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Performance Evaluation of VITEK MS for the Identification of a Wide Spectrum of Clinically Relevant Filamentous Fungi Using a Korean Collection

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The correct identification of filamentous fungi is challenging. We evaluated the performance of the VITEK MS v3.0 system (bioMérieux, Marcy-l'Étoile, France) for the identification of a wide spectrum of clinically relevant filamentous fungi using a Korean collection. Strains that were added to the upgraded v3.2 database were additionally identified by the VITEK MS v3.2 system. Of the 105 tested isolates, including 37 *Aspergillus* (nine species), 41 dermatophytes (seven species), and 27 other molds (17 species), 43 (41.0%) showed "no identification" or "multiple species identification" results at the initial VITEK MS testing; these isolates were retested using the same method. Compared with sequence-based identification, the correct identification rate using VITEK MS for *Aspergillus*, dermatophytes, other molds, and total mold isolates was 67.6%, 56.1%, 48.1%, and 58.1% at the initial testing and 94.6%, 78.0%, 55.6%, and 78.1% with retesting, respectively. Following retesting, 19 (18.1%) and two (1.9%) isolates showed "no identification" and "misidentification" results, respectively. VITEK MS reliably identified various filamentous fungi recovered in Korea, with a very low rate of misidentification.

Key Words: Filamentous fungi, Mass spectrometry, VITEK MS, *Aspergillus*, Dermatophytes, Evaluation, Performance, Identification

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Fungi have increasingly been shown to cause various serious infections owing to the growing number of immunocompromised patients receiving chemotherapy, immunosuppressive agents, or medical intervention [1-3]. *Candida* species are the most common invasive fungal infection-causing pathogens; however, filamentous fungi, such as *Aspergillus* species, also increasingly cause severe fungal infections with fatal outcomes [1-3]. Although immediate and accurate identification of the pathogen is critical for the treatment and management of fungal infections, conventional morphological examination has some limitations such as difficult differentiation of less common species, relatively complex identification training, and new emerging pathogens [4, 5]. Molecular identification is used as the reference method for fungal identification; however, it requires expertise in interpretation, thus hindering its routine use in clinical laboratories [4]. In contrast, the recently introduced matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS) method is less labor intensive and can provide rapid identification results [4, 5].

MALDI-TOF MS VITEK MS (bioMérieux, Marcy-l'Étoile, France) recently introduced an update of its knowledge base version 3.0 (v3.0) database, version 3.2 (v3.2). To date, only

three studies have evaluated the performance of VITEK MS v3.0 system for the identification of filamentous fungi, showing that it may be influenced by the examined species distribution [6-8]. Furthermore, in contrast to the study by Rychert, *et al*. [7], few dermatophytes were evaluated in the other two studies [6, 8]. In addition, the performance may vary depending on the instrument and database [9, 10]. This study evaluated the performance of VITEK MS v3.0 system to identify 105 clinical filamentous fungi isolates using a Korean collection, representing 33 species from 14 genera, including various dermatophyte isolates. Owing to a database upgrade, strains that were added to the v3.2 database were additionally identified by the VITEK MS v3.2 system. This study was conducted with approval of the Institutional Review Board of Chonnam National University Hwasun Hospital, Hwasun, Korea (IRB CNUHH-2017-098).

All 105 filamentous fungi isolates were obtained from 12 Korean hospitals from 2016 to 2019, and duplicate isolates were excluded. Isolates were recovered from skin/tissue (N=48), wound/pus (N=22), respiratory specimens (N=21), body fluids (N=4), and other non-sterile specimens (N=10). After sequencing the internal transcribed spacer or D1/D2 region of the 28S ribosomal DNA with additive sequencing of β-tubulin or calmodulin genes for *Aspergillus* species [10, 11], 37, 41, and 27 isolates were molecularly identified as *Aspergillus* (nine species), dermatophytes (seven species), and other molds (17 species), respectively. The isolates were cultured on potato dextrose agar (PDA) or Sabouraud dextrose agar (SDA) and incubated for 2-23 days to acquire colonies at least 1 cm in diameter. Further, the isolates were prepared using the VITEK MS MOULD KIT (bioMérieux) and tested using the VITEK MS v3.0 system according to the manufacturer's protocol. Additionally, only the species included in the v3.2 database were identified using the VITEK MS v3.2 system which was installed during the revision of this study. All isolates showing "no identification" or "multiple species identification" (two or more species were proposed) results at the initial VITEK MS testing were subcultured onto the initial culture media except two isolates (*Aspergillus fumigatus* and *Trichophyton interdigitale*), which were cultured on PDA for the initial testing and on SDA for the retesting. All isolates were retested following the same method using the VITEK MS MOULD KIT. The final additive testing results included the retesting results of the isolates initially showing "no identification" or "multiple species identification," as well as the initial results of the other isolates showing acceptable identifications other than "no identification" or "multiple species identification."

The VITEK MS results were compared with the sequence-based identification results and assigned to one of the four categories: (i) correct identification (identical to sequence-based identification), (ii) incomplete identification (either only the genus level was correctly identified or two or more species were proposed and one was correct), (iii) misidentification (none of the proposed species were correct), or (iv) no identification. As VITEK MS only displays species-complex-level identifications for some species, these were considered as the correct identification. McNemar's, chi-square, and Fisher's exact tests were performed to compare the correct identification rates. IBM SPSS Statistics for Windows version 25.0 (IBM Corp., Armonk, NY, USA) was used, and $P<0.05$ was considered statistically significant.

Table 1 shows the results of 105 clinical filamentous fungi isolates identified using VITEK MS. At the initial testing, VITEK MS correctly identified 67.6% of *Aspergillus*, 56.1% of dermatophytes, 48.1% of other molds, and 58.1% of the total mold isolates. Of the 105 isolates, 43 (41.0%) isolates had "no identification" (41 isolates) or "multiple species identification" (two isolates) results. These 43 isolates were retested using the same method; the correct identification rates for *Aspergillus*, dermatophytes, other molds, and total mold isolates were 94.6%, 78.0%, 55.6%, and 78.1%, respectively, yielding a statistically significant increase for Aspergillus, dermatophytes, and total mold isolates compared with the initial testing ($P<0.05$). Two isolates (*Trichophyton verrucosum* and *Alternaria astragali*) showed "incomplete identification" (genus-level identification), and only two dermatophytes (*Trichophyton rubrum* and *Microsporum gypseum*) showed "misidentification." The "no identification" rate was 5.4%, 14.6%, 40.7%, and 18.1% for *Aspergillus*, dermatophytes, other molds, and total mold isolates, respectively.

Of the 33 species tested in this study, *Aspergillus tubingensis*, *Aspergillus westerdijkiae*, and *Cladosporium sphaerospermum* were not included in the v3.0 database, but were included in the v3.2 database, whereas *Alternaria astragali* and *Cunninghamella bertholletiae* were not included in either database. All these isolates were not correctly identified using the VITEK MS v3.0 system; however, *A. tubingensis* and *A. westerdijkiae* were correctly identified using the VITEK MS v3.2 system. Nevertheless, the database needs continuous update and inclusion of additional species because it represents only a minor fraction of the filamentous fungi [12].

According to three recent studies on the performance evaluation of VITEK MS v3.0 system for identification of filamentous fungi, the correct identification rate varied, ranging from 51.0% to 91.3%, most likely owing to the different composition of the

Table 1. Clinical filamentous fungi isolates identified using the VITEK MS v3.0 system in comparison with sequence-based identification

Sequence-based identification (N of isolates)		N (%) of isolates at initial testing				N (%) of isolates at additive testing†			
		Correct ID	Incomplete ID	Mis-ID	No ID	Correct ID	Incomplete ID	Mis-ID	No ID
Aspergillus species (37)									
	Aspergillus flavus/oryzae (9)	7 (77.8)	0	0	2 (22.2)†	9 (100)	0	0	0
	Aspergillus fumigatus (8)	6 (75.0)	0	0	2 (25.0)†	7 (87.5)	0	0	1 (12.5)
	Aspergillus niger (6)	3 (50.0)	1 (16.7)†	0	2 (33.3)†	5 (83.3)	0	0	1 (16.7)
	Aspergillus terreus (4)	4 (100)	0	0	0	4 (100)	0	0	0
	Aspergillus sydowii (3)	2 (66.7)	0	0	1 (33.3)†	3 (100)	0	0	0
	Aspergillus tubingensis (3)*	1 (33.3)	0	0	2 (66.7)†	3 (100)	0	0	0
	Aspergillus nidulans (2)	1 (50.0)	0	0	1 (50.0)†	2 (100)	0	0	0
	Aspergillus lentulus (1)	0	0	0	1 (100)†	1 (100)	0	0	0
	Aspergillus westerdijkiae (1)*	1 (100)	0	0	0	1 (100)	0	0	0
	Subtotal (37)	25 (67.6)	1 (2.7)	0	11 (29.7)	35 (94.6)	0	0	2 (5.4)
Dermatophytes (41)									
	Trichophyton rubrum (12)	8 (66.7)	1 (8.3)†	0	3 (25.0)†	10 (83.3)	0	1 (8.3)	1 (8.3)
	Trichophyton interdigitale (11)	7 (63.6)	0	0	4 (36.4)†	9 (81.8)	0	0	2 (18.2)
	Trichophyton tonsurans (3)	0	0	0	3 (100)†	3 (100)	0	0	0
	Trichophyton verrucosum (3)	1 (33.3)	0	0	2 (66.7)†	1 (33.3)	1 (33.3)	0	1 (33.3)
	Microsporum canis (5)	4 (80.0)	0	0	1 (20.0)†	5 (100)	0	0	0
	Microsporum gypseum (4)	1 (25.0)	0	1 (25.0)‡	2 (50.0)†	1 (25.0)	0	1 (25.0)‡	2 (50.0)
	Epidermophyton floccosum (3)	2 (66.7)	0	0	1 (33.3)†	3 (100)	0	0	0
	Subtotal (41)	23 (56.1)	1 (2.4)	1 (2.4)	16 (39.0)	32 (78.0)	1 (2.4)	2 (4.9)	6 (14.6)
Other molds (27)									
	Penicillium citrinum (5)	1 (20.0)	0	0	4 (80.0)†	1 (20.0)	0	0	4 (80.0)
	Penicillium camemberti (1)	0	0	0	1 (100)†	0	0	0	1 (100)
	Penicillium chrysogenum (1)	1 (100)	0	0	0	1 (100)	0	0	0
	Penicillium expansum (1)	0	0	0	1 (100)†	0	0	0	1 (100)
	Fusarium solani (4)	3 (75.0)	0	0	1 (25.0)†	4 (100)	0	0	0
	Fusarium proliferatum (1)	1 (100)	0	0	0	1 (100)	0	0	0
	Alternaria alternata (3)	3 (100)	0	0	0	3 (100)	0	0	0
	Alternaria astragali (1)*	0	0	0	1 (100)†	0	1 (100)	0	0
	Scedosporium apiospermum (2)	1 (50.0)	0	0	1 (50.0)†	2 (100)	0	0	0
	Scedosporium boydii (1)	0	0	0	1 (100)†	0	0	0	1 (100)
	Cladosporium cladosporioides (1)	0	0	0	1 (100)†	0	0	0	1 (100)
	Cladosporium sphaerospermum (1)*	0	0	0	1 (100)†	0	0	0	1 (100)
	Acremonium sclerotigenum (1)	1 (100)	0	0	0	1 (100)	0	0	0
	Cunninghamella bertholletiae (1)*	0	0	0	1 (100)†	0	0	0	1 (100)
	Lichtheimia corymbifera (1)	0	0	0	1 (100)†	0	0	0	1 (100)
	Paecilomyces variotii (1)	1 (100)	0	0	0	1 (100)	0	0	0
	Purpureocillium lilacinum (1)	1 (100)	0	0	0	1 (100)	0	0	0

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Table 1. Continued

Sequence-based identification (N of isolates)		N (%) of isolates at initial testing				N (%) of isolates at additive testing†			
		Correct ID	Incomplete ID	Mis-ID	No ID	Correct ID	Incomplete ID	Mis-ID	No ID
	Subtotal								
	Only database (25)	13 (52.0)	0	0	12 (48.0)	15 (60.0)	0	0	10 (40.0)
	All species (27)	13 (48.1)	0	0	14 (51.9)	15 (55.6)	1 (3.7)	0	11 (40.7)
Total molds (105)									
	Only database (103)	61 (59.2)	2 (1.9)	1 (1.0)	39 (37.9)	82 (79.6)	1 (1.0)	2 (1.9)	18 (17.5)
	All species (105)	61 (58.1)	2 (1.9)	1 (1.0)	41 (39.0)	82 (78.1)	2 (1.9)	2 (1.9)	19 (18.1)

**Aspergillus tubingensis*, *A. westerdijkiae*, and *Cladosporium sphaerospermum* were not included in the v3.0 database but were included in the v3.2 database, whereas *Alternaria astragali* and *Cunninghamella bertholletiae* were not included in either database. *A. tubingensis*, *A. westerdijkiae*, and *C. sphaerospermum* were identified using the VITEK MS v3.2 system; †Isolates with an initial testing result of "no identification" or "multiple species identification" were retested following the same VITEK MS method. The additive testing results included the retested results, as well as the initial results of all acceptable identifications; ‡One isolate of *Microsporum gypseum* was misidentified as *Epidermophyton floccosum*.
Abbreviations: ID, identification; Mis-ID, misidentification.

tested isolates in each study [6-8]. In the present study, for all 105 filamentous fungi isolates representing commonly isolated species from Korean hospitals, the correct identification rate was 58.1% at the initial testing and 78.1% with retesting using VITEK MS v3.0 and v3.2 systems. The correct identification rate of dermatophytes was 78.0%, like the previous finding (84.5%) [7]. In line with previous studies [7, 8], retesting filamentous fungi isolates improved the correct identification rate, indicating the necessity for retesting. The reasons for the improvement following retesting are poorly understood; however, they may be attributed to the characteristics of the filamentous fungi. In contrast to bacteria, it can be difficult to obtain uniform conidia for testing from filamentous fungi colonies on solid media, depending on culture conditions. However, given the fact that misidentification rate was only 1.9%, filamentous fungi isolates that remain unidentified after repeated VITEK MS testing can be further evaluated by sequence analysis or other morphological evaluation without the risk of misidentification.

The detailed VITEK MS retesting results for the 43 isolates, including 12 *Aspergillus*, 17 dermatophyte, and 14 other molds, are shown in Table 2. Of the 43 isolates, 10 (83.3%) *Aspergillus*, nine (52.9%) dermatophyte, and two (14.3%) other molds were correctly identified. Other molds, including *Penicillium*, *Cladosporium*, *Cunninghamella*, and *Lichtheimia* species, were not identified despite retesting. The correct identification rate after retesting was significantly higher for isolates cultured on SDA ($P=0.012$) but were similar irrespective of increased or decreased incubation time. This difference might be due to species selection bias, as other mold isolates were mostly cultured on PDA.

VITEK MS correctly identified commonly isolated *Aspergillus* species, as well as some clinically relevant species showing antifungal resistance such as *Aspergillus terreus* and *Aspergillus lentulus* [13]. In the case of *Fusarium* species, which are multiresistant organisms and the second most common filamentous fungi causing invasive fungal infections in immunocompromised patients [14], VITEK MS correctly identified all five *Fusarium* isolates, showing a higher rate of correct identification than that in previous studies (93.0% and 65.4%) [7, 8].

T. rubrum is the most frequently isolated dermatophyte in Korea [15]. Rychert, *et al*. [7] demonstrated that dermatophytes other than *T. rubrum* are not always correctly identified at the species level using the VITEK MS v3.0 system. In the present study, the correct identification rate with retesting for *T. rubrum* was 83.3%, while that for dermatophytes other than *T. rubrum* was 75.9%. Furthermore, the correct identification rate for other molds was significantly lower than that for *Aspergillus* and dermatophytes ($P<0.05$). However, the correct identification rate for other molds increased from 55.6% to 76.5%, excluding clinically insignificant species such as *Penicillium* and *Cladosporium* species, which are often regarded as contaminants [16, 17]. VITEK MS correctly identified all *Alternaria alternata*, *Acremonium sclerotigenum*, *Paecilomyces variotii*, and *Purpureocillium lilacinum* isolates. VITEK MS seems to provide a correct identification for most clinically relevant filamentous fungi.

This study represents the first performance evaluation of the VITEK MS v3.0 system for the identification of clinically relevant filamentous fungi using the Korean collection, some of which were supplemented by the VITEK MS v3.2 system. VITEK MS provided 94.6% and 78.0% correct identification rates for *Aspergillus* and dermatophytes, respectively, which were commonly recovered in Korea, with only 1.9% rate of misidentification. In addition, it could differentiate clinically critical species

Table 2. Clinical filamentous fungi isolates that were retested using the VITEK MS v3.0 system

Sequence-based identification (N of isolates)		Initial testing		Retesting†		
		Culture medium	Incubation time (days)	ID results	Culture medium	Incubation time (days)
Aspergillus species (12)						
	Aspergillus flavus/oryzae	SDA	5	*Aspergillus flavus*	SDA	6
	Aspergillus flavus/oryzae	SDA	7	*Aspergillus flavus*	SDA	3
	Aspergillus fumigatus	PDA	13	*Aspergillus fumigatus*	SDA	3
	Aspergillus fumigatus	PDA	2	No ID	PDA	4
	Aspergillus niger‡	SDA	14	*Aspergillus niger complex*	SDA	4
	Aspergillus niger	SDA	5	*Aspergillus niger complex*	SDA	2
	Aspergillus niger	PDA	2	No ID	PDA	4
	Aspergillus sydowii	PDA	7	*Aspergillus sydowii*	PDA	18
	*Aspergillus tubingensis**	PDA	5	*Aspergillus niger complex*	PDA	5
	*Aspergillus tubingensis**	PDA	7	*Aspergillus niger complex*	PDA	5
	Aspergillus nidulans	PDA	3	*Aspergillus nidulans*	PDA	4
	Aspergillus lentulus	PDA	8	*Aspergillus lentulus*	PDA	6
Dermatophytes (17)						
	Trichophyton rubrum‡	SDA	9	No ID	SDA	13
	Trichophyton rubrum	PDA	13	*Trichophyton rubrum*	PDA	17
	Trichophyton rubrum	PDA	16	*Trichophyton rubrum*	PDA	12
	Trichophyton rubrum	SDA	9	*Fusarium proliferatum*	SDA	13
	Trichophyton interdigitale	PDA	10	*Trichophyton interdigitale*	PDA	6
	Trichophyton interdigitale	PDA	20	*Trichophyton interdigitale*	SDA	12
	Trichophyton interdigitale	PDA	16	No ID	PDA	12
	Trichophyton interdigitale	PDA	17	No ID	PDA	12
	Trichophyton tonsurans	SDA	9	*Trichophyton tonsurans*	SDA	13
	Trichophyton tonsurans	SDA	9	*Trichophyton tonsurans*	SDA	13
	Trichophyton tonsurans	SDA	9	*Trichophyton tonsurans*	SDA	20
	Trichophyton verrucosum	PDA	14	*Trichophyton interdigitale*	PDA	8
	Trichophyton verrucosum	PDA	14	No ID	PDA	12
	Microsporum canis	SDA	9	*Microsporum canis*	SDA	23
	Microsporum gypseum	SDA	9	No ID	SDA	13
	Microsporum gypseum	SDA	9	No ID	SDA	13
	Epidermophyton floccosum	SDA	13	*Epidermophyton floccosum*	SDA	14
Other molds (14)						
	Penicillium citrinum	PDA	10	No ID	PDA	8
	Penicillium citrinum	PDA	10	No ID	PDA	8
	Penicillium citrinum	PDA	10	No ID	PDA	8
	Penicillium citrinum	PDA	13	No ID	PDA	8
	Penicillium camemberti	PDA	3	No ID	PDA	14
	Penicillium expansum	PDA	14	No ID	PDA	14
	Fusarium solani	SDA	5	*Fusarium solani complex*	SDA	6

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Table 2. Continued

Sequence-based identification (N of isolates)		Initial testing		Retesting†		
		Culture medium	Incubation time (days)	ID results	Culture medium	Incubation time (days)
	*Alternaria astragali**	PDA	4	*Alternaria alternata*	PDA	4
	Scedosporium apiospermum	SDA	5	*Scedosporium apiospermum*	SDA	6
	Scedosporium boydii	PDA	10	No ID	PDA	8
	Cladosporium cladosporioides	PDA	7	No ID	PDA	14
	*Cladosporium sphaerospermum**	PDA	16	No ID	PDA	5
	*Cunninghamella bertholletiae**	PDA	2	No ID	PDA	4
	Lichtheimia corymbifera	PDA	6	No ID	PDA	4
Total (43)						

**Aspergillus tubingensis* and *Cladosporium sphaerospermum* were not included in the v3.0 database but were included in the v3.2 database, whereas *Alternaria astragali* and *Cunninghamella bertholletiae* were not included in either database. *A. tubingensis* and *C. sphaerospermum* were identified using the VITEK MS v3.2 system; †Isolates with an initial testing result of "no identification" or "multiple species identification" were retested following the same VITEK MS method; ‡All except these two isolates showed "no identification" results at the initial testing. The Aspergillus niger isolate showed "multiple species identification" result as *Candida haemulonii*, *Aspergillus niger complex*, *Candida rugosa*, and *Candida parapsilosis*. The *Trichophyton rubrum* isolate showed "multiple species identification" result as *Trichophyton rubrum* and *Trichophyton violaceum*.
Abbreviations: ID, identification; PDA, potato dextrose agar; SDA, Sabouraud dextrose agar.

exhibiting antifungal resistance such as *A. terreus*, *A. lentulus*, and *Fusarium solani*. Although VITEK MS has some limitations, such as its narrow-spectrum database and limited identification of rarely isolated species, it can help overcome the disadvantages of conventional methods, especially for *Aspergillus* species and dermatophytes.

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AUTHOR CONTRIBUTIONS

JHS[1] and DL performed the experiments. SHK supervised the experiments. SYL, SC, JHL, EJW, HJC, HWC, SJK, SHK, MGS, and JHS[2] provided advice regarding interpretation. JHS[1] and SHK wrote the manuscript. SHK was responsible for conceiving this study and provided critical contributions to this manuscript. All authors provided critical feedback and approved the final manuscript.

CONFLICTS OF INTEREST

No potential conflicts of interest relevant to this paper were reported

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