Evaluation of a New Commercially Available Rapid Assimilation of Trehalose (RAT) Test for the Identification of *Candida glabrata*

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**ABSTRACT**

**BACKGROUND:** As the occurrence and significance of *C. glabrata* in clinical specimens increases it is ever more important that a prepared, reliable, and inexpensive RAT test be available for routine use. This study evaluates a new commercial RAT test and compares results of isolates taken from 2 prominent isolation media.

**METHODS:** 40 *C. glabrata* and 40 other *Candida* species (12 *C. albicans*, 12 *C. parapsilosis*, 9 *C. tropicalis*, 4 *C. lusitaniae*, 3 *C. krusei*) were inoculated onto CHROMagar Candida (CAC) and Sabouraud Dextrose Agar (SDA) (both from Becton Dickinson [BD], Cockeysville, MD) and incubated for 48 hours at 30°C. The Trehalose Screen (Scientific Device Laboratory [SDL], Des Plaines, IL) consists of a slide with 4 substrate lined wells. Each well was rehydrated with 100 µL of sterile water; a heavy inoculum (3-5 colonies) from CAC and SDA was then mixed into each well to form a homogenous suspension. The tests were incubated at 37°C and examined at 1 & 4 hours. A positive reaction was indicated by a change in suspension color from green to yellow.

**RESULTS:** The reactions were very clear and easy to read. All *C. glabrata* tested from CAC were RAT test positive at 1 and 4 hours (sensitivity 100%). The same isolates from SDA yielded 4 false negatives (sensitivity 90%). Of the other *Candida* species, all were RAT test negative from CAC at 1 and 4 hours and from SDA at 1 hour (specificity 100%). However, 7 isolates from SDA (5 *C. albicans*, 2 *C. tropicalis*) produced false positive results at 4 hours (specificity 83% with extended incubation).

**CONCLUSION:** The new SDL RAT assay is very useful for distinguishing *C. glabrata* from other *Candida* species. Optimal results are obtained with isolates from BD’s CAC (sensitivity & specificity 100%). One hour incubation is best, as isolates from SDA may give false positives if incubated for 4 hours. Additionally, isolates of *C. glabrata* from SDA may give false negative results and require further testing for identification. Of note, the SDL assay closely mimics the previously reported results of the NCCLS (M35-P) proposed method and has a more rapid reaction time and is more reasonably priced than other products currently on the market.

**INTRODUCTION**

*C. glabrata*, once considered to be a nonpathogenic saprophyte, has become an increasing cause of systemic and mucosal fungal infections following increased use of immunosuppressive therapy and broad spectrum antimicrobial drugs. In our institution, *C. glabrata* is the 2nd most common clinically encountered yeast. The organism is known to exhibit increased resistance to fluconazole with previous exposure to the drug. The infections caused by *C. glabrata* may have a high mortality rate in immunocompromised patients. Therefore, early and accurate identification has a significant effect on patient management. *C. glabrata* can be distinguished from other *Candida* species by its ability to rapidly assimilate trehalose. As the importance of *C. glabrata* in the clinical setting becomes more apparent, it is necessary that a prepared, reliable, and inexpensive RAT test be in routine use. This study evaluates a new commercially available RAT test and compares results of isolates when taken from 2 prominent isolation media.
MATERIALS AND METHODS

• 40 C. glabrata and 40 isolates of other Candida species from clinical stock cultures were inoculated onto CHROMagar Candida (CAC) and Sabouraud Dextrose Agar (SDA) and incubated at 30°C for 48 hours.

• The Trehalose Screen (Scientific Device Laboratory [SDL], Des Plaines, IL) was performed on each of the 80 yeast isolates; the organisms were tested from both CAC and SDA.

• The RAT screen test consists of 4 substrate-lined wells which were rehydrated with 100 µL of sterile water.

• 3-5 colonies from CAC and from SDA were mixed into individual wells to form a homogenous suspension.

• Tests were incubated at 37°C in ambient air and examined at 1 and 4 hours.

• A positive reaction was indicated by a color change from blue to clear yellow or tan-yellow. Blue, green, or greenish-yellow indicated a negative reaction.

RESULTS

![Images of yeast cultures on CAC and SDA](images)

- C. glabrata/C. parapsilosis
- C. albicans/C. tropicalis

RAT test slide (before rehydration)

RAT test slide wells rehydrated with 100 µL sterile H₂O

Inoculate each well with 3-5 colonies of yeast

Appearance of inoculated RAT tests after 1 hour incubation

- C. glabrata
- C. parapsilosis
- C. albicans
- C. tropicalis

Isolates from SDA

Isolates from CAC
• Reactions were clear and easy to read with optimal reaction time of 1 hour.
• Positive reactions on isolates from CAC were yellow with a beige tinge while those from SDA were bright yellow.
• Table 1 shows the RAT test results for *C. glabrata* and the other *Candida* species.

Isolates grown on CAC yielded 100% sensitivity and 100% specificity for all *Candida glabrata* and other *Candida* spp.

Isolates grown on SDA yielded 90% sensitivity at both 1 & 4 hours; The specificity was 100% at 1 hour and 83% at 4 hours.

Table 1. RAT test results for *C. glabrata* and other *Candida* species from CAC vs SDA

<table>
<thead>
<tr>
<th>Organism</th>
<th>#</th>
<th>CAC 1 hr</th>
<th>CAC 4 hr</th>
<th>SDA 1 hr</th>
<th>SDA 4 hr</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>40</td>
<td>0</td>
<td>40</td>
<td>0</td>
<td>40</td>
</tr>
<tr>
<td><em>C. albicans</em></td>
<td>12</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td><em>C. parapsilosis</em></td>
<td>12</td>
<td>0</td>
<td>12</td>
<td>0</td>
<td>12</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>9</td>
<td>0</td>
<td>9</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td><em>C. lusitaniae</em></td>
<td>4</td>
<td>0</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td><em>C. krusei</em></td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
</tbody>
</table>

CAC = CHROMagar Candida;  
SDA = Sabouraud Dextrose Agar

Table 2. Calculation of Sensitivity & Specificity or RAT test from CAC vs SDA

<table>
<thead>
<tr>
<th></th>
<th>CAC</th>
<th>SDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 HR</td>
<td>4 HR</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td>Specificity</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>

DISCUSSION

• As compared to SDA, CAC is the better medium from which to take isolates for the SDL RAT test (as seen in the Tables).
• One hour incubation is best for the SDL RAT test, as isolates from SDA may give false positives if incubated for 4 hours and lead to misidentification (as observed in isolates of *C. albicans* and *C. tropicalis*).
• Isolates of *C. glabrata* from SDA may give false negative results at both 1 & 4 hours and require further testing for identification; this would not lead to misidentification.
• The SDL RAT assay closely mimics the reported results of the NCCLS (M35-P) proposed method developed at Mayo Clinic (Stockman and Roberts, 1985).
• We now routinely use the SDL Trehalose Screen in our clinical mycology laboratory.
**CONCLUSIONS**

- Optimal results on the SDL Trehalose Screen are obtained from BD's CAC (sensitivity and specificity 100%) as isolates from SDA may give false negative results (sensitivity 90%).

- 1 hour incubation is optimal as isolates from SDA may give false positive results if incubated longer (specificity 83% at 4 hours).

- The SDL Trehalose Screen is easy to read, yields excellent results, is inexpensive, and has a more rapid reaction time than current products on the market.

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**ACKNOWLEDGEMENTS**

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