



BD™ Sabouraud GC Agar / CHROMagar™ Candida Medium (Biplate)

INTENDED USE

BD Sabouraud GC Agar / CHROMagar Candida Medium (Biplate) is used for the selective isolation of fungi and for the isolation and identification of *Candida albicans*, *C. tropicalis* and *C. krusei* from clinical specimens.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.

Sabouraud Agar with glucose is a widely used medium which, due to its low pH and the high glucose concentration, is partially selective for fungi. As many bacteria tolerate the low pH and the high glucose concentration and will grow on Sabouraud agar, especially during the prolonged incubation period often necessary for fungal isolation, several formulations containing antibacterial inhibitors have been developed. Antimicrobials like penicillin, chloramphenicol, aminoglycosides, or combinations of these have been shown to be effective in inhibiting bacteria without affecting fungal growth.¹⁻⁶

In Sabouraud GC Agar the peptones are sources of nitrogen. Glucose (=dextrose) is an energy source for the growth of fungi. Chloramphenicol and gentamicin are broad-spectrum antibiotics inhibitory to a wide range of gram-negative and gram-positive bacteria.

CHROMagar Candida Medium is a selective and differential medium for the isolation of fungi. With the inclusion of chromogenic substrates in the medium, the colonies of *C. albicans*, *C. tropicalis* and *C. krusei* produce different colors, thus allowing the direct detection of these yeast species on the isolation plate.⁷⁻¹² Colonies of *C. albicans* appear light to medium green, *C. tropicalis* colonies appear blue-greenish to metallic-blue, and *C. krusei* colonies appear light rose with a whitish border. Other yeast species may develop either their natural color (cream) or appear rose or light to dark mauve [e.g., *Candida (Torulopsis) glabrata* and other species]. An additional advantage of the medium is the easy detection of mixed yeast cultures due to their colony appearance in different colors.^{7,9-12}

Specially selected peptones supply the nutrients in **CHROMagar Candida Medium**. The proprietary chromogen mix consists of artificial substrates (chromogens), which release differently colored compounds upon degradation by specific enzymes. This permits the differentiation of certain species, or the detection of certain groups of organisms, with only a minimum of confirmatory tests. Chloramphenicol inhibits most bacterial contaminants.

CHROMagar Candida Medium was developed by A. Rambach and is sold by BD Diagnostic Systems under a licensing agreement with CHROMagar, Paris, France.

REAGENTS

Formulas* Per Liter Purified Water

Sabouraud Agar with Gentamicin and Chloramphenicol		CHROMagar Candida Medium	
Pancreatic Digest of Casein	5.0 g	Chromopeptone	10.0 g
Peptic Digest of Animal Tissue	5.0	Glucose	20.0
Glucose	40.0	Chromogen Mix	2.0
Agar	15.0	Chloramphenicol	0.5
Gentamicin	0.04	Agar	15.0
Chloramphenicol	0.4	pH 6.0+/- 0,3	
pH 5.6 +/- 0.2			

*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.

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

Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store plates **in the dark** at 2 to 8° C, in their original sleeve wrapping until just prior to use. Avoid freezing and overheating. The plates may be inoculated up to the expiration date (see package label) and incubated for the recommended incubation times.

Plates from opened stacks of 10 plates can be used for one week when stored in a clean area at 2 to 8° C.

Minimize exposure to light before and during incubation, since light may destroy the chromogens.

USER QUALITY CONTROL

Inoculate representative samples with the following strains (for details, see **GENERAL INSTRUCTIONS FOR USE** document). Incubate the plates aerobically for 20 to 48 hours at 35 +/- 2° C.

Strains	Sabouraud GC Agar	CHROMagar Candida Medium
<i>Candida albicans</i> ATCC™ 60193	Growth good to excellent; white colonies	Growth good to excellent; light to medium green colonies
<i>Candida krusei</i> ATCC 34135	Growth good to excellent; white to creme, flat colonies	Growth good to excellent; light rose to pink, flat colonies with a whitish border
<i>Candida tropicalis</i> ATCC 1369	Growth good to excellent; white to creme colonies	Growth good to excellent; gray blue to blue-greenish or metallic blue colonies with or without violet halos in the surrounding medium
<i>Pseudomonas aeruginosa</i> ATCC 27853	Inhibition partial to complete	Inhibition partial to complete
* <i>Aspergillus niger</i> ATCC 16404	Growth good to excellent	Growth good to excellent
Uninoculated	Light amber, transparent	Light amber, transparent

* may be incubated for up to 4 days

PROCEDURE

Materials Provided

BD Sabouraud GC Agar / CHROMagar Candida Medium (90 mm **Stacker™** biplates).

Microbiologically controlled.

For identification of the media of this biplate, Sabouraud GC Agar is labelled with a black dot.

Materials Not Provided

Ancillary culture media, reagents and laboratory equipment as required.

Specimen Types

The media in this biplate are used for the isolation of fungi and for isolation and identification of *Candida albicans*, *C. tropicalis*, and *C. krusei* from all types of clinical specimens (see also **PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE**).

Test Procedure

Streak the specimen or culture for isolation onto the surface of each medium. If the specimen is cultured from a swab, roll the swab gently over a small area of each surface at the edge, then

streak from these areas with a loop. Incubate plates aerobically at 35 +/- 2° C. Minimize exposure to light both before and during incubation. Read **CHROMagar Candida Medium** after 42 to 48 h. As some slow-growing filamentous fungi may require a longer incubation, return the plate to the incubator until day 4 or longer. After this time, inspect Sabouraud GC Agar for additional isolates not yet found on **CHROMagar Candida Medium**, but do not read **CHROMagar Candida Medium** again after the extended incubation. Isolates on Sabouraud GC Agar must be further differentiated for complete identification.³⁻⁶ Occasional isolates, such as *Cryptococcus neoformans* and filamentous fungi, will require a longer incubation time and possibly a lower incubation temperature. Therefore, it is recommended to inoculate a **BD Sabouraud Agar with Gentamicin and Chloramphenicol** plate with the specimen and to incubate this plate at 25 to 30° C, if fungi requiring a lower incubation temperature are expected.

Results

After sufficient incubation, examine Sabouraud GC Agar for fungal colonies exhibiting typical color and morphology. Biochemical tests and microscopical and serological procedures should be performed for complete identification of isolates.³⁻⁶

CHROMagar Candida Medium: It is recommended to read this medium on a white background. If *Candida* species are present, colonies will appear light to medium green (*C. albicans*), light rose to pink with a whitish border (*C. krusei*), or blue-greenish to metallic blue with or without violet halos (*C. tropicalis*). Other *Candida* species and other yeasts appear light to dark mauve (rose to violet) or, if none of the chromogenic substrates is utilized, will assume their natural colony color (cream to white).

Data from various studies indicate that further identification tests are not necessary for *Candida albicans*, *C. tropicalis*, and *C. krusei*.^{7,9-11}

Colonies that appear light to dark rose or mauve to violet, or appear in their natural cream color must be identified using standard methods.⁷⁻¹¹

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Sabouraud GC Agar / CHROMagar Candida Medium (Biplate) is used for the selective isolation of fungi (Sabouraud GC Agar) and for the isolation and identification of *C. albicans*, *C. krusei*, and *C. tropicalis* (**CHROMagar Candida Medium**).

Sabouraud GC Agar is a conventional medium, widely used for the selective isolation of fungi. Isolates from this medium must be further differentiated using the classical procedures for fungal identification.¹⁻⁶

Nocardia and *Actinomyces* are filamentous bacteria (no fungi!) and, therefore, do not grow on Sabouraud media containing bacterial inhibitors.

Consult appropriate references for detailed information and recommended procedures for the identification of isolates.^{3-6,8}

Use of **CHROMagar Candida Medium** for direct identification of *C. albicans*, *C. krusei*, and *C. tropicalis* has been documented in several studies and manuals which can also be consulted for additional information on the recommended procedures.^{7,9-12} Results of a recent performance evaluation of **BD CHROMagar Candida Medium** are reported by Jabra-Rizk and coworkers.¹¹

Candida (Torulopsis) glabrata usually produces mauve to dark mauve colonies on this medium.⁹ However, it is recommended that organisms appearing in this color should be confirmed with additional biochemical tests since this colony color can be produced by a variety of yeast species.

Colonies that appear rose or light to dark mauve, or appear in their natural cream color on this medium must be identified using standard methods.³⁻⁵

Fungi other than yeasts can also be isolated on this medium if incubated at a temperature and for a time appropriate for these organisms.

Since filamentous fungi may metabolize the chromogenic substrates, the colors exhibited by these organisms on **CHROMagar Candida Medium** may differ from those exhibited on other

fungal media. Do not use the appearance of growth of filamentous fungi on this medium for traditional morphological identification.

It has been reported that *C. dubliniensis* produces a distinctive dark green color on primary isolation with **CHROMagar Candida Medium**.¹³⁻¹⁵ However, this property may not be retained in subculture. Additional phenotypic and genotypic tests are necessary for confirmation of *C. dubliniensis*. Simple phenotypic tests, e.g., growth of the isolate at 45° C (*C. dubliniensis*: negative; *C. albicans*: positive) may be used for the differentiation of the two species.¹²

Before using **BD CHROMagar Candida Medium** for the first time, we recommend to train the typical colony appearance with defined strains of *C. albicans*, *C. krusei*, and *C. tropicalis*, e.g., the strains mentioned under **USER QUALITY CONTROL**.

Several filamentous fungi require lower incubation temperatures than those necessary for **BD Sabouraud GC Agar / CHROMagar Candida Medium (Biplate)**. Incubation of this biplate at temperatures lower than 35° C, however, may delay the chromogenic reactions on **CHROMagar Candida Medium**.

REFERENCES

1. MacFaddin, J.F. 1985. Media for the isolation – cultivation – maintenance of medical bacteria. Volume 1. Williams and Wilkins, Baltimore, London
2. Atlas, R.M. 1993: Handbook of Microbiological media; CRC Press, Boca Raton
3. Ajello, L., L.K. Georg, W. Kaplan, and L. Kaufman. 1963. CDC laboratory manual for medical mycology. PHS Publication No. 994, U.S. Government Printing Office, Washington, D.C.
4. Larone, D.H. 1995: Medically important fungi - a guide to identification. Third edition. American Society for Microbiology Press, Washington.
5. Merz, W.G., Roberts, G.D. 1995: Detection and recovery of fungi from clinical specimens. In: Manual of Clinical Microbiology (eds. Murray, P.R. et al.) , p. 709-722. ASM Press, Washington D.C.
6. Weitzman, I., J. Kane, and R.C. Summerbell. 1995. Trichophyton, Microsporum, Epidermophyton and agents of superficial mycoses, p. 791-808. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Tenover (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
7. Odds, F.C., and R. Bernaerts. 1994. CHROMagar Candida, a new differential isolation medium for presumptive identification of clinically important *Candida* species. J. Clin. Microbiol. 32: 1923-1929.
8. Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
9. Pfaller, M.A., A. Huston, and S. Coffman. 1996. Application of CHROMagar Candida for rapid screening of clinical specimens for *Candida albicans*, *Candida tropicalis*, *Candida krusei*, and *Candida (Torulopsis) glabrata*. J. Clin. Microbiol. 34: 56-61.
10. Beighton, D., R. Ludford, D.T. Clark, S.R. Brailsford, C.L. Pankhurst, G.F. Tinsley, J. Fiske, D. Lewis, B. Daly, N. Khalifa , V. Marren, and E. Lynch. 1995. Use of CHROMagar Candida medium for isolation of yeasts from dental samples. J. Clin. Microbiol. 32: 3025-3027.
11. Jabra-Rizk, M.A. et al. 2001. Evaluation of a reformulated CHROMagar Candida Medium. J. Clin. Microbiol. 30: 2015-2016.
12. Hazen, K.H., and S.A. Howell. 2003. *Candida*, *Cryptococcus*, and other yeasts of medical importance. In: Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Tenover (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
13. Schoofs, A., F.C. Odds, R. Coleblunders, M. Ieven, and H. Goosens. 1997. Use of specialised isolation media for recognition and identification of *Candida dubliniensis* isolates from HIV-infected patients. Eur. J. Clin. Microbiol. Infect. Dis. 16:296-300.
14. Kirkpatrick, W.R., S.G. Revankar, R.K. McAtee, J.L. Lopez-Ribot, A.W. Fothergill, D.I. McCarthy, S.E. Sanche, R.A. Cantu, M.G. Rinaldi, and T.F. Patterson. 1998. Detection of *Candida dubliniensis* in oropharyngeal samples from Human Immunodeficiency Virus-

infected patients in North America by primary CHROMagar Candida screening and susceptibility testing of isolates. J. Clin. Microbiol. 36:3007-3012.
15. Odds, F.C., L. Van Nuffel, and G. Dams. 1998. Prevalence of *Candida dubliensis* isolates in a yeast stock collection. J. Clin. Microbiol. 36:2869-2873.

PACKAGING/AVAILABILITY

BD Sabouraud GC Agar / CHROMagar Candida Medium (Biplate)

Cat. No. 254515 Ready-to-use Plated Media, cpu 20

FURTHER INFORMATION

For further information please contact your local BD representative.



BD Diagnostic Systems

Tullastrasse 8 – 12

D-69126 Heidelberg/Germany

Phone: +49-62 21-30 50 Fax: +49-62 21-30 52 16

Reception_Germany@europe.bd.com

BD Diagnostic Systems Europe

Becton Dickinson France SA

11 rue Aristide Bergès

38800 Le Pont de Claix/France

Tel: +33-476 68 3636 Fax: +33-476 68 3292 <http://www.bd.com>

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