PROVIDENCE
Sacred Heart
Medical Center &
Children's Hospital

CHROMagar™ Candida Procedure

Department of Microbiology

Effective date: 08/2008

Last Revision: 08/04/2008

Last reviewed: 04/2014

Table of Contents

1.0 Principle

BBL™ CHROMagar™ Candida is comprised of peptones, glucose, chloramphenicol, and a chromogenic mix, consisting of artificial substrates. These chromogens release different colored compounds upon degradation by specific enzymes. This permits the differentiation of certain species of *Candida*. Due to the differences in morphology and colors of yeast colonies, this medium also facilitates the detection of mixed yeast cultures. After 48 h incubation, *C. albicans*, *C. krusei*, and *C. tropicalis* produce unique colonies that permit identification without further testing. Other *Candida* species require further testing for identification.

2.0 Clinical Significance

BBL™ CHROMagar™ Candida is a selective and differential medium for the isolation and identification of *Candida* species from clinical specimens.

3.0 Scope

This procedure is classified under CLIA as Highly Complex. It should be carried out by technical personnel familiarized and trained to identify yeast. Testing includes but is not limited to: morphologic recognition, confirmatory testing, and Quality Control testing of media.

4.0 Specimen Requirements

Specimens submitted for fungal culture or yeast screen should be inoculated onto CHROMagar™ Candida. Refer to specimen setup charts and procedures for appropriate media selection and incubation.

5.0 Materials

- Aerobic (non-CO_2) incubator set at 35 ± 2°C
- Sterile inoculation loop
- BBL™ CHROMagar™ Candida Agar
  Store in the dark at 2 to 8°C in original sleeve wrapping and original cardboard box until time of inoculation. Plates may be inoculated up to the expiration date.

6.0 Procedure

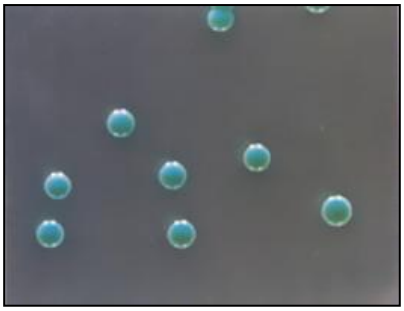
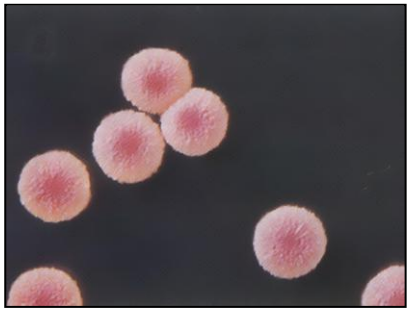
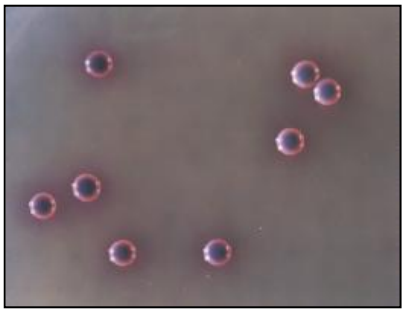
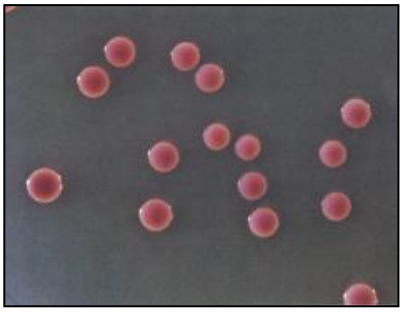
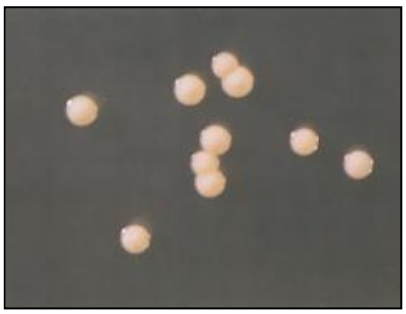
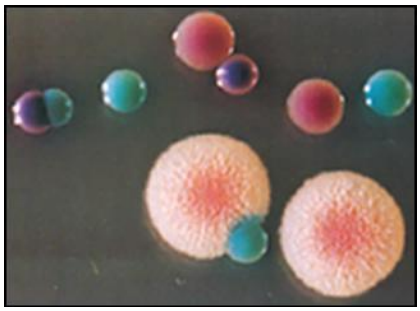
1. Inoculate specimen onto BBL CHROMagar™ Candida plate and streak for isolation. If specimen is received on a swab, **roll** the swab gently over a small area of the surface at the edge.
2. Streak for isolation using a sterilized loop.
3. Incubate the plates aerobically at 35 ± 2°C for 36 to 48 h in an inverted position.

7.0 Interpretation of Results

After proper incubation, examine plates over a white background. Expected colors and morphologies can be found in Table 1 below.

C. albicans, *C. krusei*, and *C. tropicalis* produce unique colonies that permit identification without further testing. Confirmatory testing should be performed on isolates that yield colonies exhibiting atypical morphology. Other *Candida* species require further testing for identification. Yeast other than *Candida* will grow on CHROMagar. See Limitations below for colony descriptions.

Table 1

C. albicans  Light to medium green	***C. krusei***  Large, spreading colonies with pink centers and rough, pale edges
C. tropicalis  Dark blue to blue-gray with a dark halo in the agar	***C. glabrata***  White, pale pink, dark pink
C. parapsilosis  White or pale pink	***C. albicans*, *C. krusei*, *C. tropicalis*, and *C. glabrata***  Mixture for relative comparison

8.0 Quality Control & Quality Assurance

Examine plates for signs of deterioration. Do not use plates if they show evidence of microbial contamination, discoloration, drying or cracking.

1. Make a basic cell suspension directly from growth of a fresh agar plate (weekly sub) and adjust the turbidity to 0.5 McFarland standard.
2. Dilute the suspension 1:10 in normal saline.
3. Using a 10-µL loop (large urine loop), inoculate the agar and streak for isolation.
4. Incubate the plates aerobically at 35 ± 2°C for 36 to 48 h in an inverted position.

Control Organism	Expected Results
Candida albicans ATCC 90028	Growth; light to medium green colonies
Candida krusei ATCC 14243	Growth; spreading, light pink colonies with rough, pale edges
Candida tropicalis ATCC 750	Growth; dark blue colonies with or without halos
Pseudomonas aeruginosa ATCC 27853	Inhibition (partial to complete)

9.0 Limitations

1. Growth and colony color development are inconsistent at 24 h incubation. If identification is required prior to 36-48 h incubation, a germ tube test may be performed to classify the isolate as *C. albicans* or *Candida* species, not *albicans*.
2. *C. glabrata* and *C. parapsilosis* cannot be differentiated using this medium. These identifications should be confirmed using other laboratory methods.
3. Isolates of *C. norvegensis* produce colonies resembling those of *C. krusei*. Some strains of *Pichia* may produce colonies that resemble those of *C. tropicalis*. However, *C. norvegensis* and *Pichia* are very rarely encountered in clinical specimens.
4. *C. dubliniensis* produces colonies similar to that of *C. albicans* (colonies may be slightly darker).
5. Yeast other than *Candida* species will grow on CHROMagar Candida. While their colonies are somewhat distinctive, they require further testing for definitive identification.
    a. *Geotrichum* species may only be pinpoint at 48 h. After 72 h they may appear as small, rough colonies that are pale to pink. Some isolates may produce green pigment in the agar beneath the colonies. This appearance is not seen with *C. albicans* for which the colonies themselves are green.
    b. *Trichosporon* species typically form small colonies after 48 h. Their color may vary from pale, “dirty pink” to “dirty grey-green” (becoming darker and rough with prolonged incubation).
    c. *Saccharomyces* species may reportedly appear as small colonies ranging in color from pale pink to purple.
    d. *Cryptococcus* species grow slowly and may require at least 72 h incubation. The color of *Cryptococcus* species varies from gray to pale pink to pink-purple.
6. Since molds and other filamentous fungi metabolize the chromogenic substrates, the colors exhibited by these organisms on CHROMagar may differ from those exhibited on conventional media. Do not use the appearance of growth on this medium for traditional descriptive identification.
7. Minimize exposure to light before and during incubation, as light may destroy chromogens.

10.0 Verification

A total of 47 *Candida* isolates, consisting of 8 different species, were tested in this evaluation of CHROMagar Candida. 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 10 *C. albicans*, 6 *C. krusei*, 5 *C. tropicalis*, 11 *C. glabrata*, 11 *C. parapsilosis*, 2 *C. guilliermondii*, 1 *C. lusitaniae*, and 1 *C. dubliniensis*.

Each isolate was subcultured to BCG agar and incubated for 48 h. A cell suspension in normal saline was made for each isolate that was equivalent to a 0.5 McFarland standard. These suspensions were then diluted 1:100 with normal saline and inoculated onto CHROMagar Candida plates using a 0.01 mL calibrated loop. The plates were then incubated aerobically for 48 h before colonies were examined.

All 10 (100%) of the *C. albicans* isolates yielded distinctively green colonies. From these data, and those reported by Odds and Bernaerts (B) in a broader evaluation, the sensitivity and specificity of a green colony color for the recognition of *C. albicans* were calculated as 100%. 4 (80%) of the 5 *C. tropicalis* isolates yielded dark blue-gray colonies while 1 (20%) isolate yielded lighter blue-gray colonies. All 5 (100%) of the isolates yielded colonies with the characteristic dark halo in the agar surrounding the colonies. All 6 (100%) of the *C. krusei* isolates yielded characteristic large colonies with pink centers and rough, spreading, pale edges. All 11 (100%) of the *C. glabrata* isolates yielded pink colonies ranging from pale pink (3) to pink (5) to dark pink (3). The *C. parapsilosis* isolates ranged from white (7) to pale pink (4). Both of the *C. guilliermondii* (2) and the *C. lusitaniae* (1) isolates were pale pink. The *C. dubliniensis* isolate was green, much like the *C. albicans* isolates.

A few non-*Candida* isolates were also tested. 1 *Saccharomyces cerevisiae* isolate yielded tiny, pale pink colonies at 48 h. 1 *Trichosporon beiglii* isolate yielded small, grey-green colonies. 2 *Cryptococcus neoformans* isolates required > 48 h incubation. These isolates both yielded variable colonies that were initially pale and turned dark blue-green with age.

11.0 References

1. Package insert: BD BBL™ CHROMagar™ Candida, 8012620, 2003/11.
2. Odds, F.C. and R. Bernaerts. 1994. CHROMagar Candida, a New Differentiation isolation Medium for Presumptive Identification of Clinically Important Candida Species. J.C.M. 32:1923-1929.

12.0 Document Control History

Reviewed by director (AR): 08/04/2008

Reviewed by J. Schappert: 03/10/2010

Reviewed by supervisor (JC): 08/06/2008, 07/2009, 04/2011, 04/2014