Evaluation of Three MALDI-TOF Mass Spectrometry Libraries for the Identification of Filamentous Fungi

M. Stein^{1,2}, V. Tran¹, K.A. Nichol², P. Lagacé-Wiens^{1,2}, P. Pieroni², H.J. Adam^{1,2}, C. Turenne^{1,2}, A.J. Walkty^{1,2}, M. Hendrickx³, A-C Normand⁴, R. Piarroux⁴, and J.A. Karlowsky^{1,2}

¹Department of Medical Microbiology and Infectious Diseases, Max Rady College of Medicine, University of Manitoba, Winnipeg, MB, Canada; ²Shared Health, MB, Canada; ³Service de Parasitologie/Mycologie, Groupe Hospitalier Pitié-Salpêtrière, AP-HP, Paris, France; and ⁴Mycology & Aerobiology, Scientific Institute of Public Health, Brussels, Belgium

Background: While MALDI-TOF has been well established in diagnostic laboratories for the identification of bacteria and yeast, its application for identification of moulds has only recently emerged, and only a limited number of publications evaluate the usefulness of MALDI-TOF in this context. In this study we evaluated the performance of the Bruker Microflex™ MALDI-TOF MS instrument (Billerica, MA, USA) to identify clinical isolates and reference strains of moulds using three libraries, the Bruker mould library, the National Institutes of Health (NIH) library, and the Mass Spectrometry Identification (MSI) online library, and compared those results to conventional (morphological) and molecular (18S/ITS; gold standard) identification methods.

Table 1: MALDI-TOF identification rates for three mould libraries

Cumulative data from all three testing laboratories	MALDI-TOF MS Library					Conventional ID
	Bruker	NIH	Bruker +NIH	MSI	Bruker +NIH +MSI	
No. of isolates with no ID	143	108	84	57	45	0
No. of isolates with species-level ID ^a	30	43	64	159	162	70
No. of isolates with genus-level ID ^a	44	68	68	N/A	9	121
No. of isolates with sp. or genus-level ID	74	111	132	159	171	191
No. of isolates that were misidentified	4	2	5	5	4	30
Total no. of IDs	78	113	137	164	175	221
Total number of isolates tested	221	221	221	221	221	221
% of total no. of IDs that were misidentifications	5.1%	1.8%	3.7%	3.1%	2.3%	13.6%
Sp. ID rate for the total no. of isolates tested ^a	13.6%	19.5%	29%	72%	73.3%	31.7%
Genus ID rate for the total no. of isolates tested ^a	33.5%	50.2%	59.7%	N/A	77.4%	86.4%
% of the total no. of IDs made that agreed with the DNA sequencing result	94.9%	98.2%	96.4%	97%	97.7%	86.4%

^aFor the Bruker Filamentous Fungi Library 1.0 and the NIH library the species and genus ID Scores were ≥ 2.0 and ≥ 1.7 respectively. The species ID Score for the MSI library was ≥ 17 .

References:

- Lau AF, Drake SK, Calhoun LB, Henderson CM, Zelazny AM. *J Clin Microbiol.* 2013;51(3):828-834.
- Gautier M, Ranque S, Normand A-C, et al. *Clin Microbiol Infect.* 2014; 20(12):1366-1371.
- Normand AC, Becker P, Gabriel F, et al. *J Clin Microbiol.* 2017;55(9):2661-2670

Material and Methods:

Fungal Isolates Tested: In total 221 moulds were tested (158 clinical isolates and 63 ATCC/CBS isolates (ATCC/CBS)).

Fungal cultures: Moulds were grown on Potato Flake Agar, or Sabouraud's Agar incubated at 30°C for 1-7 days.

DNA sequencing: ITS2, Bt2a, or EF1 DNA sequencing was performed at the Genome Quebec Innovation Center.

MALDI-TOF MS sample preparation: pieces of mould grown on solid medium were washed in ethanol, and extracted with 70% formic acid and an equal volume of acetonitrile. 1µl of extract supernatant was spotted on the MS target plate in triplicate, air dried, and overlaid with 1µl of HCCA (α -cyano-4-hydroxycinnamic acid) matrix. MALDI-TOF MS spectra were generated using the Bruker Microflex™ system.

Table 2: MALDI-TOF Misidentifications

ID Method	Isolate ID	MALDI-TOF Score	Molecular ID (Gold Standard)
Bruker library	<i>Curvularia pallescens</i>	2.04	<i>Curvularia lunata</i>
	<i>Mucor ramosissimus</i>	2.13	<i>Mucor circinelloides</i>
	<i>Scydaliidum sp.</i>	1.79	<i>Neoscytalidium dimidiatum</i>
NIH library	<i>Trichophyton tonsurans</i>	2.10	<i>Trichophyton interdigitale/mentagrophytes</i>
	<i>Scydaliidum sp.</i>	1.79	<i>Neoscytalidium dimidiatum</i>
MSI library	<i>Emericella quadrilineata</i>	2.07	<i>Aspergillus nidulans</i>
	<i>Chalastospora gossypii</i>	18.61	<i>Alternaria chlamydospora</i>
	<i>Eurotium minus</i>	18.66	<i>Aspergillus glaucus</i>
	<i>Fusarium petrophilum</i>	28.46	<i>Fusarium oxysporum</i>
	<i>Fusarium falciforme</i>	25.19	<i>Fusarium solani</i>
	<i>Aphanocladium album</i>	31.02	<i>Lecanicillium aphanocladii</i>

Conclusions:

- MALDI-TOF is a useful tool for the identification of moulds in the clinical microbiology laboratory and demonstrates superior accuracy (>94%) when compared to conventional mould identification methods (86%).
- The relatively few misidentifications occurred on the species level of closely related species or on genus level of rarely isolated environmental moulds that were underrepresented in the libraries.
- The identification rate for the MSI online library on the species-level was 72%, versus 13.6% and 19.5% for the Bruker and NIH libraries, respectively. The latter two libraries were generated using different protocols.