# Rapid Pathogen Identification by MALDI-TOF Mass Spectrometry/SARAMIS Database in Clinical Microbiological Routine Diagnostics

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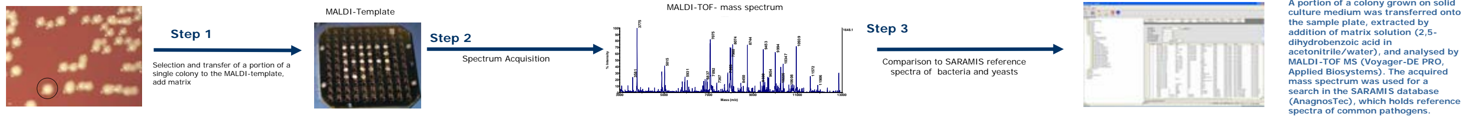
## Background

Automated identification systems are widely-used in medium-to-high-throughput clinical microbiology laboratories. However, such systems are relatively slow because they depend on bacterial growth and metabolic activity. Bacterial identification by mass spectrometry provides a promising way to accelerate pathogen identification, since it can be performed in a few minutes from small samples. In this study we compared the performance of MALDI-TOF MS coupled to SARAMIS (Spectral ARchiving And Microbial Identification System, AnagnosTec) with established methods (VITEK2/API, BioMérieux) in the clinical microbiology routine diagnostics.

## Materials and Methods – Workflow of MALDI-TOF/ SARAMIS Pathogen Identification Procedure




A portion of a colony grown on solid culture medium was transferred onto the sample plate, extracted by addition of matrix solution (2,5-dihydrobenzoic acid in acetonitrile/water), and analysed by MALDI-TOF MS (Voyager-DE PRO, Applied Biosystems). The acquired mass spectrum was used for a search in the SARAMIS database (AnagnosTec), which holds reference spectra of common pathogens.

## Materials and Methods – Isolates used in this Study

Bacterial and yeast isolates were obtained from routine diagnostics and tested by conventional methods (Vitek2/API, BioMérieux) and MALDI-TOF MS/SARAMIS in parallel.

In a three month period we compared 1575 isolates without pre-selection or bias from different standard solid culture media.

In the PILOT PHASE 221 isolates were processed to adjust MALDI-Instrument parameters, teach personnel, and customize automation. Spectra obtained in this period were used for the accommodation of SARAMIS reference spectra.

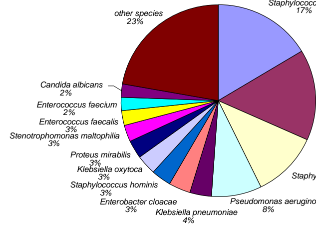
In the TEST PHASE 1354 isolates were identified. Ten samples were excluded due to preanalytical mistakes (i.e. mixture of several pathogens, sample confusion).

In case of non-concordant results the identification was confirmed by additional biochemical methods (API) or PCR/Sequencing.

### Distribution of pathogens by group

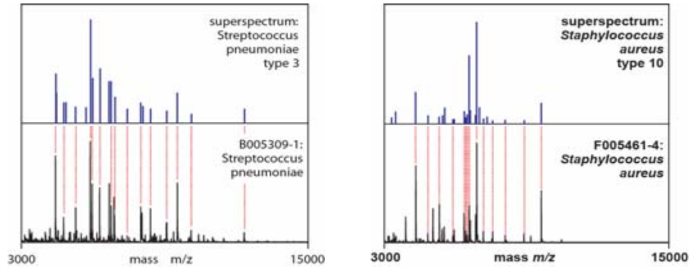
| Group | % |
| --- | --- |
| Enterobacteriaceae | 34.6% |
| Nonfermenting Gram - | 13.8% |
| Staphylococci | 35.8% |
| Streptococci | 7.3% |
| Yeasts | 5.4% |
| Other Bacteria | 3.0% |
| Other fungi | 0.1% |

### Distribution of pathogens by species




## Materials and Methods – Pathogen Identification by reference Spectra (Superspectra)

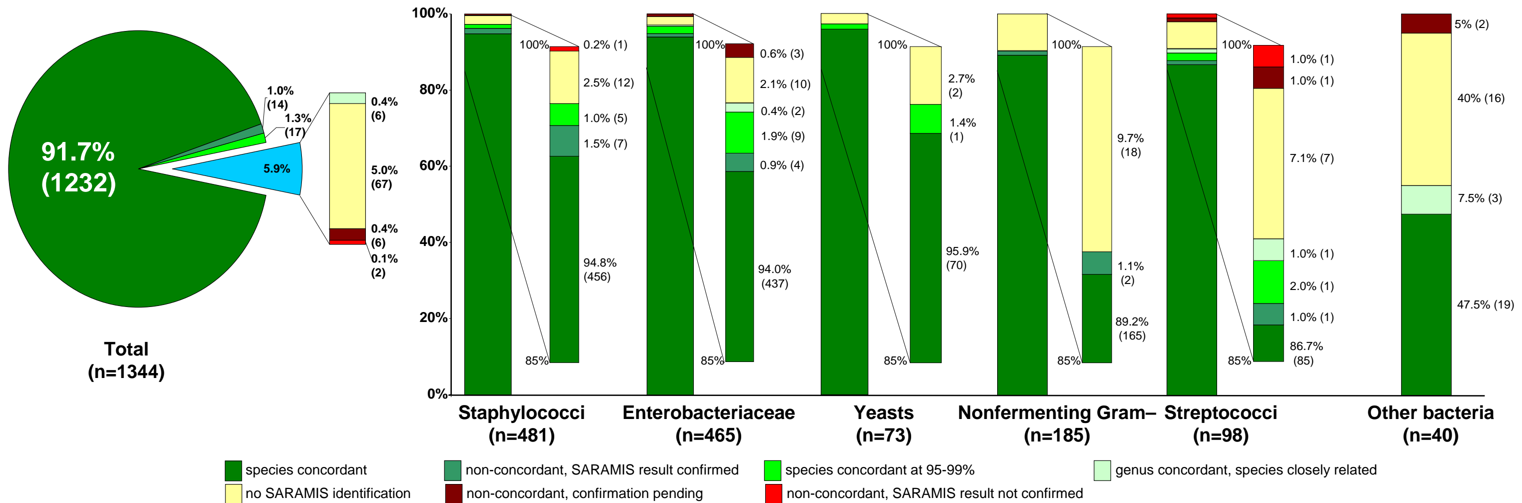
The SARAMIS database and software contains more than 633 species- or genus-specific reference spectra (so called superspectra) which are consensus spectra of multiple mass spectra of reference strains of individual serotypes, species or genera, respectively.




Superspectrum of *Streptococcus pneumoniae* type 3 (upper panel) and fingerprint mass spectrum of clinical isolate (lower panel). Dotted lines indicate matching masses in the fingerprint spectra compared to the superspectrum.

Superspectrum of *Staphylococcus aureus* type 10 (upper panel) and a fingerprint mass spectrum of clinical isolate (lower panel). Dotted lines indicate matching masses in the fingerprint spectra compared to the superspectrum.

## Results – Identification




## Results – VITEK2 and SARAMIS discrepancies

### SARAMIS identification confirmed

| Isolate No. | VITEK2 Identification | SARAMIS Identification | Biochemical methods/ PCR /Sequencing |
| --- | --- | --- | --- |
| B003514-2 | Klebsiella pneumoniae | Klebsiella oxytoca | Klebsiella oxytoca |
| A005127-1 | Klebsiella pneumoniae | Klebsiella oxytoca | Klebsiella oxytoca |
| B005416-1 | Klebsiella pneumoniae | Klebsiella oxytoca | Klebsiella oxytoca |
| B005416-3 | Klebsiella pneumoniae | Klebsiella oxytoca | Klebsiella oxytoca |
| C002171-3 | Sphingomonas paucimobilis | Stenotrophomonas maltophilia | Stenotrophomonas maltophilia |
| C002891-3 | Sphingomonas paucimobilis | Stenotrophomonas maltophilia | Stenotrophomonas maltophilia |
| F002963-1 | Staphylococcus hominis | Staphylococcus epidermidis | Staphylococcus epidermidis |
| B003236-1 | Staphylococcus epidermidis | Staphylococcus haemolyticus | Staphylococcus haemolyticus |
| A004420-1 | Staphylococcus aureus | Staphylococcus haemolyticus | Staphylococcus haemolyticus |
| C003694-1 | Staphylococcus lugdunensis | Staphylococcus aureus | Staphylococcus aureus |
| C003754-1 | Staphylococcus warneri | Staphylococcus hominis | Staphylococcus hominis |
| F004513-2 | Staphylococcus hominis | Staphylococcus epidermidis | Staphylococcus epidermidis |
| B005502-2 | Staphylococcus lentus | Staphylococcus haemolyticus | Staphylococcus haemolyticus |
| F005461-1 | Streptococcus oralis | Streptococcus parasanguinis | Streptococcus parasanguinis |

### SARAMIS identification not confirmed

| Isolate No. | VITEK2 Identification | SARAMIS Identification | PCR/Sequencing |
| --- | --- | --- | --- |
| F004476-1 | Staphylococcus warneri | Staphylococcus capitis | Staphylococcus caprae |
| F005258-1 | Streptococcus parasanguinis | Streptococcus pneumoniae | Streptococcus parasanguinis |

## Advantages of MALDI-TOF MS / SARAMIS identification

Advantages:
- no special culture media
- no fresh overnight cultures required
- no OD-measurement/McFarland-standard required
- universal gram-independent sample preparation for all bacteria (additional preparation step for fungi)
- fast identification (15min/ 1 sample; 3-4h/ 30 samples, including sample preparation)
- robust
- low consumable costs

Disadvantages:
- additional susceptibility testing required
- high initial investment

## Conclusion

MALDI-TOF MS / SARAMIS is a straightforward, rapid, robust, and, in the long run, inexpensive method for the routine identification of bacteria and fungi in clinical microbiology laboratories