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# Prospective Evaluation of the New Chromogenic Medium Candida ID, in Comparison with Candiselect, for Isolation of Molds and Isolation and Presumptive Identification of Yeast Species

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**We conducted a prospective evaluation of Candida ID chromogenic medium (bioMérieux, Marcy l'Etoile, France) with 786 clinical specimens in comparison with Candiselect medium (Bio-Rad, Marnes la Coquette, France). Candida ID chromogenic medium identified 97.7% of *Candida albicans* strains; enabled presumptive identification of *C. tropicalis*, *C. lusitaniae*, *C. guillermondii*, and *C. kefyr* and better detection of yeast combinations (11.4% more often); and was more sensitive for the isolation of filamentous fungi (17.7% more often). However, Candida ID chromogenic medium appeared to be less selective vis-à-vis bacteria, with bacterial colonies sometimes pigmented blue.**

The increasing frequency of fungal infections is being accompanied by a diversification in the fungal species involved (2, 7). While *Candida albicans* is still the most commonly isolated yeast, the frequencies of isolation of non-*C. albicans* yeast species are steadily increasing (1). Rapid and accurate diagnosis of mycological infections is required before the appropriate treatment can be instigated. Several chromogenic media for isolation and identification of *C. albicans* in a single step according to the characteristic pigmentation of the colonies are available; the use of such media also makes it easier to detect yeast combinations (4). Candida ID chromogenic medium (CAID; bioMérieux, Marcy l'Etoile, France) has recently been developed and marketed for the identification of *C. albicans* (blue colonies) and the presumptive identification of the yeasts *Candida tropicalis*, *Candida lusitaniae*, *Candida kefyr*, and *Candida guillermondii* (pink colonies). Our aim was to make a prospective evaluation of the performance of CAID in comparison with that of Candiselect medium (CS; Bio-Rad, Marnes la Coquette, France) with clinical specimens. CS is the routine medium used in our laboratory and was considered the reference medium in this study.

Seven hundred eighty-six clinical samples (192 respiratory samples, 182 urine samples, 95 stool samples, 149 samples from the mouth and nose, 68 vaginal samples, 34 catheters, 18 skin swabs, 20 peritoneal fluid samples, 28 samples from other sources) were prospectively inoculated on both CS and CAID at the same time. The plates were incubated at 35°C for 7 days and were read every 24 h. All isolates were analyzed macroscopically according to the manufacturers' recommendations and were then identified by conventional methods. The yeasts were identified on the basis of their microscopic morphologies on potato-carrot-bile medium (Bio-Rad) after 48 h at 27°C and on the basis of their physiological characteristics (Galeries Auxacolor [Bio-Rad] and, if necessary, ID 32C [bioMérieux]) and/or immunological characteristics (Bichrolatex albicans and Krusei color; Fumouze, Levallois-Perret, France). Filamentous fungi were identified on the basis of their morphological characteristics directly on the primary culture or after reinoculation at 27°C on 2% malt medium. For each medium, we noted the following parameters: number of positive cultures, number of colonies, macroscopic appearance of the colonies (morphology and pigmentation), and the final identities of all isolates. Data were statistically analyzed by the chi-square test.

Of the 786 samples inoculated, cultures were positive for 300 samples on CAID and 301 samples on CS. For the two media, these represented 332 yeast and 17 filamentous fungus isolates.

Significantly more different yeast strains were isolated in 48 h on CAID than on CS (323 of 332 [97.3%] versus 309 of 332 [93.1%] isolates; $P = 0.011$) (Table 1). The proliferation rate and the number of colonies per plate were similar for both media. The sensitivity of identification of *C. albicans* by blue staining of the colonies was 97.7% (170 of 174 isolates) for CAID and 90% (154 of 171 isolates) for CS. The difference was statistically significant ($P = 0.003$), and the blue staining was stronger after 24 h of culture on CAID. These results are consistent with those of other investigators (3, 5, 6, 8). The nonpigmented *C. albicans* isolates usually grew as abundant cultures with confluent colonies which more often remained white on CS than on CAID. All except one of the white strains of *C. albicans* stained blue after reisolation. On CAID, 98.8% of *C. tropicalis* strains (23 of 24 strains) were pink within 48 h, with 12 being dry, an appearance that we did not observe for any of the other species isolated in this series. On CS, the majority of *C. tropicalis* isolates were easily recognizable due to their dry, pleated, white appearance, and 82.6% (19 of 23) of the isolates gradually turned blue from the center of the colony. This appearance was very characteristic and differed from that of the *C. albicans* colonies. On CAID the colonies of *C. kefyr* (five of five colonies), *C. lusitaniae* (three of three colonies), and *C. guillermondii* (two of three colonies) were pink and shiny within 48 h, as expected. Except for two strains of

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TABLE 1. Coloration of colonies obtained on CAID and CS after 48 h of incubation at 35°C

| Species (no. of isolates on any medium; $n$ = 332) | No. of isolates | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | CAID ($n$ = 323) | | | | | CS ($n$ = 309) | | | |
| | Blue | White | Pink | Other | Total | Blue | White | Other | Total |
| *C. albicans* (179) | 170 | 4 | 0 | 0 | 174 | 154 | 17 | 0 | 171 |
| *C. tropicalis* (24) | 0 | 1 | 23 | 0 | 24 | 19[a] | 4 | 0 | 23 |
| *C. kefyr* (5) | 0 | 0 | 5 | 0 | 5 | 0 | 1 | 0 | 1 |
| *C. guillermondii* (3) | 0 | 1 | 2 | 0 | 3 | 0 | 3 | 0 | 3 |
| *C. lusitaniae* (3) | 0 | 0 | 3 | 0 | 3 | 0 | 1 | 0 | 1 |
| *C. glabrata* (76) | 0 | 73 | 0 | 0 | 73 | 0 | 70 | 0 | 70 |
| *C. parapsilosis* (8) | 0 | 7 | 0 | 0 | 7 | 0 | 7 | 0 | 7 |
| *C. inconspicua* (2) | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 0 | 2 |
| *C. krusei* (2) | 0 | 2 | 0 | 0 | 2 | 0 | 2 | 0 | 2 |
| *C. famata* (2) | 0 | 0 | 2 | 0 | 2 | 0 | 1 | 0 | 1 |
| *S. cerevisiae* (25) | 0 | 25 | 0 | 0 | 25 | 0 | 25 | 0 | 25 |
| *Rhodotorula* sp. (2) | 0 | 0 | 0 | 2 | 2 | 0 | 0 | 2 | 2 |
| *Trichosporon cutaneum* (1) | 1[a] | 0 | 0 | 0 | 1 | 1[a] | 0 | 0 | 1 |

[a] Rough colonies.

TABLE 2. Detection of multiple yeast species on CAID and CS after 48 h of incubation at 35°C

| Species | No. of mixed cultures detected on: | | | |
|---|---|---|---|---|
| | Any medium | Both media | CAID only | CS only |
| *C. albicans* + *C. glabrata* | 36 | 29 | 4 | 3 |
| *C. albicans* + *C. tropicalis* | 5 | 4 | 1 | 0 |
| *C. albicans* + *C. guillermondii* | 1 | 1 | 0 | 0 |
| *C. albicans* + *S. cerevisiae* | 6 | 6 | 0 | 0 |
| *C. albicans* + *Rhodotorula* sp. | 1 | 1 | 0 | 0 |
| *C. tropicalis* + *C. glabrata* | 2 | 2 | 0 | 0 |
| *S. cerevisiae* + *C. glabrata* | 5 | 5 | 0 | 0 |
| *S. cerevisiae* + *C. kefyr* | 4 | 0 | 4 | 0 |
| *S. cerevisiae* + *C. lusitaniae* | 1 | 0 | 1 | 0 |
| *C. guillermondii* + *Rhodotorula* sp. | 1 | 1 | 0 | 0 |
| *C. guillermondii* + *C. famata* | 1 | 0 | 1 | 0 |
| *C. albicans* + *C. glabrata* + *S. cerevisiae* | 3 | 3 | 0 | 0 |
| *C. albicans* + *C. glabrata* + *C. tropicalis* | 3 | 3 | 0 | 0 |
| *C. albicans* + *C. glabrata* + *T. cutaneum* | 1 | 1 | 0 | 0 |
| Total | 70 | 56 | 11 | 3 |

*Candida famata* and *Rhodotorula* sp., the other yeast species isolated were white on CAID. A pink pigmentation of some strains has already been reported for several other species, such as *Candida sphaerica*, *Candida pelliculosa*, *Candida utilis*, *Candida glabrata*, and *Saccharomyces cerevisiae* [5, 8; A. Freydières, F. Parant, C. C., and Y. Gille, abstract from the 10th European Congress of Clinical Microbiology and Infectious Diseases, Clin. Microbiol. Infect. **6**(Suppl. 1)**:**181, 2000]. We did not observe this staining for our *C. glabrata* and *S. cerevisiae* isolates. Pink isolates on CAID require further conventional tests for definite identification because four common non-*C. albicans Candida* species (*C. tropicalis*, *C. kefyr*, *C. lusitaniae*, and *C. guillermondii*) and, according to the literature (5), some rarer species can produce such colored colonies. However, in our series, the pink coloration of the colonies provided a helpful presumptive identification for *C. lusitaniae*, *C. kefyr*, and *C. guillermondii*, whose morphological characteristics are often poor. Other species showed characteristic and evocative macroscopic appearances on both media. *C. krusei* constantly presented very characteristic, dry, dirty white colonies. Only one strain of *Trichosporon cutaneum* was isolated, and colonies of *T. cutaneum* had a cerebriform beige-white appearance and were tinted blue at the center.

Seventy of 301 samples (23.2%) contained two (63 times) or three (7 times) yeast species in combination (Table 2). These combinations were statistically more often detected (11.4% more often; $P = 0.024$) on CAID (67 times) than on CS (59 times). Combinations with *C. kefyr* (four times), *C. lusitaniae* (one time), or *C. guillermondii* (one time) were detected only on CAID by pink staining of the colonies. It was easy to detect *C. tropicalis* in the mixed cultures on both media.

A filamentous fungus was isolated from 17 samples (Table 3). Although the difference was not statistically significant ($P = 0.070$), CAID was shown to perform better than CS, with a higher isolation yield (CAID, 17 isolates; CS, 14 isolates), more numerous colonies, and more rapid growth. The presence of a filamentous fungus was visible on CAID before it was visible on CS, saving 24 h in the time required to obtain the result. Although the chromogenic media were not initially designed to improve the recovery of molds, good sensitivity is of major importance for clinical laboratories processing specimens from patients at high risk of invasive mold infections.

Bacterial colonies grew from 23 samples, with 8 isolates growing on CS and 20 isolates growing on CAID, which would thus appear to be less selective vis-à-vis bacterial contamination ($P = 0.0007$). On CS, the bacterial colonies could be easily identified by their translucent whitish appearance. However, on CAID we observed not only translucent white bacterial colonies ($n = 10$) but also pink ones ($n = 5$) and blue ones ($n = 5$). The latter were sometimes strongly stained and were macroscopically very similar to *C. albicans*, and the only way of determining whether these were bacteria was by direct examination of these colonies. In addition, we noticed that stool samples cultured on CAID frequently stained blue, which also proved to be a source of error. In two cases, the massive proliferation of bacteria totally inhibited the growth of yeasts on CAID, whereas the yeasts were detected on CS.

In conclusion, this prospective evaluation of CAID in comparison with CS with clinical samples demonstrated that CAID is an effective and useful new chromogenic medium for clinical laboratories. CAID (i) identified *C. albicans* with a sensitivity of 97.7% after culture for 48 h; (ii) enabled better detection of combinations of yeasts; (iii) provided an improved ability to identify yeast species that are sometimes difficult to identify, such as *C. lusitaniae*, *C. kefyr*, and *C. guillermondii*; and (iv) had

TABLE 3. Filamentous fungi detected on CAID and CS

| Species | No. of isolates observed on: | | | |
|---|---|---|---|---|
| | Any medium | Both media | CAID | CS |
| *Aspergillus fumigatus* (more than one colony) | 7 | 6 | 7 | 6 |
| *Geotrichum candidum* | 5 | 4 | 5 | 4 |
| *Mucor racemosus* | 4 | 3 | 4 | 3 |
| *Fusarium proliferatum* | 1 | 1 | 1 | 1 |
| Total | 17 | 14 | 17 | 14 |

an improved yield and speed of isolation of a filamentous fungus. However, we consider CAID to be insufficiently selective vis-à-vis bacteria, with a resulting risk of inhibition of fungal growth in specimens with polymicrobial growth and a risk of confusion between blue-stained bacterial colonies and *C. albicans*.

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