</i>	<i>infantis</i>	1	0	0%	1	-
<i>Bacillus</i>	<i>lentus</i>	1	0	0%	1	-
<i>Bacillus</i>	<i>licheniformis</i>	4	4	100%		
<i>Bacillus</i>	<i>megaterium</i>	2	2	100%		
<i>Bacillus</i>	<i>mycoides</i>	2	2	100%		
<i>Bacillus</i>	<i>pumilus</i>	1	1	100%		
<i>Bacillus</i>	<i>simplex</i>	3	3	100%		
<i>Bacillus</i>	<i>subtilis</i>	4	4	100%		
<i>Bacillus</i>	<i>circulans</i>	1	1	100%		
<i>Bacillus</i>	<i>clausii</i>	1	1	100%		
<i>Bacillus</i>	<i>horneckiae</i>	1	1	100%		
<i>Brachybacterium</i>	<i>nesterenkovi</i>	1	1	100%		
<i>Brevibacillus</i>	<i>agri</i>	1	1	100%		
<i>Brevibacillus</i>	<i>laterosporus</i>	1	0	0%	1	-
<i>Brevibacterium</i>	<i>sanguinis</i>	1	1	100%		species BadID
<i>Brevibacterium</i>	<i>casei</i>	2	2	100%		
<i>Brevibacterium</i>	<i>luteolum</i>	2	2	100%		
<i>Brevundimonas</i>	<i>diminuta</i>	1	1	100%		
<i>Brevundimonas</i>	<i>olei/naejangsanensis</i>	1	1	100%		species BadID
<i>Brevundimonas</i>	<i>vesicularis</i>	1	0	0%	1	-
<i>Burkholderia</i>	<i>cepacia group</i>	3	3	100%		
<i>Burkholderia</i>	<i>multivorans</i>	1	1	100%		

## No ID results : details

Genus	species	Claimed in IVD V3.0	ID	Total isolates
<i>Asaia</i>	<i>lannensis</i>	no	0	1
<i>Bacillus</i>	<i>assamensis</i>	no	0	1
<i>Bacillus</i>	<i>circulans</i>	yes	1	2
<i>Bacillus</i>	<i>firmus</i>	yes	0	2
<i>Bacillus</i>	<i>graciliciensis</i>	no	0	1
<i>Bacillus</i>	<i>infantis</i>	no	0	1
<i>Bacillus</i>	<i>lentus</i>	no	0	1
<i>Brevibacillus</i>	<i>laterosporus</i>	mix taxon	0	1
<i>Brevundimonas</i>	<i>vesicularis</i>	yes	0	1
<i>Corynebacterium</i>	<i>coylae</i>	yes	0	1
<i>Globicatella</i>	<i>species</i>	no	0	1
<i>Lactobacillus</i>	<i>harbinensis</i>	no	0	1
<i>Leifsonia</i>	<i>species</i>	no	0	1
<i>Methylobacterium</i>	<i>aquaticum</i>	yes	0	3
<i>Methylobacterium</i>	<i>extorquens</i>	no	0	1
<i>Methylobacterium</i>	<i>rhodesianum</i>	no	0	1
<i>Microbacterium</i>	<i>laevaniformans</i>	no	0	1
<i>Microbacterium</i>	<i>luteolum</i>	no	0	1
<i>Microbacterium</i>	<i>oleivorans</i>	no	0	1
<i>Microbacterium</i>	<i>species</i>	no	0	1
<i>Micrococcus</i>	<i>cohnii</i>	no	0	1
<i>Paenibacillus</i>	<i>provencensis</i>	no	0	1
<i>Paenibacillus</i>	<i>chitinolyticus</i>	no	0	1
<i>Sphingomonas</i>	<i>capsulata</i>	no	0	1
<i>Sphingomonas</i>	<i>dokdonensis</i>	no	0	3

6 Claimed taxa (9 isolates)

19 Unclaimed taxa (21 isolates)  
(including 3 spp.)

25 taxa	1	31
3 species	NoID	30
<b>Claimed</b>		<b>9</b>
<b>UnClaimed</b>		<b>21</b>

10%

3%

7%

## No ID results : details

In total, No ID results were obtained for 25 taxa (30 isolates, 10%), but only 6 of them were included in the IVD database (9 isolates) while the remaining 19 taxa (21 isolates) were not claimed in the IVD database.

These results are very important since they demonstrated that the system did not generate bad ID for most of the species not claimed in the database. The system is robust regarding unclaimed species ID.

Hence, the « No ID » results drop down to 3 % (9 isolates out of 300) when considering claimed species only.

Nevertheless, 6 claimed taxa (*B. circulans*, *B. firmus*, *B. laterosporus*, *B. vesicularis*, *C. coylae*, *Mtb. aquaticum*) were not identified and generally this was correlated with poor spectra made up of limited number of peaks or weak peaks and linked to poor growth in 24-48h



## Bad ID at the species level results : details

Genus	species	isolates tested	%ID	Claimed in IVD V3.0	Positive	Vitek MS ID	Comment
<i>Brevibacterium</i>	<i>sanguinis</i>	1	100%	no	1	<i>Brevibacterium casei</i>	Bad ID
<i>Brevundimonas</i>	<i>olei/naejangsanensis</i>	1	100%	no	1	<i>Brevundimonas diminuta</i>	Bad ID
<i>Corynebacterium</i>	<i>minutissimum</i>	1	100%	no	1	<i>Coryn.aurimucosum</i>	Bad ID
<i>Corynebacterium</i>	<i>mucifaciens</i>	1	100%	yes	1	<i>Coryn.afermentans</i>	Bad ID
<i>Corynebacterium</i>	<i>ureicelerivorans</i>	1	100%	no	1	<i>Coryn.mucifaciens</i>	Bad ID
<i>Cupriavidus</i>	<i>metallidurans</i>	1	100%	no	1	<i>Cup.pauculus</i>	Bad ID
<i>Pantoea</i>	<i>conspicua</i>	1	100%	no	1	<i>Pantoea agglomerans</i>	Bad ID

Bad ID at the species level but Good ID at the genus level :

7 isolates out of 300 (2%)

7 taxa

1 claimed taxa

6 unclaimed taxa

# MALDI-TOF VITEK® MS Sytem Evaluation

- **Multiple isolates :**

Numerous taxa investigated were tested with more than 1 isolates (from 2 to 9 isolates (n=68 taxa)).

Only 5 taxa (7 %) (*B. circulans*, *Mtb. Aquaticum*, etc...) gave less than 100 % of good ID.

To the contrary, for the remaining 63 taxa (93 %) which included multiple isolates, 100% Good ID were obtained for all the isolates tested (e.g. *B. cereus* group 7/7, *B. subtilis* group 4/4, *C. tuberculostearicum* 3/3, *D. acidovorans* 3/3, *E. coli* 4/4, *Mtb. Radiotolerans* 2/2, *M. luteus* 9/9; *P. aeruginosa* 5/5, *R. pickettii* 4/4, *S. epidermidis* 6/6, *S. haemolyticus* 7/7, *S. maltophilia* 5/5, etc...)

- This further demonstrated a very good robustness of the system, and a very good representativity of the reference spectra that permitted to take into account the variability of the isolates inside a species. This also demonstrated the interest of evaluating numerous isolates per taxa to determine the performances of the system.

## ■ Validation of the VITEK<sup>®</sup> MS system for rapid environmental bacteria identification (Eurofins IDmyk data DV-L-003-MC)

- Specificity: 60 isolates, 60 species (taxa)

- ⇒ 30 isolates of 30 representative species of Gram negative bacteria

- Good ID : 100 %
- No ID : 0 %
- Bad ID : 0 %

- ⇒ 30 isolates of 30 representative species of Gram positive bacteria

- Good ID : 93.4 %
- No ID : 6.6 %
- Bad ID : 0 %

- Robustness

- ⇒ Matrix shelf life variations
- ⇒ Operators variations
- ⇒ Cultures (duration) variations
- ⇒ Simpicate, duplicate and triplicate testing variations

- Rapidity and ease of use recognized by operators

- ⇒ 192 isolates from fresh cultures analyzed in less than 1h (+preparation)



## Identification of environmental bacteria by MALDI-TOF VITEK® MS

- Acceptance in the Industry
  - USP General Information Chapter <1113> recognizes MALDI-TOF as a suitable phenotypic approach
    - ⇒ Provides description of limitations
    - ⇒ Manufacturers have developed systems that minimize these influences
    - ⇒ Describes qualification considerations
  - Qualification efforts should demonstrate suitable performance when challenged with routinely encountered conditions
  - Phenotypic methods including MALDI-TOF provide sufficient information for use in environmental monitoring, indicator organism screening, and known culture confirmations
- Observed performances are congruent with data from the literature regarding clinal isolates, however this is the first large study about relevant environmental bacteria from the pharmaceutical industries

## Conclusions :

MALDI-TOF Mass Spectrometry using the VITEK® MS (bioMérieux) is a suitable system for rapid identification of environmental bacteria

We can expect to well identify roughly 88-90 % of the routine most frequently encountered species in pharma industries

We can expect very few misidentification at the species level (< 2,5%)

We can expect roughly 10 % of no ID for which we can use molecular methods for identification as an alternative

Cultures must not be more than 3-5 days old to be analyzed

Samples must be tested in duplicate



## Conclusions :

MALDI-TOF Mass Spectrometry using the VITEK<sup>®</sup> MS (bioMerieux) is a suitable system for rapid identification of environmental bacteria

Updates and/or use of the open database can be effective to improve :

- ⇒ Missing species, and unique strains from various environmental sources
- ⇒ No public reference spectra available
- ⇒ Creation of new spectra through purchase of type strains, or sequencing analysis of isolates (requires comprehensive reference database), multiple strains must be included in order to take care of intra-species variability (strains)

## Extension of the system for yeast and molds identification

- ⇒ Perform the same study with representative isolates of yeasts and molds (ongoing)
- ⇒ Already used for yeast and molds of clinical significance, no extensive data for environmental isolates from pharma



**Thanks to :**

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**Victoria GIRARD (bioMerieux)**

**thank you for your attention**