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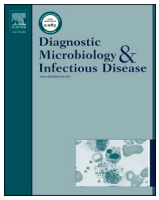

Technical Note

Comparison of MBT biotargets with MBT steel targets for matrix-assisted laser desorption/ionization time of flight mass spectrometry identifications of microorganisms in a clinical microbiology laboratory

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ABSTRACT

MALDI-TOF MS identifications of microorganisms in a clinical laboratory were investigated, comparing steel targets with MBT Biotargets. By using MBT Biotargets, the score values of yeast identifications increased, whereas the score values of Gram-negative bacteria decreased. Switching to MBT Biotargets did not negatively impact overall frequencies of high confidence identifications.

Previously, Bruker Matrix Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry (MALDI-TOF MS) systems introductions involved reusable steel targets. A major disadvantage in the use of steel targets is the requirement of meticulous cleaning, which involves toxic chemicals. The use of disposable targets markedly increases material costs but eliminates target cleaning. At our laboratory, MALDI-TOF MS identifications were performed using the direct transfer method *in duplicate* on polished steel targets for bacteria or on ground steel targets for yeasts [4]. Increased costs of switching to MBT Biotargets are balanced in our lab, if identifications using MBT Biotargets are performed as *single* spot measurements, at similar identification quality and requirement of repeat identifications.

The UMCU Department of Clinical Microbiology serves a university affiliated tertiary care teaching hospital, a university affiliated children's hospital and a centralized children's cancer referral center. This laboratory performs further identifications for non-clinical samples from environmental cultures for microbiological monitoring of pharmaceutical and cell therapy production facilities, and for the Veterinary Department of the Utrecht University. MBT Smart or MBT Sirius mass spectrometers in combination with Compass software and MBT 8468 MSP Library are used. For this study, MALDI-TOF MS score values >1.99 are considered high confidence identifications, regardless of consistency categorizations or matching hints. Score values between 1.69 and 2.00 are low confidence identifications.

We initially performed a pilot experiment in which we measured score values among ten different microorganisms obtained using MBT Biotargets (single spot measurements) in comparison with steel targets (duplicate measurements). Differences in score values ranged from 0.20 (favoring MBT Biotarget) in a *Candida glabrata* strain to -0.12 (favoring steel target) in a *Bacillus subtilis* strain (Supplementary table 2).

In a before-after study, the before period included all consecutive identifications from October 30th, 2020 to March 14th, 2021, performed on steel targets in duplicate. The after period included all consecutive identifications from March 15th, 2021, to July 15th, 2021, performed on MBT Biotargets on single spots. For the before-after study, identifications for the Veterinary Department of the Utrecht University and all systems controls were removed from the data set. Quality controls were performed using *E. coli* controls according to local procedures and with Bruker Bacterial Test Standard (BTS).

Low confidence identifications are always accepted without further testing in yeast identifications [5] and sometimes in bacterial identifications. Indications to refrain from retesting did not differ between the different study periods. A "clinically acceptable" result is defined as either a "high confidence" identification (scorevalue >1.99), or a "low confidence" identification (scorevalue 1.70-1.99) without further MALDI-TOF MS retesting attempts (Supplementary table 1.)

During the period in which steel targets were used (i.e. before-period), 21128 MALDI-TOF MS *duplicate* measurement were included on 17496 isolates. To obtain a clinically acceptable result, 14806 (84.63%) isolates required one duplicate MALDI-TOF MS measurement and 2690 (15.37%) isolates required repeat measurements (Table 1, Supplementary figure 1).

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In the after period, during which MBT Biotargets were used, 19529 MALDI-TOF MS single spot measurements were performed on 16368 isolates, (Table 1, Supplementary figure 2). To obtain clinically acceptable results, 14030 (85.72%) isolates required one MALDI-TOF MS single spot measurement, and 2338 (14.28%) isolates required repeat single spot measurements ($p<0.05$, Fisher's exact test, Supplementary figure 3, Table 1). More frequent repeat measurements were required to obtain clinically acceptable results in subgroups of "other aerobic Gram-positive rods", enterobacterales and Pseudomonas species (Table 1). Enterococci and yeasts required less frequent repeat measurements on MBT Biotargets to obtain clinically acceptable results (Table 1).

In the before period using steel targets, the median scorevalue of the best measurement was 2.25 (range 1.70-2.74), versus 2.23 (range 1.70-2.69) in the after period using MBT Biotargets (Table 2, $p<0.05$, Mann-Whitney). Using MBT Biotargets, scorevalues were higher in yeasts and lower in all Gram-negative organism subgroups (Table 2). In the before period using steel targets, the frequency of high confidence identifications was 80.5%, in the after period using MBT Biotargets, this frequency increased to 82.1% (Supplementary figure 4). Excluding yeast identifications, the frequencies of high confidence identifications didn't differ between the before and after periods at 87.2% and 87.1% respectively (Supplementary figure 5).

We conclude that switching from testing in duplicate on steel targets, to single spot testing on MBT Biotargets does not affect overall identification quality. Nevertheless, we found that by using MBT biotargets in single measurements, yeast identification is minorly improved with respect to three parameters: Firstly, repeat measurements were required less frequently to obtain a clinically acceptable result. Secondly, higher median MALDI-TOF MS score values were obtained using MBT biotargets and, thirdly, the frequency of high confidence MALDI-TOF MS identifications were increased. A similarly minor improvement was found in some Gram-positive bacteria. In contrast, a marginally decreased identification quality in Enterobacterales and Pseudomonas species was found, due to an increased frequency of repeat measurement requirement and lower median MALDI-TOF MS score values.

Table 1
Percentage (number) of measurements required to obtain a clinically acceptable result within subgroups of organisms.

	Steel target*		MBT Biotarget		Steel target vs MBT Biotarget	
Organism group	% single measurement (n)	% repeat measurement (n)	% single measurement (n)	% repeat measurement (n)	difference in % (Steel target-MBT Biotarget)	p-value (Fisher's exact)
Staphylococci	83.22 (3641)	16.78 (734)	83.93 (3395)	16.07 (650)	0.71 (↓)	0.39
Beta hemolytic streptococci	87.56 (183)	12.44 (26)	92.56 (224)	7.44 (18)	5.00 (↓)	0.08
Viridans streptococci	75.59 (223)	24.41 (72)	72.98 (208)	27.02 (77)	-2.61 (↑)	0.51
Enterococci	80.58 (664)	19.42 (160)	87.99 (901)	12.01 (123)	7.41 (↓)	<0.0001
Streptococci other	67.42 (60)	32.58 (29)	70.41 (69)	29.59 (29)	2.99 (↓)	0.75
Bacillus species	93.37 (324)	6.63 (23)	91.10 (266)	8.90 (26)	-2.27 (↑)	0.3
Corynebacteria	73.51 (136)	26.49 (49)	73.53 (125)	26.47 (45)	0.02 (↓)	>0.9999
Aerobic Gram-positive rods other	85.71 (132)	14.29 (22)	75.00 (117)	25.00 (39)	-10.71 (↑)	0.02
Enterobacterales	96.65 (3841)	3.35 (133)	94.61 (3403)	5.39 (194)	-2.04 (↑)	<0.0001
Pseudomonas species	92.90 (877)	7.10 (67)	89.25 (822)	10.75 (99)	-3.65 (↑)	0.006
Acinetobacter	92.62 (113)	7.38 (9)	89.38 (101)	10.62 (12)	-3.24 (↑)	0.49
Aerobic Gram-negative other	92.03 (1177)	7.97 (102)	89.79 (871)	10.21 (99)	-2.24 (↑)	0.07
Anaerobic Gram-positive	69.11 (255)	30.89 (114)	71.17 (311)	28.83 (126)	2.06 (↓)	0.54
Anaerobic Gram-negatives	76.59 (193)	23.41 (59)	72.07 (271)	27.93 (105)	-4.52 (↑)	0.23
Yeasts	72.51 (2348)	27.49 (890)	81.64 (2326)	18.36 (523)	9.13 (↓)	<0.0001
Other	52.63 (10)	47.37 (9)	85.71 (6)	14.29 (1)	33.08 (↓)	0.19
No reliable ID	74.68 (472)	25.32 (160)	76.18 (515)	23.82 (161)	1.50 (↓)	0.56
No peaks found	83.07 (157)	16.93 (32)	90.00 (99)	10.00 (11)	6.93 (↓)	0.12
Total	**84.63 (14806)**	**15.37 (2690)**	**85.72 (14030)**	**14.28 (2338)**	1.09 (↓)	0.005

*Polished steel targets except for yeast identifications in which ground steel targets were used.
(↓) downward arrow indicates a lesser frequency of required repeat measurement in the after period on MBT Biotargets
(↑) upward arrow indicates a higher frequency of required repeat measurements in the after period on MBT Biotargets

Table 2
Comparison of score values between the before (steel target) and after (MBT Biotarget) periods.

Organism group	Median Scorevalue Steel target (range)	Median Scorevalue MBT Biotarget (range)	Difference median (Steel target - MBT Biotarget)	p-value (Mann-Whitney U-test)
Staphylococci	2.19 (1.70-2.53)	2.20 (1.70-2.56)	0.01	0.07
Beta hemolytic streptococci	2.35 (1.88-2.62)	2.33 (1.75-2.50)	-0.02	0.33
Viridans streptococci	2.19 (1.74-2.51)	2.21 (1.70-2.53)	0.02	0.18
Enterococci	2.37 (1.83-2.62)	2.38 (1.81-2.65)	0.01	0.27
Streptococci other	2.16 (1.71-2.49)	2.15 (1.77-2.50)	-0.02	0.48
Bacillus species	2.06 (1.70-2.53)	2.06 (1.71-2.47)	0.00	0.39
Corynebacteria	2.24 (1.70-2.50)	2.17 (1.70-2.54)	-0.07	0.20
Aerobic Gram-positive rods other	2.12 (1.71-2.53)	2.07 (1.70-2.55)	-0.05	0.03
Enterobacterales	2.40 (1.75-2.74)	2.35 (1.75-2.69)	-0.05	<0.0001
Pseudomonas species	2.38 (1.73-2.65)	2.30 (1.70-2.60)	-0.08	<0.0001
Acinetobacter	2.31 (1.79-2.54)	2.22 (1.87-2.55)	-0.09	0.0004
Aerobic Gram-negative other	2.26 (1.71-2.65)	2.20 (1.70-2.69)	-0.06	<0.0001
Anaerobic Gram-positives	2.20 (1.71-2.53)	2.19 (1.72-2.53)	-0.01	0.75
Anaerobic Gram-negatives	2.28 (1.71-2.59)	2.20 (1.70-2.56)	-0.08	0.0002
Yeasts	2.00 (1.70-2.43)	2.03 (1.70-2.56)	0.03	<0.0001
All isolates	2.25 (1.70-2.74)	2.23 (1.70-2.69)	-0.02	<0.0001

One study found concordant benefit from MBT biotargets in direct identifications of yeasts from blood cultures [2]. In contrast, one study found no differences in score values using ground steel targets versus MBT Biotargets among 149 yeast isolates (*Candida albicans* n=65) [3].

Limitations of this study include that our definition of a "clinically acceptable result" is not a validated measure. Comparisons of the best identifications from double spot attempts on steel targets with single spot identifications on MBT Biotargets is ambiguous: differences may be caused by both the nature of the target and the number of spots tested. Thus, this study is not generalizable to laboratories that already employ single spot measurements from steel targets, nor to laboratories that use extended direct transfer or full extraction sample preparation. The mix and relative frequency of different species encountered in a laboratory might affect the net equation of advantages versus disadvantages for the use of disposable targets. Finally, before-after studies may suffer from additional biases that are not controlled for in this study.

The strength of our study is the consecutive inclusion of a large number of isolates reflecting real-world MALDI-TOF MS identifications, sufficiently powered to detect small differences in subgroup analyses.

Other disposable targets might become available, such as those made by 3D printing, possibly purposed for research use only [1].

Switching to MBT Biotargets resulted in overall comparable quality of MALDI-TOF MS identifications, with less laboratory technician hands-on time at increased cost for disposables. These results might help laboratories to assess whether switching to MBT Biotargets could be cost effective.

CRediT authorship contribution statement

Robert K.P. Schouten: Conceptualization, Validation, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Supervision, Project administration. **Valentijn A. Schweitzer:** Validation, Formal analysis, Data curation, Writing – review & editing, Visualization. **Sebastian van Marm:** Conceptualization, Writing – original draft, Visualization, Supervision, Project administration. **Rob J. Rentenaar:** Conceptualization, Validation, Formal analysis, Data curation, Writing – original draft, Writing – review & editing, Visualization, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.diagmicrobio.2024.116270.

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