Communication

Performance of the VITEK MS System for the Identification of Filamentous Fungi in a Routine Microbiological Laboratory in Chile

Lorena Porte *, Rodrigo Cruz, Inia Pérez, Carmen Varela, Cristina Díaz, Patricia García, Paulette Legarraga, Francisca Valdivieso, Thomas Weitzel

Posted Date: 8 August 2024

doi: 10.20944/preprints202408.0457.v1

Keywords: Diagnosis; MALDI-TOF; fungal infections; mycology; mass spectrometry

Article

Performance of the VITEK MS System for the Identification of Filamentous Fungi in a Routine Microbiological Laboratory in Chile

Lorena Porte [1,*], Rodrigo Cruz [2], Inia Pérez [3], Carmen Varela [1], Cristina Díaz [4,†], Patricia García [6], Paulette Legarraga [1], Francisca Valdivieso [5] and Thomas Weitzel [1,7]

[1] Laboratorio Clínico, Clínica Alemana, Facultad de Medicina Clínica Alemana, Universidad del Desarrollo, Santiago, 7630000, Región Metropolitana, Chile; lporte@alemana.cl (L.P.); cvarelaa@alemana.cl (C.V.); plegarraga@alemana.cl (P.L.); tweitzel@alemana.cl (T.W.)
[2] Centro de Diagnóstico e Investigación de Enfermedades Infecciosas. Universidad de Valparaíso, Valparaíso, 2340000, Quinta Región, Chile; rodrigo.cruz@uv.cl
[3] Servicio de Infectología, Departamento de Medicina Interna, Clínica Alemana, Facultad de Medicina Clínica Alemana, Universidad del Desarrollo, Santiago, 7630000, Región Metropolitana, Chile; iperez@alemana.cl
[4] Programa de Microbiología y Micología, ICBM, Facultad de Medicina, Universidad de Chile, Santiago, 8380000, Región Metropolitana, Chile
[5] Laboratorio de Microbiología, Hospital Luis Calvo Mackenna, Santiago, 7500000, Región Metropolitana, Chile; fvaldivieso@calvomackenna.cl
[6] Departamento de Laboratorios Clínicos. Escuela de Medicina. Pontificia Universidad Católica de Chile, Santiago, 8320000, Región Metropolitana, Chile; pgarciacan@uc.cl
[7] *Instituto de Ciencias e Innovación en Medicina* (ICIM), Facultad de Medicina Clínica Alemana, Universidad del Desarrollo, Santiago, 7550000, Región Metropolitana, Chile; t.weitzel@udd.cl
* Correspondence: lporte@alemana.cl
† Deceased in July 23, 2021.

Abstract: Background: Filamentous fungi are an emergent cause of severe infections in immunocompromised patients. Timely and accurate identification is crucial to initiate appropriate therapy. Traditional identification methods are time-consuming, labor-intensive, and operator-dependent. Matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry is a rapid and easy-to-perform identification method. This study evaluates the effectiveness of a commercial MALDI-TOF MS platform to identify filamentous fungi in a routine laboratory. **Material and Methods:** We included 67 fungal isolates from 35 species/species complexes within 15 genera, confirmed in mycology reference laboratories. 33 were from clinical samples and 34 from strain collections. The study used the VITEK MS system (v3.2.0 database), after sample extraction by VITEK MS Mould Kit. Results were classified into categories: 'correct species', 'correct species complex', 'correct genus', 'incorrect identification', and 'no identification'. We also evaluated the practicality of the kit. **Results:** VITEK MS correctly identified 91.0% of isolates (58.2% to species, 29.9% to species complex, and 1.5% to genus level). In 82%, the result matched the species/species complex identified by reference methods. No misidentifications were observed. The kit was rapid and easy to use. **Conclusion:** The VITEK MS system showed a high capability to accurately identify filamentous fungi in a routine laboratory.

Keywords: Diagnosis; MALDI-TOF; fungal infections; mycology; mass spectrometry

 doi:10.20944/preprints202408.0457.v1

1. Introduction

Filamentous fungal infections are an emerging problem in clinical practice, ranging from mild onychomycosis to severe invasive disease in immunocompromised patients. The high mortality of systemic mould infections requires prompt initiation of specific antifungal therapy. Since some species are intrinsically resistant to certain antifungals and susceptibility testing is not widely available, rapid and accurate species identification is crucial for the selection of appropriate antifungal treatment [1]. This diagnostic goal, however, is increasingly challenged due to the description and emergence of new opportunistic fungal species [2,3].

Traditional diagnostic techniques for filamentous fungi are based on morphological and physiological characteristics and, therefore, require experienced mycologists, a resource that is scarce in most microbiological laboratories [4,5]. Additionally, these methods are time-consuming and not always accurate [6–8]. DNA amplification and sequencing is an additional diagnostic tool; however, this approach is not commercially available and mostly used in reference laboratories [4].

Matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry has revolutionized the diagnosis of bacteria and yeast, but its capacity to identify filamentous fungi has been less studied [7]. Early reports showed poor performance due to non-optimized extraction procedures and limited pathogen databases [9]. However, subsequent studies have demonstrated that with an appropriate reference database and extraction protocol, MALDI-TOF can provide rapid identification results for filamentous fungi and has the potential to become a standard identification method [10,11]. For example, the comparison of three pretreatment procedures applied to different MALDI-TOF instruments showed better diagnostic efficacy when cell wall disruption agents were used before analysis [12].

In 2017, the VITEK MS 3.0 system received FDA approval for a Mould Kit (inactivation/extraction reagents] and a database version, which showed promising results in initial evaluations [11]. An updated version of the database (Knowledge Base v3.2.0) was released in 2018, containing a broader spectrum of fungal pathogens (55 additional taxa). The present study evaluated the specificity and practicality of this new kit together with the updated database on the VITEK MS platform to identify filamentous fungi in a microbiology laboratory in Chile.

2. Materials and Methods

2.1. Mold Isolates

Most included filamentous fungal isolates derived from routine clinical samples examined between November 2017 and December 2018 in four healthcare centers in Santiago, Chile, the Clínica Alemana, Hospital Clínico Red de Salud UC-CHRISTUS, Hospital Militar, and Hospital Luis Calvo Mackenna. After incubation at 30-35°C, fungal cultures were sent to the microbiology laboratory of Clínica Alemana, where they were stored at room temperature until analysis by VITEK MS. After this, all strains were sent to the reference mycology laboratory at Universidad de Valparaíso, where they were identified using traditional morpho-physiological methods. For this, strains were tested for growth on specific culture media at selected temperatures. Macroscopic characteristics included growth pattern, surface and reverse colony color, texture, grooves, detection of survival and/or sexual structures, pigment production, exudates, and presence of conidial chains were part of the analysis. Microscopic examination included the measurement of the distinct structures of each species (average), hyphae, conidiophores, branches, vesicles, phialides, metulae, micro and macroconidia and the description of their shapes and surface appearance (smooth, rough, finely rough or spiny). Dichotomous reference keys were used to achieve final identification [13–15].

We also included isolates from strain collections of mycology reference laboratories at Universidad de Valparaíso (n=28) and Universidad de Chile in Santiago (n=6), which were diagnosed by traditional methods and confirmed by molecular techniques. One strain (*Coccidioides posadasii*) was tested by VITEK MS on its initial isolation in our laboratory and subsequently confirmed by PCR and sequencing in the Mycology Section, Robert-Koch Institute (Berlin, Germany), as previously reported [16]. We also included one ATCC reference strain (*Aspergillus brasiliensis* 16404).

 doi:10.20944/preprints202408.0457.v1

2.2. VITEK MS System

Before testing, isolates were subcultured on potato dextrose agar (bioMérieux, Marcy-l'Étoile, France) at 35°C or 30°C (dermatophytes) and tested by MALDI-TOF, when first growth was visible. As a first step, fungal colonies were inactivated and extracted with the VITEK MS Mould Kit (bioMérieux), which includes solutions R1 to R3, following the manufacturer´s instructions. In brief, the mycelium was collected under a biosafety cabinet with a sterile cotton swab (Classiqswabs, Copan Italia SpA, Brescia, Italy), wetted with sterile deionized water, and inoculated into 0.9mL of R1 (70% ethanol), vortexed, and centrifuged at 12,000 x g for 2 minutes. The supernatant was discarded, and the pellet was suspended in 40µL of R2 (70% formic acid) and vortexed. Finally, 40µL of R3 (100% acetonitrile) were added and mixed, followed by centrifugation at 12,000 x g for 2 minutes. One microliter of the supernatant was transferred to the target slide, dried at room temperature, and covered with 1µL of VITEK MS-CHCA matrix solution. After single extraction, isolates were tested on the VITEK MS system (bioMérieux) in a double spot manner. If no identification was achieved, the extraction and identification process was repeated. The VITEK MS v3.2.0 database was used for the analysis. As stated by the manufacturer, confidence values between 60.0 and 99.9 indicated a reliable discrimination to species or species complex level. *Escherichia coli* ATCC 8739 was used as a calibrator and internal control for each acquisition group, as recommended by the manufacturer.

2.3. Classification of Reslts

MALDI-TOF results were compared to the identification by reference laboratories and classified into the categories ‘correct identification of species’, ‘correct identification of species complex’, ‘correct identification of genus’, ‘incorrect identification’ and ‘no identification’, as described previously [17]. Isolates from species/species complexes, which were within the database, but did not yield identification upon repeated testing, were classified as ‘no identification’. For species not included in the VITEK MS database, results were interpreted as ‘no identification ‘.

2.4. Practicality

Practicality of the VITEK MS Mould Kit was assessed by two independent users, who rated procedure complexity, hands-on time, and workflow integration of the identification process in our laboratory.

3. Results

The study included a total of 67 isolates of filamentous fungi; 33 derived from clinical samples and 34 from strain collections (including one ATCC strain). Strains belonged to 30 species and 5 species complexes within 15 genera; *Aspergillus* was the predominant genus. Of these 30 species, 20 (66.7%) were correctly identified by VITEK MS; 3 (10%) (*Aspergillus flavus*, *Rhizopus microspores*, and *Sporothrix globosa*) and 1 (*Coccidioides posadasii*) (3.3%) were correctly classified within the species complex and genus, respectively (Table 1). Six species were not identified, of which 4 (*Aspergillus tritici*, *Penicillium canescens*, *Rhizopus delemar*, and *Sporothrix chilensis*) were not included in the database; *Penicillium brevicompactum* and *Penicillium expansum* were not identified, albeit being part of the library database. All 5 species complexes were correctly diagnosed by VITEK MS (Table 1).

 doi:10.20944/preprints202408.0457.v1

Table 1. Identification 67 isolates belonging to 35 species/species complexes of filamentous fungi by VITEK MS system.

Reference identification	N	VITEK MS identification			
		Correct species	Correct species complex	Correct genus	No ID
Alternaria alternata	1	1	0	0	0
Aspergillus brasiliensis	1	1	0	0	0
Aspergillus calidoustus	2	2	0	0	0
Aspergillus flavus	2	0	2	0	0
Aspergillus fumigatus	12	12	0	0	0
Aspergillus nidulans	1	1	0	0	0
Aspergillus niger complex	6	0	6	0	0
Aspergillus sydowii	1	1	0	0	0
Aspergillus terreus complex	4	0	4	0	0
*Aspergillus tritici**	1	0	0	0	1
Aspergillus versicolor	1	1	0	0	0
Coccidioides posadasii	1	0	0	1	0
Curvularia spicifera	1	1	0	0	0
Epidermophyton floccosum	1	1	0	0	0
Fusarium oxysporum complex	2	0	2	0	0
Fusarium proliferatum	2	2	0	0	0
Fusarium solani complex	3	0	3	0	0
Lichtheimia corymbifera	2	2	0	0	0
Mucor velutinosus	1	1	0	0	0
Penicillium brevicompactum	1	0	0	0	1
*Penicillium canescens**	1	0	0	0	1
Penicillium chrysogenum	2	2	0	0	0
Penicillium expansum	1	0	0	0	1
Penicillium roqueforti	1	1	0	0	0
Pseudallescheria boydii	1	1	0	0	0
Purpureocillium lilacinum	1	1	0	0	0
Rhizopus arrhizus complex	1	0	1	0	0
*Rhizopus delemar**	1	0	0	0	1
Rhizopus microsporus	2	0	2	0	0
Sarocladium kiliense	1	1	0	0	0
Sarocladium strictum	1	1	0	0	0
*Sporothrix chilensis**	1	0	0	0	1

 doi:10.20944/preprints202408.0457.v1

Sporothrix globosa	1	0	1	0	0
Trichophyton rubrum	5	5	0	0	0
Trichophyton tonsurans	1	1	0	0	0
Total	**67**	**39**	**21**	**1**	**6**

ID, identification. *not within database.

Of the 67 isolates, 61 (91.0%) were correctly identified by VITEK MS; 39 (58.2%) to the species level, 21 (29.9%) to the species complex level, and 1 (1.5%) to the genus level (Table 2). Six strains (9.0%) were not recognized by VITEK MS. Overall, in 55 isolates (82.0%; 39 species and 16 species complex) VITEK MS result coincided with the identification of the reference laboratories (Table 2). No misidentifications were observed.

Table 2. Diagnostic performance of VITEK MS system in 67 isolates of filamentous fungi.

Reference standard	**VITEK MS**			
	Identification level			
	Correct species	Correct species complex	Correct genus	No ID
Species (n=51)	39 (76.5%)	5 (9.8%)	1 (2.0%)	6 (11.8%)
Species complex (n=16)	0	16 (100%)	0	0
Total (n=67)	39 (58.2%)	21 (29.9%)	1 (1.5%)	6 (9.0%)

ID, identification.

The performance of the VITEK MS system showed variations among the tested genera. The genus Aspergillus accounted for 31 (46.3%) of isolates, with Fumigati as the predominant section (n=12). All Aspergillus fumigatus isolates were identified to the species level by VITEK MS. Other clinically important, but less frequent members of the genus Aspergillus, such as Aspergillus flavus, Aspergillus niger, and Aspergillus terreus were identified to the species complex level (Table 1). Fusarium was the second most frequent genus comprised in our study. All isolates of the 3 included Fusarium species or species complexes were correctly identified. Of the 5 Penicillium species, 2 were correctly identified; the other 3 were not recognized by VITEK MS, although 2 were present in the database. Most members of the Mucorales (i.e., Lichtheimia corymbifera, Mucor velutinosus, Rhizopus arrhizus complex, and Rhizopus microsporus) were identified and only R. delemar, which was not included in the library, failed to be recognized.

Our laboratory personnel categorized the Mould Kit as easy-to-use, which permitted an easy workflow integration. Since all reagents were provided as ready-to-use components, hands-on time of the inactivation/extraction steps was short (30 to 60 minutes). The smear preparation was the same as for bacteria or yeasts, including the calibration step using Escherichia coli ATCC 8739 strain. All VITEK MS results were unambigous; all given identifications were provided with the maximum discrimination value (99.9%). In case of endemic fungi identification (Coccidioides posadasii), the system gave a biohazard warning, which helped to rapidly implement laboratory security measures.

4. Discussion

The present study examined a broad spectrum of filamentous fungi, including various clinically relevant species such as *Aspergillus* spp., *Fusarium* spp. and species of Mucorales and dematiaceous fungi. Timely and correct identification of such fungal isolates is of high clinical priority, allowing a prompt adaptation of antimycotic therapy. This goal is often not achievable using traditional methods, which often require weeks until the necessary growth of mature fungal colonies [6]. Besides, the shortage of well-trained and experienced mycologists further prolongs the time to diagnosis,

 doi:10.20944/preprints202408.0457.v1

since difficult-to-identify isolates must be sent to reference centers [4]. Under these aspects, the reduction of time to reliably identify mould species in a routine laboratory is potentially the major advantage of commercial MALDI-TOF platforms.

Since its implementation, VITEK MS has increased the diagnostic speed and accuracy of our laboratory, especially in cases of difficult-to-identify microorganisms [17,18]. The present study showed similar results for filamentous fungi, which were processed and identified after 48-72 hours of incubation, with the initial growth of mycelia. This is in accordance with previous studies, in which identification was achieved after 48 hours [7,8,10]. In early reports, the use of intact fungal cells hindered mass spectrometry processing due to the rigid cell walls of moulds [19]. Protocols that use an on-plate formic acid extraction method have shown insufficient results [12,20]. However, the development of specific extraction solutions, such as the here tested VITEK MS Mould Kit, aimed to overcome this limitation [19,21].

Another crucial factor for the identification of less frequent pathogens is the system´s database. Previous diagnostic studies of filamentous fungi using the former database version (v3.0) showed high rates of misidentifications [11,22,23] or identification only to the species complex level [24]. The v3.2.0 database utilized in our study exhibited a better performance and coincided with the reference identification in over 80% of isolates. *Fusarium* spp., for example, were correctly identified in the present study (n = 7), while in studies, using the older library version, only 65%-70% were accurately diagnosed [22,24]. Furthermore, we correctly detected cryptic species such as *Aspergillus sydowii* and *Aspergillus versicolor*, which pose a treatment challenge due to their azole resistance [25]. Of notice, we did not observe any misidentification, which is of clinical importance, avoiding incorrect or sub-optimal treatment, which is in accordance with previous data [12].

Aspergillus fumigatus, grouped in the *Fumigati* section, is the most prevalent cause of invasive mold infection [26]. This species was the most common in our study and correctly identified in all cases. Accurate diagnosis of this species has become crucial due to the emergence of azole-resistant cryptic species within this section, such as *Aspergillus lentulus, Aspergillus novofumigatus, Aspergillus fumigatiaffinis, Aspergillus thermomutatus* (*Neosartorya pseudofischeri*), and *Aspergillus viridinutans* [27]. These and other members of this section associated with invasive fungal diseases were not reliably identified by the earlier version (v3.0) of the platform [27]. Those species are now included in the new database version. However, our study did not include such cryptic *Fumigati* species. Isolates belonging to the *Aspergillus niger* and *Aspergillus terreus* complexes were correctly identified to the complex level, as with the older library (v3.0) [24]. The v3.2.0 database now also includes some cryptic *A. niger* complex species such as *A. tubingensis*. Our work included cryptic species of other sections (*Aspergillus calidoustus* and *Aspergillus sydowii*), which were correctly identified. *A. tritici* was not included in the database and, therefore, not identified.

The genus *Fusarium* is mainly described as a phytopathogenic fungi. However, *Fusarium oxysporum* complex and *Fusarium solani* complex are able to cause local (e.g., ocular) to life-threatening infections in immunocompromised patients [28]. *Fusarium solani* complex was most virulent in animal models; furthermore it exhibits amphotericin B, voriconazole, and posaconazole resistance [24,29]. The rapid and accurate identification of this complex is therefore clinically relevant. This and previous studies demonstrated that the newer VITEK MS system exhibits a better performance than other MALDI-TOF instruments [20].

Penicillium spp. are of low human pathogenicity. *Penicillium chrysogenum* is the main species isolated from dwellings and considered an important cause of allergic rhinitis and asthma [30]. Of the *Penicillium* species included in this study, the VITEK MS only identified *P. chrysogenum,* while *P. brevicompactum* (agent of hypersensitivity pneumonitis for wood workers) and *P. expansum* were not identified, although within the database. *Penicillium canescens* was not part of the database, so no identification was obtained. These limitations within the *Penicillium* genus have been described previously and might require further updates of the database [23].

The most common Mucorales involved in human disease are *Rhizopus, Mucor* and *Rhizomucor* [31]. An accurate identification of Mucorales is clinically relevant due to species-specific clinical presentations (e.g. *Rhizopus arrhizus* causing rhinocerebral invasion). VITEK MS correctly identified

 doi:10.20944/preprints202408.0457.v1

the included Mucorales to the species/species complex level [32]. However, only a low number of Mucorales strains were included, so further studies are warranted. This was also the case with dematiaceous fungi.

Coccidioides posadasii, a dimorphic fungus, was correctly identified as *C. immitis/posadasii* and signaled by the device as a biosafety risk. The capability of MALDI-TOF to identify this fungus permitted timely measures for the prevention and control of laboratory acquired infections, as reported previously [16]. This is an important add-on for laboratories in non-endemic countries, since clinical information leading to the suspicion of endemic mycosis, are often not available. Though dimorphic fungi are identifiable by VITEK MS and it is the only FDA approved database for their diagnosis,, the need for an inactivation step in a biosafety level 3 (BSL-3) facility and the requirement of a minimal amount of culture biomass for identification, will probably preclude its use in a routine BSL-2 facility [33].

Other advantages of fungal identification by MALDI-TOF include its technical simplicity, short time for sample preparation and data analysis, and economical savings in laboratory costs [4,34]. Until recently, sequencing was the only alternative for unidentifiable mould strains, resulting in much higher expenses and time losses compared to MALDI-TOF [35]. A major drawback to the implementation of the VITEK MS Mould Kit is the large package size (100 tests per box) together with a short expiry period after opening (4 weeks).

Main limitations of our study were the limited sample size and the incomplete spectrum of species, especially among cryptic *Aspergillus* species, Mucorales, dematiaceous molds, and dermatophytes.

5. Conclusions

The study demonstrated the capability of the VITEK MS system to successfully identify a broad spectrum of filamentous fungi in a routine microbiological laboratory. Overall, the system diagnosed over 90% of the isolates without misidentifications.

Author Contributions: Conceptualization, L.P. and T.W; methodology, L.P., T.W. and R.C.; validation, T.W., L.P., C.D. and P.L.; formal analysis, T.W, L.P., R.C., C.D., I.P., P.G., P.L., F.V. and T.W; investigation, C.V. and I.P.; resources, C.V., R.C., C.D., P.G. and F.V.; data curation, I.P., R.C. and L.P.; writing—original draft preparation, L.P., R.C. and I.P.; writing—review and editing, L.P., R.C., I.P., C.V., P.G., P.L., F.V. and T.W; supervision, L.P., R.C., P.G., F.V. and T.W.; project administration, L.P. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: The original contributions presented in the study are included in the article/supplementary material; further inquiries can be directed to the corresponding author.

Acknowledgements: Parts of the work were presented at the XXXV Congreso Chileno de Infectología (2018) in Coquimbo, Chile (poster N° 65) and IDWeek 2018 in San Francisco, USA (poster N° 48).

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Wiederhold, N. Antifungal resistance: Current trends and future strategies to combat. *Infect Drug Resist* **2017**, *Volume 10*, 249-259. https://doi.org/10.2147/idr.s124918.
2. Kohler, J.R.; Casadevall, A.; Perfect, J. The Spectrum of Fungi That Infects Humans. *Cold Spring Harb Perspect Med* **2015**, *5*, a019273-a019273. https://doi.org/10.1101/cshperspect.a019273.
3. Friedman, D.Z.P.; Schwartz, I.S. Emerging Fungal Infections: New Patients, New Patterns, and New Pathogens. *J Fungi* **2019**, *5*, 67. https://doi.org/10.3390/jof5030067.
4. Wickes, B.L.; Romanelli, A.M. Diagnostic Mycology: Xtreme Challenges. *J Clin Microbiol* **2020**, *58*. https://doi.org/10.1128/jcm.01345-19.
5. Bourbeau, P.P.; Ledeboer, N.A. Automation in Clinical Microbiology. *J Clin Microbiol* **2013**, *51*, 1658-1665. https://doi.org/10.1128/jcm.00301-13.

6. Iriart, X.; Lavergne, R.-A.; Fillaux, J.; Valentin, A.; Magnaval, J.-F.; Berry, A.; Cassaing, S. Routine Identification of Medical Fungi by the New Vitek MS Matrix-Assisted Laser Desorption Ionization–Time of Flight System with a New Time-Effective Strategy. *J Clin Microbiol* **2012**, *50*, 2107-2110. https://doi.org/10.1128/jcm.06713-11.
7. Gautier, M.; Ranque, S.; Normand, A.C.; Becker, P.; Packeu, A.; Cassagne, C.; L'Ollivier, C.; Hendrickx, M.; Piarroux, R. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry: Revolutionizing clinical laboratory diagnosis of mould infections. *Clin Microbiol Infect* **2014**, *20*, 1366-1371. https://doi.org/10.1111/1469-0691.12750.
8. De Carolis, E.; Posteraro, B.; Lass-Flörl, C.; Vella, A.; Florio, A.R.; Torelli, R.; Girmenia, C.; Colozza, C.; Tortorano, A.M.; Sanguinetti, M.; et al. Species identification of Aspergillus, Fusarium and Mucorales with direct surface analysis by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. *Clin Microbiol Infect* **2012**, *18*, 475-484. https://doi.org/10.1111/j.1469-0691.2011.03599.x.
9. Zvezdanova, M.E.; Escribano, P.; Ruiz, A.; Martínez-Jiménez, M.C.; Peláez, T.; Collazos, A.; Guinea, J.; Bouza, E.; Rodríguez-Sánchez, B. Increased species-assignment of filamentous fungi using MALDI-TOF MS coupled with a simplified sample processing and an in-house library. *Med Mycol* **2019**, *57*, 63-70. https://doi.org/10.1093/mmy/myx154.
10. Patel, R. A Moldy Application of MALDI: MALDI-ToF Mass Spectrometry for Fungal Identification. *J Fungi* **2019**, *5*, 4. https://doi.org/10.3390/jof5010004.
11. Rychert, J.; Slechta, E.S.; Barker, A.P.; Miranda, E.; Babady, N.E.; Tang, Y.-W.; Gibas, C.; Wiederhold, N.; Sutton, D.; Hanson, K.E. Multicenter Evaluation of the Vitek MS v3.0 System for the Identification of Filamentous Fungi. *J Clin Microbiol* **2018**, *56*. https://doi.org/10.1128/jcm.01353-17.
12. Choi, Y.; Kim, D.; Choe, K.W.; Lee, H.; Kim, J.-S.; Ahn, J.-Y.; Lee, M.-K. Performance Evaluation of Bruker Biotyper, ASTA MicroIDSys, and VITEK-MS and Three Extraction Methods for Filamentous Fungal Identification in Clinical Laboratories. *J Clin Microbiol* **2022**, *60*. https://doi.org/10.1128/jcm.00812-22.
13. de Hoog GS, G.J., Gené J, Ahmed SA, Al-Hatmi AMS, Figueras MJ, Vitale RG. *Atlas of clinical fungi*, 4th ed.; Westerdijk Institute, Utrecht, the Netherlands, 2019.
14. Mycobank.org. Fungal databases, nomenclature & species banks. Available on line: Mycobank.org (accessed on 1 december 2023).
15. Piontelli Laforet, E. Manual de microhongos filamentosos comunes. I, 3ra ed.; Viña del Mar, Chile, 2017.
16. Porte L, V.F., Wilmes D, Gaete P, Díaz MC, Thompson L, Munita JM, Alliende R, Varela C, Rickerts V, Weitzel T. Laboratory exposure to Coccidioides: Lessons learnt in a non-endemic country. *J Hosp Infect* **2019**, *102*, 461-464. https://doi.org/10.1016/j.jhin.2019.03.006.
17. Porte, L.; García, P.; Braun, S.; Ulloa, M.T.; Lafourcade, M.; Montaña, A.; Miranda, C.; Acosta-Jamett, G.; Weitzel, T. Head-to-head comparison of Microflex LT and Vitek MS systems for routine identification of microorganisms by MALDI-TOF mass spectrometry in Chile. *PLoS ONE* **2017**, *12*, e0177929. https://doi.org/10.1371/journal.pone.0177929.
18. Salvador, F.; Porte, L.; Durán, L.; Marcotti, A.; Pérez, J.; Thompson, L.; Noriega, L.M.; Lois, V.; Weitzel, T. Breakthrough bacteremia due to Clostridium tertium in a patient with neutropenic fever, and identification by MALDI-TOF mass spectrometry. *Int J Infect Dis* **2013**, *17*, e1062-e1063. https://doi.org/10.1016/j.ijid.2013.03.005.
19. L'Ollivier C, R.S. MALDI-TOF-Based Dermatophyte Identification. *Mycopathologia* **2017**, *182(1-2)*, 183-192. https://doi.org/10.1007/s11046-016-0080-x.
20. Sun Y, G.J., Chen R, Hu L, Xia Q, Wu W, Wang J, Hu F. Multicenter evaluation of three different MALDI-TOF MS systems for identification of clinically relevant filamentous fungi. *Med Mycol* **2021**, *59*, 81-86. https://doi.org/10.1093/mmy/myaa037.
21. Wilkendorf, L.S.; Bowles, E.; Buil, J.B.; Van Der Lee, H.A.L.; Posteraro, B.; Sanguinetti, M.; Verweij, P.E. Update on Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry Identification of Filamentous Fungi. *J Clin Microbiol* **2020**, *58*. https://doi.org/10.1128/jcm.01263-20.
22. McMullen, A.R.; Wallace, M.A.; Pincus, D.H.; Wilkey, K.; Burnham, C.-A.D. Evaluation of the Vitek MS Matrix-Assisted Laser Desorption Ionization–Time of Flight Mass Spectrometry System for Identification of Clinically Relevant Filamentous Fungi. *J Clin Microbiol* **2016**, *54*, 2068-2073. https://doi.org/10.1128/jcm.00825-16.
23. Shin, J.H.; Kim, S.H.; Lee, D.; Lee, S.Y.; Chun, S.; Lee, J.H.; Won, E.J.; Choi, H.J.; Choi, H.W.; Kee, S.J.; et al. Performance Evaluation of VITEK MS for the Identification of a Wide Spectrum of Clinically Relevant Filamentous Fungi Using a Korean Collection. *Ann Lab Med* **2021**, *41*, 214-220. https://doi.org/10.3343/alm.2021.41.2.214.
24. Pinheiro D, M.C., Faria MA, Pinto E. Vitek® MS v3.0 System in the Identification of Filamentous Fungi. *Mycopathologia* **2019**, *184*, 645-651. https://doi.org/10.1007/s11046-019-00377-0.
25. Van Der Linden, J.W.M.; Warris, A.; Verweij, P.E. Aspergillusspecies intrinsically resistant to antifungal agents. *Med Mycol* **2011**, *49*, S82-S89. https://doi.org/10.3109/13693786.2010.499916.

26. Atalay A, K.A., Suel A, Sav H, Demir G, Elmali F, Cakir N, Seyedmousavi S. Conventional Morphology Versus PCR Sequencing, rep-PCR, and MALDI-TOF-MS for Identification of Clinical Aspergillus Isolates Collected Over a 2-Year Period in a University Hospital at Kayseri, Turkey. *J Clin Lab Anal* **2016**, *30*, 745-750. https://doi.org/10.1002/jcla.21932.
27. M Américo F, P.M.S.L., B Del Negro GM, M Favero Gimenes V, S Trindade MR, L Motta A, Santos de Freitas R, Rossi F, L Colombo A, Benard G, N de Almeida Júnior J. Evaluating VITEK MS for the identification of clinically relevant Aspergillus species. *Med Mycol* **2020**, *58*, 322-327. https://doi.org/10.1093/mmy/myz066.
28. Hof, H. The Medical Relevance of Fusarium spp. *J Fungi* **2020**, *6*, 117. https://doi.org/10.3390/jof6030117.
29. Menu, E.; Filori, Q.; Dufour, J.-C.; Ranque, S.; L'Ollivier, C. A Repertoire of Clinical Non-Dermatophytes Moulds. *J Fungi* **2023**, *9*, 433. https://doi.org/10.3390/jof9040433.
30. Reboux G, R.S., Vacheyrou M, Millon L. Identifying indoor air Penicillium species: A challenge for allergic patients. *J Med Microbiol* **2019**, *68*, 812-821. https://doi.org/10.1099/jmm.0.000960.
31. Julie M. Steinbrink, M.H.M. Clinical Review of Mucormycosis. *Infect Dis Clin North Am* **2021**, *35*, 435-452. https://doi.org/10.1016/j.idc.2021.03.009.
32. Dannaoui, E. Antifungal resistance in mucorales. *Int J Antimicrob Agents* **2017**, *50*, 617-621. https://doi.org/10.1016/j.ijantimicag.2017.08.010.
33. Dingle, T.; Dufresne, P. Discontinuation of DNA Probes for Identification of Dimorphic Fungi Growing in Culture: What's a Lab To Do? *Clin Microbiol Newsl* **2022**, *44*, 191-197. https://doi.org/10.1016/j.clinmicnews.2022.11.001.
34. Sarvestani HK, R.A., Getso MI, Razavyoon T, Javidnia J, Golrizi MB, Saboor-Yaraghi AA, Ansari S. Mass spectrometry in research laboratories and clinical diagnostic: A new era in medical mycology. *Braz J Microbiol* **2022**, *53*, 689-707. https://doi.org/10.1007/s42770-022-00715-3.
35. Rychert, J. Benefits and Limitations of MALDI-TOF Mass Spectrometry for the Identification of Microorganisms. *J Infectiology* **2019**, *2*, 1-5.