

The Nuts and Bolts of Proteomics in Microbiology, MALDI-TOF in Microbiology

Nathan A Ledeboer
Associate Professor of Pathology
Medical College of Wisconsin

Medical Director, Microbiology and Molecular Pathology
Dynacare Laboratories and Froedtert Hospital

Medical Director, Laboratory Outreach, Logistics, Client Services and
Reference Services
Dynacare Laboratories
Milwaukee, WI

Financial Disclosures

- Consultant
 - Nanosphere
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 - Meridian, Quidel, IMDx, Cepheid, BD, bioMérieux, Bruker Daltonics, Nanosphere, Seegene, Life Technologies, Prodesse, Great Basin Corp, iCubate, Biohelix, BioRad
- Will discuss applications/products that are not FDA approved

Outline

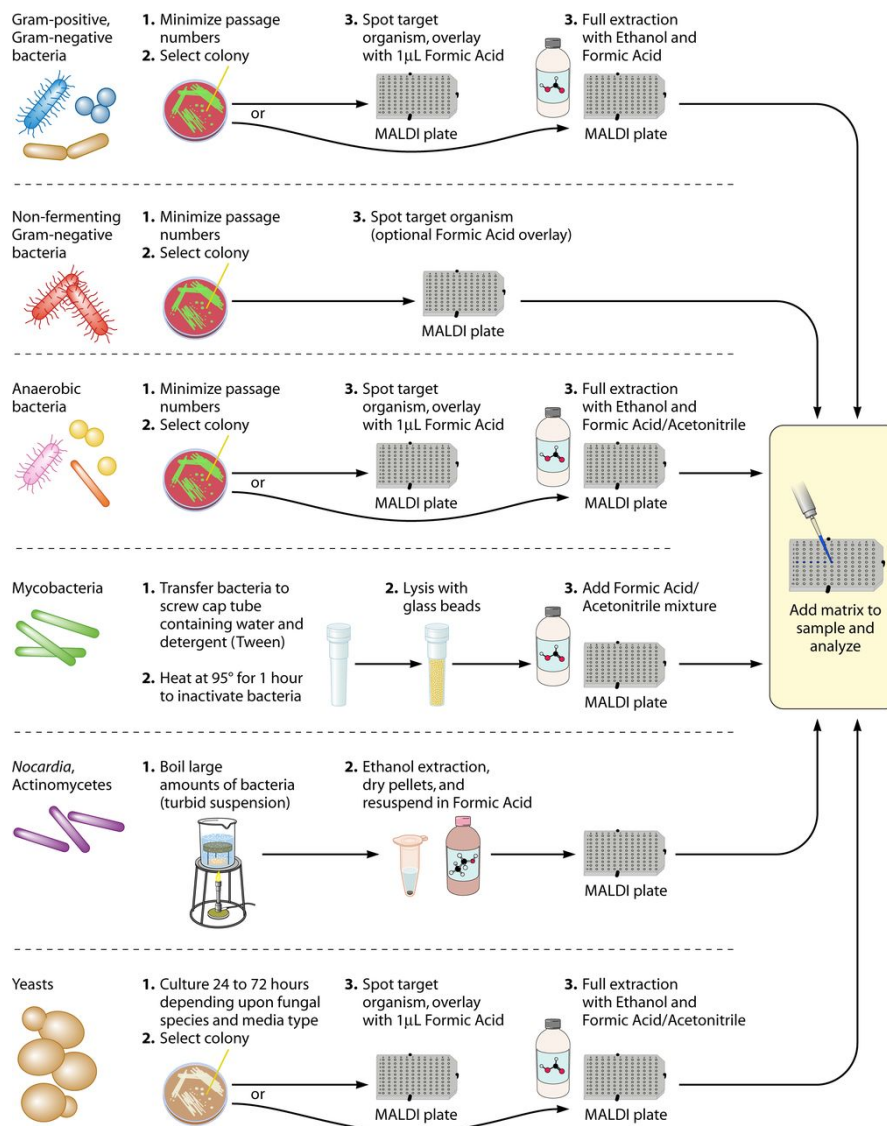
- Introduction to MALDI-TOF
- Performance of MALDI on Bacteria and Yeast
- Performance improvement on turnaround
- Performance improvement on rare isolates
- Future of Mass Spectrometry in Clinical Microbiology

Bruker Biotyper or Vitek MS

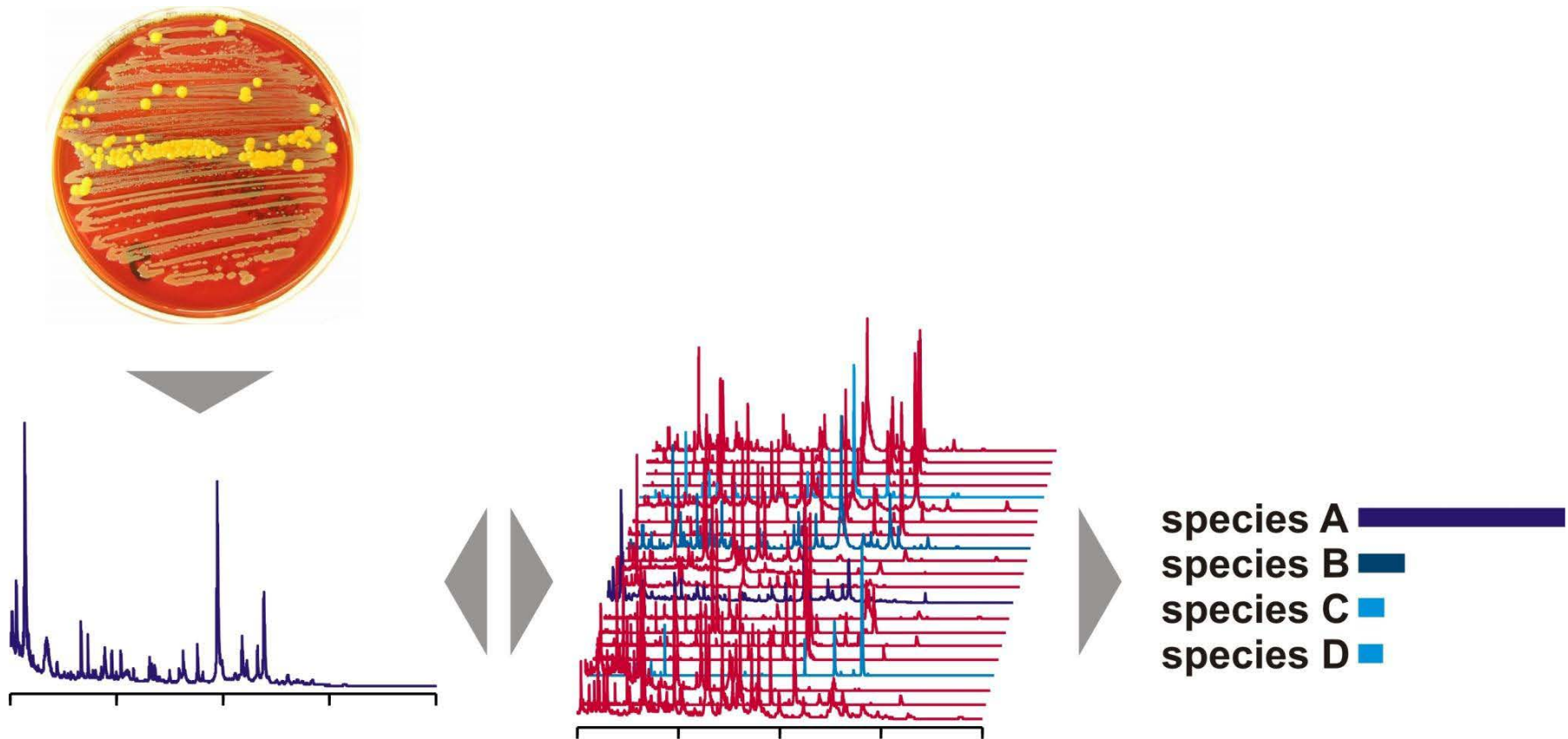


Both systems are FDA approved, but approved databases are different and continue to change.

Additional suggestions for MALDI-TOF MS sample preparations for use with different classes of microbes.

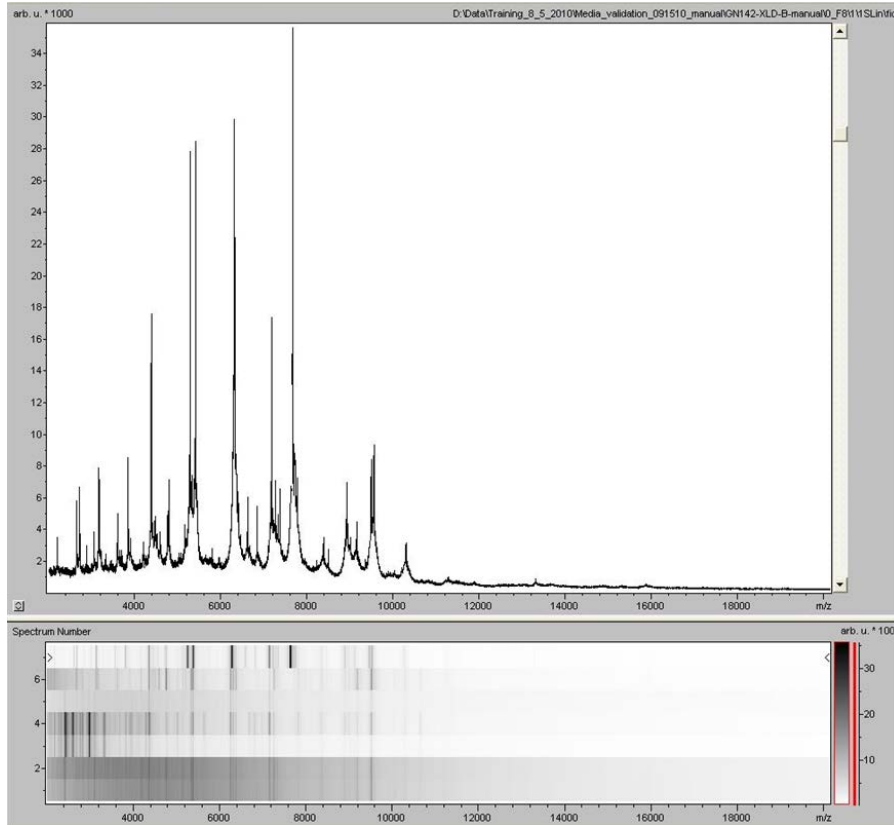


The Process of Mass Spectrometry

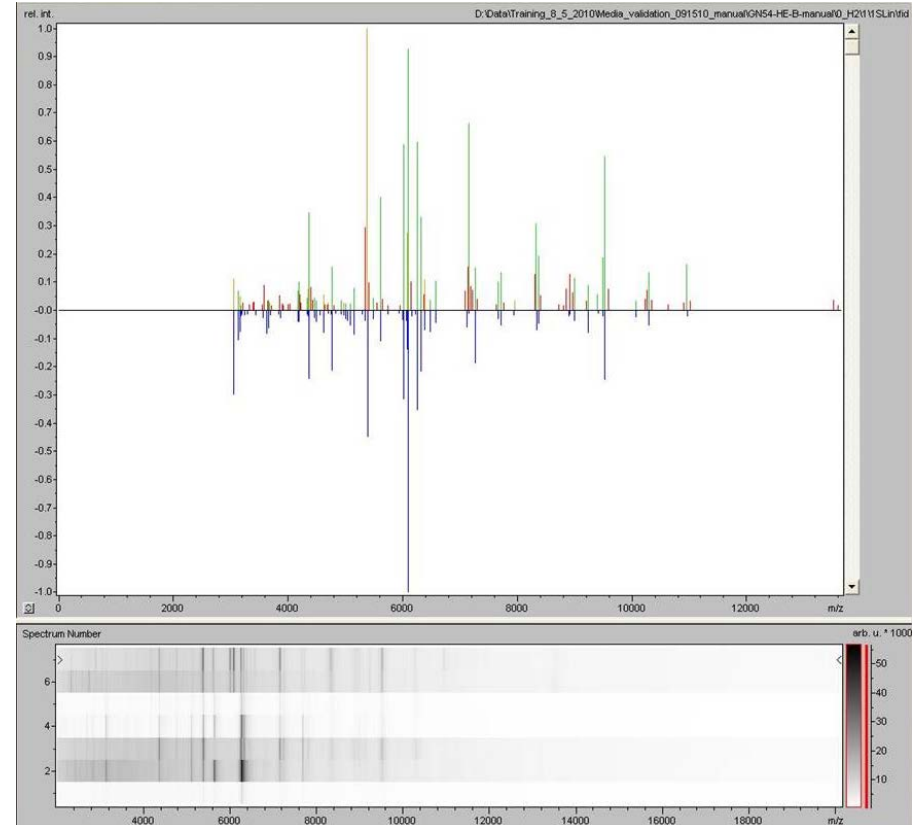


Biotyper: Results output

Raw profile spectrum



Refined profile spectrum



Results are analyzed by a computer, cleaned-up and the spectrum is searched against a database with known spectra.

MALDI identification result

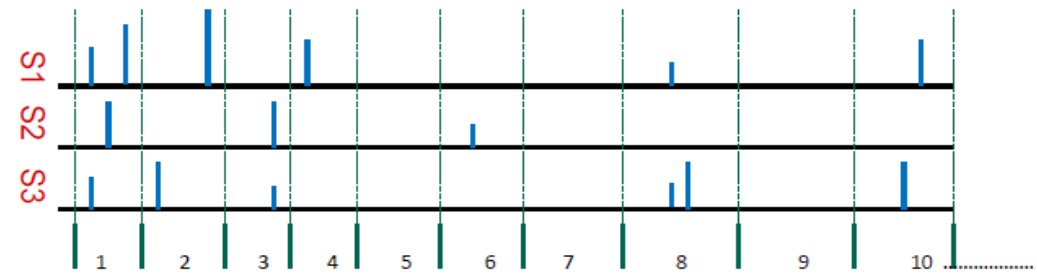
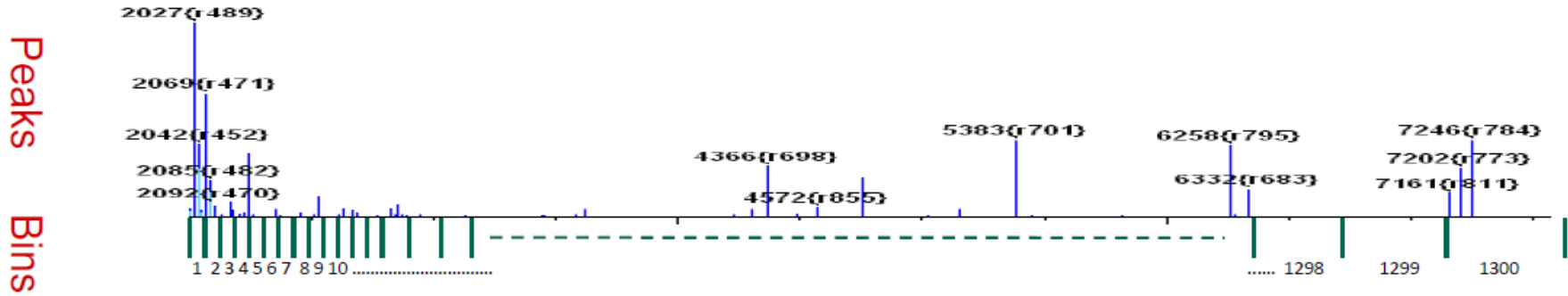
Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (++)	Staphylococcus aureus ssp aureus DSM 3463 DSM	2.194	46170
2 (++)	Staphylococcus aureus ATCC 33862 THL	2.121	1280
3 (+)	Staphylococcus aureus ssp aureus DSM 4910 DSM	1.974	46170
4 (+)	Staphylococcus aureus ssp aureus DSM 20491 DSM	1.887	46170
5 (+)	Staphylococcus aureus ssp aureus DSM 11822 DSM	1.843	46170
6 (+)	Staphylococcus aureus ATCC 29213 THL	1.787	1280
7 (+)	Staphylococcus aureus ssp aureus DSM 346 DSM	1.765	46170
8 (+)	Staphylococcus aureus ATCC 33591 THL	1.745	1280
9 (-)	Staphylococcus aureus ATCC 25923 THL	1.688	1280
10 (-)	Staphylococcus aureus ssp aureus DSM 20652 DSM	1.528	46170

 2.0-3.00 Secure genus and species identification

 1.7-1.99 Probable genus identification

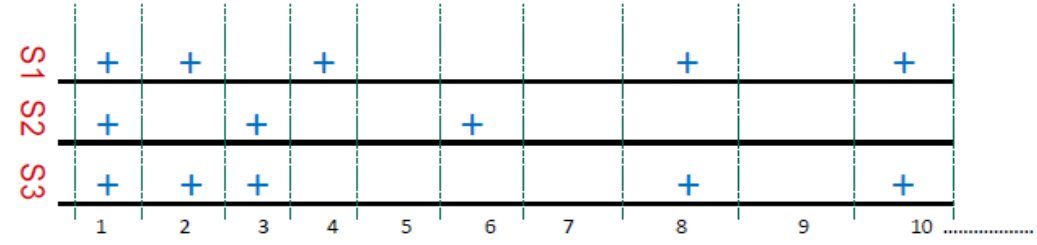
 0.0- 1.69 Unreliable identification

Vitek MS Binning

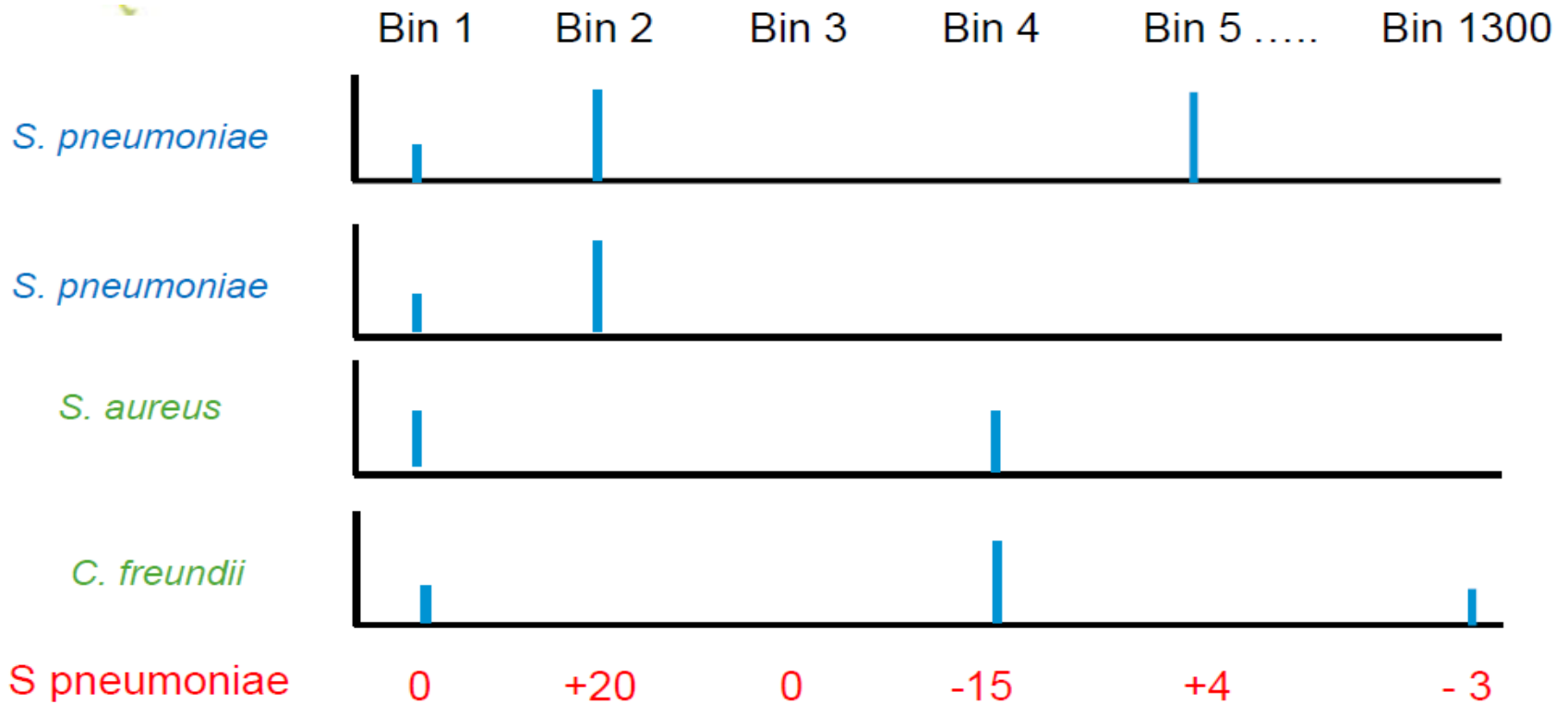


Absence

Presence /



Vitek MS Binning



=> weights for each bin:

++ : highly species-specific (+15, +20....)

+ : moderately species-specific (+3, +4....)

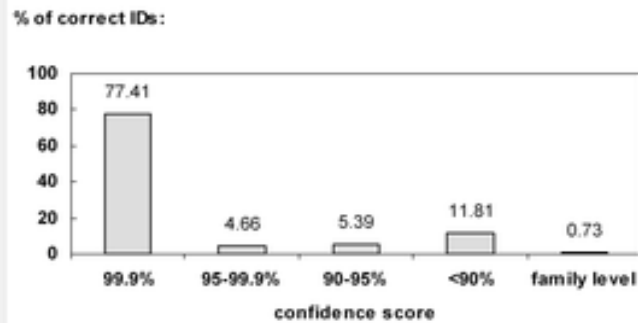
- : peak absent (-3, -4....)

-- : Absent and peak important for other species (-15)

Bacteriology and Yeast

Isolates studied
by MALDI-TOF MS and biochemical
methods:
(N=1,019)

Concordant ID:
94.7% (965 isolates)



Discordant ID tested by 16S:
5.3% (54 isolates)

Agreement of IDs with 16S:
MALDI-TOF MS 63% (34 isolates)
PHOENIX® 11% (9 isolates)
API® 17% (9 isolates)

In a study by Benagli *et al.*,
the authors compared
performance of MALDI-
TOF to biochemical ID and
resolved discrepancies
with sequencing. The
results follow.

Jamal, J Medical Microbiology (2013)

Genus and species ID by API 20AN	No. (%) of isolates	Bruker MS			VITEK MS		
		No. with score <1.7	No. with score 1.7–1.999	No. with score 2–2.299	No. with score ≥3.00	No. with score <85	No. with score 85–90
<i>Bacteroides fragilis</i>	113 (41.2)	1	2	34	76	0	113
<i>Bacteroides ovatus</i>	8 (2.9)	1	1	4	2	0	8
<i>Bacteroides thetaiotaomicron</i>	15 (5.5)	1	0	13	1	0	15
<i>Bacteroides uniformis</i>	5 (1.8)	0	0	3	2	0	5
<i>Bacteroides vulgatus</i>	10 (3.6)	1	2	7	0	0	10
<i>Clostridium butyricum</i>	1 (0.4)	0	0	1	0	0	1
<i>Clostridium difficile</i>	70 (25.5)	1	10	51	8	0	70
<i>Clostridium histolyticum</i>	2 (0.7)	0	2	0	0	0	2
<i>Clostridium perfringens</i>	14 (5.1)	0	2	1	11	0	2
<i>Clostridium sporogenes</i>	1 (0.4)	1	0	0	0	0	1
<i>Prevotella bivia</i>	31 (11.3)	1	3	16	11	0	31
<i>Prevotella disiens</i>	1 (0.4)	0	0	1	0	0	1
<i>Peptostreptococcus asaccharolyticus</i>	2 (0.7)	1	0	1	0	0	2
<i>Veillonella parvula</i>	1 (0.4)	0	0	1	0	0	1
Total no. (%)	274	8 (2.9)	22 (8)	133 (48.5)	111 (40.5)	0	274 (100)

TABLE 1 Performance characteristics of the Vitek MS system in identifying clinically relevant *Candida* species

Organism	No. (%) of isolates				
	Total	Identified correctly to genus	Identified correctly to species	Unidentified	Misidentified
<i>Candida albicans</i>	58	57 (98.3)	57 (98.3)	0 (0)	1 (1.7) ^a
<i>Candida dubliniensis</i>	34	34 (100)	34 (100)	0 (0)	0 (0)
<i>Candida famata</i>	29	29 (100)	28 (96.6)	0 (0)	0 (0)
<i>Candida glabrata</i>	62	62 (100)	62 (100)	0 (0)	0 (0)
<i>Candida guilliermondii</i>	36	35 (97.2)	35 (97.2)	1 (2.8)	0 (0)
<i>Candida haemulonii</i>	12	12 (100)	12 (100)	0 (0)	0 (0)
<i>Candida inconspicua</i>	23	23 (100)	23 (100)	0 (0)	0 (0)
<i>Candida intermedia</i>	7	7 (100)	7 (100)	0 (0)	0 (0)
<i>Candida kefyr</i>	30	30 (100)	30 (100)	0 (0)	0 (0)
<i>Candida krusei</i>	53	53 (100)	53 (100)	0 (0)	0 (0)
<i>Candida lambica</i>	9	9 (100)	9 (100)	0 (0)	0 (0)
<i>Candida lipolytica</i>	28	28 (100)	28 (100)	0 (0)	0 (0)
<i>Candida lusitanae</i>	33	30 (90.9)	29 (87.9)	3 (9.1)	0 (0)
<i>Candida norvegensis</i>	30	29 (96.7)	29 (96.7)	1 (3.3)	0 (0)
<i>Candida parapsilosis</i>	73	72 (98.6)	72 (98.6)	0 (0)	1 (1.4) ^b
<i>Candida pelliculosa</i>	33	33 (100)	33 (100)	0 (0)	0 (0)
<i>Candida rugosa</i>	6	6 (100)	6 (100)	0 (0)	0 (0)
<i>Candida tropicalis</i>	54	51 (94.4)	49 (90.7)	3 (5.6)	0 (0)
<i>Candida utilis</i>	8	8 (100)	8 (100)	0 (0)	0 (0)
<i>Candida zeylanoides</i>	8	8 (100)	8 (100)	0 (0)	0 (0)
Total	626	616 (98.4)	612 (97.8)	8 (1.3)	2 (0.3)

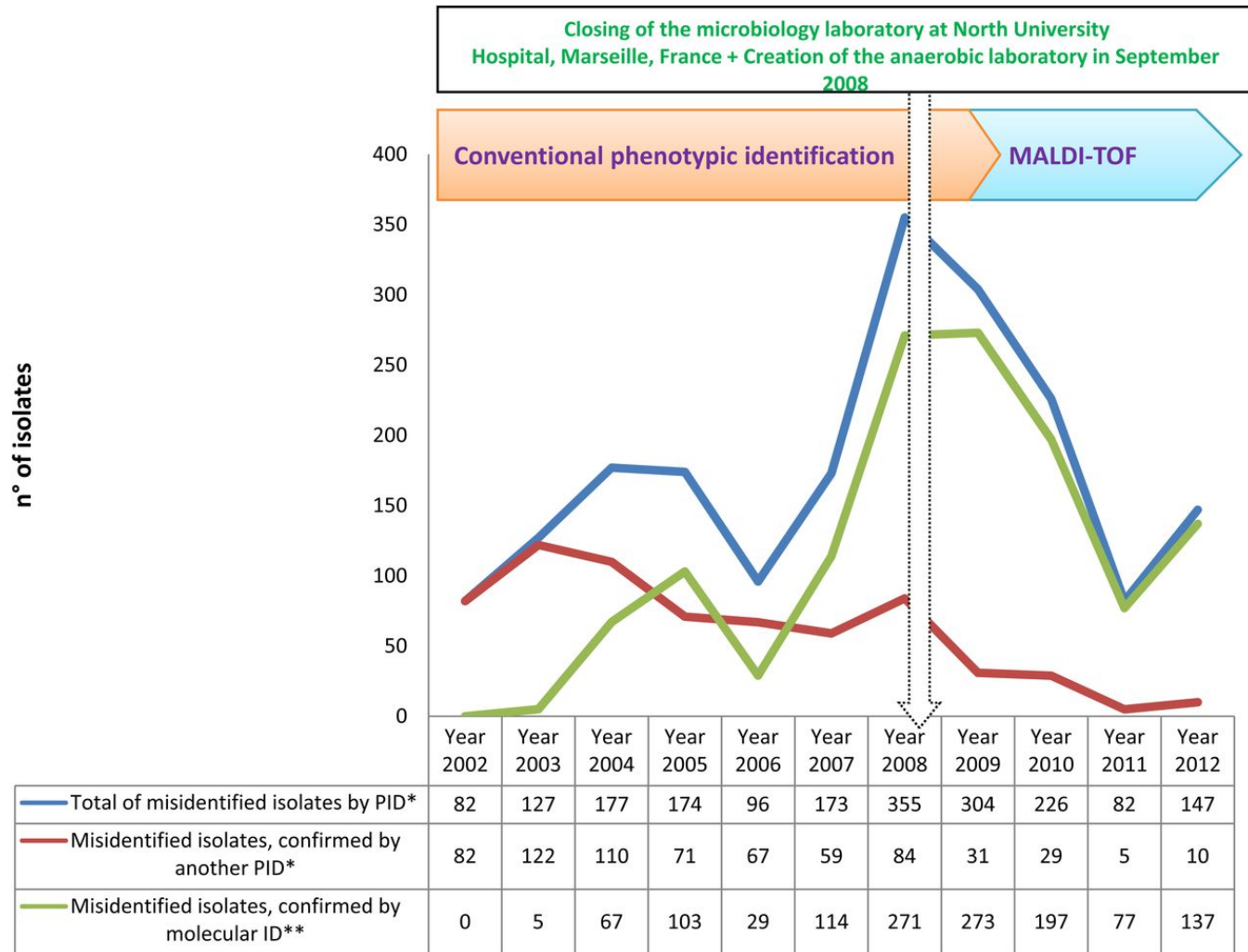
^a Isolate misidentified as *C. dubliniensis*.

^b Isolate misidentified as *C. pelliculosa*.

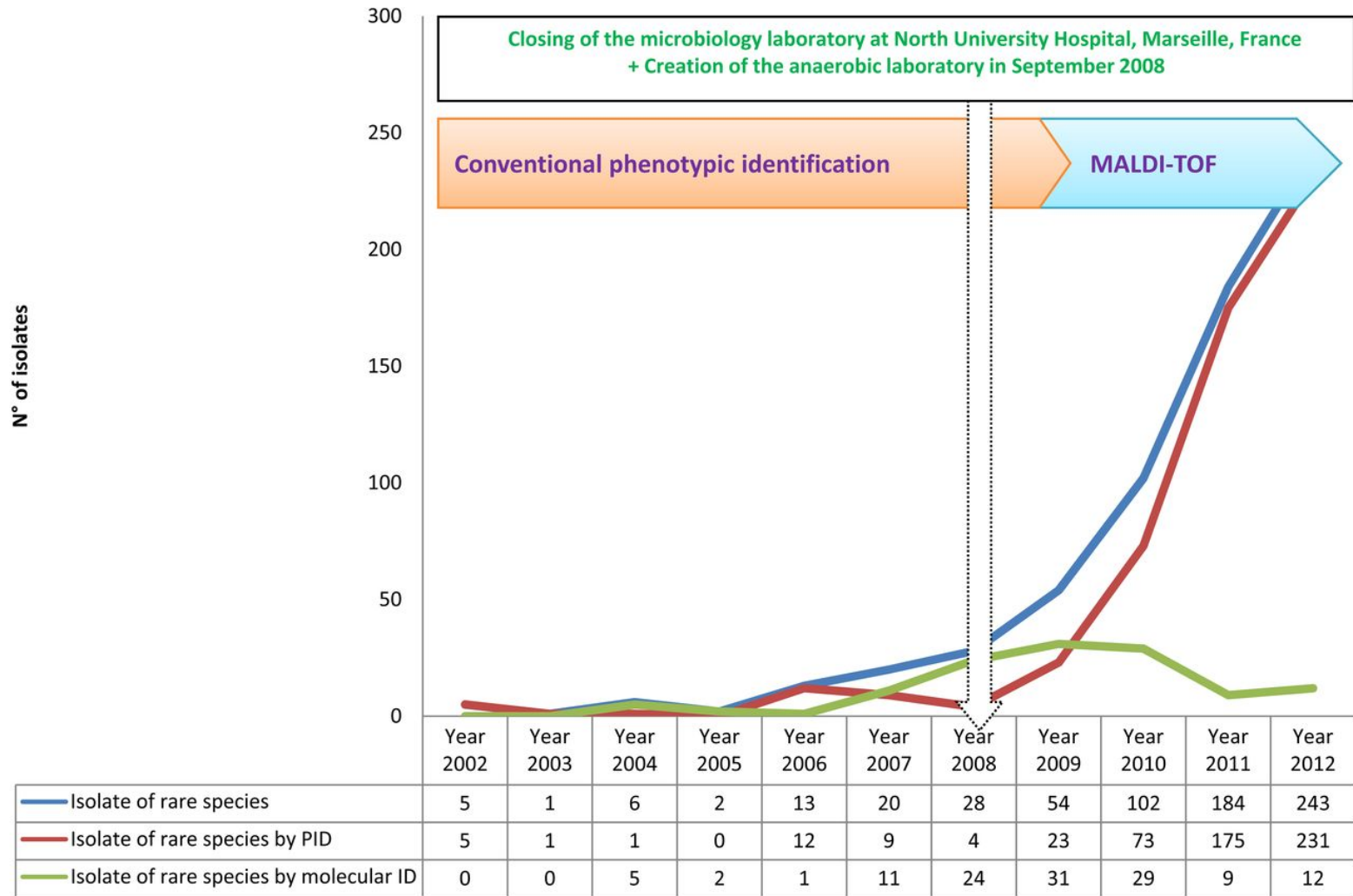
Biotyper and Vitek MS for Yeast

- *Candida*
 - **Vitek MS**: 87.3% species level identification, 3.8% no identification, **2.5% misidentification**
 - **Bruker Biotyper**: 92.3% species level identification, 6.4% no identification, **0% misidentification**
- Non-*Candida* isolates
 - **Vitek MS**: 72.5% species level identification, 2.5% no identification, **12.5% misidentification**
 - **Bruker Biotyper**: 80% species level identification, 20% no identification, **0% misidentification**
- Overall, the rate of correct identification to species level was comparable between the two systems

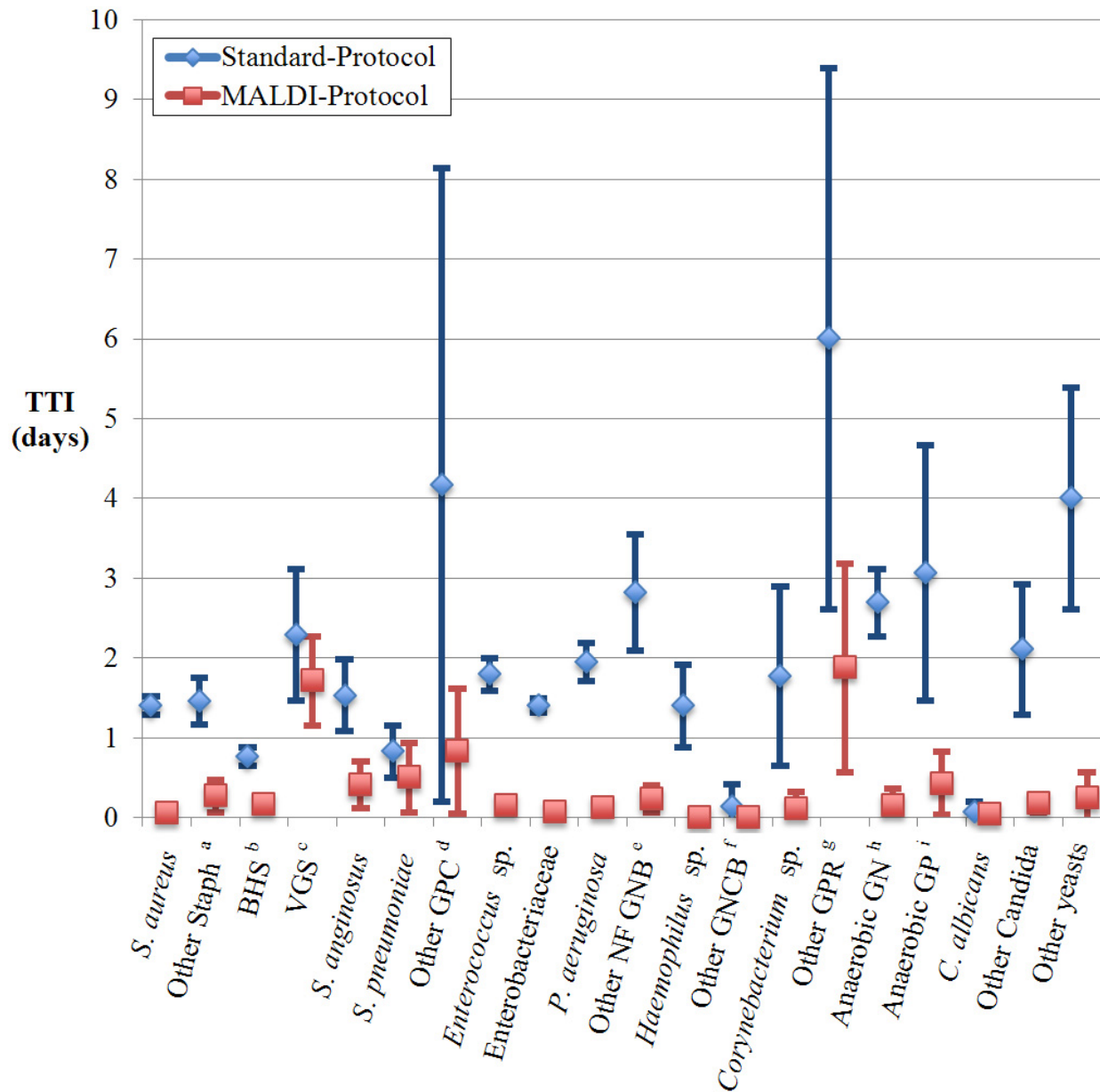
Time course of the numbers of total isolates misidentified using phenotypic identification (PID*), isolates confirmed by a second PID* and isolates confirmed by molecular identification (ID) over 11 years of routine identification in our clinical laboratory.**



Time course of the numbers of isolates of 128 rare species, 48 of which were identified using phenotypic identification (PID), and 75 of which were identified using molecular identification (ID).



Turnaround



Tan KE, et al, JCM– Kindly provided by K. Carroll, MD

Organism-group	n	Mean # of days isolate identified earlier	Proportion identified earlier by MALDI-protocol, by number of days of workup								
			<0d ^a	0d ^b	1d	2d	3d	4d	5d	6d	>6d
		(days)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)	(%)
<i>S. aureus</i>	109	1.35		1.8	66.1	28.4	2.8	0.9			
Other Staph ^c	26	1.19		7.7	65.4	26.9					
BHS ^d	72	0.60	1.4	38.9	58.3	1.4					
VGS ^e	7	0.57		42.9	57.1						
<i>S. anginosus</i>	17	1.12		41.2	29.4	5.9	23.5				
<i>S. pneumoniae</i>	6	0.33		66.7	33.3						
Other GPC ^f	6	3.33		33.3	16.7	16.7				16.7	16.7
Enterococcus sp.	78	1.64		1.3	51.3	34.6	9.0	2.6	1.3		
Enterobacteriaceae	284	1.34		2.8	69.4	23.2	2.1	1.1	1.1	0.4	
<i>P. aeruginosa</i>	77	1.82			41.6	49.4	2.6	1.3	2.6	2.6	
Other NF GNB ^g	39	2.59		2.6	30.8	35.9	2.6	15.4	5.1	5.1	2.6
<i>Haemophilus</i> sp.	10	1.40			80.0		20.0				
Other GNGB ^h	7	0.14		85.7	14.3						
<i>Corynebacterium</i> sp.	9	1.67		22.2	33.3	22.2		22.2			
Other GPR ⁱ	8	4.13	12.5		37.5			12.5			37.5
Anaerobic GN ^j	26	2.54		3.8		65.4	7.7	19.2		3.8	
Anaerobic GP ^k	14	2.64		21.4	14.3	28.6	7.1	7.1		14.3	7.1
<i>C. albicans</i>	52	0.04	3.8	92.3	1.9		1.9				
Other <i>Candida</i> sp.	56	1.93		8.9	67.9	7.1	3.6	3.6	3.6		5.4
Other yeasts	8	3.75				25.0	37.5	12.5		12.5	12.5
All organisms	911	1.45	0.4	13.5	52.7	23.6	3.7	2.7	1.1	1.1	1.1

Cost-effectiveness of switching to MALDI-TOF MS for routine bacterial identification

- September 2009
 - Switched from conventional biochemicals (Vitek 2 and API) to MALDI-TOF MS (Bruker)
- Cost analysis performed

	October 2008-September 2009	October 2009-September 2010
Isolates Tested	33,320	38,624
Biochemical Costs	\$193,754	\$5,374
MALDI-TOF	-	\$15,836
TOTAL	\$193,754	\$21,210
Avg Cost/ID	\$5.81	\$.54

Annual Savings = \$177,090

“allowed decrease of 89.3% of the cost of bacterial identification in the first year.”

In addition:

Decreased waste from 1,424kg to 44kg

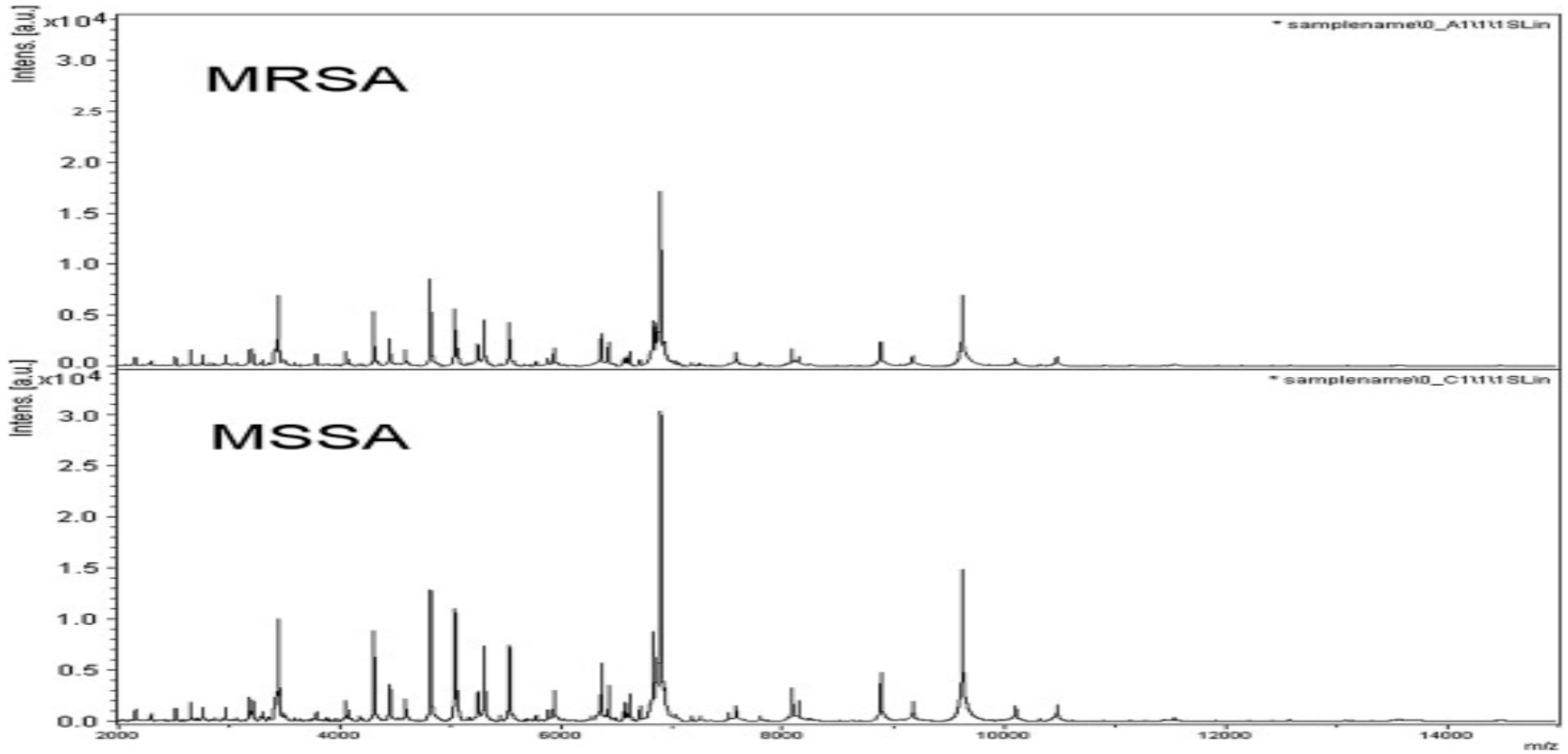
Decreased subculture media of \$1,102

Decreased sequencing cost of \$1,650

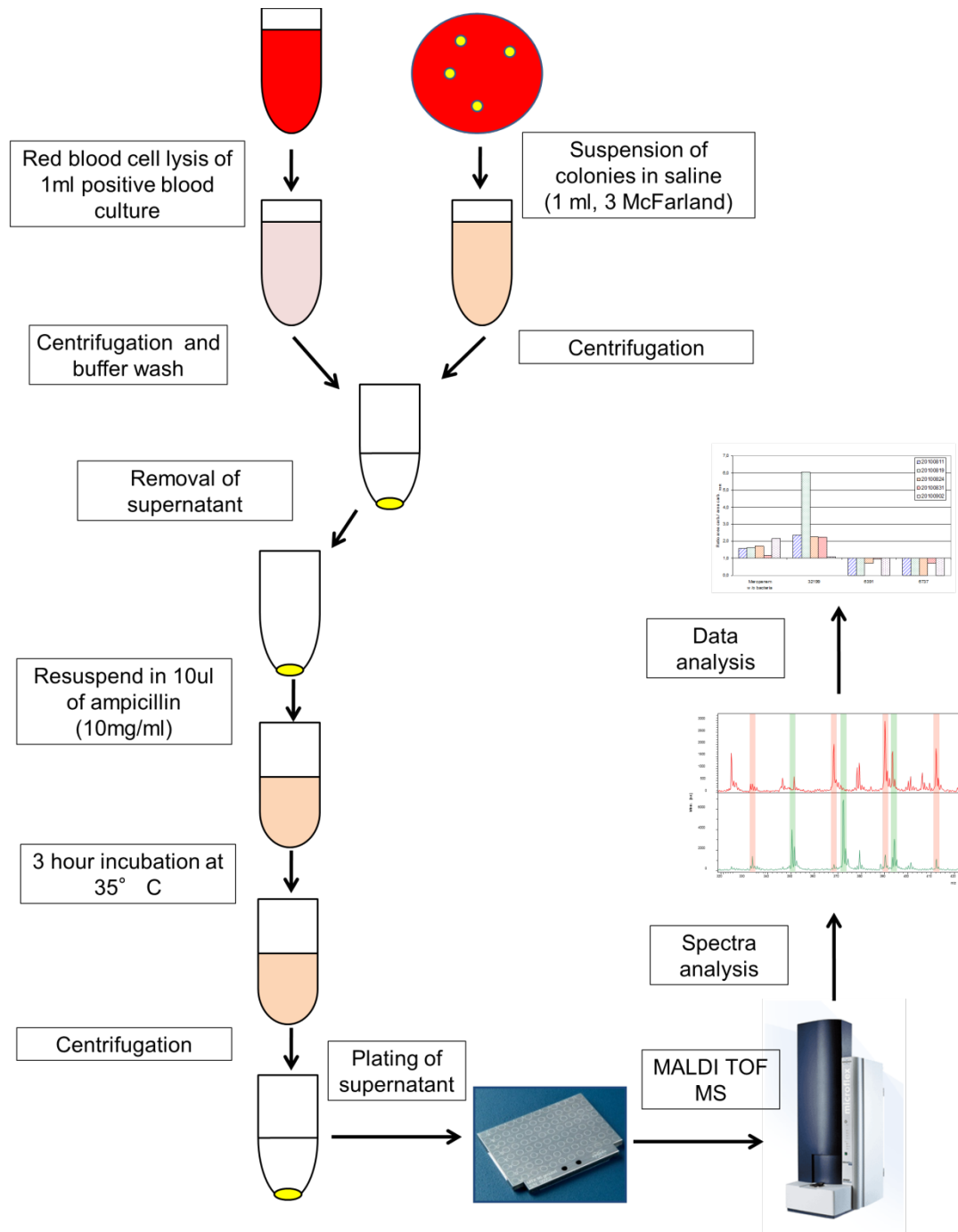
What about Susceptibility Testing?

Table 1. Previous studies investigating MRSA and MSSA associated peaks by mass spectrometry.						
Study	Sample number	Sample preparation	Peak evaluation	MRSA falsely identified	Peaks associated with MRSA (Da)	Peaks associated with MSSA (Da)
Edward-Jones et al	14	Formic acid	Presence of peaks	NA	511, 563, 640, 743, 767, 773, 854, 891, 999, 1026, 1140, 1165, 1229, 2127	2548.2647
Du et al	76	None	Presence of peaks	7 out of 43	1834, 1874, 2413, 2453, 2490	2093, 2308, 2345, 2547, 2585, 2686, 2723
Shah et al	99	Lysis by urea, Lysostaphin, mechanical disruption	Peak intensity	26 out of 50	5709, 7694, 15, 308, 18, 896	3081, 5893, 9580
Majcherczyk et al	4	None	Presence of peaks	NA	peaks around 2450	NA
Sun et al	34	NA	NA	None	NA	NA

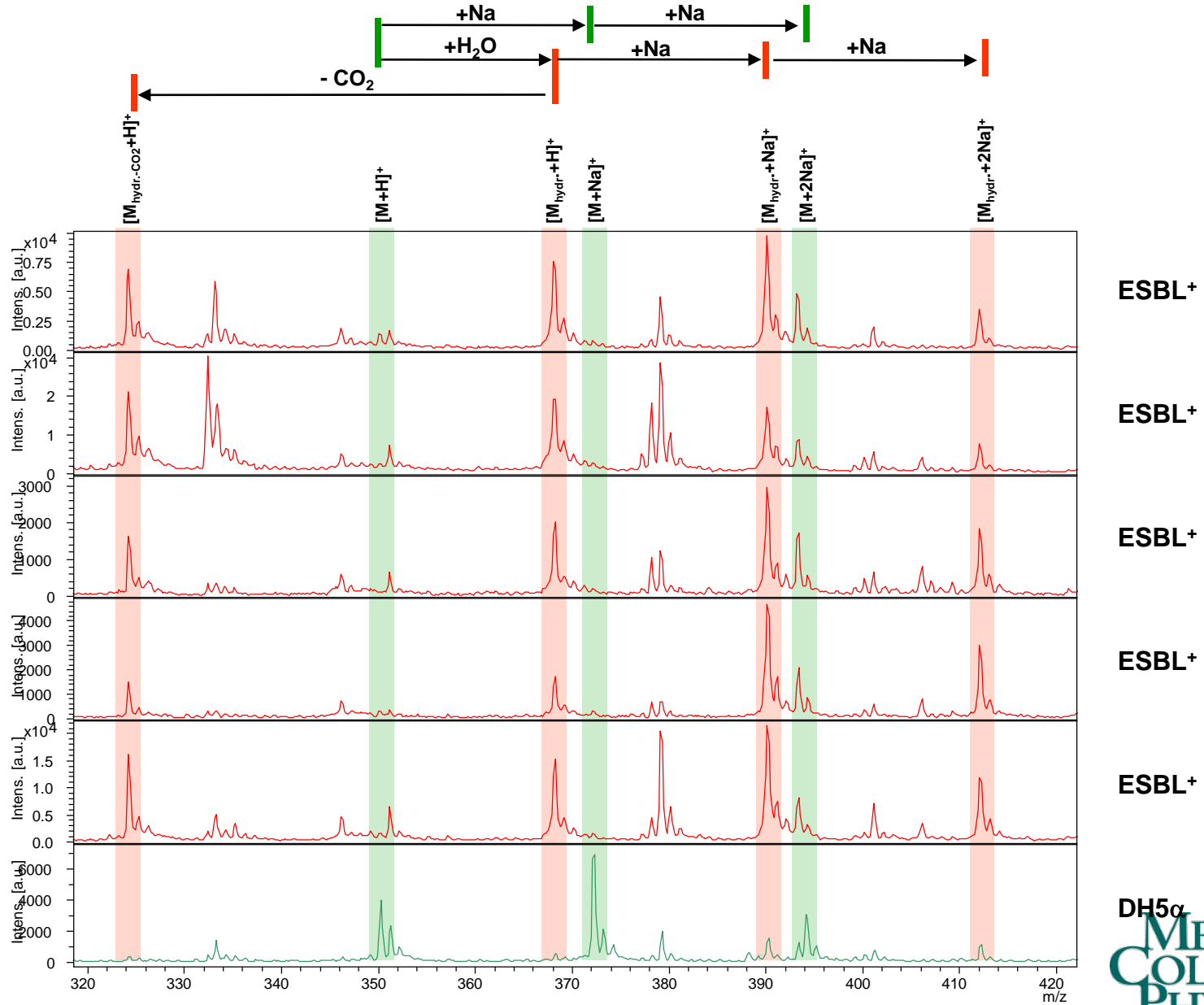
Szabados F. et al. 2012. **Identical MALDI TOF MS-derived peak profiles in a pair of isogenic SCCmec-harboring and SCCmec-lacking strains of Staphylococcus aureus.** *J Infection.* 65: 400-405.



Szabados F. et al. 2012. Identical MALDI TOF MS-derived peak profiles in a pair of isogenic SCCmec-harboring and SCCmec-lacking strains of *Staphylococcus aureus*. *J Infection*. 65: 400-405.

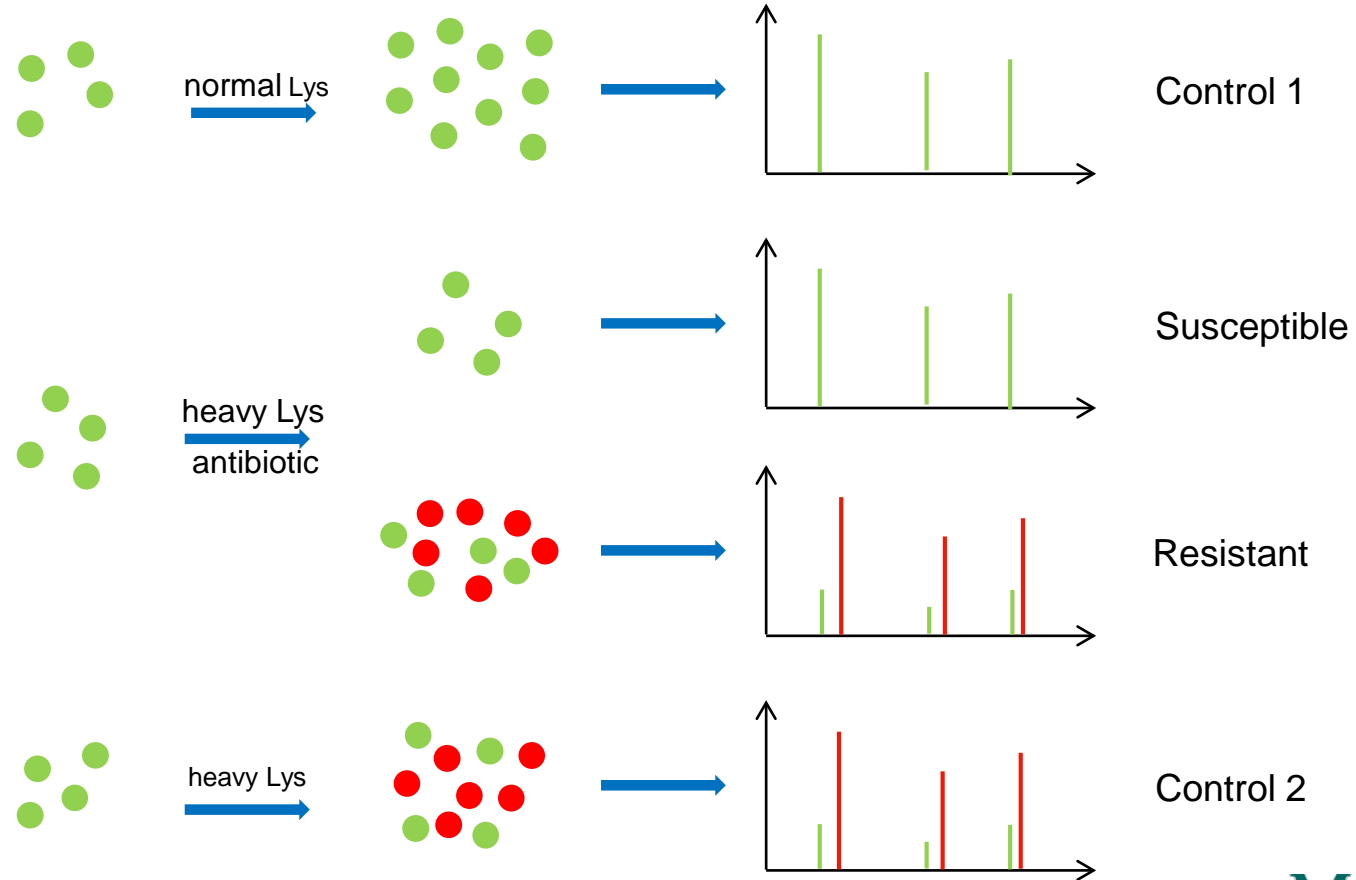


Conversion of Ampicillin by *E.coli*



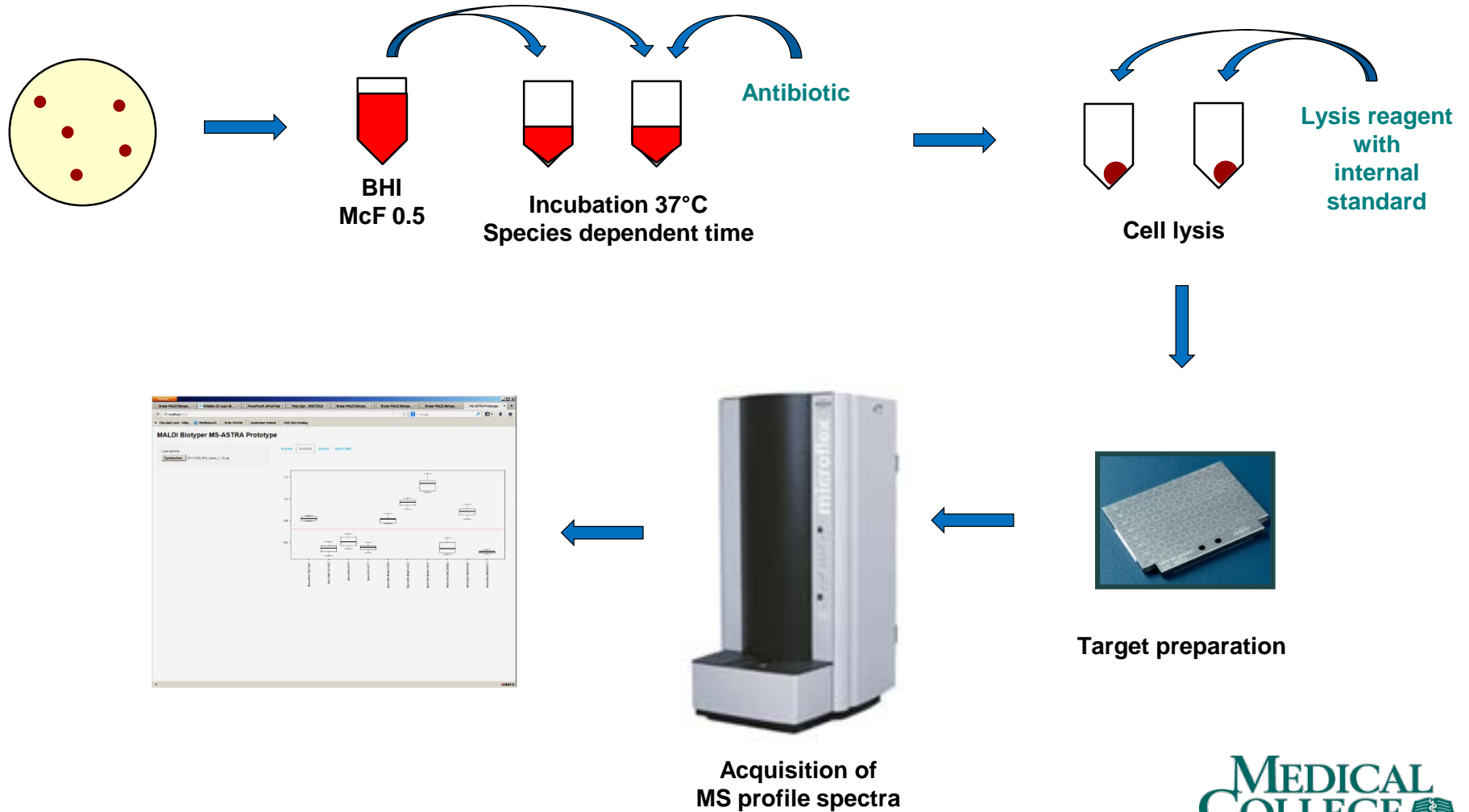
MS-RESIST

Resistance detection through stable isotopes



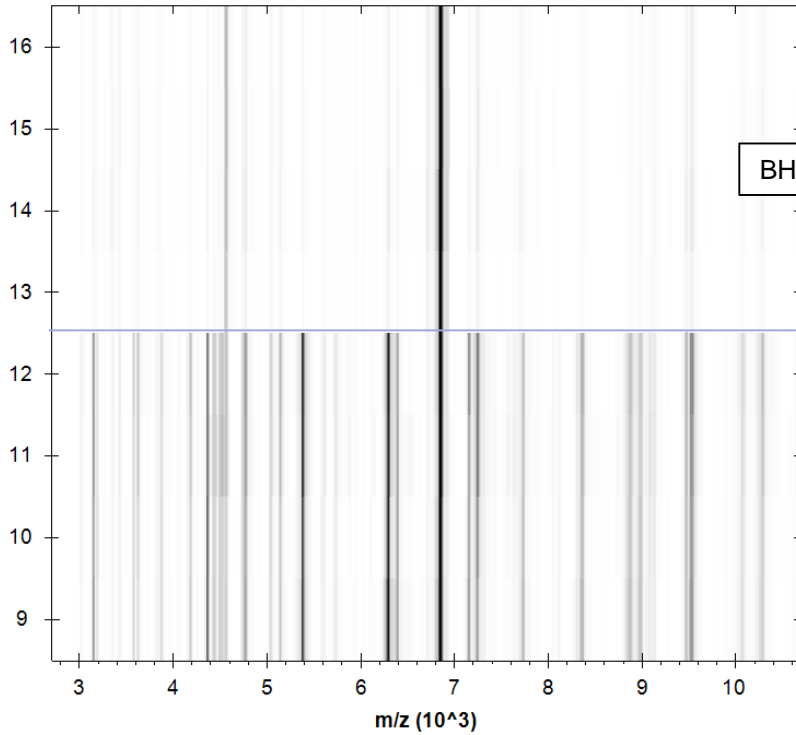
MS-ASTRA

MALDI-TOF MS as quantitative growth monitor



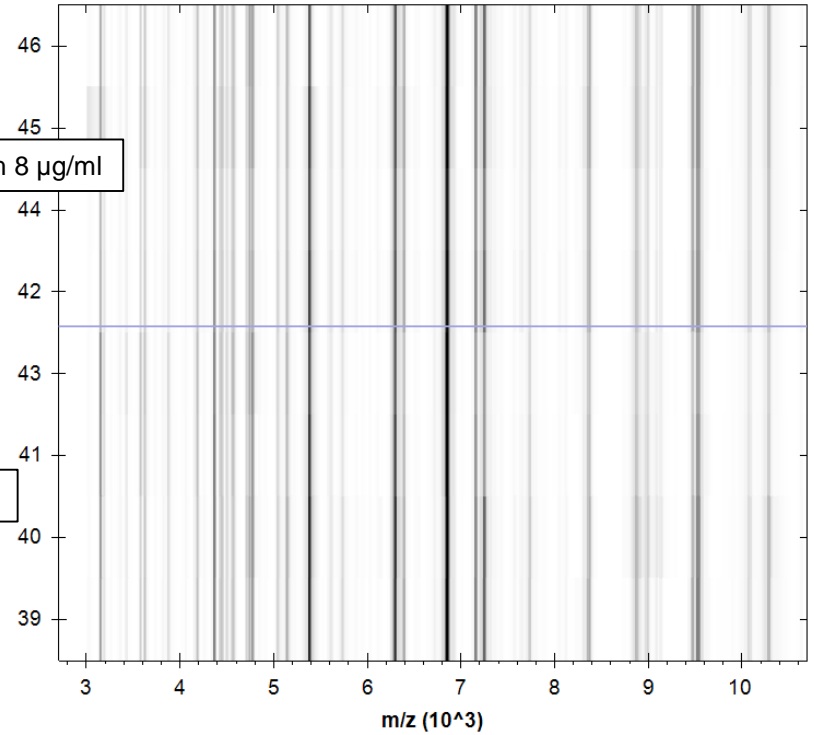
MS-ASTRA pseudo gel view

Standard $[M+H]^{2+}$



Susceptible

Standard $[M+H]^{2+}$



Resistant

Conclusions

- Data demonstrates excellent performance of MALDI-TOF MS for identification of bacteria and yeast
 - Current IVD indications are limited, but RUO databases are comprehensive and IVD continues to expand
- High Capital Cost can be overcome by consumable savings and turnaround improvement
- Susceptibility testing methods being developed.
- MALDI-TOF and current technologies represent the beginning of protein revolution