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Comparison of the Bruker Biotyper and VITEK MS Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry Systems Using a Formic Acid Extraction Method to Identify Common and Uncommon Yeast Isolates

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Background: Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) allows rapid and accurate identification of clinical yeast isolates. In-tube formic acid/acetonitrile (FA/ACN) extraction is recommended prior to the analysis with MALDI Biotyper, but the direct on-plate FA extraction is simpler. We compared the Biotyper with the VITEK MS for the identification of various clinically relevant yeast species, focusing on the use of the FA extraction method.

Methods: We analyzed 309 clinical isolates of 42 yeast species (four common *Candida* species, *Cryptococcus neoformans*, and 37 uncommon yeast species) using the Biotyper and VITEK MS systems. FA extraction was used initially for all isolates. If 'no identification' result was obtained following the initial FA extraction, these samples were then retested by using FA (both systems, additive FA) or FA/ACN (Biotyper only, additive FA/ACN) extraction. These results were compared with those obtained by sequence-based identification.

Results: Both systems correctly identified all 158 isolates of the four common *Candida* species after the initial FA extraction. The Biotyper correctly identified 8.7%, 30.4%, and 100% of 23 *C. neoformans* isolates after performing initial FA, additive FA, and FA/ACN extractions, respectively, while VITEK MS identified all *C. neoformans* isolates after the initial FA extraction. Both systems had comparable identification rates of 37 uncommon yeast species (128 isolates), following the initial FA (Biotyper, 74.2%; VITEK MS, 73.4%) or additive FA (Biotyper, 82.0%; VITEK MS, 73.4%).

Conclusions: The identification rate of most common and uncommon yeast isolates is comparable between simple FA extraction/Biotyper method and VITEK MS methods, but FA/ACN extraction is necessary for *C. neoformans* identification by Biotyper.

Key Words: Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS), VITEK MS, Biotyper, Yeast, Candida, Formic acid extraction

INTRODUCTION

The incidence of invasive fungal infections caused by yeast pathogens has increased significantly over the last two decades [1-4]. The most well-known causes of severe yeast infections include four common *Candida* species (*Candida albicans, C. tropicalis, C. parapsilosis*, and *C. glabrata*) and *Cryptococcus neoformans*, but the proportion of the infections caused by other uncommon yeasts has increased as well [1-6]. Because each yeast species may naturally have a different degree of drug suscepti-

bility or resistance, rapid and accurate identification of yeast and yeast-like organisms has become an increasingly important role of clinical microbiology laboratories, which can guide the application of the appropriate antifungal treatment regimens [3-6]. Recently, the technology used in the identification of yeasts has improved, especially following the introduction of matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), which allows rapid and accurate identification of the clinical isolates of yeast species [7-14].

Two commercial MALDI-TOF MS systems, MALDI-TOF Biotyper (Bruker Daltonics, Billerica, MA, USA) and VITEK MS (bioMérieux, Durham, NC, USA), have been assessed in several previous studies by using different databases, sample extraction methods, cut-off scores, and fungal panels [7-12]. For the analysis using the MALDI-TOF Biotyper, in-tube formic acid/acetonitrile (FA/ACN) extraction is recommended prior to the analysis [7], but the use of a simple FA extraction method is preferable, in order to facilitate the routine use in clinical microbiology laboratories. While the results obtained by using the complete FA/ACN extraction are significantly better than those obtained by using FA extraction alone [13], due to the simplicity of the latter procedure, FA remains the preferred method [14]. For the analysis by the Biotyper system, smaller volumes of FA and fewer laboratory consumables are used in the on-plate extraction than in tube extraction, and the isolate preparation is generally more environment-friendly [14].

In this study, we evaluated the performances of two commercial MALDI-TOF MS systems for the identification of a wide spectrum of yeast species, focusing on the use of the FA extraction method in clinical laboratories. Therefore, the aims of this study were to evaluate the performance of the Biotyper system, preceded by the application of the FA, additive FA, and FA/ACN extraction methods, in the identification of four common *Candida* species, *C. neoformans*, and several uncommon yeast species, and to compare the efficacy of the Biotyper system with that of the VITEK MS in the identification of a wide spectrum of clinically relevant yeast species.

METHODS

1. Clinical yeast isolates

We tested 309 clinical isolates of 42 yeast species, including four common *Candida* species (57 *C. albicans*, 47 *C. parapsilosis*, 29 *C. tropicalis*, and 25 *C. glabrata* isolates), *C. neoformans* (23 isolates), and 37 uncommon yeast species (128 isolates). Of the total 309 isolates, 62 "Challenge" isolates were obtained from

BD Diagnostics (Sparks, MD, USA), 147 isolates from a Korean collection, and 100 isolates from routine blood cultures [15]. These BD Diagnostics "Challenge" isolates of 32 species were obtained to increase the total number of evaluated species. The Korean collection included 147 isolates of 33 species submitted between 1996 and 2011 to the Chonnam National University Hospital (CNUH), Gwangju, Korea, from several other Korean hospitals, either to confirm their identities or to test their drug susceptibility. The routine blood culture isolates included 100 non-duplicate isolates of 10 species obtained from two Korean hospitals during 2011. All 309 isolates were identified by sequencing of the internal transcribed spacer (ITS) and/or the D1/D2 domains of 26S ribosomal DNA [15]. This study was approved by the institutional review board of Chonnam National University Hospital (IRB CNUH-2012-117). A waiver of consent was granted given the observational nature of the project. The study involved only the results of the species identification of yeast isolates, and no information was used that could lead to patient identification.

2. Yeast identification using the Bruker Biotyper system

For all 309 isolates, direct on-plate FA smearing was initially used for protein extraction. A single colony was directly smeared onto the target plate, and 0.5 μL of 70% FA (Sigma-Aldrich, St. Louis, MO, USA) was applied. After air drying of the samples at room temperature, 1 μL of α-cyano-4-hydroxycinnamic acid (HCCA) matrix solution (Bruker Daltonics) was applied to the target plate and dried at room temperature. Each sample was analyzed by Microflex LT MALDI-TOF MS in linear positive-ion mode across a mass-to-charge ratio range between 2,000 and 20,000. The obtained information was analyzed automatically by using the MALDI Biotyper automation control and Bruker Biotyper 3.1 software, library v. 3.3.1.0 (Bruker Daltonics). The identification cut-off scores were interpreted as follows: scores ≥1.7 indicated confident species level identification, whereas cut-off scores <1.7 indicated no reliable identification [7, 13, 14]. The isolates for which cut-off scores <1.7 were obtained following the initial FA extraction were re-identified by repeating the same FA extraction process or using the FA/ACN extraction method. In the FA/ACN extraction, a single colony was mixed with 300 μL of distilled water and 900 μL of ethanol in a 1.5 mL Eppendorf tube. The samples were pelleted by centrifuging them at 3,000g for 2 min, dried, and reconstituted in 50 μL of 70% FA. After vortexing, 50 μL of ACN was added, and the suspension was centrifuged at 3,000g force for 2 min. A 1-μL volume of supernatant was applied to the target plate, air dried at room temperature,

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and 1 μL of HCCA matrix solution was applied to the target plate and dried at room temperature. All results obtained in the repeated runs using Biotyper were accepted and included in the final results. That is, additive FA results included all initially identified samples, with cut-off scores ≥1.7, and the results obtained following the FA extraction retest, while the results of additive FA/ACN included the initially identified samples, with cut-off scores ≥1.7 in addition to those identified following the FA/ACN extraction.

3. Yeast identification using the VITEK MS system

Here, the extraction was performed by using the FA method for all isolate samples. A single colony was directly smeared onto the target plate, and 0.5 μL of 25% FA (bioMérieux) was applied. After air drying at room temperature, 1 mL of α-cyano-4-hydroxy-cinnamic acid (CHCA) matrix solution (bioMérieux) was applied to the target plate and dried at room temperature. The samples were analyzed by using the VITEK MS in linear positive-ion mode across a mass-to-charge ratio range between 2,000 and 20,000. The sample information was transferred to the VITEK MS acquisition station using Myla v3.2 Middleware (bioMérieux). The results were automatically calculated and presented by using VITEK MS IVD Knowledgebase v.2.0, and it was reported as a confidence value; up to 99.0%). The non-identified isolates (confidence value <60) after the initial FA extraction were re-identified by using another FA extraction [16]. The results obtained in the repeated runs were included in the final additive FA results. That is, the additive FA results included all initial identification results showing the confidence value ≥60, as well as the results obtained in repeated tests.

4. Data and statistical analyses

The results obtained by using MALDI-TOF MS systems were compared with those obtained by sequence-based identification, and they were assigned to one of four categories: (i) correct identification (results identical to sequence-based identification results), (ii) incomplete identification, (iii) misidentification (either one wrong species was identified, or two or three incorrect species were proposed), or (iv) no identification. The 'incomplete identification' category included (i) a low level of discrimination group (two or three different species were proposed, and one was correct), (ii) identification of the species complex (e.g., *Candida parapsilosis* or *Candida haemulonii* complexes), and (iii) correct identification of non-*Candida* yeasts to the genus level [15]. The 'no identification' category included (i) a low level of discrimination group (score <1.7), (ii) failure to identify the isolate, and (iii) failure to obtain a peak sufficient to identify the iso-

late (confidence value <60). Fisher exact and χ^2 tests were used for the comparisons of the identification rates. Statistical analyses were performed by using PASW version 18.0 (SPSS Inc., Chicago, IL, USA), and *P* values <0.05 were considered statistically significant.

RESULTS

1. Evaluation of the efficacy of the Biotyper system combined with different extraction methods

After the initial FA extraction, the Biotyper correctly identified all 158 isolates of four common *Candida* species, but it correctly identified only 8.7% (2/23) of *C. neoformans* isolates and 79.2% (95/120) of 32 uncommon yeast species. Of 301 isolates, 44 (14.6%) isolates, including 21 *C. neoformans* and 23 uncommon yeast isolates, were not identified by using this method (cut-off scores <1.7). These 44 isolates were then retested following and additional FA or in-tube FA/ACN extraction. Compared with the results obtained following the initial FA extraction alone, no significant differences in the rates of correct identification of *C. neoformans* (initial FA, 8.7%; additive FA, 30.4%) or 32 uncommon yeast species (initial FA, 79.2%; additive FA, 87.5%) were observed after the Biotyper analysis combined with the additive FA. The Biotyper analysis with additive FA/ACN had a higher rate of correct identification of *C. neoformans* (initial FA, 8.7%; additive FA/ACN, 100%, *P*<0.0001), but it showed a comparable rate of correct identification of 32 uncommon yeast species (initial FA, 79.2%; additive FA/ACN, 89.2%). Overall, the Biotyper analysis with additive FA/ACN yielded better identification results than that performed with the initial FA extraction alone (initial FA, 84.7%; additive FA/ACN 95.7%; *P*<0.0001), and had fewer 'no identification' results (initial FA, 14.6%; additive FA/ACN 2.6%; *P*<0.0001) for all isolate samples.

2. Performance comparisons between the Biotyper and VITEK MS systems

In Table 1, the identification results and extraction methods used for the analyses with Bruker Biotyper and VITEK systems are presented. Using the initial FA extraction, both systems correctly identified all 158 isolates of four common *Candida* species. However, the Biotyper had poorer performance in *C. neoformans* identification than VITEK MS ('no identification', Biotyper 91.3% vs VITEK MS 0%; *P*<0.0001), in addition to a lower rate of correct identification (Biotyper 8.7% vs VITEK MS 100%; *P*<0.0001). In the analysis of 37 uncommon yeast species, the Biotyper showed a rate of correct identification comparable to that obtained with

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Table 1. Identification of 309 yeast isolates using the Biotyper and VITEK MS systems combined with different extraction methods, in comparison with the sequence-based identification

System	Extraction method*	Species (N of isolates tested)	N (%) of isolates			
			Correct identification	No identification	Incomplete identification‡	Misidentification
Biotyper	Initial FA	Four common *Candida* species (158)†	158	0	0	0
		Cryptococcus neoformans (23)	2	21	0	0
		Uncommon 37 yeast species (128)	95	30	2	1
		Total (309)	255 (82.5)	51 (16.5)	2 (0.6)	1 (0.3)
	Additive FA	Four common *Candida* species (158)	158	0	0	0
		Cryptococcus neoformans (23)	7	16	0	0
		Uncommon 37 yeast species (128)	105	19	2	2
		Total (309)	270 (87.4)	35 (11.3)	2 (0.6)	2 (0.6)
	Additive FA/ACN	Four common *Candida* species (158)	158	0	0	0
		Cryptococcus neoformans (23)	23§	0§	0	0
		Uncommon 37 yeast species (128)	107	15	4	2
		Total (309)	288 (93.2)§	15 (4.9)§	4 (1.3)	2 (0.6)
VITEK MS	Initial FA	Four common *Candida* species (158)	158	0	0	0
		Cryptococcus neoformans (23)	23ǁ	0ǁ	0	0
		Uncommon 37 yeast species (128)	94	20	12ǁ,¶	2
		Total (309)	275 (89.0)ǁ	20 (6.5)ǁ	12 (3.9)ǁ,¶	2 (0.6)
	Additive FA	Four common *Candida* species (158)	158	0	0	0
		Cryptococcus neoformans (23)	23ǁ	0ǁ	0	0
		Uncommon 37 yeast species (128)	94	16	15ǁ,¶	3
		Total (309)	275 (89.0)	16 (5.2)ǁ	15 (4.9)ǁ,¶	3 (1.0)

*Initial extraction was performed by using a simple formic acid (FA) method for all isolates (initial FA). The isolates that were not identified by using this method were retested by using the same FA extraction (both systems, additive FA) or an in-tube FA and acetonitrile method (Bruker Biotyper only, additive FA/ACN). The additive FA or FA/ACN results include the results of repeated tests, as well as the initial results of all acceptable identifications (scores ≥1.7).
†Including 57 *Candida albicans*, 47 *Candida parapsilosis*, 29 *Candida tropicalis*, and 25 *Candida glabrata* isolates.
‡Incomplete identification included (i) correct identification but a low level of discrimination group (two or three species were proposed, one of them was correct), (ii) identification of the species complex (e.g., the *Candida parapsilosis* or *Candida haemulonii* complexes), and (iii) correct identification of non-*Candida* yeasts to the genus level.
§$P < 0.05$, between the results obtained with the Biotyper (initial FA vs additive FA or initial FA vs additive FA/ACN) within the same identification category.
ǁ$P < 0.05$, between the results obtained with Biotyper or VITEK MS (both with initial FA or additive FA) within the same identification category.
¶$P < 0.05$, between the results obtained with Biotyper preceded with additive FA/ACN and those obtained with the VITEK MS (initial FA or additive FA), within the same identification category.

VITEK (Biotyper, 74.2%; VITEK MS, 73.4%), and a lower rate of incomplete identification (1.6% vs 9.4%, $P = 0.011$). Following the initial FA extractions, the Biotyper showed a lower rate of correct identification of all 309 isolates than VITEK MS (82.5% vs 89.0%, $P = 0.028$), a higher rate of no identification (16.5% vs 6.5%, $P < 0.0001$), and a lower rate of incomplete identification (0.7% vs 3.9%, $P = 0.012$). The Biotyper analysis combined with the additive FA showed a lower rate of correct identification (Biotyper, 31.4%; VITEK MS, 100%) of *C. neoformans* as well, with a higher rate of no identification. However, using the Biotyper analysis with additive FA/ACN, we identified all 23 isolates of *C. neoformans*, same as when using the VITEK MS after the initial FA extraction (100% correct identification). Additionally,

The VITEK MS analysis with additive FA showed a higher rate of incomplete identification for uncommon yeast species than the Biotyper analysis with additive FA (Biotyper, 1.6%; VITEK MS, 11.7%; $P = 0.002$) or additive FA/ACN (Biotyper, 3.2%; VITEK MS, 11.7%; $P = 0.015$). For the identification of all 309 isolates, both systems in the combination with additive FA extraction method showed no differences in the rates of correct identification or misidentification, but the Biotyper analysis showed a lower rate of incomplete identification (Biotyper 0.6%; VITEK MS, 4.9%; $P = 0.002$) and a higher rate of no identification (Biotyper 11.3%; VITEK MS, 5.2%; $P = 0.008$). In contrast to this, Biotyper analysis combined with the additive FA/ACN extraction showed correct and no-identification rates comparable to the results obtained

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Table 2. Detailed identification of 309 isolates belonging to 42 yeast species using two MALDI-TOF MS systems in combination with additive formic acid extraction and in comparison with sequence-based identification

Final identification	N of isolates	N (%) of isolates							
		Correct identification		No identification		Incomplete identification		Misidentification	
		Biotyper	VITEK MS	Biotyper	VITEK MS	Biotyper	VITEK MS	Biotyper	VITEK MS
Species included in databases in both systems									
Candida albicans	57	57	57	0	0	0	0	0	0
Candida tropicalis	29	29	29	0	0	0	0	0	0
Candida parapsilosis	47	47	47	0	0	0	0	0	0
Candida glabrata	25	25	25	0	0	0	0	0	0
Cryptococcus neoformans	23	7[‡]	23	16[‡]	0	0	0	0	0
Candida guilliermondii	20	19	20	1	0	0	0	0	0
Candida krusei	12	12	12	0	0	0	0	0	0
Candida pelliculosa	9	9	9	0	0	0	0	0	0
Saccharomyces cerevisiae	7	7	6	0	1	0	0	0	0
Candida haemulonii	6	6	6	0	0	0	0	0	0
Candida lipolytica	6	6	6	0	0	0	0	0	0
Candida lusitaniae	6	6	6	0	0	0	0	0	0
Trichosporon asahii	6	6	6	0	0	0	0	0	0
Candida catenulata	3	3	3	0	0	0	0	0	0
Candida intermedia	3	3	2	0	1	0	0	0	0
Malassezia pachydermatis	3	2	2	1	1	0	0	0	0
Candida norvegensis	2	2	2	0	0	0	0	0	0
Candida rugosa	2	2	2	0	0	0	0	0	0
Candida utillis	2	2	2	0	0	0	0	0	0
Kodamaea ohmeri	2	0	2	2	0	0	0	0	0
Rhodotorula minuta	2	0	0	1	0	0	0	1	2
Blastoschizomyces	1	1	1	0	0	0	0	0	0
Candida dubliniensis	1	1	1	0	0	0	0	0	0
Candida kefyr	1	1	1	0	0	0	0	0	0
Candida pulcherrima	1	0	0	1	1	0	0	0	0
Candida sphaerica	1	1	1	0	0	0	0	0	0
Cryptococcus humicolus	1	0	1	1	0	0	0	0	0
Rhodotorula mucilaginosa	1	1	1	0	0	0	0	0	0
Total, 28 species	279	255 (91.4)[‡]	273 (97.8)	23 (8.2)[‡]	4 (1.4)	0	0	1 (0.4)	2 (0.7)
Total, all									
Only species included in each system's database*		270 (89.7)	275 (97.2)	28 (9.3)	5 (1.8)	1 (0.3)	1 (0.4)	2 (0.7)	2 (0.7)
Only species not included in each system's database[†]		0 (0)	0 (0)	7 (87.5)	11 (42.3)	1 (12.5)	14 (53.8)	0 (0)	1 (3.8)
All 42 species	309	270 (87.4)	275 (89.0)	35 (11.3)[‡]	16 (5.2)	2 (0.6)[‡]	15 (4.9)	2 (0.6)[§]	3 (1.0)[ǁ]

*Of 42 uncommon species, 37 (301 isolates) and 31 (283 isolates) species were included in the Biotyper and VITEK MS databases, respectively.
[†]Of 42 species, 5 (8 isolates) and 11 (26 isolates) species were not included in the Biotyper and VITEK databases, respectively.
[‡]*P* < 0.05, between the results obtained with Biotyper and VITEK MS systems, within the same identification category.
[§]One isolate of *Rhodotorula minuta* and one isolate of *Candida viswanathii* were misidentified as *Candida catenulate* and *Candida tropicalis*, respectively, by the Biotyper.
[ǁ]Two isolates of *Rhodotorula minuta* were misidentified as *Candida haemulonii* and *Candida catenulate*, and one isolate of *Candida metapsilosis* was misidentified as *Geotrichum capitatum* by the VITEK MS.

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by VITEK MS in the analysis of all isolates, and a lower incomplete identification (Biotyper, 1.3%; VITEK MS, 4.9%; *P*=0.017). Of all isolate samples, only three (1.0%) were misidentified by using the VITEK MS analysis with additive FA extraction, which was comparable to the rate of misidentification yielded by the Biotyper with additive FA (0.6%) or additive FA/ACN (0.6%).

3. Detailed identification of 42 yeast species using two MALDI–TOF systems

Detailed results obtained by using the Bruker Biotyper and VITEK MS systems for the identification of 309 yeast isolates using the additive FA method are shown in Table 2. Of the 42 yeast species tested in this study, 37 (301 isolates) and 31 (283 isolates), respectively, were included in the Biotyper and VITEK MS system databases. Taking into the consideration only the species included in the databases, the Biotyper demonstrated a lower rate of correct identification than VITEK MS (89.7% [270/301] vs 97.2% [275/283], *P*<0.001) and a higher rate of no identification (9.3% [28/301] vs 1.8% [5/283], *P*<0.001). However, when *C. neoformans* isolates were excluded from the latter assessment, the analysis using Biotyper yielded correct (94.6% [263/278] vs 96.9% [252/260]) and no identification (4.3% [12/278] vs 1.9% [5/260]) results comparable to those obtained with VITEK MS. The analysis of five and 11 species, respectively, that were not included in the Biotyper and VITEK MS databases, showed that the Biotyper analysis had a lower rate of incomplete identification (12.5% [1/8] vs 53.8% [14/26], *P*=0.039) but a higher rate of no identification (87.5% [7/8] vs 42.3% [11/26], *P*=0.025) than VITEK MS, while they had comparable misidentification rates (0% [0/8] vs 3.8% [1/16]).

DISCUSSION

In a previous report, the VITEK MS system was shown to allow the identification of significantly more isolates than the Biotyper system, following the application of the on-plate FA method (94.9% vs 82.9%, respectively). However, when on-plate FA and in-tube FA extractions were combined, followed by the Biotyper analysis, the identification rates were comparable between the two platforms (94.9% vs 96.6%) [7]. Similarly, the results of our study demonstrated that the analysis with Biotyper combined with the initial on-plate FA extraction yielded a lower rate of correct identification than the analysis with VITEK MS (82.5% vs 89.0%, respectively) for all 309 isolates, but the analysis with Biotyper and additive FA showed comparable rates of correct identification for all isolates (87.4% vs 89.0%, respectively). These results sug-

gest that a repeated testing with Biotyper may be required for non-identified isolate samples (scores <1.7) after the initial FA extraction.

The analysis performed by using the Biotyper with initial FA extraction alone showed much lower rates of successful identification of *C. neoformans* and uncommon yeasts, than VITEK MS. Using the VITEK MS system, an increase in the confidence value from 60% to 99% yielded the same rate of 'no identification' (6.5%). In contrast to this, adjusting Biotyper cut-off score to 2, a total of 100 (32.4%) samples, including 21 common *Candida* isolates, 23 *C. neoformans*, and 56 uncommon yeast isolates, were not identified, which support the use of optimal cut-off value of 1.7 for routine yeast identification [7]. To overcome this problem, we retested these isolates using the same FA extraction or in-tube FA/ACN extraction. Additive FA extraction did not significantly improve the Biotyper identification rate compared with the analysis using the initial FA extraction alone, but the additive FA/ACN extraction resulted in a significantly better identification rates and fewer 'no identification' results in the analysis of all isolates. Considering that the FA/ACN method is more time-consuming and laborious, these results suggest that the initial FA and subsequent additive FA/ACN extraction methods may provide a cost-effective and reliable solution for yeast identification using the Biotyper.

Notably, our results showed that, out of 23 *C. neoformans* isolates, 21 and 16, respectively, were not identified by the Biotyper system following the initial FA extraction alone or additive FA, while all of these isolates were correctly identified after FA/ACN extraction. Unreliable identification of *C. neoformans* by the Biotyper has been reported (identification rates of 50–66%) [7, 17, 18]. This may be due to the polysaccharide capsule of this pathogen, which renders the extraction and solubilization of proteins difficult, or due to insufficient database entries that would enable spectral matches [19]. We confirmed that the Biotyper system has some difficulties identifying *C. neoformans* when using direct FA extraction, and that an improved rate of the identification of *C. neoformans* isolates can be observed following the FA/ACN extraction, as reported previously [7, 18]. Therefore, we recommend initial in-tube FA/ACN extraction before proceeding to the Biotyper analysis for all clinical cases, in which the infection with *C. neoformans* is suspected on the basis of colony morphology, microscopic findings, or clinical conditions.

The analysis using Biotyper showed a lower rate of the incomplete identification of uncommon yeast species than that using the VITEK MS system, irrespective of the extraction method used. This may be partly due to the database differences between the

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two systems. The information about more species identified here was included in the Biotyper database (37 species) than in the VITEK MS database (31 species). In particular, we found that some cryptic species, such as *Candida pseudohaemulonii* (*Candida haemulonii* complex), *Candida metapsilosis*, and *Candida orthopsilosis* (*Candida parapsilosis* complex) were correctly identified at the species level by the Biotyper, as reported previously [9]. Additionally, Biotyper yielded a lower rate of the incomplete identification of species not included in the databases, and a higher rate of no identification results than VITEK MS. These findings suggest that the analysis using the VITEK MS system is more likely to yield an incomplete identification of the uncommon yeasts not included in the databases, while the Biotyper is more likely to provide no identification. However, the use of both systems seldom led to the misidentification due to the database limitations.

To date, few studies have analyzed the effectiveness of FA extraction for the identification of a wide spectrum of clinically relevant yeast species using the Biotyper. We showed that using simple FA extraction in combination with the Biotyper, the identification performance is comparable to that of the VITEK MS analyses for most common and uncommon yeast isolates, except for *C. neoformans*. When considering only species included in the database, and excluding *C. neoformans*, the rates of correct and no identification yielded by the application of Biotyper and VITEK MS were comparable. A recent report also showed that the Biotyper use, preceded by the FA extraction, can correctly identify over 97% of yeast species [20]. The researchers analyzed 190 isolates comprising 19 yeast species, but they did not include any *C. neoformans* isolates [20]. These data suggest that the lower correct identification rate obtained here using the Biotyper, in comparison with that obtained using the VITEK MS after the FA extraction, may reflect the high rate of non-identified *C. neoformans* isolates. These results, together with Korean data showing that < 1% of clinical yeast isolates from hospitals were *C. neoformans* samples [21], suggest that the Biotyper system, in combination with the initial FA extraction alone, may be suitable for the routine use. Additionally, repeated testing using the additive FA/ACN method may provide a cost-effective and reliable method to identify a wide spectrum of clinically relevant yeast species, including *C. neoformans*.

Authors' Disclosures of Potential Conflicts of Interest

No potential conflicts of interest relevant to this article were reported.

Acknowledgments

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