Multicenter Evaluation of the New VITEK 2 Advanced Colorimetric Yeast Identification Card[∇]

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The performance of the new VITEK 2 Advanced Colorimetry yeast identification (YST) card for use with the VITEK 2 system (bioMérieux, Inc., Hazelwood, MO) was compared to that of the API 20C AUX (API) system (bioMérieux SA, Marcy-l'Etoile, France) in a multicenter evaluation. A total of 12 quality control, 64 challenge, and 623 clinical yeast isolates were used in the study. Comparisons of species identification, platform reliability, and substrate reproducibility were made between YST and API, with API considered the reference standard. Quality control testing to assess system and substrate reproducibility matched expected results ≥95% of the time. The YST card correctly identified 100% of the challenge strains, which covered the species range of the manufacturer's performance claims. Using clinical isolates, the YST card correctly identified 98.5%, with 1.0% of isolates incorrectly identified and 0.5% unidentified. Among clinical isolates, the YST card generated fewer low-discrimination results (18.9%) than did API (30.0%). The time to identification with YST was 18 h, compared to 48 to 72 h with API. The colorimetric YST card used with the VITEK 2 provides a highly automated, objective yeast identification method with excellent performance and reproducibility. We found this system useful for timely and accurate identification of significant yeast species in the clinical microbiology laboratory.

Candida species have emerged as notable pathogens over the last decade, especially among hospitalized and immunosuppressed populations. Recent studies have implicated Candida spp. as one of the leading causes of nosocomial fungemia, with a crude mortality rate of approximately 40% (5). In addition, there has been a shift in the dominant causative agent from C. albicans to non-C. albicans species (i.e., C. parapsilosis, C. glabrata, and C. tropicalis) (14). Concern about antifungal resistance, particularly with agents of the azole class of antifungals and amphotericin B (3, 13, 15), necessitates the rapid and accurate identification of yeasts to the species level by the clinical microbiology laboratory. Although nonculture methods (e.g., PCR and antigen detection) (12, 17, 18, 22) have been applied to Candida diagnostics, they exhibit variable sensitivities, can be time- and labor-intensive, and may not be applicable for routine clinical use (19). A number of commercial systems which use enzymatic reactivity or carbohydrate utilization as the basis for yeast identification have been developed (2, 20).

VITEK 2 (bioMérieux, Inc. Hazelwood, MO) is a fully automated microbiology identification system that evaluates an optical signal generated by individual biochemical reactions contained within a variety of microbe identification cards. After inoculation with a standardized suspension of the unknown organism, each self-contained card is incubated and read by the instrument's internal optics. Comparison of results to

This study utilized a new colorimetric yeast identification (YST) card designed to replace the fluorimetric yeast identification (ID-YST) card. The colorimetric card has been developed for use with the VITEK 2 system in conjunction with updated instrument optics and identification software. Results from the 46 tests contained in the card were compared to an identification database which included 52 taxa belonging to the following genera: Candida, Cryptococcus, Geotrichum, Kloeckera, Kodamaea, Malassezia, Pichia, Rhodotorula, Saccharomyces, Sporobolomyces, Stephanoascus, Trichosporon, and Zygosaccharomyces. Prototheca, an alga occasionally causing infection and encountered in clinical specimens, was also included in the database. Identification to species level for clinical specimens was generated after 18 h of incubation.

The purpose of this multicenter study was to evaluate the reliability and reproducibility of the YST card compared to the API 20C AUX (API) for the routine identification of yeasts and other yeast-like organisms in the setting of the clinical microbiology laboratory. The study consisted of three components which were examined using the YST card in parallel with API: (i) quality control (QC) testing evaluated system and substrate reproducibility, (ii) challenge testing covered the species range of YST performance claims, and (iii) the use of clinical isolates and stock cultures obtained from each of the three participating centers tested the utility in the clinical laboratory.

MATERIALS AND METHODS

Organisms. The 12 QC strains that were tested included *C. albicans* ATCC 14053 (American Type Culture Collection, Manassas, VA), *C. glabrata* ATCC MYA2950, *Candida guilliermondii* ATCC 6260, *Candida kefyr* ATCC 204093, *Candida lipolytica* ATCC 9773, *Candida lusitaniae* ATCC 3449, *Candida utilis*

known species-specific reactions in the VITEK 2 database yields organism identification.

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TABLE 1. Challenge organisms tested with the YST card at three trial sites

	No. of	No. of	No. of correct results		
Challenge organism	isolates	tests	One choice	Low discrimination	
Candida albicans	10	30	30	0	
Candida colliculosa	1	3	3	0	
Candida dubliniensis	3	9	9	0	
Candida famata	1	3	2	1	
Candida glabrata	8	24	24	0	
Candida kefyr	1	3	3	0	
Candida krusei	5	15	0	15	
Candida lambica	1	3	2	1	
Candida lipolytica	1	3	3	0	
Candida lusitaniae	1	3	3	0	
Candida parapsilosis	6	18	15	3	
Candida pelliculosa	2	6	6	0	
Candida tropicalis	7	21	19	2	
Candida utilis	1	3	3	0	
Cryptococcus albidus	1	3	0	3	
Cryptococcus neoformans	2	6	6	0	
Geotrichum capitatum	1	3	1	2	
Kloeckera sp.	1	3	3	0	
Pichia farinosa	2	6	6	0	
Prototheca zopfii	1	3	3	0	
Rhodotorula minuta	1	3	3	0	
Rhodotorula mucilaginosa	1	3	3	0	
Saccharomyces cerevisiae	4	12	10	2	
Trichosporon asahii	2	6	5	1	
Total (%)	64	192	162 (84.4)	30 (15.6)	

ATCC 9950, Cryptococcus albidus ATCC 34140, Geotrichum capitatum ATCC 28576, Kloeckera apis ATCC 32857, Prototheca wickerhamii ATCC 16529, and Trichosporon mucoides ATCC 204094. A set of 64 challenge isolates was supplied by bioMérieux (Table 1). In addition, a total of 471 fresh and 152 stock clinical isolates (623 total) representing 13 genera and 39 yeast species were used in this study (Table 2). Testing was performed on primary subcultures grown overnight at 30°C on Sabouraud dextrose agar.

YST card. Pure subcultures of QC, challenge, clinical, and stock organisms were suspended in aqueous 0.45% (wt/vol) NaCl to achieve a turbidity equivalent to that of a McFarland 2.0 standard (range, 1.80 to 2.20), as measured by the DensiChek (bioMérieux) turbidity meter. The VTTEK 2 instrument automatically filled, sealed, and incubated the individual test cards with the prepared culture suspension. Cards were held at 35.5°C for 18 h, with optical readings taken automatically every 15 min. Based on these readings, an identification profile was established and interpreted according to a specific algorithm. Final profile results were compared to the database, generating identification of the unknown organism. Final identifications listed as "excellent," "very good," "good," "acceptable," or "low discrimination" were considered correct. The data summary (Table 2) indicates one-choice correct results separately from low-discrimination correct results.

API. The API is composed of a 20-cupule plastic strip containing dehydrated carbohydrate substrates. Strips were inoculated with a 1:70 dilution of an organism suspension corresponding to a McFarland 2.0 standard and were incubated according to manufacturer's recommendations at 30°C for 48 to 72 h. After the recommended incubation, individual cupules in each strip were visually examined by the investigators for the presence of turbidity, which is indicative of carbohydrate assimilation. An additional 100 µl of the organism suspension was plated on cornmeal agar with Tween 80 (Remel, Lenexa, KS) and incubated at 30°C. Cornmeal plates were examined microscopically for the presence of hyphae or pseudohyphae. Profile numbers were generated based upon strip reactions and hyphal characteristics observed after 48 to 72 h of incubation at 30°C. Identification was made by profile number comparison to the APILAB plus V. 3.3.2 program. Final results were listed as "excellent," "very good," "good," "acceptable," "genus level," "low-discrimination," "presumptive," "doubtful," or "unacceptable" identifications. No distinction was made between excellent, very good, good, and acceptable matches. Additional supplemental tests were performed as directed by the manufacturer to confirm genus level, low-discrimination, presumptive, questionable, or doubtful identifications. Supplemental tests included rapid assimilation of trehalose, fermentation (glucose, maltose, sucrose, lactose, galactose, and trehalose), pigment production, phenol oxidase production, assimilation of nitrate and lactate, microscopic morphology, and growth with and without a fatty acid source (olive oil).

QC and challenge testing. Substrate reproducibility and QC testing were performed with the YST card using a set of 12 QC organisms, which were evaluated for 20 consecutive days. System reproducibility was assessed by testing each QC strain a total of 20 times to determine if the identification of the organism would be as expected $\geq\!95\%$ of the time, within a $\geq\!95\%$ confidence level. Challenge testing using the YST card was performed once using the 64 supplied isolates at each of the three sites. Personnel were blinded as to the identity of the challenge organisms. The API system was not tested against the challenge organisms at individual study sites, as all supplied challenge strains were previously characterized by the manufacturer.

Clinical and stock isolate testing. The API was considered the reference standard for this study. Concordant identifications between the YST card and API were considered correct. The API and YST identifications were made independently of each other, and personnel were blinded to the identity of the clinical and stock isolates. The purity of culture isolates was confirmed by streaking a portion of the suspensions used for each YST card and API strip to a Sabouraud dextrose agar plate, followed by 24 hours of incubation at 30°C. Repeat testing of clinical and stock isolates using both YST and API was performed once in instances of card termination, mixed morphology on purity plates, or a result of "no identification." Low-discrimination (slashline) results occur when the generated biopattern is insufficient to discriminate between two or more taxa. Low-discrimination calls on YST and API containing the species in question were considered correct for overall calculations of accuracy. Thirty-four isolates generating discrepant results between YST and API were blind coded and sent to a confirmatory laboratory (bioMérieux, Inc., Hazelwood, MO) for additional evaluation. Discrepancies were resolved based upon Wickerham liquid assimilation and fermentation tests in conjunction with macroscopic and microscopic morphology. Samples with incomplete identifications by multiple methods or species not among those organisms claimed to be identified by the YST database were excluded from the study.

Statistical analysis. Data from each of the three participating centers were combined. Determination of statistical significance was performed using McNemar's test with continuity correction. All tests of statistical significance were two tailed.

RESULTS

Testing of the YST card met substrate and system reproducibility criteria (\geq 95% correct with \geq 95% confidence intervals) with all QC organisms at each of the three sites. Of the 12 QC isolates tested, 3 (*C. guilliermondii*, *C. lipolytica*, and *G. capitatum*) gave low-discrimination correct results, while the remaining 9 isolates gave one-choice correct results (data not shown).

All 64 challenge strains were successfully identified by YST at each testing site (192/192; 100%) (Table 1). Of these, 30/192 (15.6%) returned a low-discrimination result. Low discrimination results were specifically noted with *Candida famata*, *Candida krusei*, *Candida lambica*, *C. parapsilosis*, *C. tropicalis*, *Cryptococcus albidus*, *G. capitatum*, *Saccharomyces cerevisiae*, and *Trichosporon asahii*.

Using clinical samples, the overall agreement between the YST card and API on initial testing was 95.0% (592/623). After confirmatory tests (Wickerham liquid assimilation, fermentation, and morphology), the YST card identified 614/623 (98.5%) clinical isolates correctly and 6/623 (1.0%) incorrectly, and 3/623 (0.5%) were unidentified. Within the 614 YST results considered correct, 118/623 (18.9%) were low-discrimination calls. When results of clinical and challenge testing were combined, YST correctly identified a total of 678/687 isolates (623 clinical and 64 challenge isolates) to the species level

TABLE 2. Results of testing clinical isolates with YST card compared to API^a

			No. of results			
Species	Total no. tested	Correct		•		
		One choice	Low discrimination	Incorrect identification	Unidentified	
Candida albicans	162	155	7	0	0	
Candida colliculosa	2	1	0	0	1	
Candida dubliniensis	9	9	0	0	0	
Candida famata	8	1	7	0	0	
Candida glabrata	82	72	9	1	0	
Candida guilliermondii	4	2	2	0	0	
Candida inconspicua	3	0	3	0	0	
Candida krusei	35	0	35	0	0	
Candida lambica	5	0	5	0	0	
Candida lipolytica	14	12	2	0	0	
Candida lusitaniae	24	22	2	0	0	
Candida magnoliae	1	1	0	0	0	
Candida norvegensis	1	1	0	0	0	
Candida parapsilosis	68	61	7	0	0	
Candida pelliculosa	1	1	0	0	0	
Candida rugosa	4	4	0	0	0	
Candida sphaerica	4	4	0	0	0	
Candida tropicalis	84	67	14	2	1	
Candida utilis	1	1	0	$\tilde{0}$	0	
Candida zevlanoides	1	1	ő	ő	0	
Cryptococcus albidus	12	10	2	0	0	
Cryptococcus laurentii	7	1	5	0	1	
Cryptococcus neoformans	16	15	0	1	0	
Cryptococcus uniguttulatus	1	0	0	1	0	
Geotrichum capitatum	1	0	1	0	0	
Geotrichum klebahnii	6	5	1	0	0	
Kloeckera spp.	1	1	0	0	0	
Kodamaea ohmeri	1	1	0	0	0	
Malassezia pachydermatis	1	1	0	0	0	
Pichia farinosa	1	1	0	0	0	
Prototheca wickerhamii	2	2	0	0	0	
Rhodotorula minuta	2	1	1	0	0	
Rhodotorula mucilaginosa	12	0	12	0	0	
	14	14	0	0	0	
Saccharomyces cerevisiae	14 1	0	0	0 1	0	
Sporobolomyces salmonicolor	3	3	0	0	0	
Stephanoascus ciferrii		•	*	*	0	
Trichosporon asahii	15	14	1	0		
Trichosporon inkin	2 12	2 10	0 2	0	0	
Trichosporon mucoides	12	10	<i>L</i>	U	U	
Total (%)	623	496 (79.6)	118 (18.9)	6 (1.0)	3 (0.5)	
Overall performance		614 (98.5)		6 (1.0)	3 (0.5)	

^a Includes confirmatory testing.

(98.7%). There were 30% low-discrimination and 1.0% unidentified results with the API (data not shown).

A detailed analysis of the 31 discrepant isolates indicates misidentification by YST and API to be 19.4% (6/31), and 54.8% (17/31), respectively. Misidentification with YST included two isolates of *C. tropicalis* and one isolate each of *C. glabrata*, *Cryptococcus neoformans*, *Cryptococcus uniguttulatus*, and *Sporobolomyces salmonicolor* (Tables 2 and 3). One isolate each of *Candida colliculosa*, *C. tropicalis*, and *Cryptococcus laurentii* was unidentified by YST. The API failed to identify one isolate each of *C. lambica*, *C. parapsilosis*, *Cryptococcus laurentii*, and *Pichia farinosa* (Table 3). The *P. farinosa* unidentified by the API is unclaimed by its database. When overall correct results were considered among discrepant isolates, the

enhanced performance of the YST card compared to API was statistically significant (P = 0.0176).

When species of *Cryptococcus* were tested, the YST card correctly identified 33/36 isolates. One isolate each of *Cryptococcus neoformans* and *Cryptococcus uniguttulatus* was incorrectly identified as *Rhodotorula glutinis/Rhodotorula mucilaginosa/Cryptococcus laurentii* by YST. One isolate of *Cryptococcus laurentii* was unidentified by the YST card (Table 3).

The YST card performed satisfactorily in the identification of non-Candida yeast species. All six isolates of Geotrichum klebahnii and a single isolate of G. capitatum were accurately identified by the YST card. However, one isolate of S. salmonicolor was incorrectly identified as Candida magnoliae/R. glutinis/R. mucilaginosa/Cryptococcus laurentii by YST.

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TABLE 3. Results of testing discrepant isolates

Referee final identification	Identification by ^a :				
Referee final identification	YST card	API			
C. colliculosa	Unidentified	C. colliculosa			
C. dubliniensis	C. dubliniensis	C. albicans			
C. famata	C. sake/ C. famata /C. lipolytica	C. sphaerica			
C. famata	C. sake/ C. famata /C. lipolytica	C. parapsilosis			
C. famata	C. sake/ C. famata /C. lipolytica	Candida spp.			
C. glabrata	Z. bailii/C. sake/C. famata/C. lipolytica	C. glabrata			
C. guilliermondii	C. guilliermondii	C. famata			
C. guilliermondii	C. guilliermondii	C. famata			
C. krusei	C. krusei/C. inconspicua/C. lambica/C. norvegenesis	C. zeylanoides			
C. lambica	C. krusei/C. inconspicua/ C. lambica	Unidentified			
C. norvegensis	C. norvegensis	C lambica/C. norvegensis			
C. parapsilosis	C. parapsilosis	C. famata			
C. parapsilosis	C. parapsilosis	S. cerevisiae			
C. parapsilosis	C. parapsilosis	Unidentified			
C. tropicalis	C. famata	C. tropicalis			
C. tropicalis	C. famata	C. tropicalis			
C. tropicalis	C. tropicalis	T. mucoides			
C. tropicalis	C. tropicalis	T. mucoides			
C. tropicalis	Unidentified	C. tropicalis			
Cryptococcus albidus ^b	Cryptococcus uniguttulatus/R. glutinis/ R. mucilaginosa/Cryptococcus laurentii	Cryptococcus laurentii			
Cryptococcus laurentii	R. glutinis/R. mucilaginosa/Cryptococcus laurentii	Unidentified (mixed)			
Cryptococcus laurentii	Unidentified	Cryptococcus laurentii			
Cryptococcus neoformans	Cryptococcus neoformans	C. glabrata			
Cryptococcus neoformans	R. glutinis/R. mucilaginosa/Cryptococcus laurentii	Cryptococcus neoformans			
Cryptococcus uniguttulatus	R. glutinis/R. mucilaginosa/Cryptococcus laurentii	Cryptococcus uniguttulatus			
G. klebahnii	G. klebahnii	C. utilis			
P. farinosa	P. farinosa	Unidentified			
R. minuta	R. minuta/M. furfur/C. sake/C. famata/C. lipolytica	R. mucilaginosa			
S. cerevisiae	S. cerevisiae	C. utilis			
S. cerevisiae	S. cerevisiae	C. pelliculosa			
S. salmonicolor	C. magnoliae/R. glutinis/R. mucilaginosa/ Cryptococcus laurentii	Candida spp.			

^a The final identification is in boldface.

DISCUSSION

The use of both the API 20C AUX and the VITEK 2 ID-YST for identification of commonly isolated yeast species in the clinical laboratory has been well established (6, 8, 10, 11, 16, 21). Particular attention has been given to the use of these two systems in the identification of non-C. albicans or emerging yeast species. Previous studies have indicated the identification of certain species (Candida dubliniensis, Candida inconspicua, Candida norvegensis, and Cryptococcus spp.) to be problematic with the VITEK Yeast Biochemical Card (YBC). For example, a study by Fenn et al. indicated that 2/6 (33.3%) of Cryptococcus albidus isolates and 1/1 (100%) of Cryptococcus laurentii isolates were incorrectly identified by YBC (7). In addition, Wadlin and colleagues reported that 3/23 (13%) of Cryptococcus neoformans isolates were incorrectly identified using the YBC system (21). The colorimetric YST card, designed to replace the fluorometric (ID-YST) card previously used with the VITEK 2 system, was evaluated here in a sideby-side comparison to API. Other studies have proven the utility of the API system as an acceptable reference method (20, 21).

Overall, the YST card performed well and demonstrated excellent reproducibility as shown by QC and challenge set testing. Seven (*C. glabrata* ATCC MYA2950, *C. lusitaniae* ATCC 3449, *C.*

utilis ATCC 9950, Geotrichum capitatum ATCC 28576, Kloeckera apis ATCC 32857, Prototheca wickerhamii ATCC 16529, and Trichosporon mucoides ATCC 204094) of the 12 QC isolates were retained for final use in product QC. Among clinical isolates, there was high accuracy with YST (98.5%) compared to the API, with fewer incorrect and unidentified results (Table 3). Lowdiscrimination results were reduced from 30.0% with API to 18.9% with YST. Low-discrimination results occur when the generated biopattern is not sufficient to discriminate between two or more taxa. For both YST and API, low-discrimination results were always observed for the following species: C. inconspicua, C. krusei, C. lambica, and G. capitatum. This is due to the fact that these species are fairly unreactive and very similar in their carbohydrate assimilation profiles. Slashline identifications were technically considered to be low discrimination and required supplemental observation or testing to resolve to a single taxon. It is important to remember that basic techniques (e.g., microscopic morphology, nitrate assimilation, and pigment production) are straightforward and should not be discounted in the resolution of low-discrimination or slashline results. In addition, the VITEK 2 software can be user configured to automatically select the most commonly occurring taxon in a specific slashline(s). In some cases (e.g., C. krusei) complete resolution is important, as it can be critical for clinical decision-making or epidemiological analysis.

b See Discussion.

In this study, YST showed definite improvement in the identification of *Cryptococcus* spp. Other than *Cryptococcus unigut-tulatus*, identification of non-*Cryptococcus neoformans* species by YST was much improved compared to that in previous studies (4, 7). This is a welcome enhancement, considering the serious clinical implications of cryptococcal infection. A single isolate of *Cryptococcus albidus* was initially misidentified as *Cryptococcus uniguttulatus/R. glutinis/R. mucilaginosa/Cryptococcus laurentii* by YST and as *Cryptococcus laurentii* by API (Table 3). However, additional biopattern analysis of the YST data from the trial site indicated *Cryptococcus albidus* as a possible choice, and thus the identification was resolved in favor of YST.

Species of *Geotrichum* have been reported as difficult to identify with the fluorometric ID-YST card due to low reactivity (8). In terms of *Geotrichum* spp. with the VITEK 2, it is important to note that the organism can be somewhat refractory to preparation of a uniform suspension in saline prior to filling the YST card. Additional technical care must be taken in order to achieve a McFarland 2.0 turbidity, increasing the probability of a successful test. In this study, identification of *Geotrichum* spp. with the YST card was excellent (7/7; 100%).

The majority of discrepant isolate identifications, as determined by confirmatory testing, were resolved in favor of the YST card. In this study, discrepant results were arbitrated through the use of Wickerham liquid assimilation and fermentation testing in combination with morphological observations. Unfortunately, this classical method may take up to a month for complete analysis (23). Confirmation of discrepant yeast identifications could be addressed by the use of molecular methods, since they can be highly accurate and can be completed in as little as 24 h. Massonet and colleagues recently published a comparison of ITS2 rRNA sequencing to use of the VITEK 2 fluorometric ID-YST card (11). In addition, Hall et al. reported use of sequencing of the D2 region of the large-subunit ribosomal DNA in the identification of uncommonly encountered clinical yeast isolates (9).

A previous study at a single site evaluated the performance of the new YST card in comparison to an older fluorimetric version of the card and the use of Chromagar as a source medium for testing with the YST card (1). However, that study did not evaluate the entire range of claims made for the YST card, and so we report here a multicenter study comparison of the VITEK 2, utilizing the updated colorimetric YST card versus the API 20 AUX system for both common and rarely observed yeast and yeast-like organisms. The YST card was found to be highly accurate, correctly identifying 98.5% of clinical isolates. A compelling advantage of YST over API was fewer misidentified or unidentified results and the reduction of turnaround times for identification from 48 to 72 h to 18 h. Because of automated interpretation, results from the YST card are objective compared to the subjective observer-driven results of API. The setup of YST is simple, requires less technologist time than the API, and is less prone to operator error. We conclude that overall, the VITEK 2 with the updated colorimetric YST card is a valuable addition in the identification of medically encountered yeast species and is a useful addition to the clinical mycology laboratory.

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