

Comparison of Chromogenic and Fluorogenic Membrane Filtration Methods for Detection of Four *Candida* Species

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CHROMagar Candida medium was inoculated with a variety of clinical samples ($n = 282$) by using membrane filtration instead of swabbing or streaking. This modified CHROMagar procedure increased the ability to detect yeast by 16.7%. Compared to an enzymatic two-step membrane filtration method with fluorogenic substrates, it yielded similar recovery but was slower.

CHROMagar Candida (CA; CHROMagar, Paris, France) is a differential medium used for isolation and presumptive identification of clinically important *Candida* spp. (1, 2, 5–7). After 24 to 48 h of incubation, contrasting colored colonies result from the cleavage of chromogenic substrates by species-characteristic enzymes. The value of this medium for the isolation of *Candida* spp. from samples containing low numbers of cells has not been systematically addressed. Inoculation by streaking or swabbing of a small sample volume is a sensitivity-limiting factor and may account for a certain false-negative rate. In addition, the incubation time required for CA is long in connection with the diagnosis of life-threatening candidemia.

We have previously improved the sensitivity and speed of enzymatic methods for detection of yeasts by an unusual two-step method (TSM), consisting of microcolony formation on a nylon membrane filter followed by an assay of enzyme activities using fluorogenic substrates in the presence of a membrane permeabilizer (3). This TSM allows the detection of low numbers of *Candida* organisms, even in a mixed flora with one predominant species, in as few as 9 to 11 h.

The aim of the present study was to evaluate a possible increase in sensitivity and speed by combining CA with membrane filtration and to compare this modified method (m-CA) with the TSM.

For the comparison of CA and m-CA, 282 clinical specimens, including oropharyngeal swabs, samples from tracheo-esophageal voice prostheses (TVPs) (4), vaginal swabs, and whole-blood samples, were used.

For comparison of m-CA and the TSM, 776 additional samples were analyzed, including oropharyngeal exudates, TVPs, and oropharyngeal and vaginal swabs.

In the CA procedure, plates were inoculated by streaking or swabbing 100 μ l of the sample onto the plate surface. For m-CA and the TSM, swabs and TVPs were extracted by vortex mixing in 10 ml of 1% aqueous peptone. Liquid samples such as exudates were used without pretreatment. The total volume was divided into two equal parts, except for blood samples, 100 μ l of which was used for each test. The parts were filtered over

47-mm-diameter, 0.45- μ m-pore-size nylon membrane filters (Nylaflo; Gelman Sciences, Ann Arbor, Mich.) to be used in the m-CA and TSM procedures, respectively. The first membrane filter was incubated for 48 h at 37°C in an air incubator on CA. Presumptive identification of *Candida albicans*, *C. glabrata*, *C. krusei*, and *C. tropicalis* was achieved after 24 to 48 h. The second membrane filter was incubated on a selective medium for 9 to 11 h at 37°C to yield microcolonies (3). The filter was subsequently cut into four quarters that were placed on absorbent fiberglass pads (Gelman Sciences), each impregnated with a specific fluorogenic 4-methylumbelliferyl substrate or no substrate, respectively, and 0.1% digitonin (Sigma, St. Louis, Mo.) acting as a membrane permeabilizer. The combination of the membrane filter and pad was incubated for 30 min, resulting in blue (*C. albicans*, *C. krusei*, and *C. tropicalis*) or orange (*C. glabrata*) fluorescent microcolonies under long-wavelength UV light.

Confirmatory tests included germ tube formation, API 20C AUX, morphology on cornmeal agar, and latex agglutination (Krusei-Color; Fumouze, Levallois Perret, France).

As m-CA and the TSM were experimental methods and no definitive reference method was available, the actual number of yeast-positive samples was unknown. Therefore, the recovery of the method that yielded the highest number of isolates, i.e., the TSM, was set at 100% and the recovery of m-CA was calculated relative to that of the TSM. Presumptive identification of isolates was based on the typical colors of the colonies in m-CA and fluorescence of the microcolonies in the TSM. Identification was considered definitive if both methods yielded the same results. In case of questionable colors or fluorescence or disagreement between the two methods in terms of numbers or identities of isolates, confirmatory tests were performed.

The CA and m-CA procedures yielded 147 and 194 *Candida*-positive samples, respectively, corresponding to a difference in recovery of 16.7% in the favor of m-CA. In 27 (13.9%) out of 194 samples, m-CA recovered multiple *Candida* species while CA detected a single one. Furthermore, in 20 (10.3%) out of 194 samples, including 10 blood samples from candidemic patients, low numbers (≤ 10 CFU per plate) of *Candida* sp. were obtained with the m-CA procedure but a zero count on CA was obtained. The superior recovery of *Candida* spp. on m-CA relative to CA stems from the ability of membrane

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filtration to handle larger sample volumes, thus lowering the detection limit and favoring the demonstration of minor species in a mixed yeast flora. However, membrane filtration did not improve the detection speed of m-CA relative to that of CA, as both procedures required 24 to 48 h before fully colored colonies became visible.

In contrast, membrane filtration is an integral part of the TSM to enable the TSM. The key to the reduction of the detection time to 9 to 11 h in the TSM is the demonstration of the enzyme activities in the microcolonies after rather than during growth so that an otherwise growth-inhibiting membrane permeabilizer can be used to enhance the rate of cellular uptake of the substrates. A second factor contributing to detection time reduction is the amplification of fluorescence at the surface of a nylon membrane (3).

Apart from a substantial difference in speed, the m-CA and TSM procedures performed remarkably similarly. The numbers of yeast isolates detected by the two methods were nearly identical, i.e., 699 and 701, respectively. The recovery by m-CA was 99.0% relative to that of the TSM, which was set at 100%. Identification was correct for 100% (m-CA) and 99.3% (TSM) of the isolates, respectively. In 5 (3.8%) out of 131 samples, *C. glabrata* was misidentified by the TSM but not by the m-CA procedure. However, this was offset by the fact that the TSM detected *C. glabrata* in five other samples with mixed *Candida*

flora while m-CA failed to do so. In 496 samples, no yeasts were found with either of the two methods.

The gain in speed afforded by the TSM would be useful in detecting cases of candidemia. The higher recovery rate of minor, potentially azole-resistant species such as *C. krusei* and *C. glabrata* from mixed flora by both m-CA and the TSM would also be significant in this context but less relevant in connection with mucosal samples.

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