# MALDI-TOF in Transfusion and Transplantation Medicine

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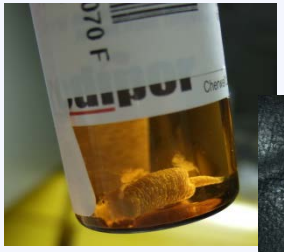
# Summary

- About the National Bacteriology Laboratory
- Introduction and the principles of MALDI
- The systems
- Development
- Benefits
- Aims of the study
- Methods
- Results
- The future

# National Bacteriology Laboratory

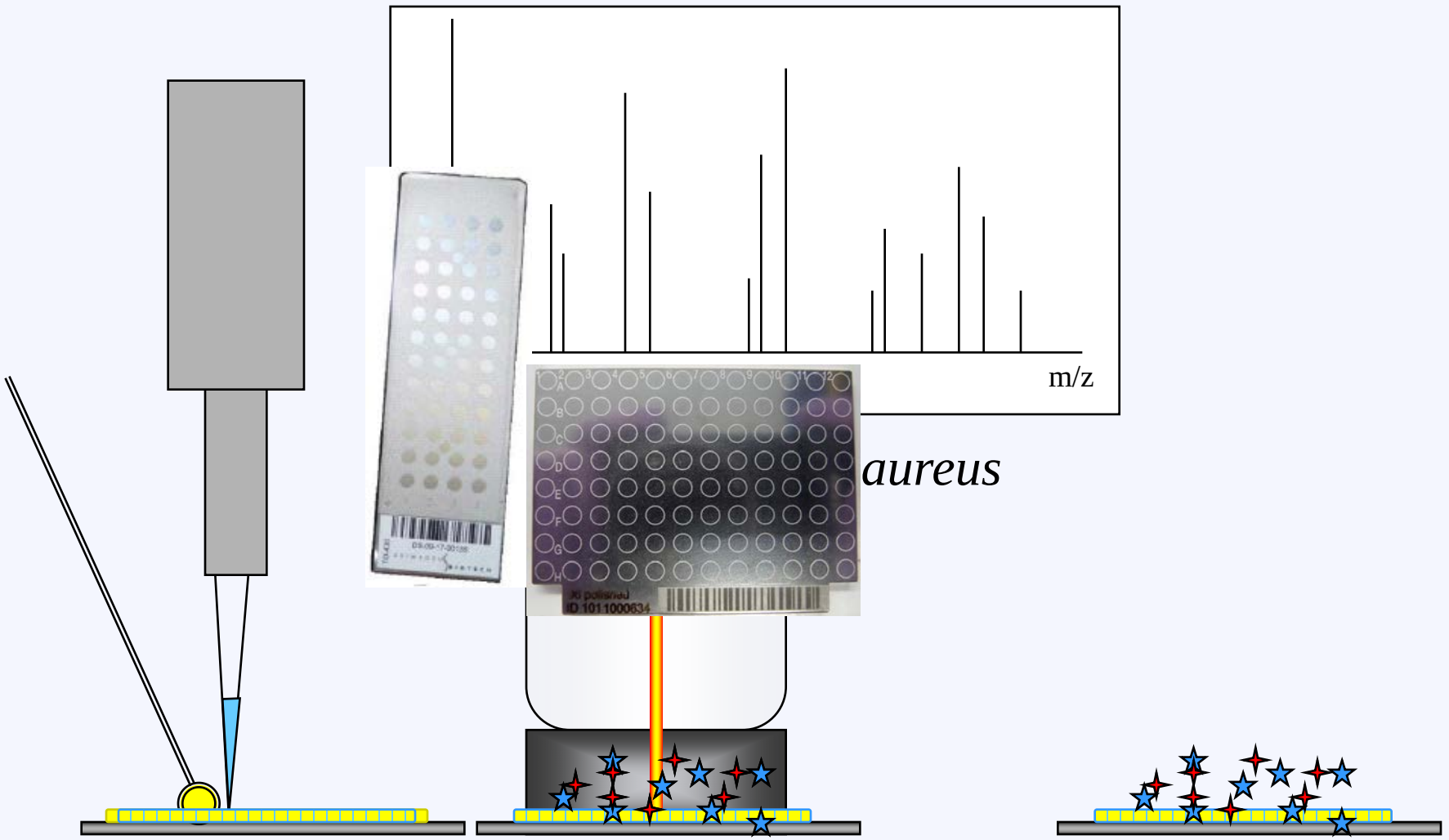
- Samples received:
  - Pre and post decontamination tissues samples
  - Cord blood, ASE and stem cell screening
  - Components involved in suspected TTI or visually abnormal products
  - Platelet screening samples (from Feb 2011)
  - Environmental monitoring isolates



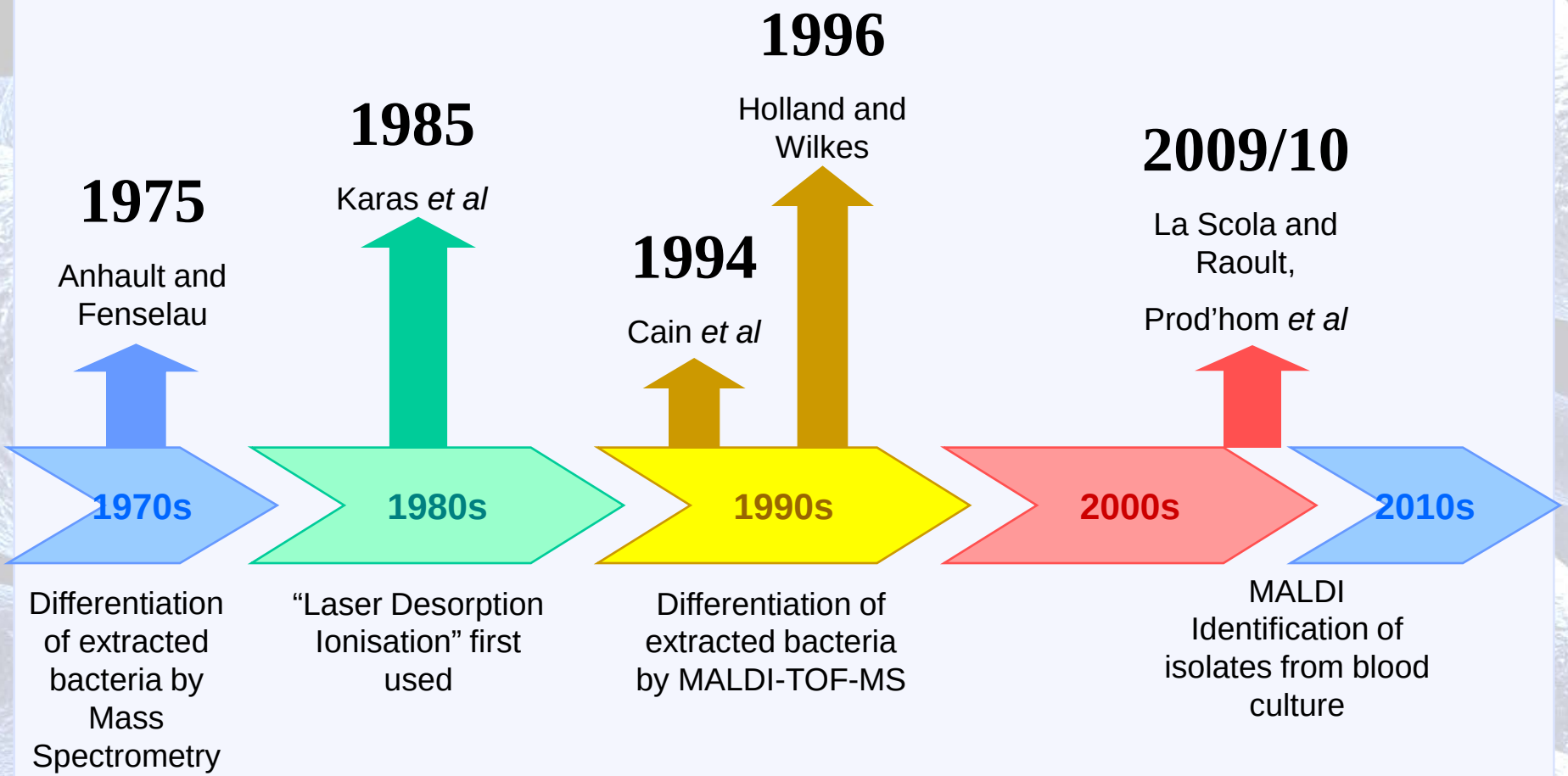

- Identification of around 2500-3000 isolates/year
- Average ~50 samples/year sent to reference laboratory (PHE) for further work
- Current systems used for identification include BD Phoenix and BBL Crystal – phenotypic identification

# MALDI-TOF-MS

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


m/z
aureus

# Development History



# Principles of Analysis

- Many spectral peaks from bacterial proteins
  - Other biomolecules may also be present
- >50% ribosomal proteins
- Many ribosomal proteins are species specific
  - 16S rRNA sequencing used in identification
- Basic in nature, possibly improving ionisation
  - allows binding to RNA

# Vitek MS


- 4 components:
  - Vitek MS: MALDI analysis
  - Prep station: input target slide information
  - Acquisition station: Display spectra
  - Myla middleware: Manages workflow and sends identification results
- Database: >25,000 spectra for clinically relevant organisms
  - Clinical and reference strains grown under a range of conditions
- Instrument can analyse 4 target slides per run
  - 48 sample spots per slide, divided into 3 groups of 16 with a central *E. coli* control

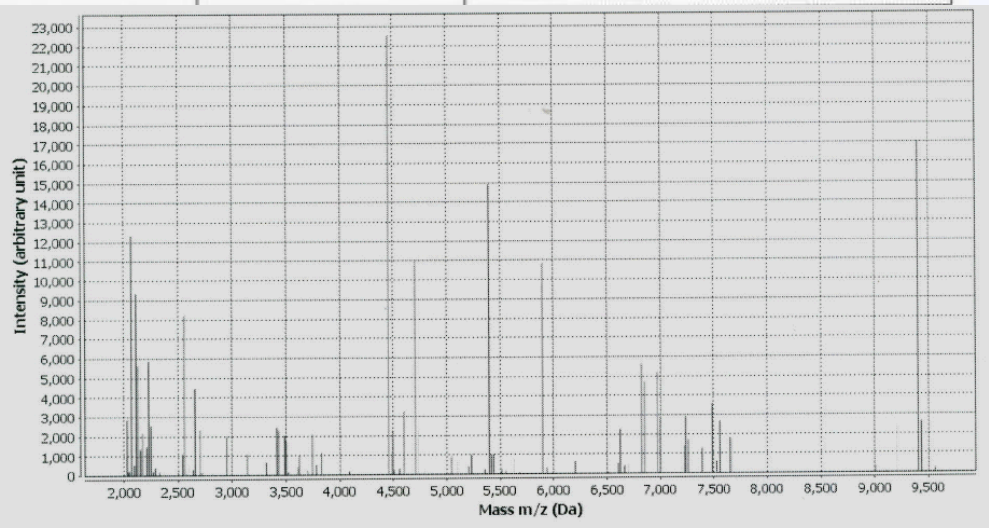
# Results presentation

- ID scored based on match to references
  - 99.9 = perfect match (1 option)
  - 60-99.8 = Good match (1 option, or 2-4 for low discrimination results)
  - “No ID” for those that could not be matched
- Also represent results visually
  - green square = good match, 1 probable species
  - orange triangle = low discrimination result, >1 possible species
  - Red circle = no peaks, no match for ID

**Number of identifications: 8**

**List of identifications**

| | Position | Analysis Date | Organism Name | Confidence Value | Confidence Level | Acquisition/Computation message(s) |
|---|---|---|---|---|---|---|
| | D2 | 12/14/12 4:21 PM | Lactobacillus paracasei | 99.9 | | |
| | D2 | 12/14/12 4:21 PM | Lactobacillus casei | 99.9 | | |
| | D3 | 12/14/12 4:21 PM | Lactobacillus casei | 99.9 | | |
| | D3 | 12/14/12 4:21 PM | Lactobacillus paracasei | 99.9 | | |
| | D4 | 12/14/12 4:21 PM | Lactobacillus paracasei | 99.9 | | |
| | D4 | 12/14/12 4:21 PM | Lactobacillus casei | 99.9 | | |
| | E1 | 12/14/12 4:40 PM | Lactobacillus paracasei | 99.9 | | |
| | E1 | 12/14/12 4:40 PM | Lactobacillus casei | 99.9 | | |



# Bruker Biotyper

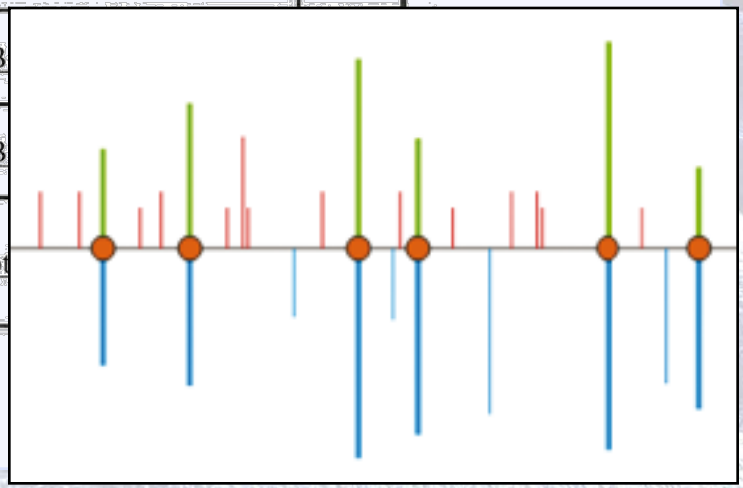
- Bench-top system
- Input sample info from LIMS, MS Excel spreadsheet or barcode reader to a separate computer
- Database comprising 5267 spectra
  - Multiple spectra from reference strains under the same conditions
- Analysis in <30 minutes
- Option for disposable (48 spot) or reusable (48 or 96 spot) target slides

# Results Presentation

- ID result is scored based on match with reference data
  - 2.300-3.000 (+++) = highly probable species ID
  - 2.000-2.299 (++) = probable species, secure genus
  - 1.700-1.999 (+) = probable genus
  - <1.699 (-) = No ID
- Also categorised based on consistency
  - A (to species), B (to genus), C (no consistency)
- Top 2 matches for each sample are displayed
  - Top 10, indicating closest match, can also be shown

## Result Overview

| Analyte Name | Analyte ID | Organism (best match) | Score Value | Organism (second best match) | Score Value |
|---|---|---|---|---|---|
| A1 (+++) (A) | 1 | Clostridium perfringens | 2.341 | Clostridium perfringens | 2.263 |
| A2 (+++) (A) | 1 | Clostridium perfringens | 2.518 | Clostridium perfringens | 2.392 |
| A3 (+++) (A) | 2 | Bacteroides fragilis | 2.399 | Bacteroides fragilis | 2.356 |
| A4 (+++) (A) | 2 | Bacteroides fragilis | 2.418 | Bacteroides fragilis | 2.387 |
| A5 (+) (B) | 3 | Bacillus subtilis | 1.733 | B | |
| A6 (+) (B) | 3 | Bacillus subtilis | 1.802 | B | |
| A7 (+++) (A) | 4 | Streptococcus pyogenes | 2.444 | Strept | |



# Development

- Direct analysis from blood culture bottles and urine samples
- Detection of resistance profiles
  - E.g. MRSA and ESBLs
- Strain typing

# How will this help NBL?

- More rapid than current techniques (BD Phoenix and BBL Crystal)
  - Improve sample TAT
- Lower cost ID per sample
- Multiple isolates can be analysed in one go
- Reduce number of isolates sent to reference laboratory for confirmation

# System Evaluation using NBL isolates

**Aims:**

- To assess the performance of each system using previously identified NBL isolates

- To compare the performance, ease of use and system requirements, to determine suitability for use within the lab

# Methods

- Panel of 164 wild-type isolates from NBL cryobank (-80°C storage)
  - Tissues, platelets, transfusion reactions, stem cells/cord bloods
- Incubated for 24-72 hours 35°C prior to testing
- Tested in triplicate on both systems

# Methods

- Testing was repeated on the same system for any samples that did not match the NBL ID to species level
- Samples were also sent for 16S sequencing identification
- Results compared against NBL/16S ID to determine performance

# Results: Initial Testing

| | | Total Tested | Consistent to Species | % | Consistent to Genus | % | Not consistent | % | No ID assigned | % |
|---|---|---|---|---|---|---|---|---|---|---|
| Vitek MS | Initial analysis | 164 | 89 | 54.3 | 131 | 79.9 | 26 | 15.9 | 7 | 4.3 |
| | Repeat analysis | 75 | 14 | 18.7 | 50 | 66.6 | 21 | 28.0 | 4 | 5.3 |
| Biotyper | Initial analysis | 164 | 94 | 57.3 | 134 | 81.7 | 18 | 11.0 | 12 | 7.3 |
| | Repeat analysis | 70 | 6 | 8.6 | 48 | 68.6 | 17 | 24.3 | 5 | 7.1 |

- "Repeat analysis": the total no. samples not consistent with NBL to species level that underwent further analysis on the system

# Comparison with 16S ID

| | Total Tested | Consistent to Species | % | Consistent to Genus | % | Not consistent | % | No ID assigned | % |
|---|---|---|---|---|---|---|---|---|---|
| Vitek MS | 75 | 59 | 78.7 | 6 | 8.0 | 6 | 8.0 | 4 | 5.3 |
| Biotyper | 70 | 59 | 79.5 | 3 | 4.1 | 3 | 5.5 | 5 | 6.8 |

- Only isolates that did not match NBL species on initial testing were analysed
- 35 isolates could not be confirmed by 16S
  - Reported by PHE to genus level only
- Probable species were taken into account (99% homology)

# Overall performance

| | Total Tested | Consistent to Species | % | Consistent to Genus | % | Not consistent | % | No ID assigned | % |
|---|---|---|---|---|---|---|---|---|---|
| Vitek MS | 164 | 152 | 92.7 | 155 | 94.5 | 7 | 4.3 | 2 | 1.2 |
| Biotyper | 164 | 155 | 94.5 | 158 | 96.3 | 4 | 2.4 | 2 | 1.2 |

- Results of repeat analysis used for any isolates that did not match NBL ID on initial testing.
- Repeat result also compared with 16S.
- 16S was the gold standard

# Discussion

- Overall consistency of 92.7% (Vitek MS) and 94.5% (Bruker)
- Isolates that grew well within 24 hours were more easily identified by the systems
  - E.g. *S. aureus, E. coli, Bacillus spp.*
- Many isolates that were not consistent to NBL included CNS and α-haemolytic Streptococci (mitis group)
  - Species very closely related with conserved ribosomal proteins
  - α-haemolytic Streptococci generally identified as *S. pneumoniae* by Bruker and *S. mitis/oralis* by Vitek MS

- Other considerations:
  - Working temperature
  - Space required
  - Additional equipment, e.g. fume hood, consumables
  - Disposable vs. re-useable target plates
  - Quality assurance
  - Database limitations
  - Need for additional tests

# Summary

- MALDI provides rapid, specific, low cost ID
- Comparison of performance showed similar results for each
- Limitations to both systems
- Problems with ID of α-haemolytic Streptococci

# The future

- Analysis of a wider range of environmental strains using MALDI
- Direct analysis of blood culture bottles
- Purchase a MALDI-TOF system

# Acknowledgements

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