PRACTICALITIES OF MALDI-TOF*

REPORTING, QC/TROUBLESHOOTING, FURTHER APPLICATIONS (AND OTHER MISCELLANY)

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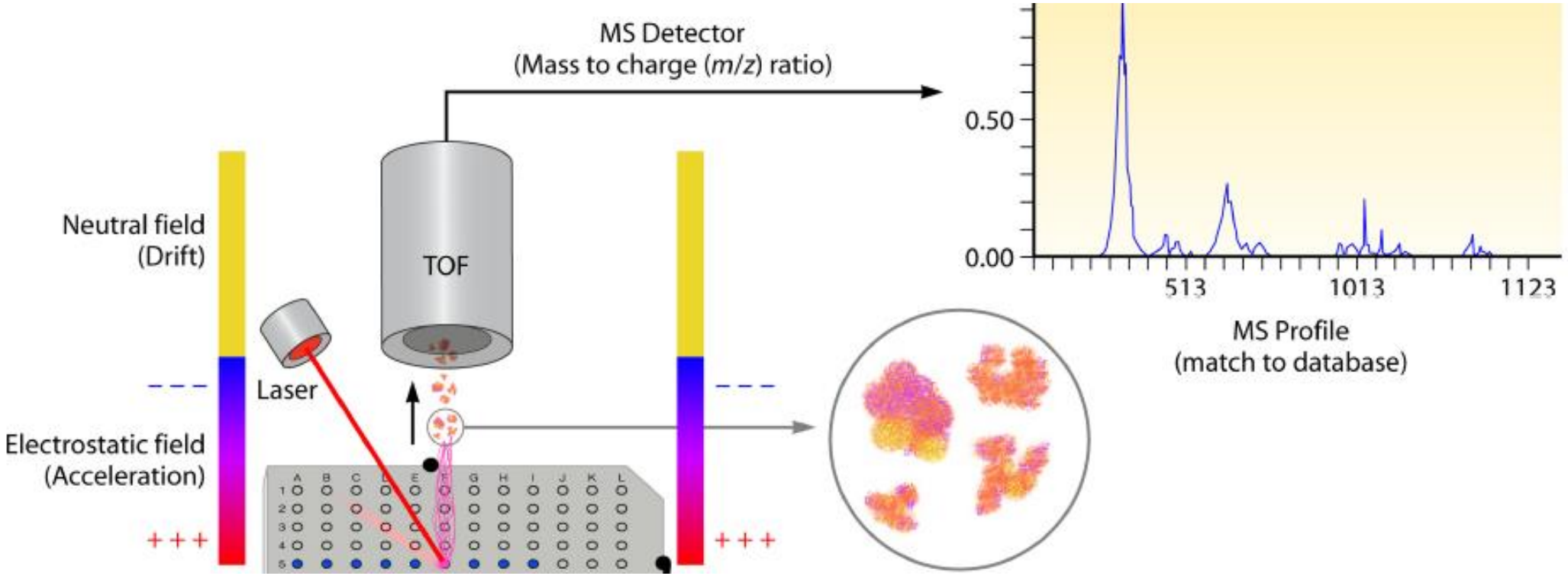
DISCLOSURES

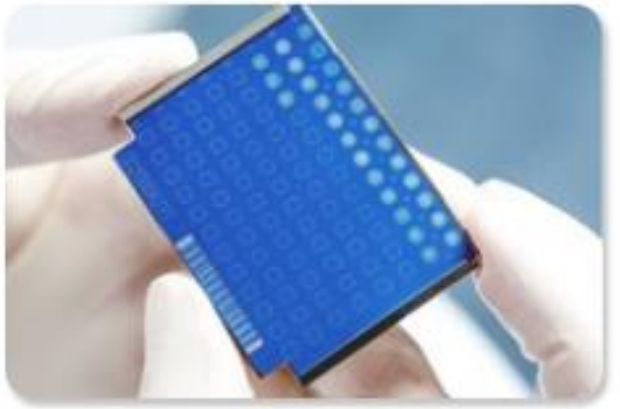
- No conflicts of interest to disclose: no affiliation with any company
- As my lab uses Bruker, will focus on this platform, I will focus on this platform.



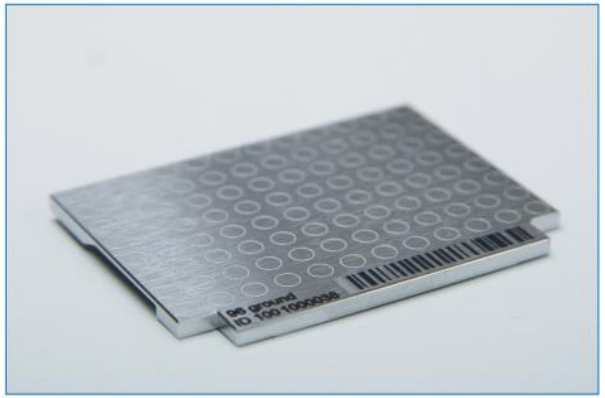
Clark AE, Kaleta EJ, Arora A, Wolk DM. Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry: a Fundamental Shift in the Routine Practice of Clinical Microbiology. Clinical Microbiology Reviews. 2013;26(3):547-603.

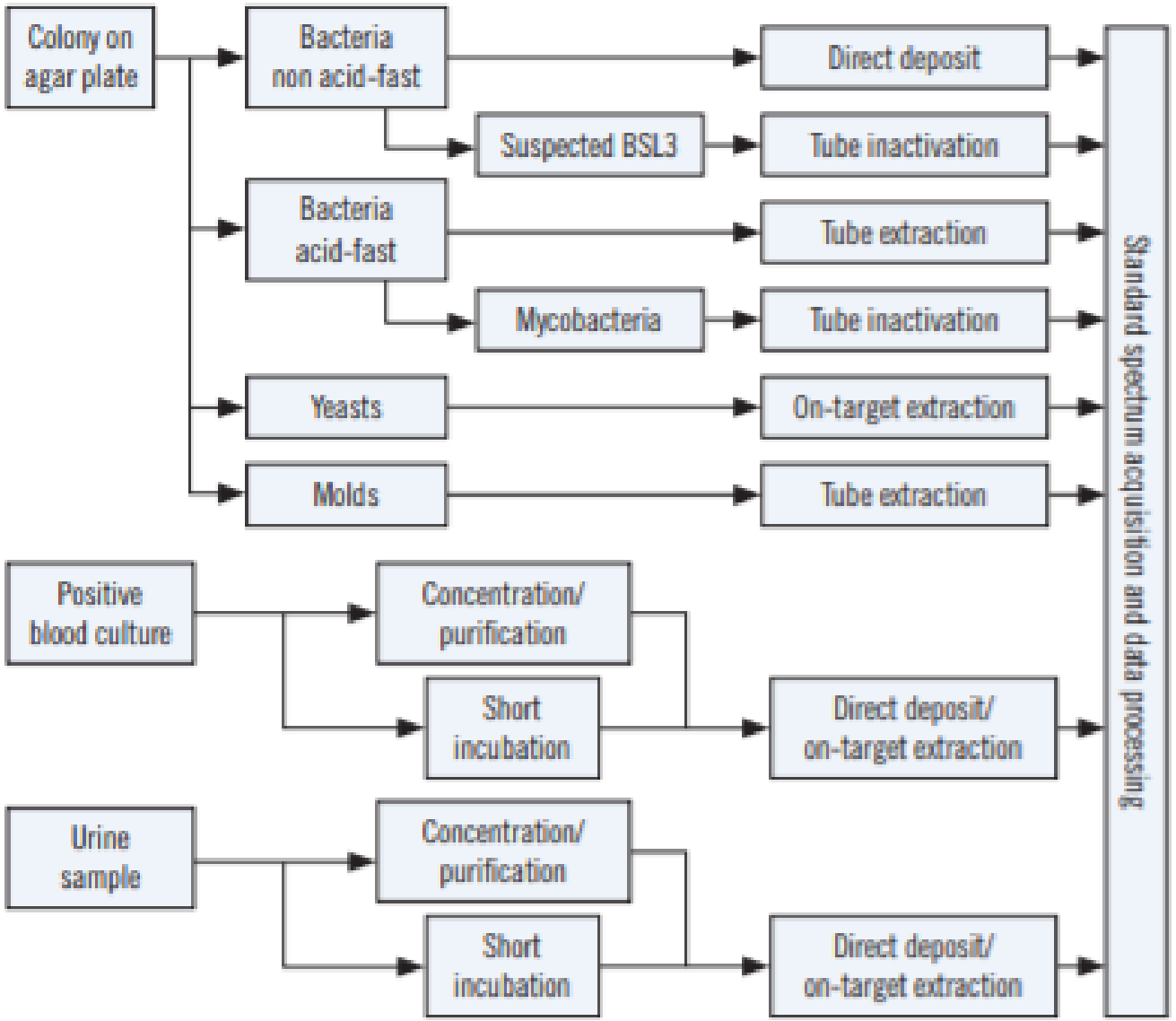
QUICK REVISION (FOR THOSE WHO MISSED TONY'S TALK)

SLIDES



- Biotyper has two types of target slides
  - disposable, single use only slides (MBT Biotarget 96)
  - Reusable, ground steel target plates with hydrophobic target rings
    - Laser requires an extremely flat surface – target plate will become **unusable** if dropped or bent
    - Needs to be thoroughly washed after use to prevent memory effects




Colony on agar plate
Bacteria non acid-fast
Direct deposit
Suspected BSL3
Tube inactivation
Bacteria acid-fast
Tube extraction
Mycobacteria
Tube inactivation
Yeasts
On-target extraction
Molds
Tube extraction
Positive blood culture
Concentration/ purification
Short incubation
Direct deposit/ on-target extraction
Urine sample
Concentration/ purification
Short incubation
Direct deposit/ on-target extraction
Standard spectrum acquisition and data processing

SAMPLE PREPARATION

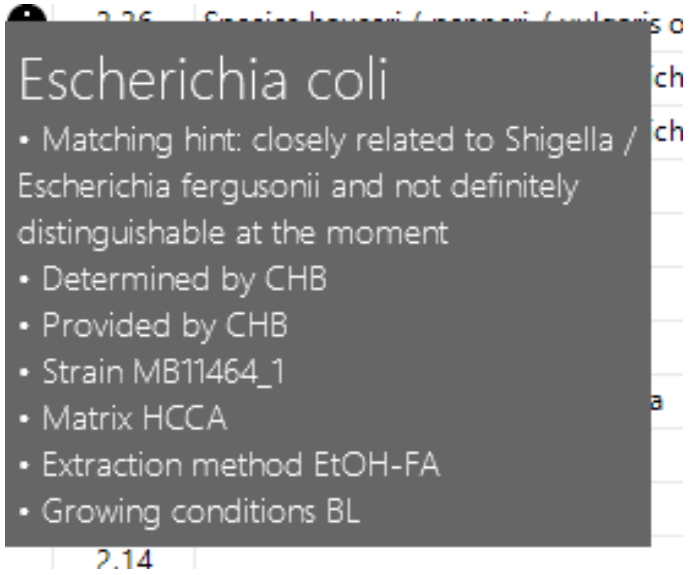
REPORTING

- Score value: log score that represents the probability of isolate ID
- SV is a composite score of 3 values
  - Number of peaks in the reference spectrum that have a closely matching partner in the test spectrum (value 0-1)
  - Number of peaks in the test spectrum that have a closely matching partner in the reference spectrum (value 0-1)
  - Peak height symmetry of the matching peaks (value 0 – 1)
- Above 3 values are multiplied together and normalised to 1000, and then the base 10 logarithm is taken to give a final Bruker score

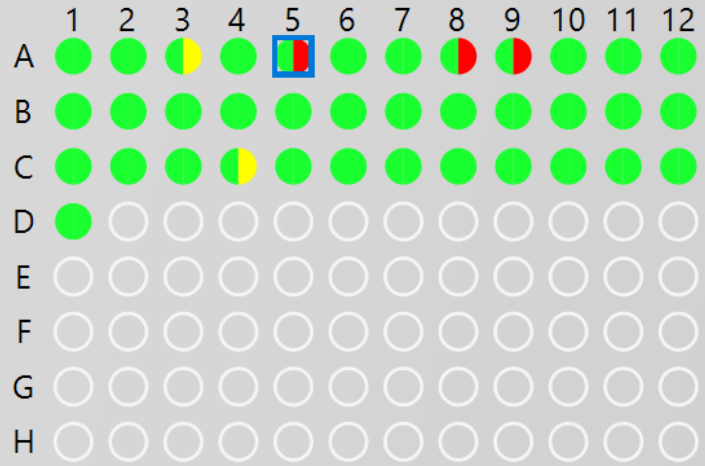
Michael A Reeve, Denise Bachmann, MALDI-TOF MS protein fingerprinting of mixed samples, *Biology Methods and Protocols*, Volume 4, Issue 1, 2019, bpz013, https://doi.org/10.1093/biomethods/bpz013

Range	Interpretation	Symbols	Color
2.00 - 3.00	High Confidence Identification	(+++)	green
1.70 - 1.99	Low Confidence Identification	(+)	yellow
0.00 - 1.69	No Organism Identification Possible	(-)	red

- In general,
  - An organism with a score value > 2.00 can be reported to the genus and species level
  - An organism with a score value between 1.70 and 2.00 can be reported to genus level only
  - SV < 1.7 – no organism ID possible
- The Biotyper also provides matching hints with further taxonomical information or caveats
- Any score should be correlated to gram stain and culture characteristics, clinical context and biochemical features



-IVD-1001003932



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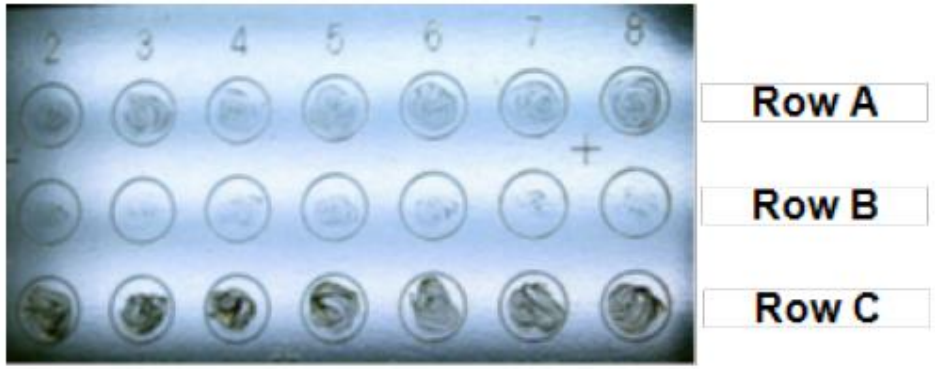
	Name	Position	Sample Type	Detected Species	Score	Comment	Consistency			Export status
	A1	A1	BTS	Escherichia coli	2.34	closely related to Shigella / Escherichia fergusonii and not definitely distinguishable at the moment	high ◉	low ○	none ○	export
	A2	A2	Standard	Actinomyces turicensis	2.32		high ○	low ○	none ◉	export
	A3	A3	Standard	Dialister micraerophilus	1.95		high ○	low ○	none ◉	export
	A4	A4	Standard	Streptococcus dysgalactiae	2.28	Species canis / dysgalactiae / pyogenes of the genus Streptococcus have very similar patterns: Therefore distinguishing their species is difficult.	high ◉	low ○	none ○	export
	A5	A5	Standard	No Identification Possible	1.31		high ○	low ○	none ◉	export
	A6	A6	Standard	Corynebacterium massiliense	2.00		high ◉	low ○	none ○	export
	A7	A7	Standard	Streptococcus agalactiae	2.39		high ◉	low ○	none ○	export
	A8	A8	Standard	No Identification Possible	1.50		high ○	low ○	none ◉	export
	A9	A9	Standard	No Identification Possible	1.63		high ○	low ○	none ◉	export
	A10	A10	Standard	Streptococcus agalactiae	2.39		high ◉	low ○	none ○	export
	A11	A11	Standard	Streptococcus vestibularis	2.35		high ○	low ◉	none ○	export
	A12	A12	Standard	Streptococcus agalactiae	2.44		high ◉	low ○	none ○	export
	B1	B1	Standard	Staphylococcus pseudintermedius	2.15		high ◉	low ○	none ○	export
	B2	B2	Standard	Staphylococcus aureus	2.33		high ◉	low ○	none ○	export
	B3	B3	Standard	Pseudomonas aeruginosa	2.09		high ◉	low ○	none ○	export
	B4	B4	Standard	Enterococcus faecalis	2.28		high ◉	low ○	none ○	export
	B5	B5	Standard	Klebsiella oxytoca	2.40	Klebsiella oxytoca and species ornithinolytica / planticola / terrigena of the genus Raoultella have very similar patterns: Therefore distinguishing th...	high ◉	low ○	none ○	export
	B6	B6	Standard	Candida albicans	2.15		high ◉	low ○	none ○	export
	B7	B7	Standard	Escherichia coli	2.41	closely related to Shigella / Escherichia fergusonii and not definitely distinguishable at the moment	high ◉	low ○	none ○	export
	B8	B8	Standard	Escherichia coli	2.39	closely related to Shigella / Escherichia fergusonii and not definitely distinguishable at the moment	high ◉	low ○	none ○	export
	B9	B9	Standard	Proteus hauseri	2.22	Species hauseri / penneri / vulgaris of the genus Proteus have very similar patterns: Therefore distinguishing their species is difficult.	high ○	low ◉	none ○	export
	B10	B10	Standard	Proteus vulgaris	2.26	Species hauseri / penneri / vulgaris of the genus Proteus have very similar patterns: Therefore distinguishing their species is difficult.	high ○	low ◉	none ○	export
	B11	B11	Standard	Escherichia coli	2.38	closely related to Shigella / Escherichia fergusonii and not definitely distinguishable at the moment	high ◉	low ○	none ○	export
	B12	B12	Standard	Escherichia coli	2.37	closely related to Shigella / Escherichia fergusonii and not definitely distinguishable at the moment	high ◉	low ○	none ○	export

Sample Name	Sample ID	Organism (best match)	Score Value	Organism (second-best match)	Score Value
A1 (+++) (A)	BTS (BTS)	Escherichia coli	2.34	Escherichia coli	2.33
A2 (+++) (C)	387047179 (Standard)	Actinomyces turicensis	2.32	Actinomyces turicensis	2.26
A3 (+) (C)	387047179 (Standard)	Dialister micraerophilus	1.95	Dialister micraerophilus	1.93
A4 (+++) (A)	m160662 (Standard)	Streptococcus dysgalactiae	2.28	Streptococcus dysgalactiae	2.27
A5 (-) (C)	387064916 (Standard)	**No Organism Identification Possible**	1.31	**No Organism Identification Possible**	1.30

A6 (-) (C)	ID of A6 (standard)	No Organism Identification Possible	1.69	No Organism Identification Possible	1.49

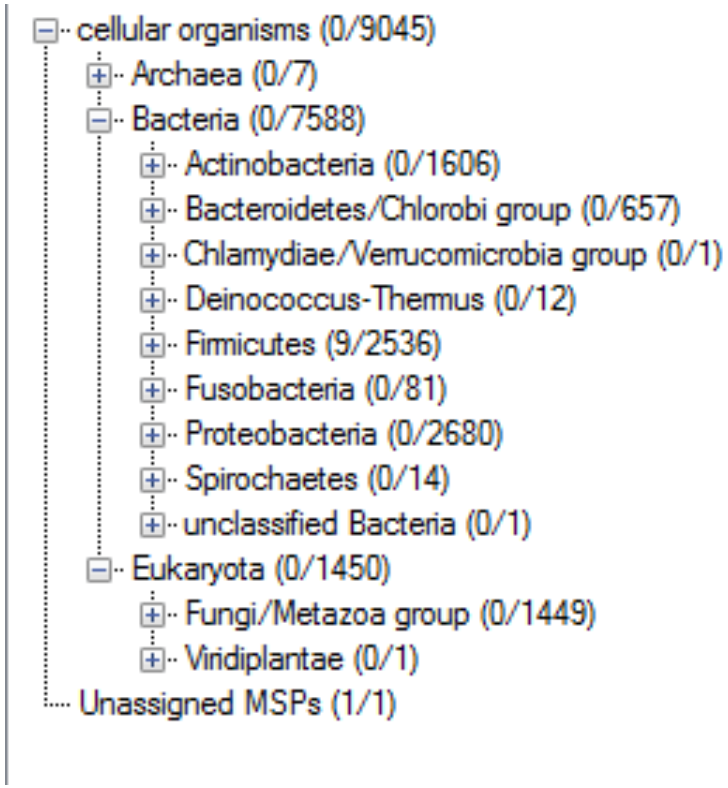
- Possible causes:
  - Organism is not in the IVD Biotyper Database
  - Mixed or suboptimal specimen
    - Ideal is 10^4 – 10^7 cells per sample position: ~ one isolated colony
    - Contamination of sample with polymers from plastic consumables
  - Organisms with rigid cell walls
  - Incorrect reagents, accidental omission of matrix
    - Matrix must be added to specimen on slide within 30 mins
  - Incorrect cleaning of the MALDI plate



Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (-)	Acidovorax defluvii DSM 12644T HAM	1.31	86669
2 (-)	Thauera phenylacetica B4P MPB	1.30	164400
3 (-)	Clostridium haemolyticum 1069_ATCC 9650T BOG	1.25	84025
4 (-)	Pseudomonas pertucinogena LMG 1874T HAM	1.23	86175
5 (-)	Bacteroides uniformis HU33120_3_13 PNU	1.20	820
6 (-)	Tatlockia micdadei AQZ_14 ALZW	1.20	451
7 (-)	Lactobacillus amylotrophicus DSM 20534T DSM	1.18	376807
8 (-)	Bacteroides vulgatus HU40347_2 PNU	1.17	821
9 (-)	Clostridium innocuum 1079_ATCC 14501T BOG	1.17	1522
10 (-)	Staphylococcus schleiferi ssp schleiferi DSM 4808 DSM	1.17	74707

Rank (Quality)	Matched Pattern	Score Value	NCBI Identifier
1 (+++)	Corynebacterium massiliense DSM 45435T DSM	2.00	53374
2 (-)	Corynebacterium tuberculostearicum DSM 44922T DSM	1.33	38304
3 (-)	Neisseria meningitidis C1 2 PGM	1.33	487
4 (-)	Corynebacterium pseudodiphtheriticum ATCC 10701 THL	1.29	37637
5 (-)	Corynebacterium tuberculostearicum DSM 44923 DSM	1.27	38304
6 (-)	Citrobacter freundii 22054_1 CHB	1.27	546
7 (-)	Corynebacterium tuberculostearicum DSM 44921 DSM	1.27	38304
8 (-)	Corynebacterium accolens 8 RLT	1.24	38284

DATABASE IS KNOWLEDGEABLE, BUT NOT OMNISCIENT



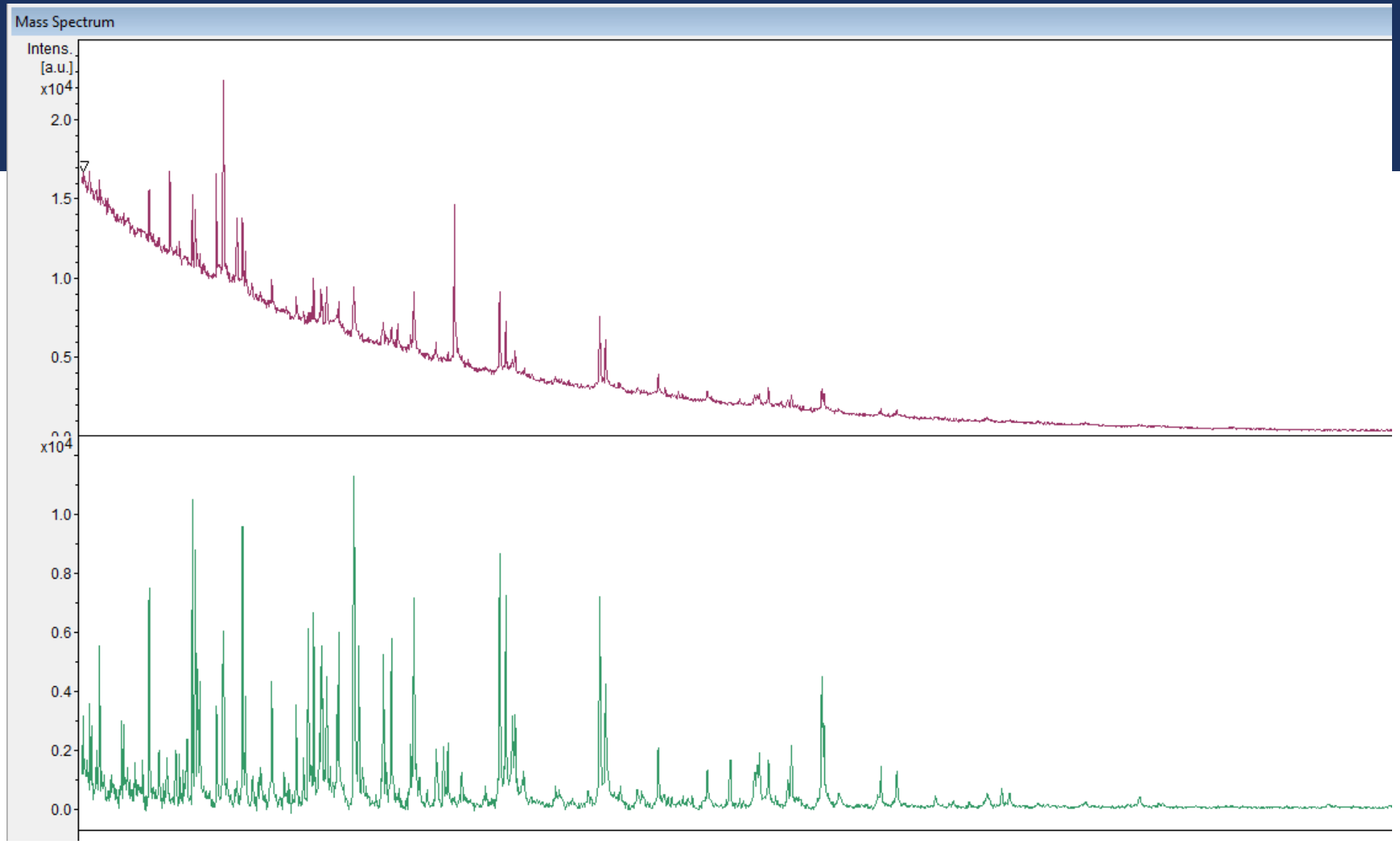
- FDA requires that approximately 30 unique isolates are required to produce a reference spectra for IVD purposes
- For rare organisms, this may be with fewer strains
- Anaerobes typically under-represented
- As taxonomy changes, the database may change with it
  - Consider reporting recently changed species with a reference to the clinically familiar name
  - Example: *Klebisella (Enterobacter) aerogenes*
  - Example: *Hungatella (Clostridium) hathewayi*

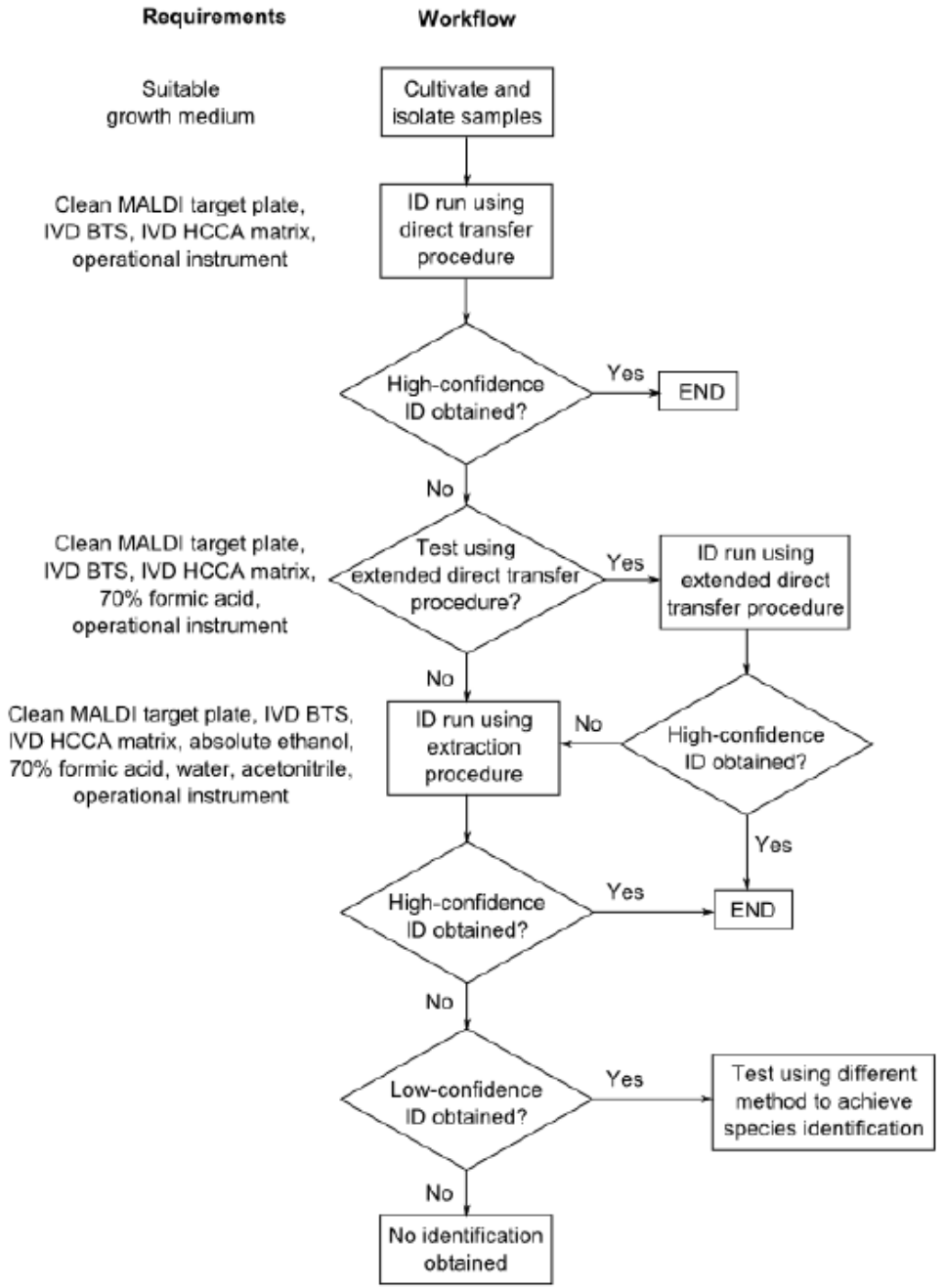
van Belkum A, et. al. Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry in Clinical Microbiology: What Are the Current Issues?. *Ann Lab Med*. 2017;37(6):475-483. doi:10.3343/alm.2017.37.6.475

NO PEAKS FOUND (SV 0.00)

- Indicates an issue with either sample preparation or MALDI function (usually the former)
- Check if sample and/or matrix was actually added to spot
- Repeat MALDI, consider using 70% formic acid overlay before adding matrix (Extended Direct Transfer Method)
- Do not perform MALDI on refrigerated isolates (4 degrees) – needs to be a fresh, pure culture or subculture

- When documenting MALDI results, it is useful to document whether the result was "No Peaks" vs "No ID Possible" rather than "No Maldi ID"
- Transcription check

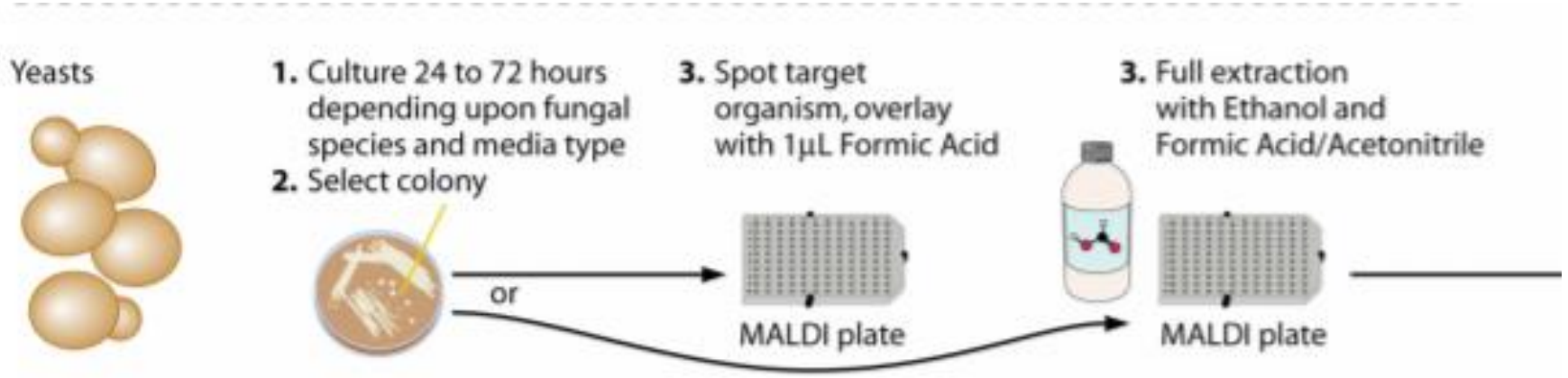

Mass Spectrum
Intens.
[a.u.]
x10^4
2.0
1.5
1.0
0.5
x10^4
1.0
0.8
0.6
0.4
0.2
0.0



MBT Compass IVD User Manual
Revision B

EXTRACTION – A RECOURSE FOR POOR SV

- Overcomes very rigid cell walls (eg yeasts)
- Also produces high quality spectra for library creation
- Summarized: Involves adding HPLC and absolute ethanol to isolate => centrifugation
  - Supernatant removed, pellet air dried, formic acid and acetonitrile added and centrifuged again
- Labor intensive (~ 30 min)



ORGANISMS NOT RELIABLY IDENTIFIED BY MALDI – LIMITED EXAMPLES

Organism/Issue	Suggested action
Shigella species: identified by MALDI as E.coli	Confirm by biochemical means, Indole test for suspected E.coli*
Salmonella typing: will usually identify to genus and species level, but not reliable for serotyping (eg *Typhi* vs *Typhimurium*)	Vi antigen, serotyping
S. pneumoniae vs S. mitis group: very similar genetically	Confirm *S. pneumoniae* with optochin susceptibility testing or bile solubility
S. argenteus/S. schweiteri – reported by Bruker to species level however can be confusing for clinicians	Report as "S. aureus complex" Be suspicious of *S. schweitzeri* identifications – mostly a pathogen of great apes
Aeromonas: cannot distinguish between *A. caviae, A. hydrophila* and *A. veronii*	Consider confirming ID with VITEK GN card

OTHER CAVEATS

- Consider reporting closely related isolates to complex level for consistency, especially for multiple serial cultures
  - Example: *E. asburiae/ E. kobei / E. nimipressuralis* => "*Enterobacter cloacae complex*"
  - *P. vulgaris* group
    - *(P. hauseri, P. penneri, P. vulgaris*)
- *N. gonorrhoea* - ideally would undergo a second biochemical confirmation rather than relying on MALDI alone
- Not reliable for identification of mixed isolates
- *Nocardia* and *Actinomyces* have tough cell walls -> often lower SV scores
- Consider " frameshift error" if your report makes no sense

SAFETY NOTE

- As sample preparation may involve manipulation of isolates on an open bench – potential for laboratory acquired infections (e.g. *Brucella*)
  - Gram-negative cocco-bacilli in gram stain: consider referring isolate.
  - Inactivation steps for MALDI identification exist (Yagupsky et. Al)
  - Traditionally MALDI-TOF was liable for mis-identifying *Brucella* as *Ochrbactrum* (VITEK MS)

OVERALL PERFORMANCE

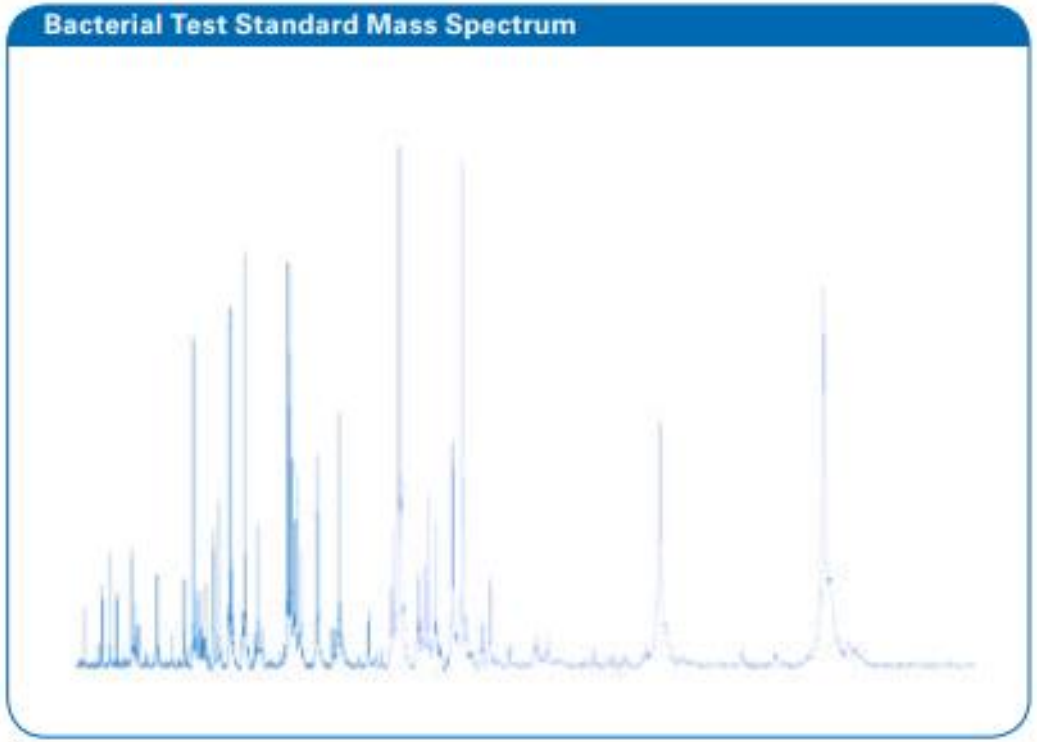
- Bizzini et. Al (JCM 2009) – n = 1371 isolates – 93.2% identified to species and additional 5.3% to genus
  - No ID in 1.5%
  - 4.9% discordance -> attributable to database related taxonomical differences in 42/63, with 14 due to poor discriminant of the spectra.
- Martiny et. Al compared Bruker and Vitek MS (JCM, 2012), n = 1129 isolates
  - Similar overall performance to species level (92.4% for Bruker and 93.4% for Vitek)
  - Rate of false identification to species level was 1.2% using Biotyper and lower than 1% for VITEK

OPERATING PARAMETERS

Dimensions & Operating Parameters			
	MALDI Biotyper System (microflex™ LT/SH)	**MALDI Biotyper smart System (microflex™ LT/SH smart)**	**MALDI Biotyper sirius System**
L x W x H	530 x 680 x 1093 mm (20.9" x 26.8" x 43")		500 x 710 x 1070 mm (19.7" x 28.0" x 42.2")
Weight	84 kg (185 lb) net weight	99 kg (218 lb) net weight	75 kg (165.4 lb) net weight
Noise	<60 dB		
Temperature Range	16-33°C (61-91°F)		16-30°C (61-86°F)
Operating Humidity	20-75% non-condensing @ 33°C (91°F)		

QC: BACTERIAL TEST STANDARD

- Bruker Biotyper utilises an *E. coli* extract with two high molecular weight proteins (BTS)
  - Represents a mass range of proteins used by the Biotyper for precise identification of micro-organisms
    - E.coli proteins PLUS myoglobulin and RNAse A
    - For each run, Biotyper will perform an automatic QC, which involves calibration of the mass spectrometer, a laser setting check and evaluation of spectrum quality
    - Performance is confirmed by match to *E. coli* identification
- By contrast, the VITEK MS uses an ATCC strain of E.coli as QC, with QC checks at the beginning and ends of runs
- Both platforms will not report any results if QC fails.



Typical mass spectrum of the Bacterial Test Standard, showing the *E. coli* peptide and protein profile with additional higher molecular weight m/z values of RNAse A and Myoglobin.

BTS – NOT A ROBUST EXTRACT

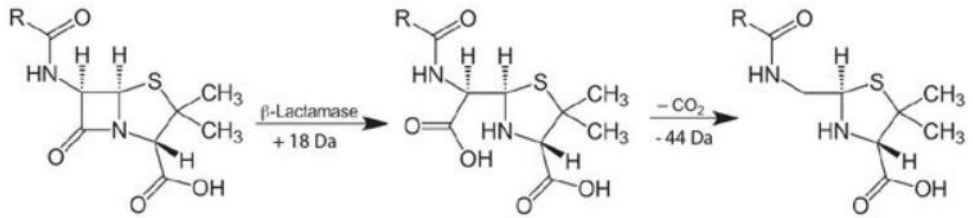
- Aliquots of BTS degrade quickly at room temperature – discard if at room temp > 8 hours
- Can be freeze/thawed twice.
- Consider including multiple BTS "spots" on your run, in case one QC fails
- The Biotyper laser takes mutliple representative "shots" with the laser – QC can fail if poorly pipetted onto the slide
- 1uL of BTS is required per "run"

ANTIMICROBIAL SUSCEPTIBILITY TESTING

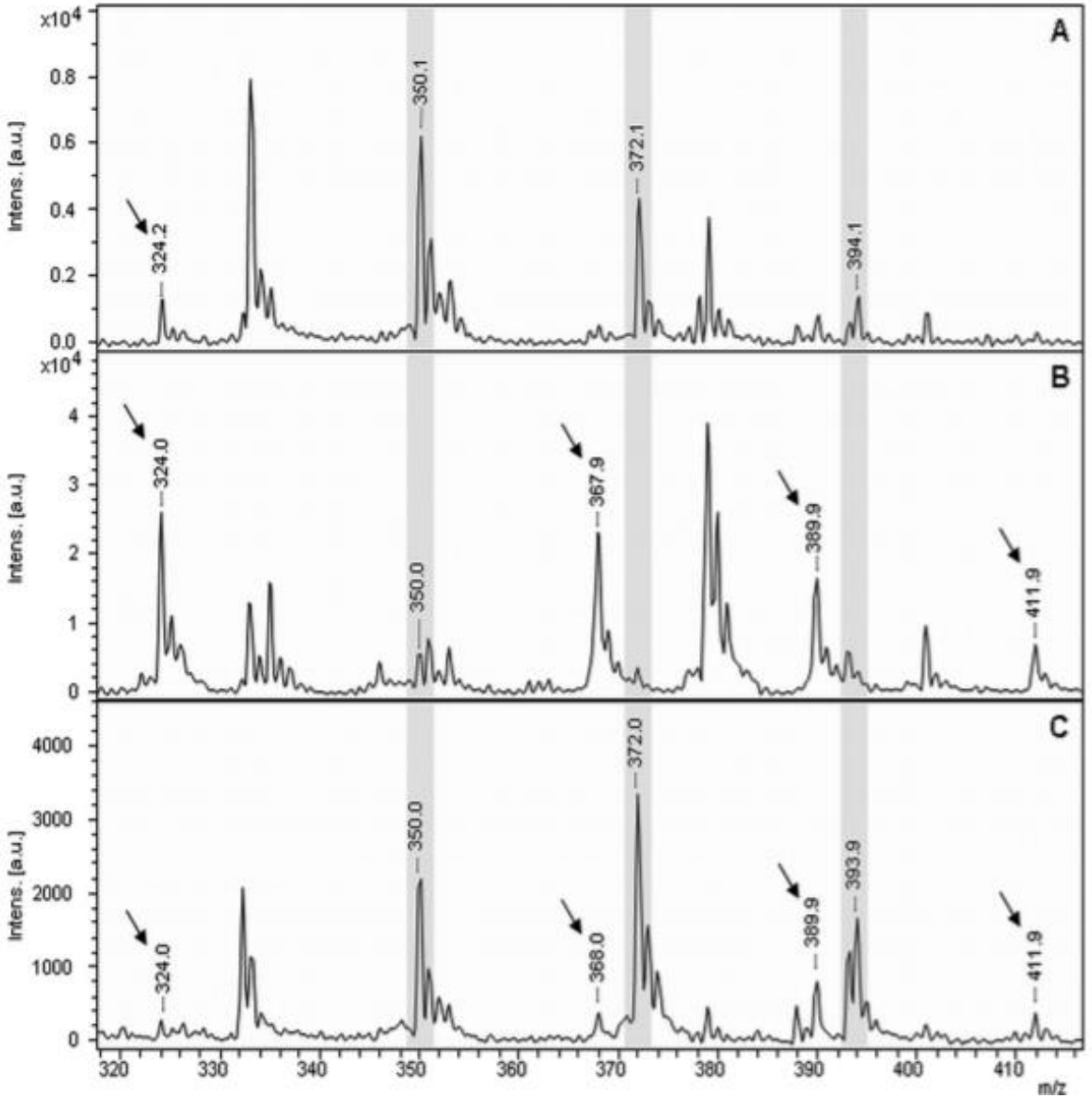
- Previously, detection of carbapenemases required culture based methods (*e.g.*: Carbapenem Inactivation Method) or disc testing +/- genotypic characterization
- Hydrolysis of carbapenems can be detected using Bruker Biotyper MBL Star Carba and BL STAR
  - Good for *Enterobacteriaceae* and *P. aeruginosa* (Class A and B producers), but
  - Less accurate for carbapenemase producing *A. baumannii* (Class D enzymes)
  - Improved detection of OXA 23 like enzymes has been described with addition of NH_4HCO_3 to buffer
- Cephalosporinase activity can be detected with the STAR-Cepha kit

Rapp E. et Al Detection of carbapenemases with a newly developed commercial assay using Matrix Assisted Laser Desorption Ionization-Time of Flight, Journal of Microbiological Methods, 146:2018,Pages 37-39

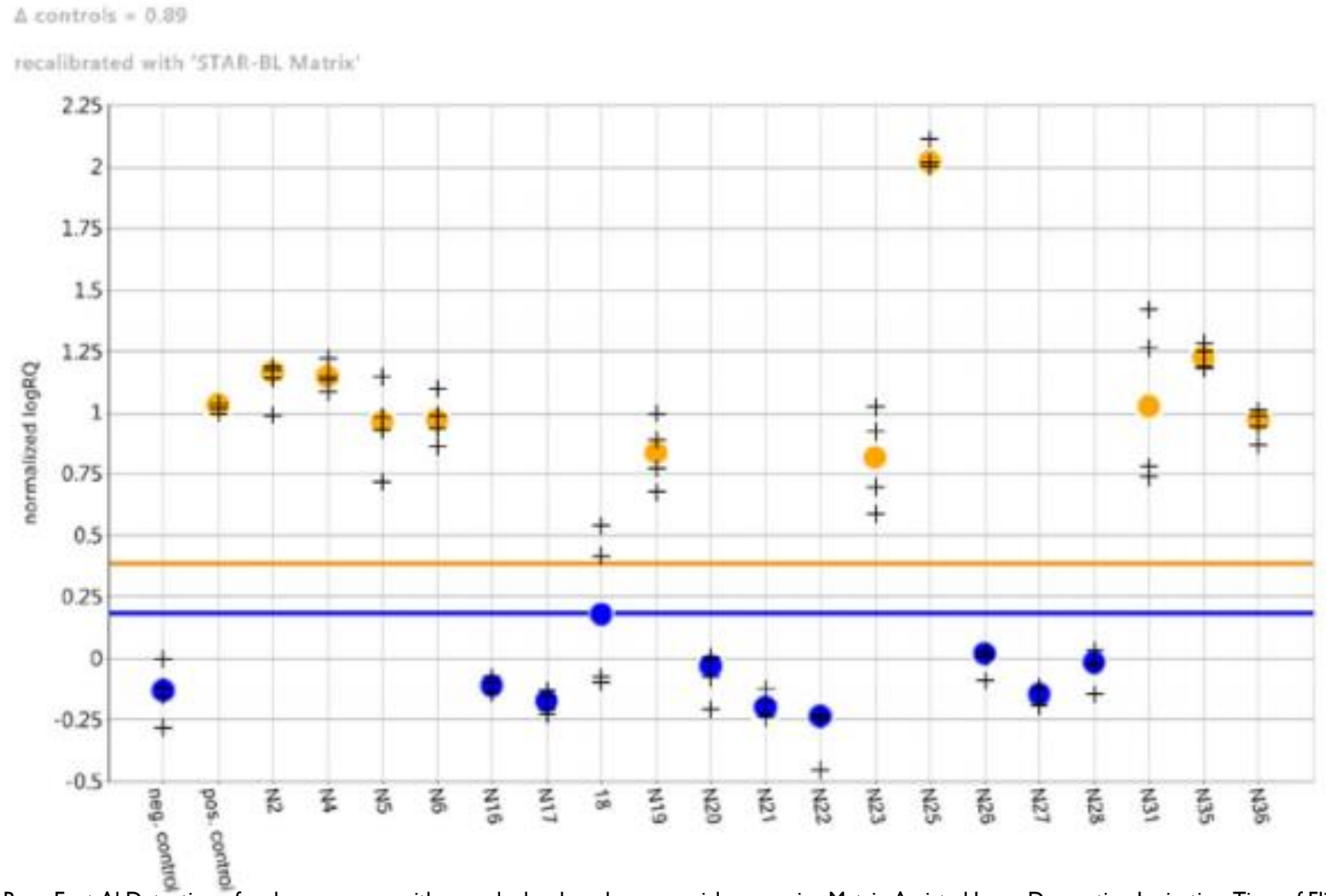
- Isolates are co-incubated with a beta-lactam antibiotic. Hydrolysis produces a change in mass



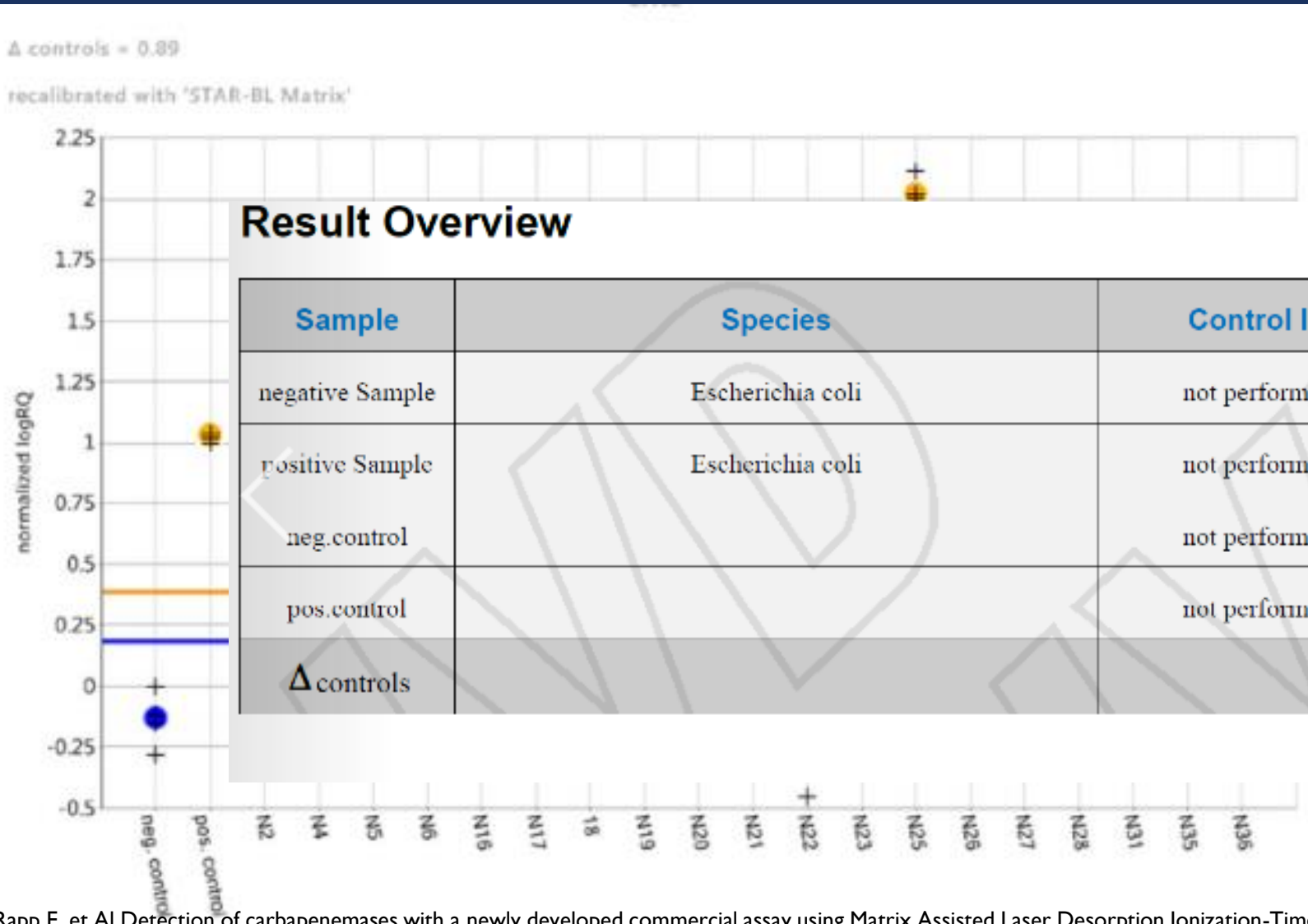
- Spectra associated non-hydrolysed antibiotics (A) are compared with those that are (B).



Sparbier K. et al: Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry-Based Functional Assay for Rapid Detection of Resistance against β-Lactam Antibiotics
Journal of Clinical Microbiology Feb 2012, 50 (3) 927-937; **DOI:** 10.1128/JCM.05737-11



Rapp E. et Al Detection of carbapenemases with a newly developed commercial assay using Matrix Assisted Laser Desorption Ionization-Time of Flight, Journal of Microbiological Methods, 146:2018,Pages 37-39



Result Overview

Sample	Species	Control ID	CARBA	
negative Sample	Escherichia coli	not performed	-0.01	NH
positive Sample	Escherichia coli	not performed	1.04	H
neg.control		not performed	-0.02	NH
pos.control		not performed	1.05	H
Δ controls			2.12	

Rapp E. et Al Detection of carbapenemases with a newly developed commercial assay using Matrix Assisted Laser Desorption Ionization-Time of Flight, Journal of Microbiological Methods, 146:2018,Pages 37-39

ADVANTAGES AND LIMITATIONS

Advantages	Limitations
Detects phenotypic hydrolysis	Only detects phenotypic hydrolysis, not presence of genotypic resistance
Can be performed on standard microflex Bruker instrument	Cannot distinguish between classes
Fast (results in ~3 hrs)	Qualitative result, not quantitative
	Requires dedicated kit
	Some OXA enzymes not well detected

FUTURE?

- Recently, investigators have developed a phenotypic screening tool (direct-on-target microdroplet growth assay)
- Correctly identifies KPC, MBL and OXA carbapenemase producing organisms
  - Co-incubated with MEM + inhibitor in different concentrations
  - MALDI used to detect growth

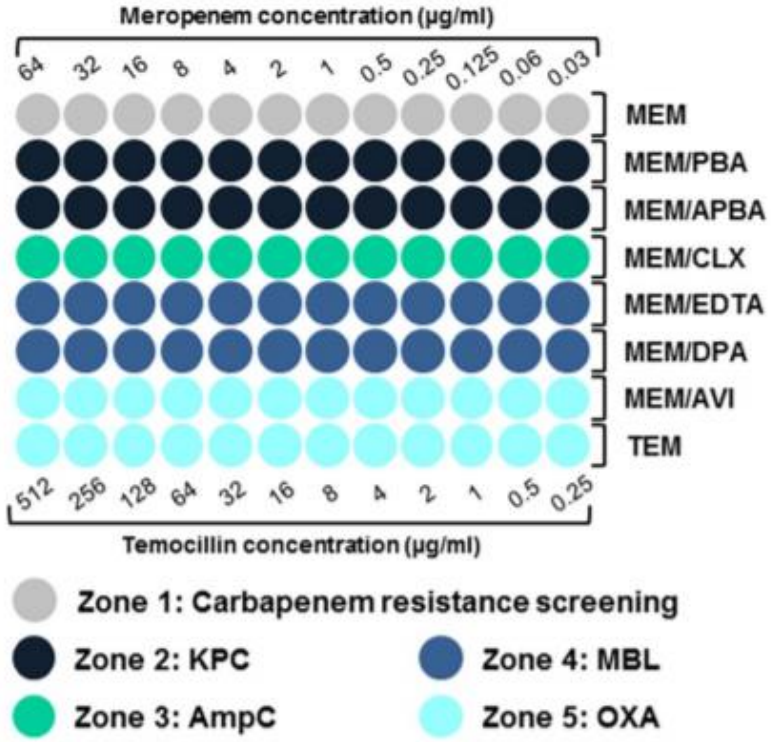


Figure 1. Layout of the DOT-MGA screening panel. The mass spectrometric assessment of bacterial growth on each spot allows the MIC determination for each row. Significant MIC decrease (8-fold or more) in zones 2–5 in relation to zone 1 indicates presence of a certain carbapenemase. Temocillin MIC > 128 µg/ml (last row) is compatible with OXA production MEM: meropenem; PBA: phenylboronic acid; APBA: aminophenylboronic acid; CLX: cloxacillin; EDTA: ethylendiamintetraacetic acid; AVI: avibactam; TEM: temocillin.

Correa-Martínez, C.L., Idelevich, E.A., Sparbier, K. *et al.* Development of a MALDI-TOF MS-based screening panel for accelerated differential detection of carbapenemases in *Enterobacterales* using the direct-on-target microdroplet growth assay. *Sci Rep* **10,** 4988 (2020). https://doi.org/10.1038/s41598-020-61890-7

MALDI-TOF DIRECT FROM BLOOD CULTURES

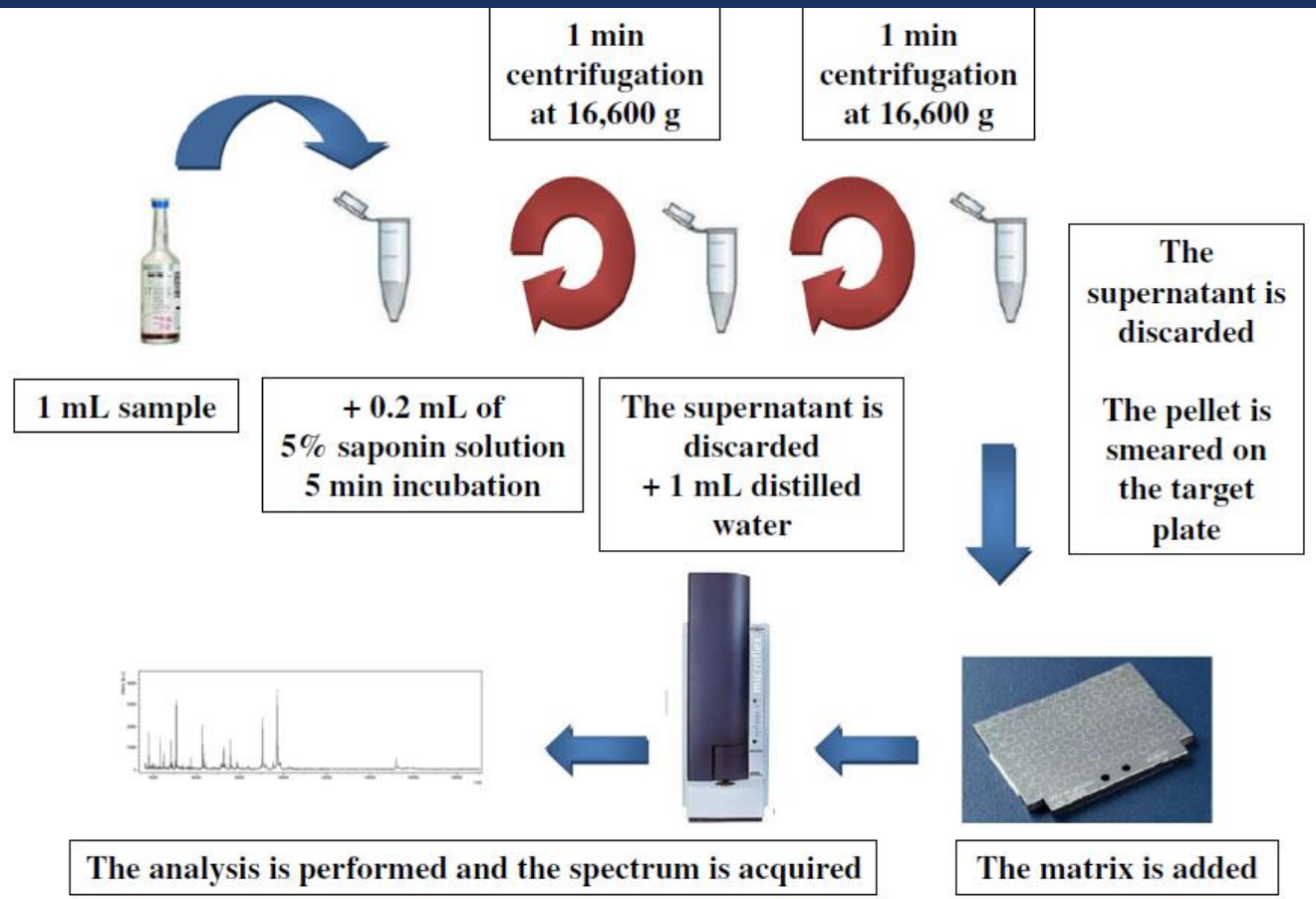


Fig. 2 IH method protocol

PERFORMANCE

- Direct MALDI from blood culture bottles in general lowers SV due to lower quality spectra compared to pure cultures
- Gram-negatives more likely to be identified compared to gram-positives
- Struggles with polymicrobial blood cultures – typically only one organism will be identified.
  - Caution with applying to positive blood cultures if gram staining is mixed.

PERFORMANCE

- Identification of organisms generally improved by the addition of 70% formic acid
- Tradeoff of speed vs. accuracy
- Less common organisms may suffer more
- Identifies definite pathogens better than contaminant species

Organism Group	DT			eDT		
	Identification			Identification		
	Correct	Incorrect	No	Correct	Incorrect	No
Gram negative bacteria (48% of all samples)	90%	0.7%	10%	88%	0.4%	12%
Enterobacteria (84% of all Gram negative bacteria)	93%	0.4%	7%	92%	0.0%	8%
Escherichia coli (47% of all Enterobacteria)	98%	0.0%	2%	98%	0.0%	2%
Klebsiella pneumoniae / variicola (29% of all Enterobacteria)	93%	0.7%	7%	95%	0.0%	5%
Gram positive bacteria (47% of all samples)	62%	2.0%	36%	78%	2.1%	20%
Staphylococci (61% of Gram positive bacteria)	68%	1.5%	30%	83%	1.9%	14%
Staphylococcus aureus (34% of Staphylococci)	88%	1.8%	10%	91%	0.0%	9%
Yeast (5% of all samples)	30%	7.0%	63%	68%	3.5%	29%
All samples (1162 samples)	74%	1.6%	24%	82%	1.3%	17%

- Bruker Daltronik:: Instructions for Use MBT Sepsityper IVD kit

IN HOUSE METHODS: SUPERIOR RESULTS, YET SIMILAR

- Martiny et. Al (2011) compared an in-house direct MALDI method to Sepsityper (59 isolates)
  - 73.7% correct to species by in-house method vs 68.4% Sepsityper, however this required lowing cut offs to 1.4-1.6 SV
  - Using recommended cutoffs, 72.9% and 47.4% IH correct to genus and species respectively, compared to 71.2% vs 49.1%
  - No false IDs but ~29% "No ID" in both arms
  - However, higher proportion of gram-positive organisms (62.7%)

Table 5 Identification results for polymicrobial cultures using both methods

Polymicrobial cultures	In-house method	Score	Sepsityper™ method	Score
More than three organisms, no ID in routine	No reliable identification	1.19	No reliable identification	1.267
S. epidermidis + *E. coli*	*E. coli*	2.119	*E. coli*	2.256
S. epidermidis + *Streptococcus* sp.	*S. epidermidis*	1.917	*S. epidermidis*	2.047
E. faecalis + *E. coli*	*E. faecalis*	2.347	No reliable identification	1.101

ID: identification

YEASTS IN BLOOD CULTURES?

- Requires full extraction for reliable identification – additional steps
- Bidart et. Al (JCM, 2015) in-house assay vs Sepsityper
  - 95/107 (88%) of isolates accurately identified by IH vs 94/115 (81.7%)

Bidart. Et, Al, An In-House Assay Is Superior to Sepsityper for Direct Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) Mass Spectrometry Identification of Yeast Species in Blood Cultures, Journal of Clinical Microbiology Apr 2015, 53 (5) 1761-1764; **DOI:** 10.1128/JCM.03600-14

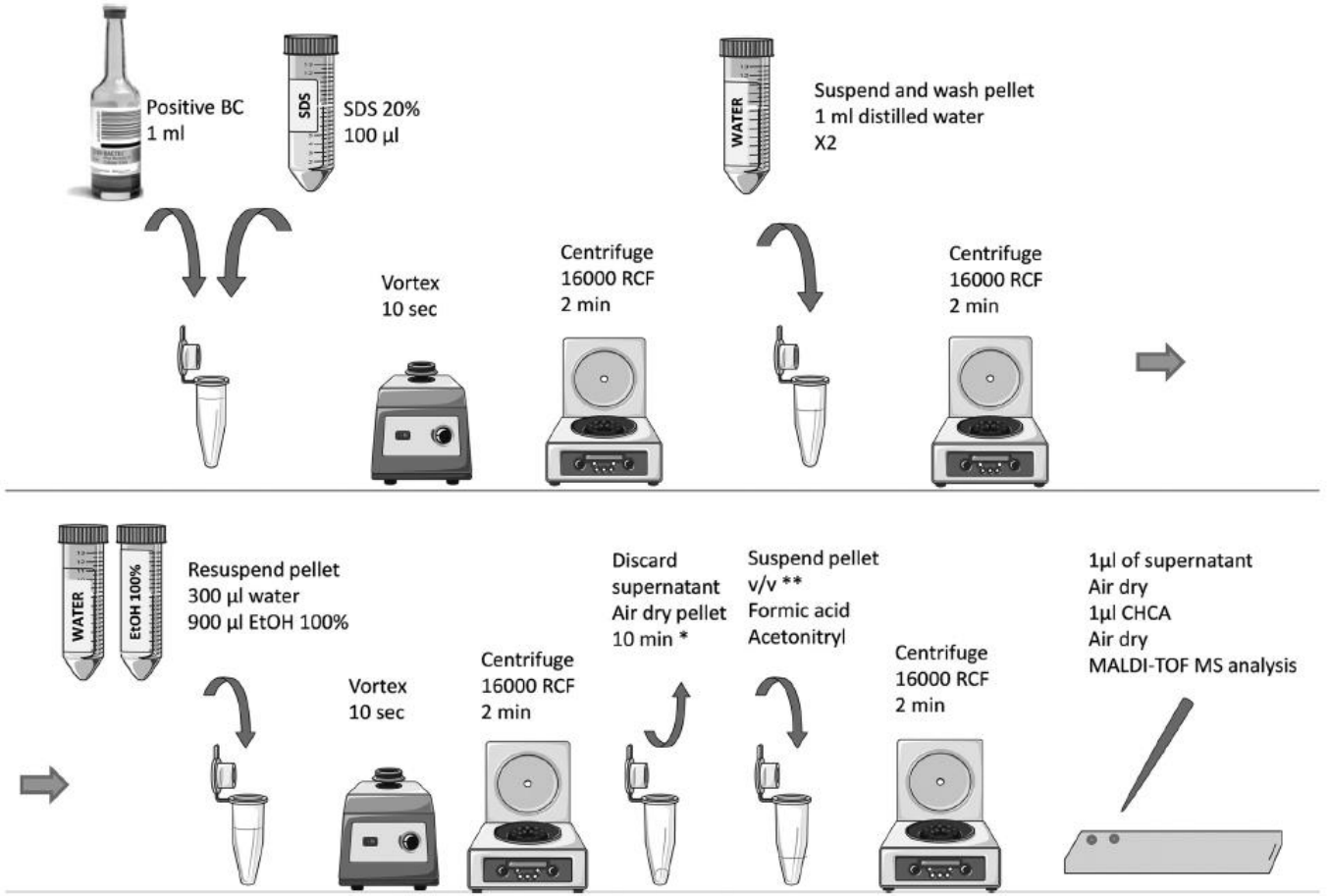


FIG 1 Workflow of the SDS1.8 protocol. BC, blood culture; SDS, sodium dodecyl sulfate; EtOH, ethanol; HCCA, α-cyano-4-hydroxycinnamic acid; *, the pellet should be as dry as possible with no residual ethanol; **, volume of formic acid depending on the size of the pellet (formic acid should entirely recover the pellet). If no pellet is clearly visible, suspend the residual biological material, which should be present on the side of the tube, with 2 µl of formic acid and proceed.

BRINGING IT ALL TOGETHER?

TABLE 5 | The overall diagnostic performances of drug hydrolysis assays for cultured isolates and blood cultures at logRQ cut-off values of 0.4 and 0.2.

	Drug hydrolysis assay											
	AMP[a]		AMP (For *E.coli* only)		PIP[b]		CTX[c]		CAZ[d]		MEM[e]	
	Sn %	Sp (%)	Sn (%)	Sp (%)	Sn (%)	Sp (%)	Sn (%)	Sp (%)	Sn (%)	Sp (%)	Sn (%)	Sp (%)
RETROSPECTIVE ISOLATES												
logRQ cut-off = 0.4	91.3 (94/103)	100 (36/36)	95.5 (63/66)	100 (35/35)	100 (31/31)	100 (30/30)	97.9 (47/48)	100 (91/91)	47.2 (25/53)	100 (147/147)	100 (47/47)	100 (153/153)
logRQ cut-off = 0.2	96.1 (99/103)	97.2 (35/36)	100 (66/66)	100 (35/35)	100 (31/31)	100 (30/30)	97.9 (47/48)	100 (91/91)	47.2 (25/53)	100 (147/147)	100 (47/47)	100 (153/153)
BLOOD CULTURE BOTTLES												
logRQ cut-off = 0.4	80.4 (90/112)	92.9 (13/14)	92.1 (58/63)	92.9 (13/14)	100 (4/4)	100 (4/4)	68.8 (22/32)	91.5 (86/94)	0 (0/4)	100 (4/4)	40 (2/5)	97.7 (126/129)
logRQ cut-off = 0.2	85.7 (96/112)	92.9 (13/14)	95.2 (60/63)	92.9 (13/14)	100 (4/4)	100 (4/4)	87.5 (28/32)	89.4 (84/94)	0 (0/4)	100 (4/4)	100 (5/5)	95.3 (123/129)
SUBCULTURED ISOLATES FROM BC												
logRQ cut-off = 0.4	91.1 (102/112)	100 (14/14)	98.4 (62/63)	100 (14/14)	100 (4/4)	100 (4/4)	96.9 (31/32)	100 (94/94)	0 (0/4)	100 (4/4)	100 (5/5)	100 (129/129)
logRQ cut-off = 0.2	96.4 (108/112)	85.7 (12/14)	100 (63/63)	85.7 (12/14)	100 (4/4)	100 (4/4)	96.9 (31/32)	93.6 (88/94)	0 (0/4)	100 (4/4)	100 (5/5)	97.7 (126/129)

[a] *In this column, the diagnostic performance of the AMP hydrolysis assay includes all Enterobacteriaceae.*

[b] *The PIP hydrolysis assay was only applied to NFGRs.*

[c] *The CTX hydrolysis assay was only applied to Enterobacteriaceae.*

[d] *In the first part of the study (retrospective isolates), a CAZ hydrolysis assay was applied to both Enterobacteriaceae and NFGR isolates, whereas in the second part of the study (blood culture), only NFGRs were included in the CAZ hydrolysis assay.*

[e] *The MEM hydrolysis assay were used for both Enterobacteriaceae and NFGR in both the cultured isolate or blood culture stages of the study.*

CLINICAL IMPACT

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Original article

Impact of MALDI-TOF-MS-based identification directly from positive blood cultures on patient management: a controlled clinical trial

M. Osthoff [1,7], N. Gürtler [1,7], S. Bassetti [2], G. Balestra [3], S. Marsch [3], H. Pargger [4], M. Weisser [1], A. Egli [5,6,*]

- Single center, open label controlled trial (n = 425) with a direct MALDI arm vs conventional arm
- Comparable duration of antibiotics (12.9 vs 13.2 days, p =0.9) and length of stay (16.1 vs 17.9 days).
- Numerically improved 30 day mortality for "clinically significant isolates" (9/114 vs 20/128, but not statistically significant)
- Reduced admission to ICU after BSI onset (36/114 vs 45/128, p = 0.02)
- Study "hampered" by a robust AMS service associated with positive blood culture notifications.

CLINICAL IMPACT?

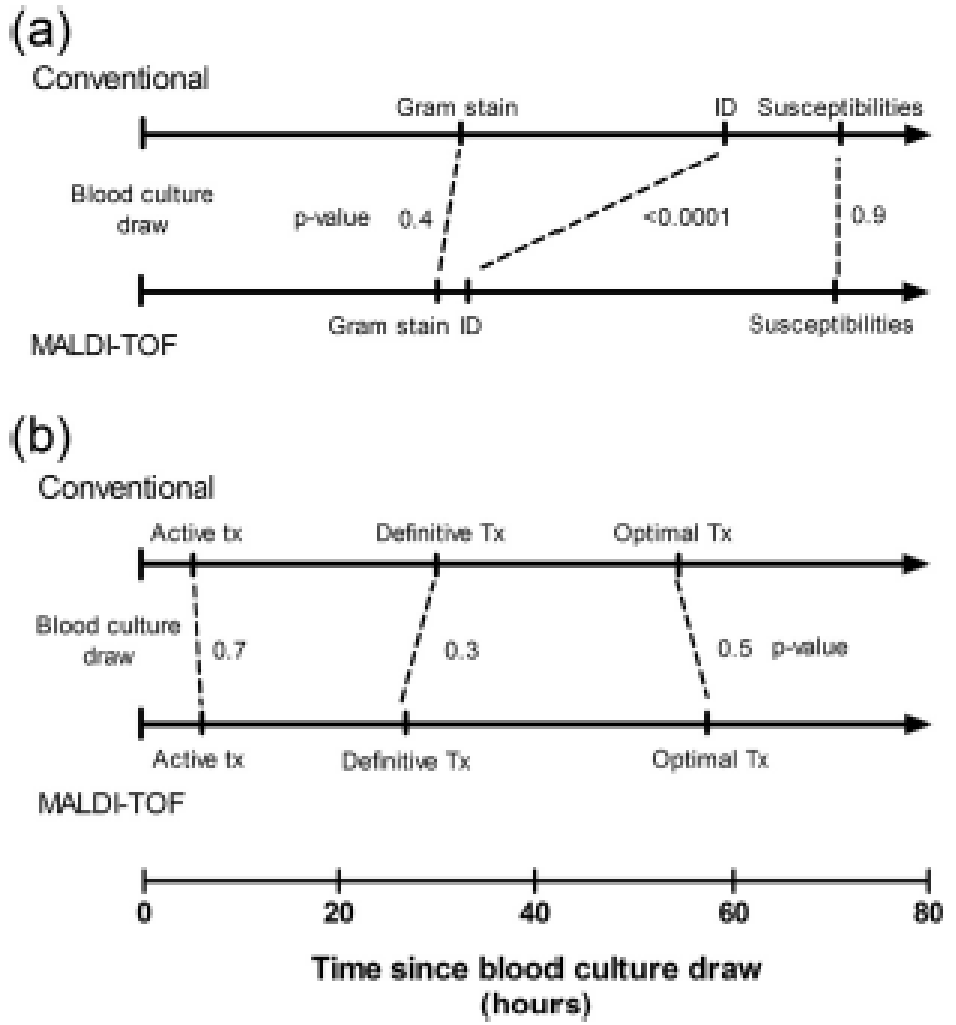

Fig. 2. Timeline (mean) of (a) microbiological procedures and (b) antibiotic management starting from blood culture collection according to study group (entire cohort). Abbreviation: MALDI-TOF, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry.

COST AND TURN-AROUND TIME

- Philippe R. S and colleagues (n = 63 positive blood cultures), using a cut-off score of 1.7, found identification for 85.2% positive blood cultures
  - Average reduction in TAT was 34.3h (all isolates), or 26.5h where organisms requiring further conventional ID were identified from subcultures
  - Came at a price

TABLE 3 Cost estimates for conventional identification of blood culture isolates in three scenarios[a] versus MALDI-TOF Sepsityper identification[b]

Conventional ID scenario	Cost (U.S. dollars)/positive culture			
	Avg	Sepsityper	Conventional ID for MS failure[c]	Net
Low cost	1.58	5.15	0.07	3.64
High cost	5.50	5.15	0.07	−0.28[d]
Best estimate	1.98	5.15	0.07	3.24

[a] Low cost, high cost, and best estimate. See text for details.
[b] Estimates assume a typical distribution of isolates (see Table 1).
[c] Assumes 14.8% failure to identify with Sepsityper and cost of conventional identification is $0.50 per failed identification. See methods for details.
[d] Net savings.

Philippe R. *S Identification of Blood Culture Isolates Directly from Positive Blood Cultures by Use of Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry and a Commercial Extraction System: Analysis of Performance, Cost, and Turnaround Time*. Journal of Clinical Microbiology Sep 2012, 50 (10) 3324-3328; DOI: 10.1128/JCM.01479-12

SEROTYPING – STILL A CHALLENGE

- Not in clinical practice yet
- Requires a large number of reproducible peaks, dependent on algorithm design
- Research is ongoing into *S. pneumoniae* strain typing but some peaks described in the literature cannot be replicated in other studies (Ercibengoa et. Al, PLOS ONE 2019)
- Successfully used for *E. coli* H antigen typing (Chui et. Al, JCM 2015), *Salmonella* (Dieckmann et. Al, AEM 2011), *S.aureus* – research use only

SUMMARY

- Powerful technology
- Advantages must be weighed with limitations
- Understanding process and evidence base helpful for reporting and implementation of IVD and in-house methods.

Thank you for your time

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