MALDI-TOF Mass Spectrometry: A New Rapid ID Method in Clinical Microbiology

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Outline

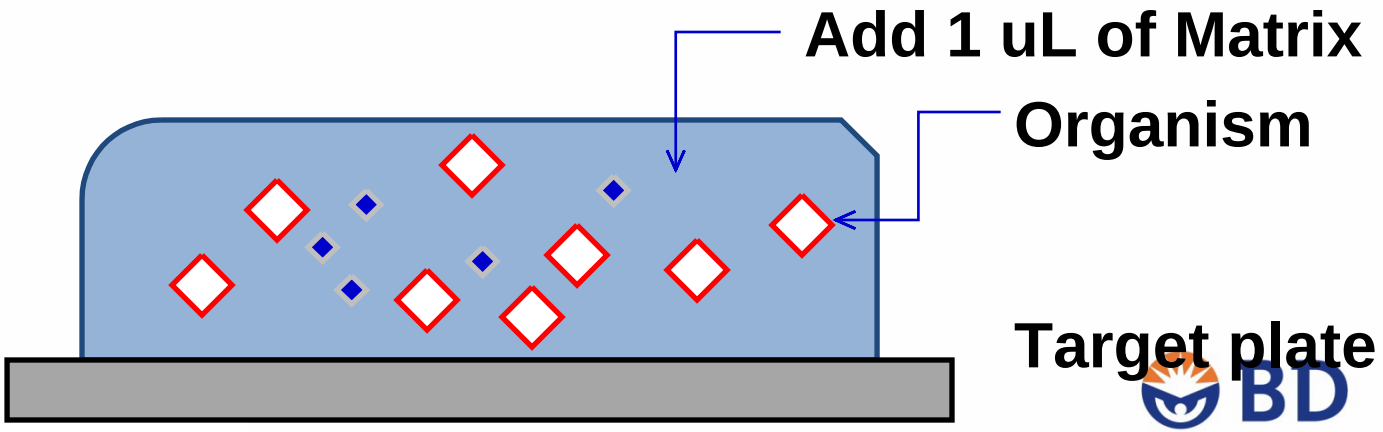
- MALDI-TOF is the most important innovation in organism identification since microorganisms were first grown in the laboratory.
- By the end of this decade, laboratories will routinely use MALDI-TOF for the routine identification of all bacteria, mycobacteria, yeasts and molds.

Mass Spectrometry

- Mass spectrometer is composed of 3 functional units: ion source, mass analyzer, and detection device
- Ion source – ionize sample molecules and transfers into gas phase
  - MALDI considered “soft ionization” (intact proteins)
- Mass analyzer separates ions by mass-to-charge (m/z) ratio, e.g. time of flight (TOF)
- Detection device used to record separated ions

MALDI-TOF Ionization of Sample

Matrix Assisted Laser Desorption/Ionization



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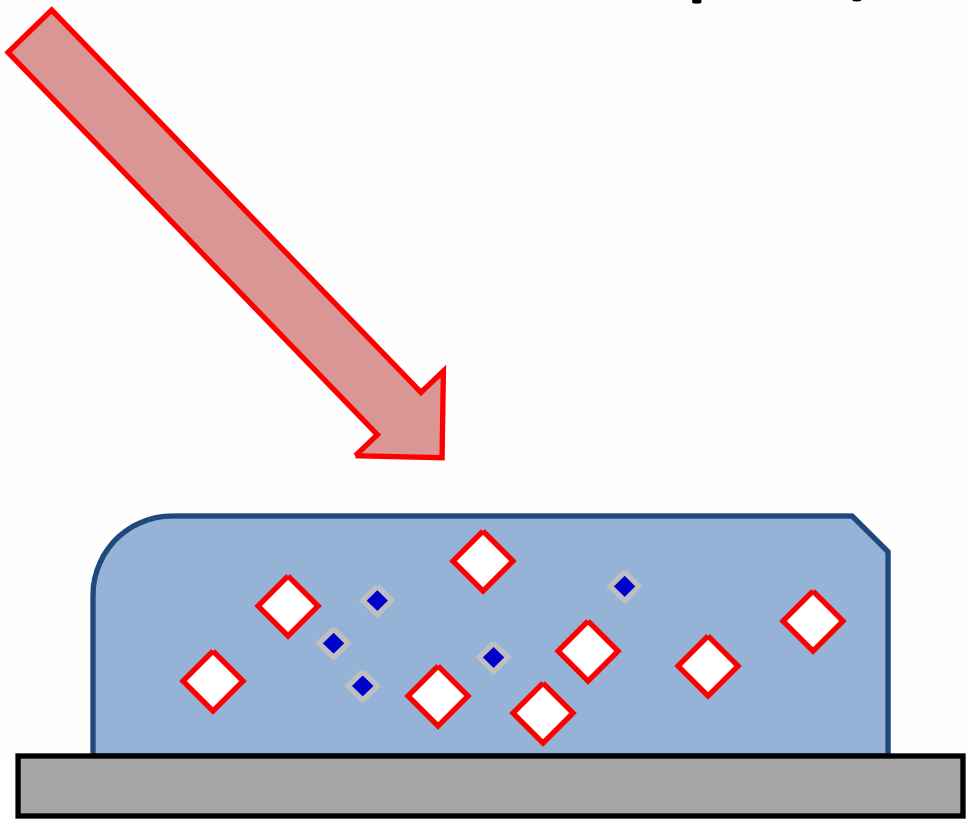
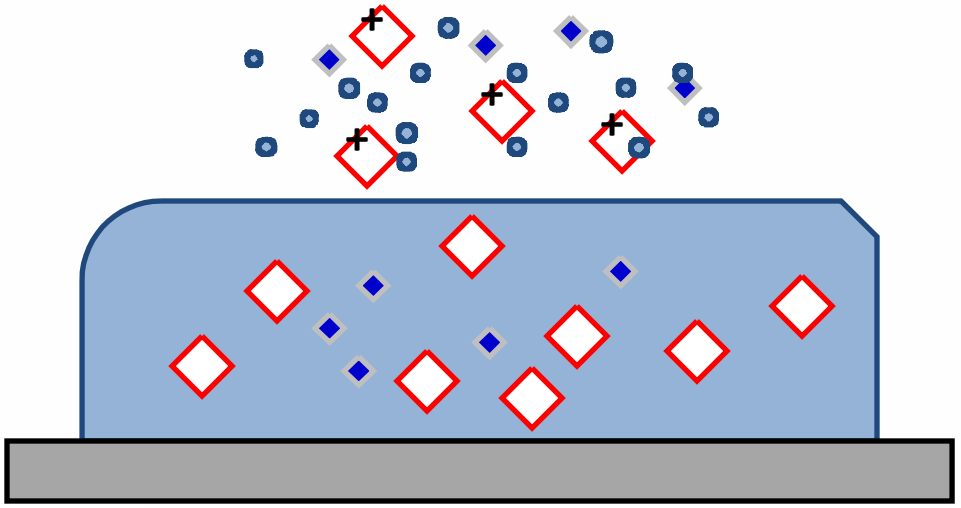


Target plate

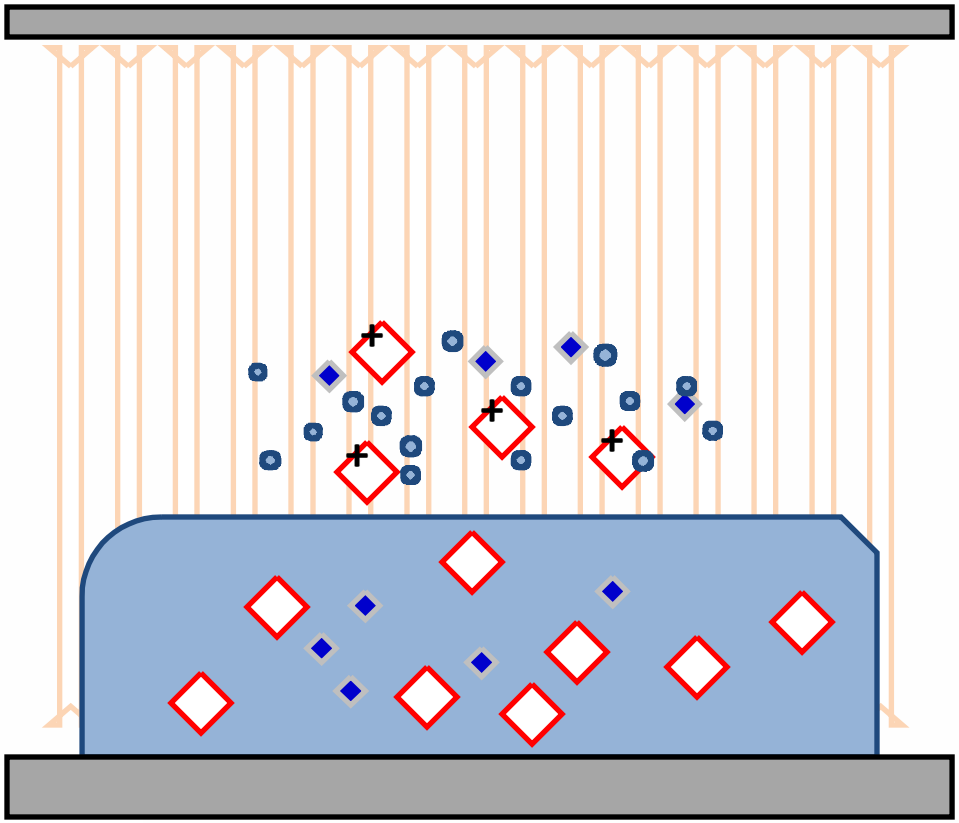
MALDI-TOF Ionization of Sample

Matrix Assisted Laser Desorption/Ionization

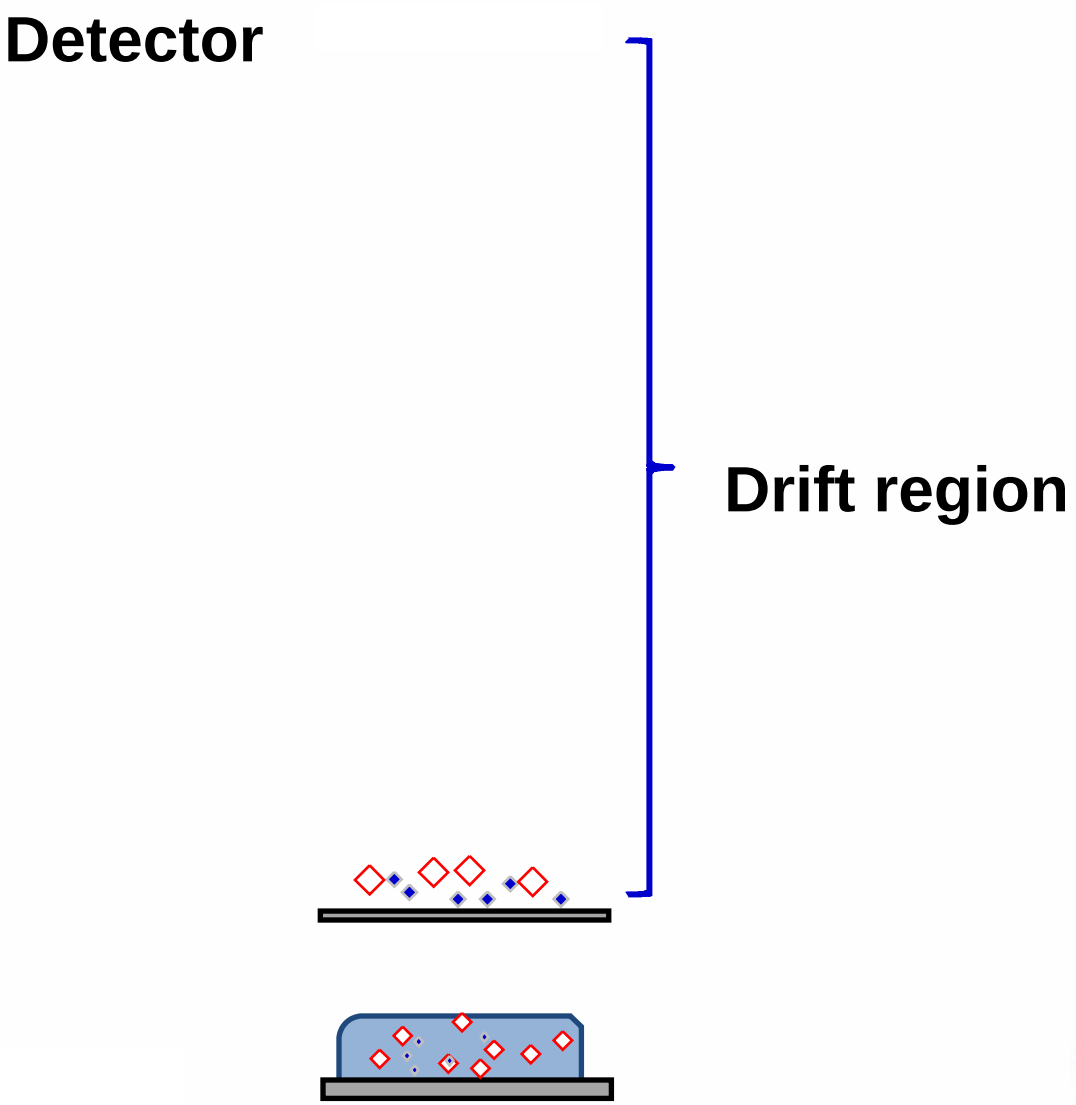


MALDI-TOF Ionization of Sample

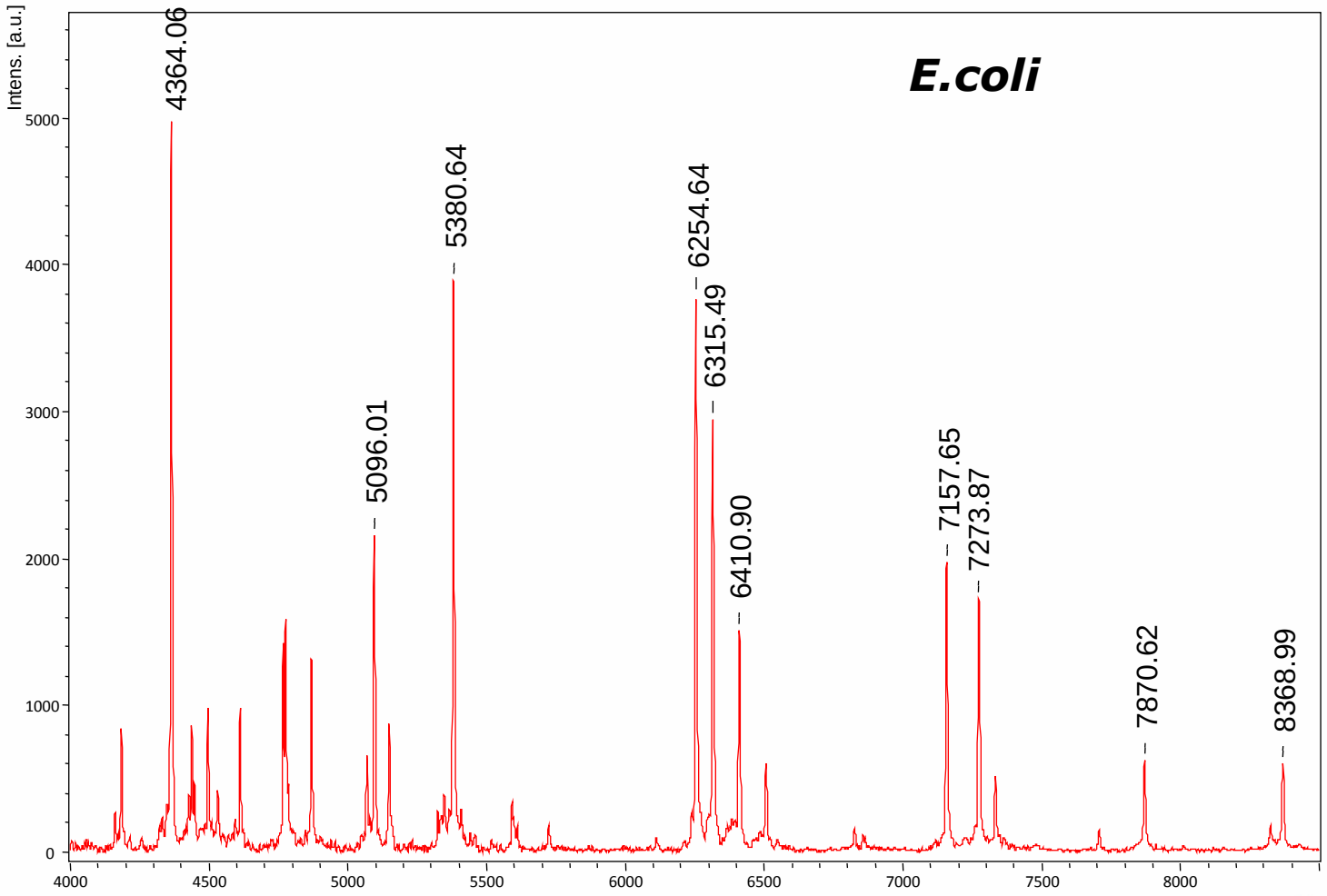
Acceleration electrode



The positively charged proteins drift in the flight tube; smaller proteins drift faster



MALDI-TOF MS profile spectrum
Mass Range: 2-20 kDa



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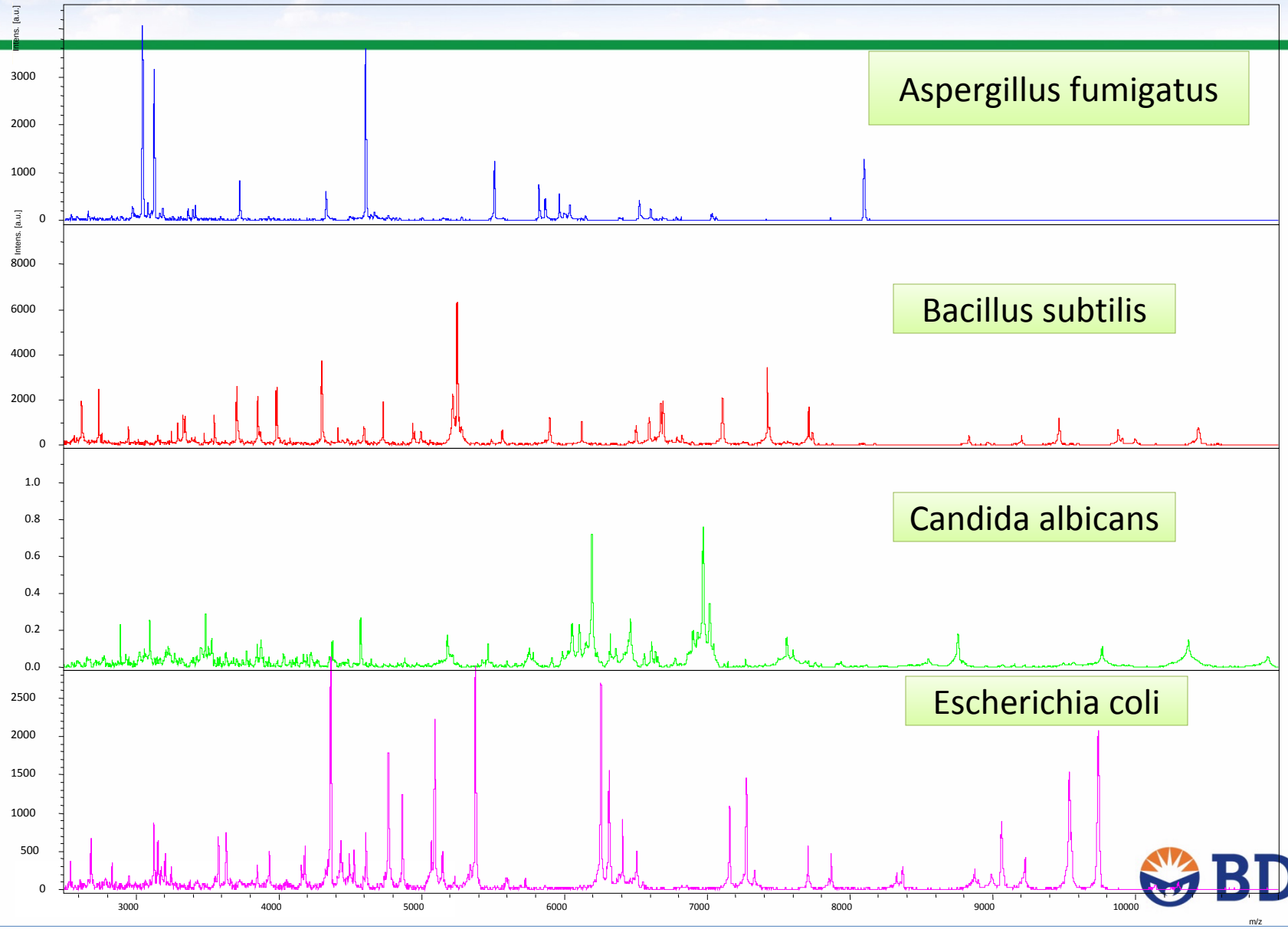
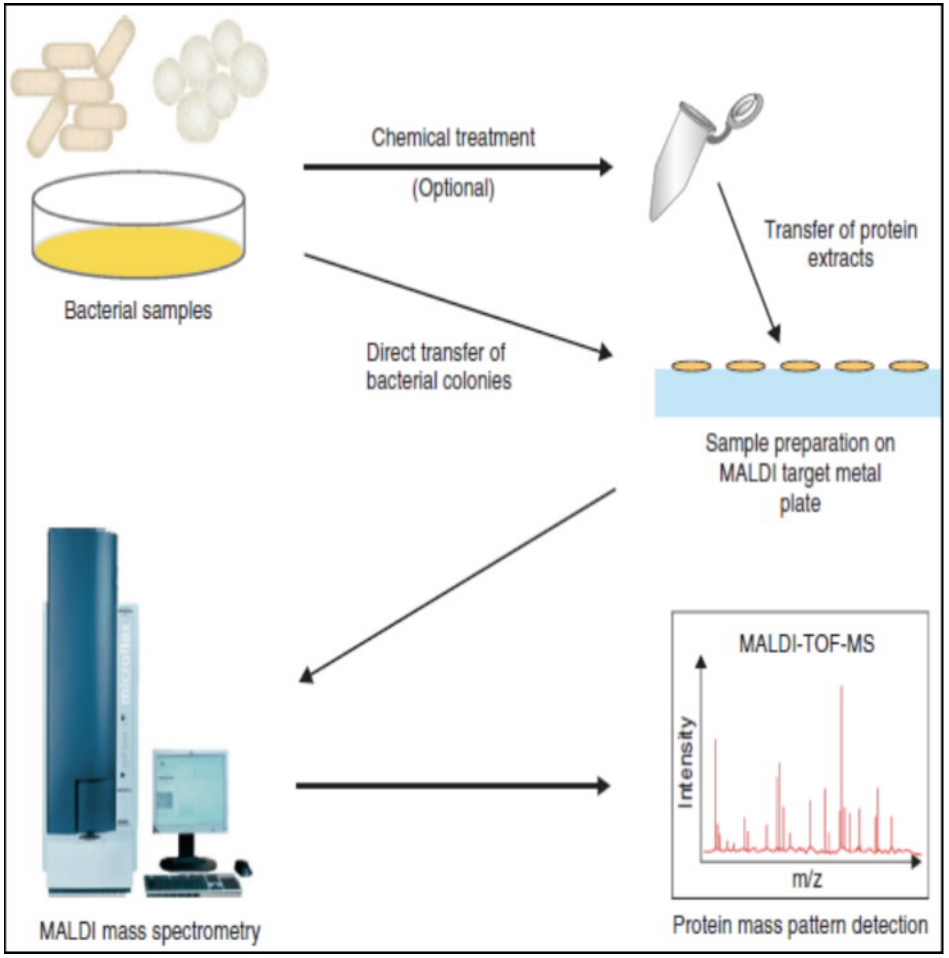
Unique Profiles for Bacteria, Yeasts and Molds



MALDI-TOF Mass Spectrometry
Pre-analytical Processing

- Single colonies can be either transferred directly to the target plate or extracted with ethanol or methanol.
- If sufficient number of organisms are present, broth cultures (e.g., blood culture broths) can be concentrated and processed.
- Cell wall structure is disrupted with a strong organic acid (e.g., formic, trifluoracetic, or acetic acid).
- Acetonitrile is used for protein extraction.
- Extracts spotted on stainless steel plates coated with Teflon (or disposable cards) and overlayed with matrix.



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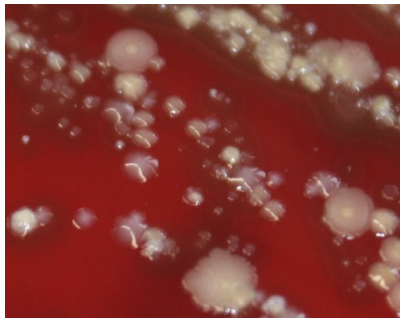
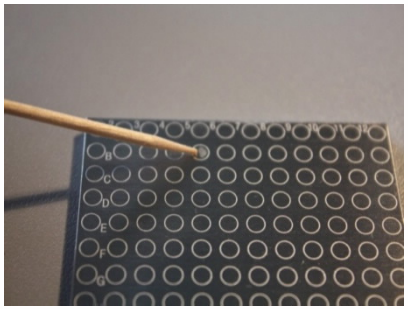
Target Preparation - Rapid

Direct Smear Method

- Touch colony with transfer device, such as toothpick
- Transfer a small amount onto spot
- Air dry
- Cover with 1 µL of matrix; air dry
- Analyze





Target Preparation - Best

Modification of Extraction Method
(Haigh et al, J Clin Microbiol 2011)

- Touch colony with transfer device, such as toothpick
- Transfer a small amount onto target plate; air dry
- Add 1 µL of absolute **formic acid** to colony; air dry
- Cover with 1 µL of matrix; air dry
- Analyze

Target Preparation – Mycobacteria

- Suspend a small loopful of growth in water and heat to 95^0C for 30 min to kill mycobacteria
- Disperse organisms with micropestle; centrifuge; remove supernatant
- Resuspend pellet in 70% formic acid and silica beads (breaks open the organisms); vortex 10 min
- Add acetonitrile (extracts proteins), vortex 10 min, centrifuge
- Apply supernatant to MALDI target plate; add matrix; analyze
- **Less than one hour from detection to ID**

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Target Preparation – Filamentous Fungi

Lau et al, J Clin Microbiol 2013

- Suspend a small colony fragment in absolute ethanol and silica beads; vortex 15 min.
- Remove ethanol by centrifugation; resuspend pellet in 70% formic acid and vortex 5 min
- Add acetonitrile, vortex for 5 min; centrifuge
- Apply supernatant to MALDI target plate; add matrix; analyze
- **Less than 30 min from detection to ID**

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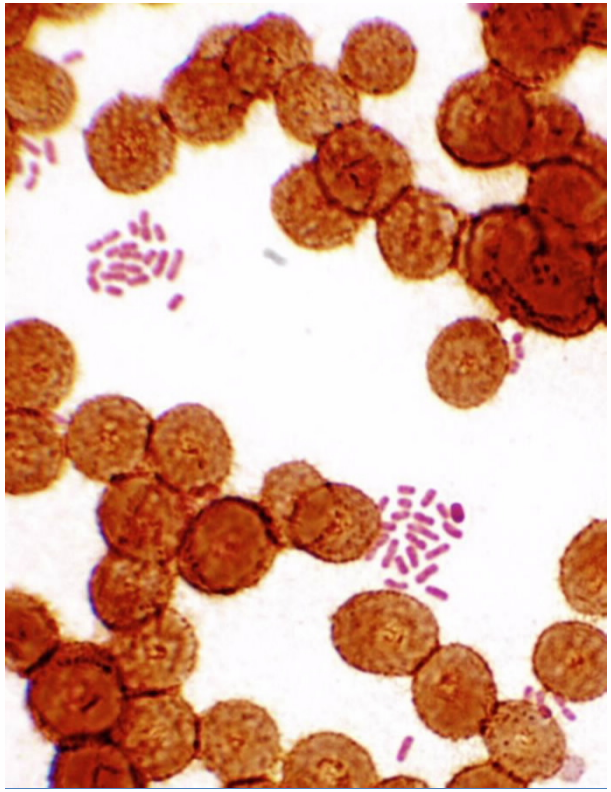
MALDI-TOF Identification
Protocol for Processing Blood Culture Broths

- Blood cells, serum proteins, and hemoglobin must be removed
- Lyse blood cells and concentrate bacteria by centrifugation.
- Wash pellet twice with sterile water to remove serum proteins
- Wash with 70% ethanol to kill bacteria
- Continue processing as with colonies from agar plates



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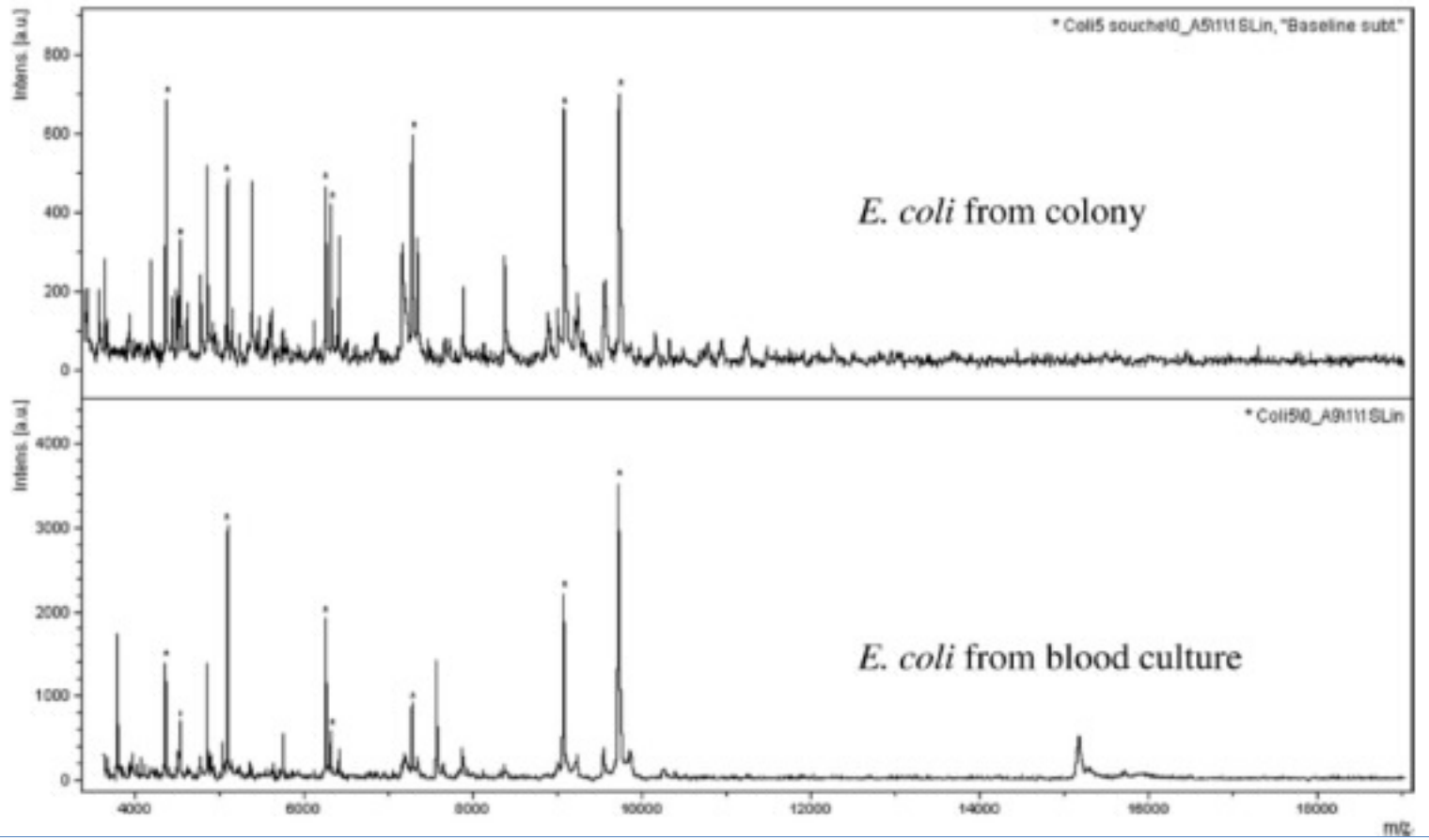
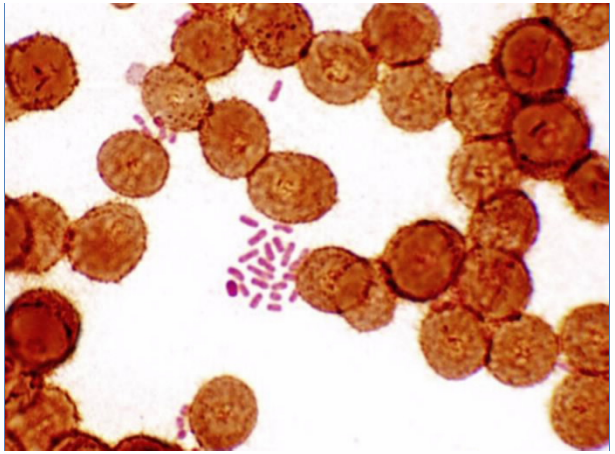
MALDI-TOF Profiles: Colonies vs. Broth



Ferroni et al, JCM 48:1542-48, 2010

MALDI-TOF
Microbial Identifications

- More than 250 publications in 2012
- Identification accuracy demonstrated for bacteria, mycobacteria, yeasts, and molds
- Accuracy determined by preanalytical processing, number of organisms analyzed, taxonomy of isolate, and database
- Most non-identifications due to:
  - too few organisms in sample
  - isolate not in database



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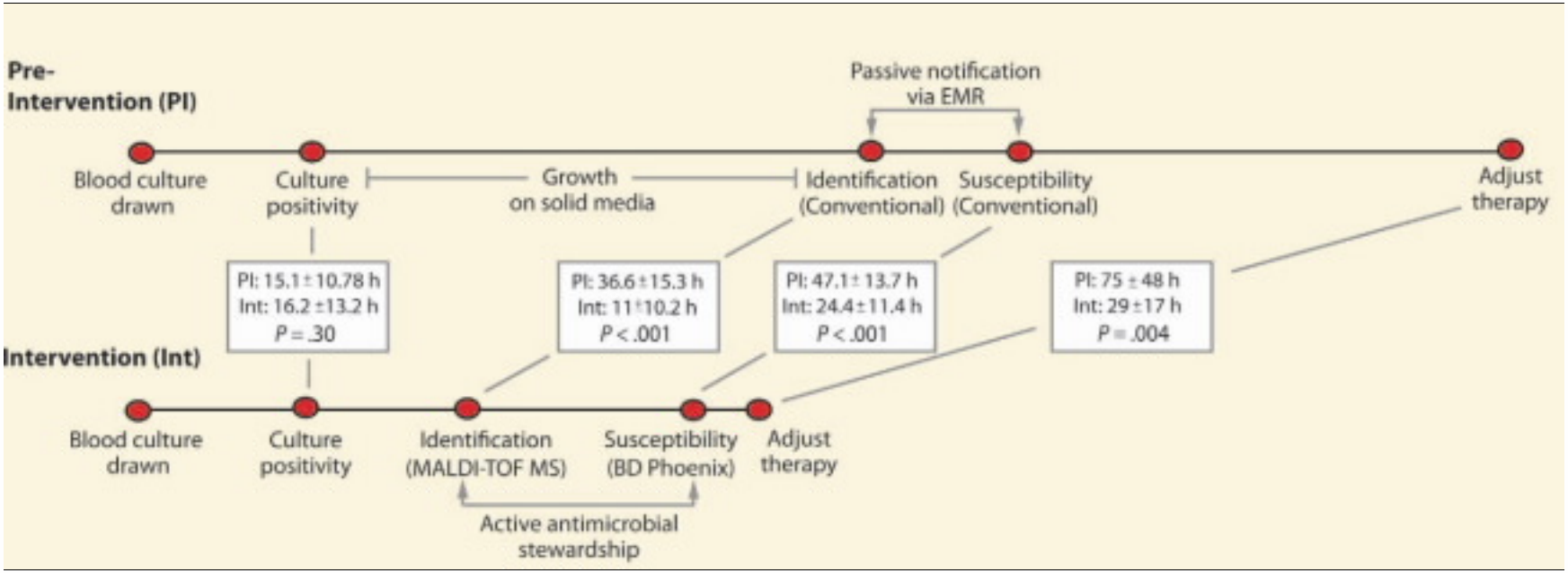
Effect of MALDI on Antibiotic Treatment of Bacteremic Patients

- Vlek et al (PLoS ONE, 2012) assessed the impact of MALDI-TOF on treatment of bacteremic patients:
  - Direct ID of blood culture isolates reduced time to ID by 28.8 hours
  - The proportion of patients receiving appropriate antibiotic therapy increased by 11.3%.
  - The effect was directly related to guided empiric therapy because time to definitive AST results was not modified in this study.

Impact of MALDI, Rapid AST, and Antibiotic Stewardship

Perez et al, Arch Pathol Lab Med, 2013



Length of Stay and Cost Outcomes			
Outcome	Preintervention Cohort (n=100)	Intervention Cohort (n=101)	P value
Hospital length of stay	11.9 days	9.3 days	0.01
ICU length of stay	7.3 days	6.3 days	0.05
Total hospital costs	$45,709	$26,162	0.009

Comparison of MALDI vs. Standard Identification Tests

Tan et al, J Clin Microbiol 2012

- Large medical center laboratory compared identification of all bacteria and yeasts isolated during a 12 week period by MALDI-TOF (Bruker) and standard ID tests in routine use (biochemical, agglutination, GLC, sequencing).
- 952 isolates from 2,214 specimens were processed.
- MALDI protocol provided ID an average of 1.45 days earlier.
- The estimated annual cost of organism identification was reduced by $102,424 or 57%.

MALDI: Workflow Impact

- MALDI can replace all biochemical and morphological identifications for bacteria, fungi, mycobacteria and blood isolates
  - Decreased consumable supplies
  - Decreased QC tests
  - Decreased repeat testing
  - Decreased supplemental tests
- Anaerobes can be identified as easily as aerobic bacteria
  - Elimination of subcultures to demonstrate strict anaerobes
  - Elimination of preliminary and definitive biochemical tests
  - Reduction to time to ID from days to minutes
- Definitive identification of molds and mycobacteria the same day growth is detected vs. days to weeks with traditional approaches

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