Faster identification method for pharmaceutical microbiology with the MALDI Biotyper®

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Introduction

The microbiological quality of drugs affects both efficacy and patient safety. Microbial contamination can cause immediate or long term adverse effects, including morbidity and mortality. Additionally, microbes can alter the chemistry and pharmacology of drugs, with a potential negative impact on their effectiveness due to the breakdown of the active ingredients, as well as on their safety due to the toxicity of potential degradation products.

Detecting microbial contamination is essential for pharmaceutical manufacturers managing controlled environments. Establishing an environmental monitoring program that measures the effectiveness of the hygienic practices is key in a manufacturing facility. It can provide information to track contamination through ingredients or biofilms in the water pipeline to help prevent possible microbial contamination in pharmaceutical products. It also fulfills a regulatory requirement.

Significant advances in microbiological methods have been achieved thanks to continuous innovations in mass spectrometry (MS) over the past decade. Nowadays, the standard in microorganism identification is matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) MS, enabling microbiology laboratories to identify microbes reliably, rapidly and cost-effectively, starting from colony material.

MALDI-TOF MS for microbial identification

In recent years MALDI-TOF MS has emerged as a reliable tool for microbial identification and in-house analysis. During the MALDI-TOF MS process, microbes are identified using either fresh cell material from non-selective or selective agar plates, or cell extracts. The process is rapid, sensitive and economical in terms of both labor and costs.

The technology has been readily adopted by microbiologists who have reported usage of MALDI-TOF MS for microbial identification and confirmation, epidemiological studies, identification of highly pathogenic microorganisms, water- and foodborne pathogens as well as skin microbiota.

Type	Microorganisms
Sporogenic *Bacillus*	*Bacillus subtilis*
Non-sporogenic gram-positive *Bacillus*	*Corynebacterium propinquum*
Gram-negative *Enterobacteriaceae*	*Pantoea agglomerans*
Gram-negative non-*Enterobacteriaceae*	*Pseudomonas koreensis*
Gram-negative coccus	*Paracoccus yeeii*
Gram-positive coccus *(Staphylococcus)*	*Staphylococcus capitis*
Gram-positive coccus *(Micrococcus)*	*Micrococcus luteus*
Gram-positive coccus *(Streptococcus)*	*Streptococcus sanguinis*
Yeast	*Candida albicans*

Table 1
The nine types of bacteria and yeast analyzed in the scope of the performance assessment.

Mold
Aspergillus brasiliensis ATCC 16404
Alternaria alternata
Aspergillus fumigatus
Chaetomium globosum
Paecilomyces lilacinus

Table 2
The five types of molds analyzed in the scope of the performance assessment.

The Bruker MALDI Biotyper solution

Bruker has successfully introduced a molecular-based mass spectrometry identification method that enables faster and more efficient analysis than conventional assays or sequencing.
The MALDI Biotyper (MBT) is a microbial identification system that allows unbiased identification of microorganisms down to the species level, within a few minutes starting from culture. It is designed to be an easy, rapid, robust, high-throughput, cost-effective and efficient identification technology, ideally suited to microbiology laboratories.

By determining an organism's unique proteomic fingerprint and matching this characteristic pattern with an extensive reference library, the MBT can reliably identify an unknown microorganism. The MALDI Biotyper currently covers close to 4,700 species (version 2022), including common environmental species and also rare microorganisms. Continuous expansion of the reference library ensures that a broad range of microorganisms can be identified easily. Additionally, the Bruker open software concept allows generation of a site-specific, in-house custom library, which can be selected together with the Bruker reference library for matching with the unknown spectrum.

Performance assessment

A major pharmaceutical manufacturer conducted microbial identification of samples derived from environmental monitoring, water monitoring and production operator monitoring, as well as screening assays for ingredients and final products performed in the laboratory. The goal was to assess the accuracy, specificity, precision and reproducibility of the Bruker MBT for bacteria, yeast and mold samples. Additional testing assessed the MBT`s robustness using mold samples.

The laboratory set up a performance qualification protocol considering the experience of other microbiology laboratories at other international sites within the company.

Chapter 1113 of the United States Pharmacopeia (USP) states different options for performance qualification:

1. Using an existing system for parallel testing of microbial isolates obtained from routine testing (the number of isolates tested may be as high as 50, and any discrepancies in identification can be arbitrated using a reference).

2. Testing 12–15 known representative stock cultures of different commonly isolated species for a total of 50 tests.

3. Confirming that 20–50 organism identifications, including 15–20 different species, agree with the results of a reference laboratory test.

Table 3
Acceptance criteria from United States Pharmacopeia, chapter 1113, PDA Technical report 33.

	Acceptance Criteria
Accuracy | Precision | Specificity	>90% correct ID
Reproducibility	>90% correct ID between 3 operators

Table 4
Accuracy and specificity results for bacteria and yeast samples. Measurement of 9 samples resulted in 100% accuracy and specificity. A score of 2.0 or above indicated correct identification at the species level, scores between 1.7 and <2.0 indicated identification at the genus level. (MO = microorganism)

MO Type	**MO inoculated**	**MO identified**	**Log(score) of best match**	**Correct ID Y/N**
Sporogenic bacillus	*Bacillus subtilis*	*Bacillus subtilis*	2.264	Y
Non-sporogenic gram-positive *Bacillus*	*Corynebacterium propinquum*	*Corynebacterium propinquum*	2.516	Y
Gram-negative *Enterobacteriaceae*	*Pantoea agglomerans*	*Pantoea agglomerans*	2.435	Y
Gram-negative non-*Enterobacteriaceae*	*Pseudomonas koreensis*	*Pseudomonas koreensis*	2.277	Y
Gram-negative coccus	*Paracoccus yeeii*	*Paracoccus yeeii*	2.412	Y
Gram-positive coccus *(Staphylococcus)*	*Staphylococcus capitis*	*Staphylococcus capitis*	2.450	Y
Gram-positive coccus *(Micrococcus)*	*Micrococcus luteus*	*Micrococcus luteus*	2.450	Y
Gram-positive coccus *(Streptococcus)*	*Streptococcus sanguinis*	*Streptococcus sanguinis*	2.210	Y
Yeast	Candida albicans	Candida albicans	2.293	Y

Protocol for bacteria and yeast

Nine different types of microorganisms mainly of environmental origin (bacteria and yeasts) were analyzed (Table 1). Each microorganism was submitted to the following assessments:

Accuracy and specificity

Each of the nine microorganisms underwent double identification with the MBT and the results were compared to a reference method.

Precision

One environmental isolate was identified ten times, in duplicate, to determine how many times the right identification was obtained.

Reproducibility

Three analysts completed the analyses of the nine microorganisms over three different days, to determine the reproducibility.

Protocol for molds

Five different types of molds – four environmental and one American Type Culture Collection (ATCC) strain – were analyzed (Table 2). Each microorganism was submitted to the following assessments:

Accuracy and specificity / Precision / Reproducibility

Analyses of the five molds was performed as described above for bacteria and yeasts.

Robustness

MBT identifications were performed and compared to the reference methods used to identify the molds. For this purpose two different batches of TSB liquid medium were used, as well as one batch of HCCA-matrix of which two tubes were used; one tube of HCCA-matrix was prepared the day of the analysis, the other one was prepared 8 days before the analysis (and hence expired).

Table 5
Reproducibility results for bacteria and yeast samples. (MO = microorganism)

	MO inoculated	MO identified	Analyst 1 Log(score) of best match	Correct ID Y/N	Analyst 2 Log(score) of best match	Correct ID Y/N	Analyst 3 Log(score) of best match	Correct ID Y/N
Sporogenic *Bacillus*	*Bacillus subtilis*	*Bacillus subtilis*	2.321	Y	2.185	Y	2.293	Y
Non-sporogenic gram-positive *Bacillus*	*Corynebacterium propinquum*	*Corynebacterium propinquum*	2.390	Y	2.487	Y	2.527	Y
Gram-negative *Enterobacteriaceae*	*Pantoea agglom-erans*	*Pantoea agglomerans*	2.436	Y	2.468	Y	2.446	Y
Gram-negative non-*Enterobacteriaceae*	*Pseudomonas koreensis*	*Pseudomonas koreensis*	2.279	Y	2.306	Y	2.209	Y
Gram-negative coccus	*Paracoccus yeeii*	*Paracoccus yeeii*	2.471	Y	2.406	Y	2.402	Y
Gram-positive coccus *(Staphylococcus)*	*Staphylococcus capitis*	*Staphylococcus capitis*	2.321	Y	2.406	Y	2.409	Y
Gram-positive coccus *(Micrococcus)*	*Micrococcus luteus*	*Micrococcus luteus*	2.504	Y	2.440	Y	2.410	Y
Gram-positive coccus *(Streptococcus)*	*Streptococcus sanguinis*	*Streptococcus sanguinis*	2.212	Y	2.290	Y	2.296	Y
Yeast	*Candida albicans*	*Candida albicans*	2.169	Y	2.256	Y	2.209	Y

Table 6
Accuracy and specificity results for mold samples.

MO inoculated	MO identified	Log(score) of best match	Correct ID Y/N
Aspergillus brasiliensis ATCC 16404	*Aspergillus niger* (renamed to *A. brasiliensis)*	1.974	Y
Alternaria alternata	*Alternaria alternata*	2.436	Y
Aspergillus fumigatus	*Aspergillus fumigatus*	2.620	Y
Chaetomium globosum	*Chaetomium globosum*	2.594	Y
Paecilomyces lilacinus	*Paecilomyces lilacinus*	2.447	Y

Acceptance Criteria

Criteria for accuracy, specificity, precision and reproducibility were as listed in Table 3, taking the following identification results on the Bruker MALDI Biotyper into account:

- Log(score) between ≥ 2.000 and 3.000: microorganism well identified to species level
- Log(score) between ≥ 1.700 and ≤ 1.999:microorganism well identified to genus level
- Log(score) between ≥ 0.000 and ≤ 1.699: microorganism NOT identified or no peaks found

Note: In the performance qualification, yellow scores were accepted if the species obtained was the same as the reference method.

Results

Bacteria & Yeast

High MBT identification scores were obtained. The accuracy and specificity resulted in 100% correct identification to the species level for all bacteria and yeast (Table 4). Log(score) values are given in the 'Log(score) of best match' column.
Repeating 10 times the analysis of one environmental isolate (*Staphylococcus capitis*), in duplicate, resulted in a precision of 100% (results not shown). Similar results were found for reproducibility for all bacteria and yeast (Table 5); 100% correct identification was observed for the 3 operators.

Mold

Also for the assessment of the mold analysis high MBT identification scores were obtained. The accuracy and specificity resulted in identification to the species level for all molds – with the exception of *Aspergillus brasiliensis* ATCC 16404, which was identified to the genus level [log(score) band 1.7-2.0] (Table 6).

Repeating 10 times the analysis of one environmental isolate (*Alternaria alternata*), in duplicate, resulted in a precision of 100% (results not shown).

Assessment of the reproducibility (Table 7) and the robustness (Table 8) resulted in high scores, again with the exception of *Aspergillus brasiliensis* ATCC 16404 (all results for *A. brasiliensis* had log(score) values >1,7). Even when using the expired tube of reconstituted HCCA-matrix, the log(score) results remained high.

Table 7
Reproducibility results for mold samples.

MO inoculated	MO identified	Analyst 1 Log(score) of best match	Correct ID Y/N	Analyst 2 Log(score) of best match	Correct ID Y/N	Analyst 3 Log(score) of best match	Correct ID Y/N
Aspergillus brasiliensis ATCC 16404	*Aspergillus niger* (renamed to *A. brasiliensis)*	2.078	Y	2.028	Y	1.886	Y
Alternaria alternata	*Alternaria alternata*	2.343	Y	2.361	Y	2.441	Y
Aspergillus fumigatus	*Aspergillus fumigatus*	2.597	Y	2.639	Y	2.73	Y
Chaetomium globosum	*Chaetomium globosum*	2.607	Y	2.617	Y	2.595	Y
Paecilomyces lilacinus	*Paecilomyces lilacinus*	2.519	Y	2.333	Y	2.552	Y

Table 8
Robustness results for mold samples.

MO inoculated	MO identified	TSB batch nº 1 – fresh HCCA matrix Log(score) of best match	TSB batch nº 1 – 8 days old HCCA matrix Log(score) of best match	TSB batch nº 2 – fresh HCCA matrix Log(score) of best match	Correct ID Y/N
Aspergillus brasiliensis ATCC 16404	*Aspergillus niger* (renamed to *A. brasiliensis*)	1.938	1.974	1.970	Y
Alternaria alternata	*Alternaria alternata*	2.428	2.412	2.482	Y
Aspergillus fumigatus	*Aspergillus fumigatus*	2.690	2.680	2.467	Y
Chaetomium globosum	*Chaetomium globosum*	2.538	2.439	2.542	Y
Paecilomyces lilacinus	*Paecilomyces lilacinus*	2.562	2.566	2.566	Y

Conclusion

Implementing the MALDI Biotyper as an in-house rapid microbiological method causes a considerable drop in cost/ID to a few EUR per sample only (Figure 1), in this particular case saving roundabout 265K€ annually, based on the assumption of about 5000 samples/year.

Significant cost savings

The excellent reliability, time advantages of an in-house method, and ease of use delivered by the Bruker MBT all add up to the significant cost savings per identification.

Reliability

Results using the Bruker MBT showed reliable correct identification scores for all tested bacteria, yeasts and molds.

Time saving

Pharmaceutical microbiology laboratories can benefit from the time savings of obtaining an identification within minutes, after only a very short hands-on time, using one system and one workflow enabling analysis of bacteria, yeast and molds in the same test run, with the capacity to process up to 96 samples per run.

Ease of use

Technicians can easily be trained on the MBT workflow and identifications are clearly shown in easy-to-interpret PDF result reports. The MBT does not require other traditional ID methods, such as gram staining or biochemical testing.

Summary

The MALDI Biotyper delivers accurate and fast results for microorganism identification, enabling pharmaceutical laboratories to quickly gain insight into environmental strains, water bacteria and common or pathogenic bacterial and fungal species.

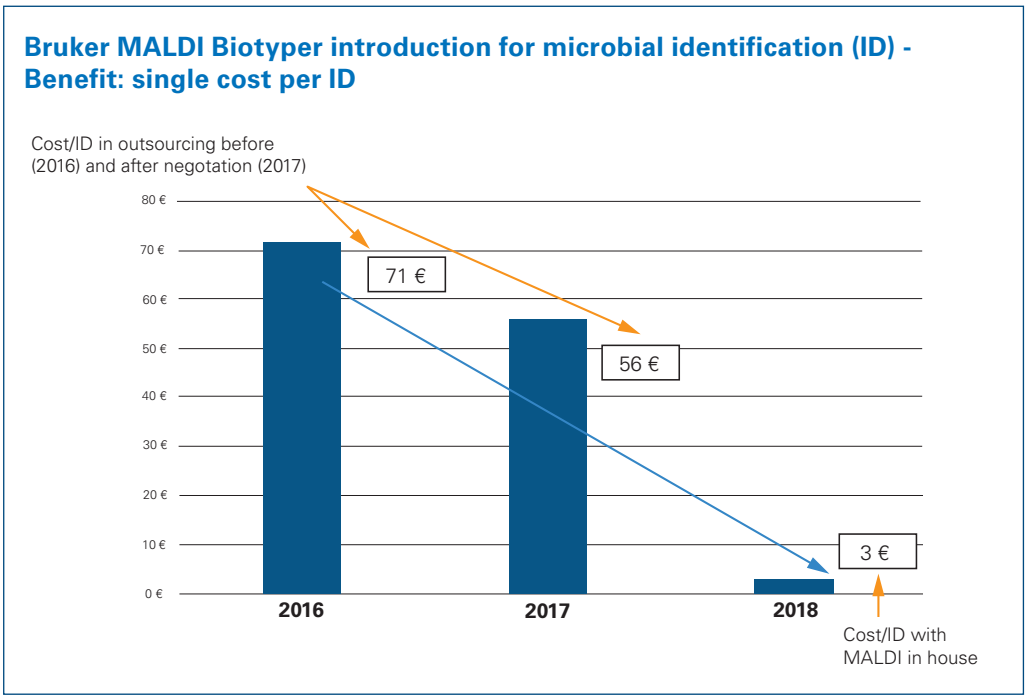

Figure 1
Cost benefit per identification using the Bruker MALDI Biotyper.

Not for use in clinical diagnostic procedures.
Please contact your local representative for availability in your country.

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