RUO

Application Note

Identifying Pathogenic Filamentous Fungi with Novel Culture Medium Id-Fungi Plates™ and the MALDI Biotyper®

Introduction

Despite the significant increase in research into invasive fungal diseases (IFD) in recent years, such infections still represent a significant challenge to healthcare institutions. High mortality rates of over 50% [1], complex identification procedures, and an increasing number of reports of emerging antifungal resistance all contribute to the complex task of tackling IFD. *Aspergillus* and *Candida* species remain the most common causes of IFD, but over the last decade a number of more uncommon filamentous fungi, such as *Scedosporium* spp., *Fusarium* spp., *Penicillium* spp., melanized molds, and basidiomycetes, have been attributed to well-characterized respiratory disorders [2].

Filamentous fungi occur commonly in the environment, and some species are capable of colonizing human hosts and causing a spectrum of disease from superficial infections in the skin and nails (dermatophytosis) to life-threatening IFD, particularly in immunocompromised individuals. Correct identification of molds is essential to distinguish dermatophytes from non-dermatophytes and establish the causative species, to inform appropriate therapeutic strategies. The number of patients with increased susceptibility to pathogenic filamentous fungi is rising due to advances in medicine, such as hematopoietic stem cell and solid organ transplantations, and use of immunomodulatory agents. Early diagnosis can improve treatment results and potentially

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Instrumentation and Software:
MALDI Biotyper
Id-Fungi Plates
MBT Compass

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reduce IFD-associated healthcare costs, so rapid, accurate and reliable identification methods are vital for positive patient and financial outcomes.

Mold identification with MALDI-TOF MS

Traditional methods of identifying IFD causative pathogens, such as microscopy and histology, rely on visualizing morphological characteristics, which requires specialized knowledge and expertise. Additionally, these methods cannot identify fungi at the species level and are often invasive. DNA sequencing, including polymerase chain reaction (PCR), is considered the gold standard for mold identification, but this method is labor- and resource-intensive. For over a decade, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been widely used in clinical microbiology research laboratories to identify bacteria and yeast, but the identification of filamentous fungi with this method has remained a challenge. Molds can grow inside the solid media and are difficult to harvest, making the workflow time-consuming and laborious and identification scores can be low due to contamination of spectra with agar. Additionally, less convenient extraction methods are frequently needed to obtain a clean and significant spectrum, further limiting the routine use of MALDI-TOF MS in mycology or microbiology laboratories.

To facilitate the growth and sampling of filamentous fungi for MALDI-TOF MS identification, a new solid medium has been developed that includes a transparent membrane deposited on the agar surface, allowing the growth of molds but impassable by fungi hyphae, enabling a clean harvest. In addition to the unique membrane, the Id-Fungi Plates™ (IDFP) use a specific culture medium with an optimized composition of antibiotics, low pH agar, peptones, and a high amount of dextrose, that promotes the growth of fungi and limits bacterial growth. The benefits of IDFP for microbiologists are two-fold:

- **Reduced incubation time** – for non-dermatophytes, IDFP require incubation of 1–2 days, compared with the industry standard media requirement of approx. 5 days. For dermatophytes, incubation time is reduced from 10–13 days to 5–7 days using IDFP.
- **Easier mold harvesting** – reduces turnaround time, simpler protocol for non-experts, and facilitates a clean harvest for improved MALDI-TOF MS identification.

Evaluation of Id-Fungi Plates with MALDI-TOF MS

The time savings associated with reduced incubation time and improved mold harvesting with IDFP not only increase turnaround time for identification, but result in significant cost savings. An economical study found that despite the higher initial cost, the more efficient workflow from sampling to MALDI-TOF MS identification resulted in a 39% reduction in the cost of filamentous fungi identification when using IDFP, compared with conventional media such as Sabouraud. Another advantage of shorter incubation time is the ability to harvest fungi mycelium. Most filamentous fungi libraries for MALDI-TOF MS identification are based on mycelium, so if the fungi are incubated for a long time period and develop spores, identification can become challenging.

Other studies have evaluated the performance of IDFP compared with other culture media, and reported a higher proportion of acceptable identifications and MALDI-TOF MS identification score values after culture on IDFP than with Sabouraud [3], as well as faster growth of isolates for dermatophyte and non-dermatophyte filamentous fungi and enhanced performance of fungal identification [4].

The aim of the current study was to evaluate the efficiency of IDFP for integration into existing MALDI-TOF MS workflows across eight independent laboratories, and evaluate the performance of IDFP in facilitating the growth and identification of filamentous fungi.

Materials and methods

Studies were performed between 2018 and 2019 at the following laboratories: Hôpital FOCH Laboratoire de Biologie Médicale, France; CHU Grenoble Alpes Institut de Biologie et de Pathologie, France; Mycology Reference Laboratory, Public Health England, Bristol, UK; Department of Laboratory Medicine, General Hospital Maria Middelares, Belgium; R&D Reference Library Laboratory, Bruker Daltonik GmbH, Bremen, Germany; Hôpital Bichat Laboratoire de parasitologie-mycologie, France; Centre Hospitalier Bethune Laboratoire de microbiologie, France; and Hôpital Universitaire de Genève, Switzerland.

Origin of isolates

Laboratories either used clinical samples, 'collection' samples (originating from reference laboratories/libraries, or both). For clinical samples, the identity was confirmed by microscopic identification and/or molecular biology (internal transcribed spacer (ITS) identification).

A total of 31 genera and 76 species (groups) were analyzed. Main genera represented:

- 10 *Aspergillus* species/groups (n= 92)
- 8 P*enicillium* species/groups (n=18)
- 5 *Fusarium* species/groups (n=15)
- 4 *Mucor/Rhizopus* species/groups (n=11)
- 2 *Scopulariopsis* species/groups (n=11)
- 5 Genera of dermatophytes (Anamorph/Teleomorph) among which are
  - 8 *Trichophyton* species/groups (n=80)
  - 3 *Nannizzia* species/groups (n=16)
  - 6 M*icrosporum* species/groups (n=21)
  - 2 *Arthroderma* species/groups (n=5)
  - 1 *Epidermophyton* species/group (n=1)

Methodology variations

Studies at the eight sites (see table 1) were carried out independently, so some variations in methodology were as follows:

- Time and temperature of incubation.
- Analysis method (MALDI-TOF MS): some methods compared direct transfer (DT) with the longer extraction procedure (EX). The results of this study are compared using only DT as a common denominator.
- MALDI-TOF MS library used: some studies compared different fungi libraries. The results of this study are compared using only the Bruker MBT Filamentous Fungi Library 2.0 as a common denominator.
- Culture medium comparisons: six laboratories compared IDFP with Sabouraud, and two compared IDFP with CAN2 and potato dextrose agar (PDA). This study grouped non-IDFP culture media together to compare with IDFP.
- Two laboratories only tested IDFP abilities to promote growth and identification of molds, rather than evaluating efficiency of IDFP for MALDI-TOF MS identification against other culture media.

Culture media

IDFP (CONIDIA, Quincieux, France) performance was compared with either Sabouraud Agar with chloramphenicol (SAB) (Becton Dickinson GmbH, Heidelberg, Germany), Sabouraud dextrose agar plates with gentamicin and chloramphenicol (SGC2) (bioMérieux, Marcy-l'Étoile, France), ChromID™ Candida Agar (CAN2) (bioMérieux, Marcy-l'Etoile, France), or PDA (Fisher Scientific; Beckton Dickinson). Protocols were carried out according to contributing laboratories' workflows, but the recommended IDFP method is described here [5].

MALDI-TOF MS analysis

All MALDI-TOF MS analyses were performed using a MALDI Biotyper RUO/GP system (based on the microflex™ LT) (Bruker Daltonics GmbH, Bremen, Germany). An identification score between 0.0 and 3.0 was obtained, depending on the degree of similarity to a given reference spectrum in the MBT Filamentous Fungi Library. Scores were categorized as follows: score ≥ 2.0, high confidence identification at the species level; score between 1.7 and 2.0, low confidence identification at the species level; and score < 1.7, no identification. Misidentifications were also recorded.

	Laboratory	Date	Comparison	Origin of isolates			Culture conditions	
				n	Clinical	Collection	Time (h)	Temp (°C)
1	Hôpital FOCH Laboratoire de Biologie Médicale	June 2019	IDFP vs Sabouraud	66	0	66	24-48	30-37
2	CHU Grenoble Alpes Institut de Biologie et de Pathologie	April–May 2018	IDFP vs Sabouraud vs CAN2	51	3	48	24-96	30-37
3	Mycology Reference Laboratory, Public Health England	Dec 2018	IDFP vs Sabouraud	22	0	22	36-60	30-37
4	Department of Laboratory Medicine, General Hospital Maria Middelares	Dec 2019	IDFP vs Sabouraud	76	25	51	24-168	25
5	R&D Reference Library Laboratory, Bruker Daltonik	April–June 2018	IDFP vs Sabouraud agar vs Sabouraud liquid	68	0	68	72	30-37
6	Hôpital Bichat Laboratoire de parasitologie-mycologie	June 2018	IDFP	11	0	11	24-96	30-37
7	Centre Hospitalier Bethune Laboratoire de microbiologie	Nov 2018	IDFP vs Sabouraud	7	1	6	24-72	20-30
8	Hôpital Universitaire de Genève Switzerland	2018	IDFP vs PDA	22	0	22	24-72	30

Table 1: Main characteristics of eight studies comparing Id-Fungi Plates (CONIDIA) with other culture media, for filamentous fungi identification using MALDI-TOF MS (MALDI Biotyper, Bruker Daltonik).

Results

MALDI-TOF MS identification scores were obtained from each laboratory and totaled for each fungi genus. Scores were then calculated as a percentage of the total number of samples measured from those genera, for IDFP and other media. IDFP outperformed other media for all genera, with the most significant improvement in identification seen for *Aspergillus* spp., where a score of ≥ 2.0 was shown in around 80% of samples for IDFP compared with around 33% for other media.

Additionally, only 2% of samples returned 'no identification' scores for IDFP, whereas other media resulted in 51% 'no identification' results (Figure 1A).

A similar trend was seen for *Fusarium* spp., with IDFP resulting in more reliable identification (≥ 2.0) at the species level for IDFP (47%) compared with other media (33%), and fewer 'no identification' results for IDFP (27%) compared with other media (47%) (Figure 1C).

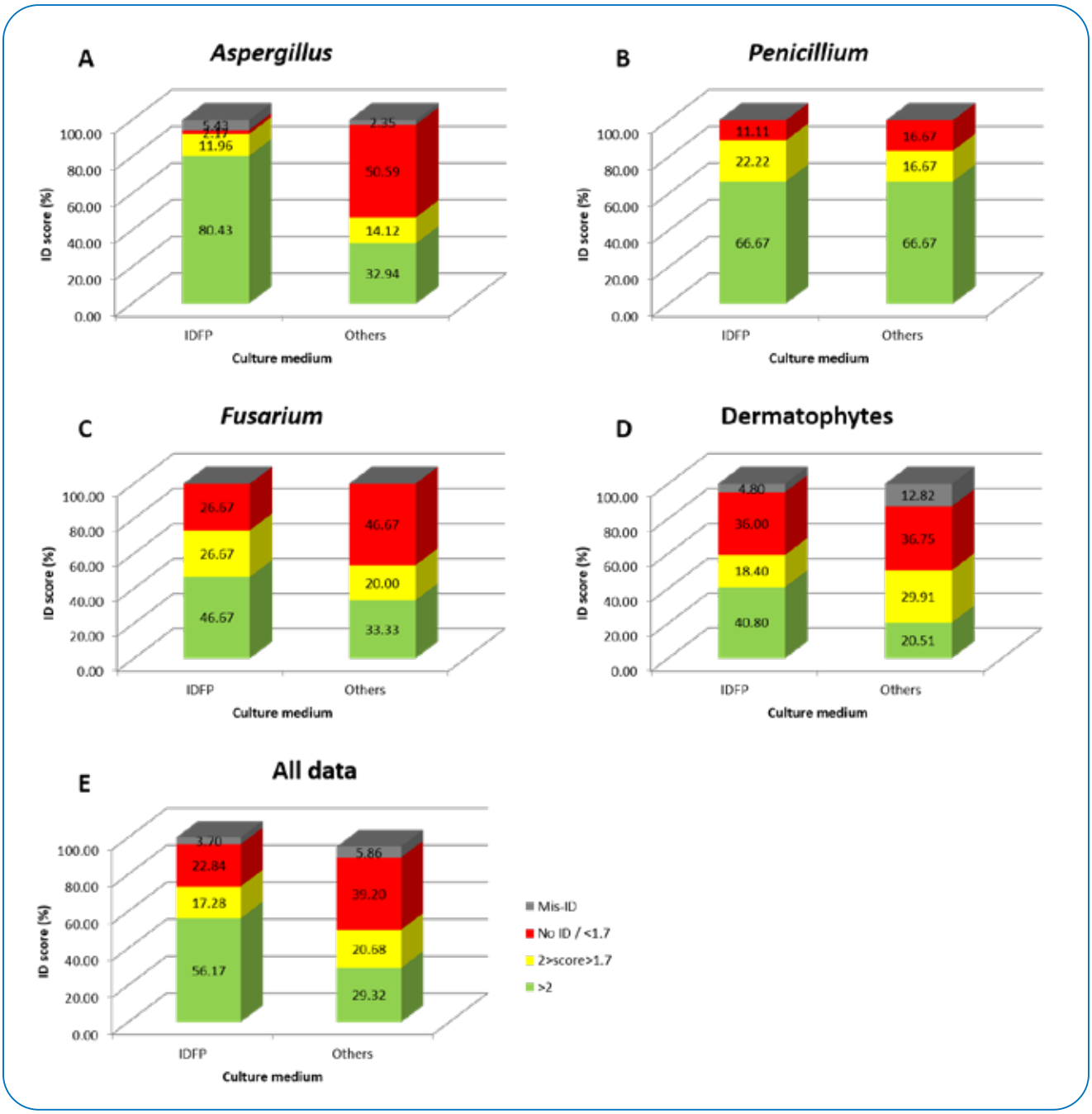

Figure 1: MALDI-TOF MS identification (ID) scores (%) comparing ID-Fungi Plates (IDFP) and other media for (A) Aspergillus *spp. (B)* Penicillium *spp. (C)* Fusarium *spp. (D) Dermatophytes (E) all genera.*

Dermatophyte identification using IDFP showed a similar rate of 'no identification' results compared with other media, but a reduced incidence of misidentification (4.8%) compared with other media (12.8%) and a higher proportion of scores above 2.0 (40.8% vs 20.1%) (Figure 1D). MALDI-TOF MS identification scores for *Penicillium* spp. showed the least difference between IDFP and other culture media, with equivalent identification scores ≥ 2.0, a higher proportion of scores between 1.7 and 2.0 for IDFP (22.2%) compared with other media (16.7%), and IDFP providing slightly fewer 'no identification' results (11.1%) than other media (16.7%) (Figure 1D). The total data for all genera and species indicates that, compared with other media, IDFP provides an improved identification for both dermatophyte and non-dermatophyte filamentous fungi (Figure 1E).

Due to the retrospective nature of the study, full alignment of protocols across the eight study sites was not possible, but would have likely provided more reliable and consistent results. For example, the total number of *Aspergillus* spp. (92 IDFP and 85 other media) and dermatophyte (125 IDFP and 117 other media) isolates were considerably greater than the number of *Penicillium* spp. (18) and *Fusarium* spp. (15) isolates. Additionally, not all laboratories compared IDFP to the same media.
One site that compared IDFP to both SAB and CAN2 reported that the difference in identification score was less obvious when IDFP was compared to CAN2 than to SAB [3].

While the results of this study could be improved by aligning protocols across the eight contributing laboratories, the overall result from 324 samples shows a considerable increase in reliable MALDI-TOF MS identification scores, and a decrease in 'no identifications' and misidentifications. This aligns with other studies reporting an improvement in correct identification using IDFP [6].

Conclusion

In comparison with traditional culture media, IDFP show an increase in reliable correct identification scores for *Aspergillus* spp., *Fusarium* spp. and dermatophyte fungi. MALDI-TOF MS reference libraries are continuously updated to reflect emerging pathogens. With more species being added to libraries such as the MBT Filamentous Fungi Library, reliability of identifications will continue to improve.
Clinical microbiology research laboratories are benefitting from the time savings associated with IDFP. Long incubation of fungi plates and laborious harvesting is no longer necessary, significantly shortening the identification workflow. Laboratories that previously relied on lengthy cultivation methods or could not support filamentous fungi identification altogether can now run these tests alongside their routine MALDI-TOF MS bacteria identification and obtain accurate, fast results, that could provide insight into current and emerging pathogens causing IFD.

About Conidia

CONIDIA company, established in 2005 by Sébastien Vacher, PhD, is an independent consulting laboratory with a specialty in the field of yeasts and molds. CONIDIA scientific teams develop specific approaches to provide dedicated solutions in many application areas, focusing on innovative R&D protocols with dedicated laboratory equipment and specific knowledge in microbiology.

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For more information on Bruker's MALDI Biotyper solutions, please visit
https://www.bruker.com/products/mass-spectrometry-and-separations/maldi-biotyper-systems.html

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