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Evaluation of the VITEK 2 System for Rapid Identification of Yeasts and Yeast-Like Organisms

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The new VITEK 2 system is a fully automated system dedicated to the identification and susceptibility testing of microorganisms. In conjunction with the VITEK ID-YST card the VITEK 2 system allows the identification of clinically important yeasts and yeast-like organisms in 15 h due to a sensitive fluorescence-based technology. The ID-YST card consists of 47 biochemical reactions. The database comprises 51 taxa, including newly described species. In this study we evaluated the reliability of the VITEK ID-YST card for the identification of yeasts and yeast-like organisms encountered in a clinical microbiology laboratory. A total of 241 strains representing 21 species were studied. The strains were isolated from clinical samples within a period of 60 days prior to the identification. The tests were performed using 24-h to 55-h subcultures on Sabouraud-gentamicin-chloramphenicol agar. Each strain was tested in parallel using the ID 32C strip as a comparison method combined with microscopic morphology and an agglutination test for *C. krusei*. Overall, 222 strains (92.1%) were unequivocally identified including 11 isolates (4.6%) identified with low discrimination resolved by simple additional tests. Ten strains (4.1%) for which results were given with low discrimination could not be unequivocally identified with supplemental tests, 4 strains (1.7%) were misidentified and 5 strains (2.1%) could not be identified. In conclusion, we found that the VITEK 2 system is a rapid and accurate method for the identification of medically important yeasts and yeast-like organisms.

The incidence of fungal infections caused by yeasts has significantly increased over the past decades in parallel with the number of patients at risk for opportunistic fungal disease (1, 4, 7). *Candida* species have emerged as common pathogens in both seriously ill and immunocompromised patients. Although *Candida albicans* remains the species most frequently encountered in a clinical laboratory, there has been an increase in the frequency of infections caused by non-*Candida albicans* species (18, 19, 23). As different *Candida* species have different intrinsic in vitro susceptibilities to fluconazole and *Candida lusitaniae* is frequently resistant to amphotericin B (3, 8, 23), a rapid and reliable identification of clinical isolates is required. Identification of yeasts using conventional methods is time-consuming and labor-intensive (17), and therefore different commercial systems for the identification of yeasts have been evaluated (2, 5, 6, 9, 10, 12, 13, 14, 15, 20, 22).

The VITEK 2 system is a fully automated instrument dedicated to the identification and susceptibility testing of microorganisms. The ID-YST database comprises 51 different taxa, including newly described species, taking into account recent advances in taxonomy. Due to the new, fluorescence-based technology of the VITEK 2 system, final identification of yeasts is available after an incubation of 15 h. In previous studies a prototype of the VITEK 2 consisting of three manually operated modules was tested using in-house strains or collection strains and clinical isolates (G. Bossy, S. Cagnes, F. Coquil, R. Cognes, S. Commenge, N. Faget, V. Montellier, and A. Rongier, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. D-56, p. 93, 1997; B. Lebeau, C. Rejasse, R. Grillot, G. Bossy, S. Cagnes, S. Commenge, A. Bassel, M. Geimer, and D. Pincus, Abstr. 98th Gen. Meet. Am. Soc. Microbiol. 1998, abstr. F-93, p. 269, 1998). This is the first evaluation using strains freshly isolated from clinical samples in a fully automated integrated VITEK 2 instrument. In our study we evaluated the reliability of the VITEK 2 ID-YST card for the identification of medically important yeasts and yeast-like organisms in a routine clinical laboratory.

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MATERIALS AND METHODS

Strains, culture conditions, and inoculum preparation. A total of 241 strains representing 21 yeast species were included in the study. All strains were isolated from clinical samples within a period of 60 days prior to the evaluation and had not been frozen. Fresh clinical samples (oral, vaginal, anorectal, urine, stool, and respiratory tract specimens) were obtained from 207 patients. Tests were performed from subcultures grown for 24 to 55 h on Sabouraud-gentamicin-chloramphenicol agar plates. Inocula were adjusted to a no. 2 McFarland standard using an ATB 1550 densitometer (bioMérieux, Marcy-l'Etoile, France) by suspending the yeast cells in 0.45% aqueous NaCl.

VITEK 2 system and ID-YST card. The VITEK ID-YST card consists of 64 wells with 47 fluorescent biochemical tests. They comprise 20 carbohydrate assimilation tests: adonitol (ribitol), D-trehalose, D-cellobiose, dulcitol, D-galactose, D-glucose, lactose, D-maltose, D-mannitol, D-melibiose, D-melezitose, palatinose, D-raffinose, L-rhamnose, sucrose, salicine, L-sorbose, D-sorbitol, D-L-lactate, and succinate. The six organic acid assimilation tests are *N*-acetyl-glucosamine, methyl-α-D-glucopyranoside, citrate, D-galacturonate, D-gluconate, and mono-methyl-ester-succinate. The eight substrates for the detection of the oxidases are coupled with 4-methylumbelliferone (4MU): α-galactoside–4MU, α-glucoside–4MU, α-mannoside–4MU, β-galactoside–4MU, β-glucoside–4MU, β-glucuronide–4MU, β-*N*-acetyl-glucosaminide–4MU, and β-xyloside–4MU. The nine substrates for the detection of arylamidases are coupled with 7-amino-methylcoumarin (7AMC): glycine-7AMC, hydroxyproline-7AMC, H-lysine-alanine–7AMC, γ-glutamyl-transferase–7AMC, H-glycine-glycine–7AMC, histidine-7AMC, isoleucine-7AMC, proline-7AMC, and valine-7AMC. The four miscellaneous tests are phosphatase, urea, nitrate, and actidione. In cases of identification with low discrimination (see below), simple additional tests (most of them can be performed the same day) are suggested by the VITEK 2 system. Additional tests were chosen to demonstrate blastospores or arthrospores, apiculated cells, polysaccharidic capsule, carotenoid pigment, convoluted colony, hyphae or pseudohyphae, sporangia, growth at 37°C, and growth without oil.

The integrated VITEK 2 instrument automatically filled, sealed and transferred the cards into an incubator (incubation temperature was 35°C). Every 15 min the cards were automatically subjected to a fluorescence measurement. Each profile was interpreted according to a specific algorithm. After an incubation

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TABLE 1. Results of the evaluation of 241 strains using the ID-YST card of the VITEK 2 system

Species	No. of strains tested	No. (%) of strains in category				
		Unequivocal identification	Low discrimination resolved	Low discrimination not resolved	No identification	Misidentification
Candida albicans	28	28	0	0	0	0
Candida colliculosa	1	1	0	0	0	0
Candida dubliniensis	2	2	0	0	0	0
Candida glabrata	36	36	0	0	0	0
Candida guilliermondii	4	2	0	1	1	0
Candida inconspicua	6	0	0	6	0	0
Candida intermedia	2	0	2	0	0	0
Candida kefyr	13	12	0	0	0	1
Candida krusei	29	20	6	1	0	2
Candida lambica	2	2	0	0	0	0
Candida lipolytica	3	3	0	0	0	0
Candida lusitaniae	14	13	0	0	1	0
Candida norvegensis	1	0	0	1	0	0
Candida parapsilosis	31	30	0	1	0	0
Candida pulcherrima	1	1	0	0	0	0
Candida rugosa	1	1	0	0	0	0
Candida tropicalis	36	36	0	0	0	0
Candida utilis	2	2	0	0	0	0
Geotrichum spp.	5	2	0	0	3	0
Kloeckera apiculata	2	0	2	0	0	0
Saccharomyces cerevisiae	21	19	1	0	0	1
Trichosporon mucoides	1	1	0	0	0	0
Total no. (%)	241	211 (87.6)	11 (4.6)	10 (4.1)	5 (2.1)	4 (1.7)

period of 15 h, the profile result was compared to the ID-YST database, which led to the final identification of the microorganism.

Comparison methods and quality controls. The same inoculum of each strain was tested in parallel using the ID 32C strip (bioMérieux) as a comparison method. The ID 32C system was used according to the manufacturer's guidelines. The strips were read automatically after 48 h using the ATB Expression and ATB Plus software (version 2.0; bioMérieux). In case of discrepant results obtained with the VITEK 2 and ID 32C systems, both methods were repeated and the results of the second run were accepted as final results. For six isolates the incubation time of the ID 32C had to be prolonged up to 96 h because of low reactivity. Strains that could not unequivocally be identified by the ID 32C system were subjected to additional tests such as microscopic morphology on rice extract-Tween 80 agar (16), an agglutination test for *Candida krusei* (Krusei Color Fumouze, Biotrin-Trignost) (11, 21), or growth at 37°C.

A set of nine quality control (QC) strains was tested once a week with the VITEK 2 system. The QC strains were *Candida tropicalis* ATCC 201380, *C. tropicalis* ATCC 201381, *Candida kefyr* ATCC 4135, *Candida magnoliae* ATCC 201379, *Trichosporon mucoides* ATCC 201382, *Candida membranaefaciens* ATCC 201377, *C. membranaefaciens* ATCC 201378, *C. lusitaniae* ATCC 34449, and *T. mucoides* ATCC 201383.

Analysis of data. Results from the VITEK 2 system were compared with the data obtained by the comparison method and assigned to one of four categories: (i) unequivocal identification, the strain was correctly identified to species level or identified with low discrimination (two to four species were proposed by VITEK 2) and resolved by simple additional tests; (ii) low discrimination, two or three species are proposed by the VITEK 2 and not resolved by simple additional tests; (iii) no identification, the strain could not be identified (e.g., unknown profile); or (iiii) misidentification, a discrepant result was obtained with regard to the comparison method.

RESULTS

When compared to the results of the ID 32C system, 222 strains out of 241 (92.1%) were unequivocally identified to the species level by the VITEK 2 system, including 11 strains (4.6%) with low discrimination resolved by simple additional tests. Ten strains (4.1%) with low discrimination could not be definitely identified to the species level by additional tests. Four strains (1.7%) were misidentified and five strains (2.1%) could not be identified (Table 1). Five strains had to be excluded from the study because it was not possible to identify these strains using the comparison method including supplemental tests.

Among the 21 species included in the evaluation, *C. albicans*, *Candida glabrata*, *C. tropicalis*, *Candida parapsilosis*, and *C. krusei* are the most common species encountered in a routine clinical laboratory. The strains belonging to these species were correctly identified at a frequency of 93.8% without supplemental tests (97.5% with additional tests).

Of 29 *C. krusei* strains tested, 20 strains were correctly identified without additional tests, 6 strains were identified with low discrimination but resolved by additional tests, 1 strain was identified with low discrimination and not resolved by supplemental tests, and 2 strains were misidentified. Interestingly, *Geotrichum capitatum* was proposed as an identification by the VITEK 2 system in all cases of misidentified *C. krusei* strains or *C. krusei* strains identified with low discrimination. However, using the ID 32C comparison method, seven out of nine *C. krusei* strains (low discrimination or misidentification) needed additional tests for unequivocal identification, indicating atypical behavior of these strains in biochemical assays. For a more detailed analysis of these strains, see Table 2.

All *Candida inconspicua* and *Candida norvegensis* strains were identified with low discrimination and not resolved, because the biochemical reactions of the VITEK ID-YST card are not appropriate for distinguishing between the two species and additional tests are not suggested by the VITEK 2 system.

Not identified or misidentified strains did not belong to a single species. Three out of five not identified isolates were *Geotrichum* spp., and two out of four misidentified strains were *C. krusei* strains. The reactions leading to misidentification of these strains are given in Table 3.

Ninety-four and four-tenths percent of the QC strains were unequivocally identified and 5.6% were identified with low

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TABLE 2. Analysis of the strains identified with low discrimination

Comparison identification	VITEK 2 results	Result of additional tests	VITEK 2 results using additional test results
C. guilliermondii	*C. guilliermondii*, *C. lusitaniae*	No additional test	*C. guilliermondii*, *C. lusitaniae*
C. inconspicua	*C. inconspicua*, *C. norvegensis*	No additional test	*C. inconspicua*, *C. norvegensis*
C. inconspicua	*C. inconspicua*, *C. norvegensis*	No additional test	*C. inconspicua*, *C. norvegensis*
C. inconspicua	*C. inconspicua*, *C. norvegensis*	No additional test	*C. inconspicua*, *C. norvegensis*
C. inconspicua	*C. inconspicua*, *C. norvegensis*	No additional test	*C. inconspicua*, *C. norvegensis*
C. inconspicua	*C. inconspicua*, *C. norvegensis*	No additional test	*C. inconspicua*, *C. norvegensis*
C. inconspicua	*C. inconspicua*, *C. norvegensis*	No additional test	*C. inconspicua*, *C. norvegensis*
C. intermedia	*C. intermedia*, *C. lusitaniae*	No growth at 37°C	*C. intermedia*
C. intermedia	*C. intermedia*, *C. lusitaniae*	No growth at 37°C	*C. intermedia*
C. krusei	*C. krusei*, *G. capitatum*, *C. inconspicua*, *C. norvegensis*	Blastoconidia	*C. krusei*, *C. inconspicua*, *C. norvegensis*
C. krusei	*C. krusei*, *G. capitatum*	Blastoconidia	*C. krusei*
C. krusei	*C. krusei*, *G. capitatum*	Blastoconidia	*C. krusei*
C. krusei	*C. krusei*, *G. capitatum*	Blastoconidia	*C. krusei*
C. krusei	*C. krusei*, *G. capitatum*	Blastoconidia	*C. krusei*
C. krusei	*C. krusei*, *G. capitatum*	Blastoconidia	*C. krusei*
C. krusei	*C. krusei*, *G. capitatum*	Blastoconidia	*C. krusei*
C. norvegensis	*C. inconspicua*, *C. norvegensis*	No additional test	*C. inconspicua*, *C. norvegensis*
C. parapsilosis	*C. parapsilosis*, *C. guilliermondii*	No additional test	*C. parapsilosis*, *C. guilliermondii*
K. apiculata	*K. apis*, *K. apiculata*	No growth at 37°C	*K. apiculata*
K. apiculata	*K. apis*, *K. apiculata*	No growth at 37°C	*K. apiculata*
S. cerevisiae	*S. cerevisiae*, *C. holmii*	Growth at 37°C	*S. cerevisiae*

discrimination. There was no misidentification or nonidentification of these strains.

DISCUSSION

In our study, we evaluated the new, fully automated VITEK 2 system for rapid identification of yeasts and yeast-like organisms. The strains were simultaneously tested by the ID 32C system. Due to its large database and accuracy, the ID 32C has been used by several authors as a reference method (12, 13, 14). The ID 32C strip consists of 32 biochemical reactions; the results were reported after incubation at 30°C for 48 h. In contrast, the ID-YST card of VITEK 2 comprises 47 biochemical tests; the results were obtained after an incubation at 35°C for 15 h. The possibility to achieve the identification within 15 h due to its more sensitive fluorescence technology represents a major progress of the new VITEK 2 system. The VITEK 2 system performed well for the correct identification with or without additional tests (92.1 and 87.6%, respectively). These data are also in agreement with recent studies of the VITEK 2 system performed with a manually operated prototype and different panels of strains (Bossy et al., 37th ICAAC; Lebeau et al., 98th Gen. Meet. Am. Soc. Microbiol.).

The most common species isolated in a routine clinical laboratory were correctly identified in 97.5 or 93.8% of cases with or without supplemental tests, respectively (*C. albicans*, *C. tropicalis*, and *C. glabrata* in 100% of cases without additional tests; *C. krusei* in 89.7 or 70% of cases with or without additional tests, respectively; and *C. parapsilosis* in 96.8% of cases without additional tests [no additional tests suggested by VITEK 2 system]). However, for 7 out of the 29 *C. krusei* strains tested, the VITEK 2 system yielded two or more species. The biochemical reaction *N*-acetyl-glucosamine assimilation, which is important for the identification of *C. krusei*, was negative in five out of these seven strains. Similarly, using the ID 32C comparison method, the same isolates failed to assimilate *N*-acetyl-glucosamine, indicating atypical behavior of these strains in biochemical assays. Therefore supplemental tests for unequivocal identification were required. Seven out of 10 strains identified with low discrimination and not resolved were *C. inconspicua* or *C. norvegensis*. The poor identification result for these species is not due to false-positive or negative biochemical reactions. Since the biochemical reactions included in the VITEK 2 system are not appropriate for discriminating between *C. inconspicua* and *C. norvegensis*, low discrimination not resolved is the highest level of identification that can be achieved using the VITEK 2 system. If required, additional tests (e.g., cellobiose assimilation, Wickerham method) can be used for unequivocal identification of these isolates.

TABLE 3. Reactions leading to misidentification of the strains tested

Comparison identification	VITEK 2 result(s)	Reaction(s) (causes)[a]
C. kefyr	*Malassezia furfur*/*Malassezia pachydermatis*, *C. colliculosa*	− (inhibition of growth by actidione; assimilation of D-galactose, D-glucose, D-raffinose, sucrose, D-sorbitol, D-L-lactate, or succinate; cleavage of isoleucine-7AMC), ± (cleavage of β-glucoside–4MU)
C. krusei	*G. capitatum*, *C. inconspicua*, *C. norvegensis*	− (assimilation of *N*-acetyl-glucosamine), + (cleavage of β-glucoside–4MU)
C. krusei	*G. capitatum*	− (assimilation of D-L-lactate), ± (assimilation of *N*-acetyl-glucosamine or mono-methyl-ester-succinate)
S. cerevisiae	*C. colliculosa*	− (assimilation of D-galactose, D-maltose, or D-L-lactate)

[a] +, false-positive reaction (with regard to the database); −, false-negative reaction (with regard to the database); ±, neither negative nor positive reaction (excluded from analysis).

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Of particular interest was the misidentification of four strains. The reason for incorrect identification was a low reactivity of the strains within the incubation time, resulting in false-negative biochemical reactions (Table 3). However, the VITEK 2 results for two out of four misidentified strains were given with an excellent confidence level. Thus, under routine conditions misidentification of these strains would not have been detected. The VITEK 2 system allows a result to be generated without the need for a morphological assessment. However, additional morphological methods could diminish the small number of misidentified strains. Thus, in our study the misidentification of three out of four strains could have been detected by such methods. Other studies evaluating the VITEK 2 system showed similar rates of misidentification (2.7 and 0.9%, respectively) (Bossy et al., 37th ICAAC; Lebeau et al., Abstr. 98th Gen. Meet. Am. Soc. Microbiol. 1998). Overall, only four strains were misidentified, and no pattern of assignment of incorrect identification to a single species was apparent.

Low reactivity of the strains was also the reason for the nonidentification of five isolates (Table 1), because the lack of positive reactions yielded profiles unknown to the database. *Geotrichum* sp. was most likely to not be identified (three out of five strains) by the VITEK 2 system, while the ID 32C system correctly identified these strains.

Due to its large database containing 51 different taxa and its full automation, the VITEK 2 system offers rapid and accurate identification within 15 h. The percentage of correct identification is similar to that by other commercial identification systems (15, 20). Occasional misidentifications could be diminished if morphological characteristics are taken into account. In conclusion, we found the VITEK 2 system suitable for the identification of yeasts and yeast-like organisms in a routine clinical mycology laboratory.

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