

Rapid Trehalose Assimilation Broth Procedure

I. Principle

Remel Rapid Trehalose Broth is a liquid medium recommended for use in qualitative procedures for the presumptive identification of *Candida (Torulopsis) glabrata*. *Candida glabrata* represents approximately 20% of all yeasts recovered from urine cultures. *C. glabrata* has also emerged as an opportunistic pathogen with cases of endocarditis, meningitis, and disseminated infection. The basal medium contains trehalose, yeast nitrogen, and bromcresol green as pH indicator. A heavy inoculum is used to facilitate rapid results. The increased incubation temperature of 42°C improves the sensitivity of the test.

II. Specimen Information

Use only pure isolates of yeast for testing. Isolates that appear white to pink on CHROMagar Candida should be screened using the Rapid Trehalose Broth. Test isolates can be up to 72 h old. Sufficient growth is needed to make a cloudy suspension.

III. Reagents & Equipment

- Remel Trehalose Assimilation Broth tube
Store at 2-8°C until used. Allow product to equilibrate to room temperature before use. Do not incubate prior to use. Do not use product if there is evidence of contamination, the color has changed, or if the expiration date has passed.
- Sterile inoculation loop
- Aerobic 42°C incubator

IV. Procedure

1. Using a sterile inoculation loop, select several colonies of the yeast isolate and emulsify in the broth to create a cloudy suspension.
2. Incubate tube(s) aerobically at 42°C. For best results, cover the opening by placing the cap on the tube without tightening.
3. Monitor tubes for up to three hours for a color change to yellow.

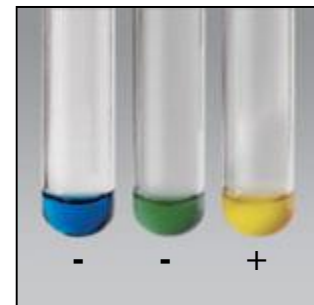
V. Interpretation

Positive Test

Broth color changes from blue to yellow or yellow-green within 3 h of incubation. White to pink colonies from CHROMagar Candida that yield a positive result may be identified as *C. glabrata*.

Negative Test

A blue, blue-green, or green color after 3 h of incubation indicates a negative result. These isolates require further testing, such as API 20C AUX or the Uni-Yeast Tek Plate, for identification.



VI. Quality Control

Each new lot or shipment of reagent should be tested for quality control by using the following test strains:

Positive: *Candida glabrata* ATCC 15126

Negative: *Candida albicans* ATCC 90028

VII. Limitations

Incubation beyond 3 hours may result in false positive reactions. False negative reactions are reported to occur with < 10% of *C. glabrata* isolates. Since isolates that yield a negative Rapid Trehalose Assimilation reaction undergo further testing, they should be properly identified.

VIII. Verification of Test Method

A total of 27 yeast isolates were tested in this evaluation of the Remel Rapid Trehalose Assimilation Broth. These included clinical isolates, isolates from previous CAP surveys, and ATCC strains. Clinical and CAP isolates were previously identified using either the API 20C AUX (bioMerieux, Durham, NC) or the Uni-Yeast Tek Plate (Remel, Lenexa, KS). The isolates tested included 11 *C. glabrata*, 12 *C. parapsilosis*, 2 *C. guilliermondii*, 1 *C. lusitanae*, and 1 *S. cerevisiae*. All of these isolates produced either white or pink colonies on BBL CHROMagar Candida.

Each isolate was subcultured to CHROMagar Candida and incubated for 48 h. Individual isolates were inoculated into separate tubes following the procedure outlined above. The tubes were examined at 1.5 h, after which all of the positive reactions had occurred. The tubes were incubated for an additional 1.5 h and examined for a final interpretation. All 11 (100%) of the *C. glabrata* isolates yielded positive results. All of the other yeast isolates tested yielded negative results. These results correlate well with larger studies that have been published. Some isolates of *C. albicans* and *C. tropicalis* are reported to yield positive results. However, these isolates would not likely be tested in our laboratory, as their morphology on CHOMagar Candida is much different than that of *C. glabrata*.

IX. References

- A. Package insert: Remel Rapid Trehalose Assimilation Broth, IFU 64859-PI, revised January 23, 2007.
- B. Fenn, J. P., E. Billedeaux, H. Segal, L. Skodack-Jones, P. E. Padilla, M. Bale, and K. Carroll. 1999. Comparison of four methodologies for rapid and cost-effective identification of *Candida glabrata*. *J. Clin. Microbiol.* 37:3387-3389.
- C. Silvius, M.A. and S. Meng. 1997. Abstract C241. Abstracts of the 97th General Meeting of the American Society for Microbiology. ASM, Washington, D.C.

X. Document Control

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