SABOURAUD DEXTROSE AGAR +CHLORAMPHENICOL

INSTRUCTIONS FOR USE THE READY-TO-USE PLATED MEDIUM

1. Intended use

Saboraud Dextrose Agar + Chloramphenicol is a selective medium used for qualitative detection isolation of dermatophytes and other fungi, including molds and yeast in human and nonhuman clinical specimens. The medium is recommended for testing specimens that contain mixed bacterial flora, such as sputum, feces and skin surface samples.

The function of Saboraud Dextrose Agar + Chloramphenicol medium is to support diagnosis in patients exhibiting symptoms of a potential fungal infection.

Fungi are opportunistic pathogens. The frequency of invasive mycoses has risen significantly in recent decades, as well as resulting morbidity and mortality, which is directly related to an increase in population of patients vulnerable to severe fungal infections. High risk groups include: patients receiving blood transfusions or transplants, patients undergoing major surgical procedures, AIDS patients, cancer patients, patients receiving immunosuppressive therapy, as well as large number of geriatric patients and premature neonates.
Among human pathogenic fungi that cause invasive mycoses, the most frequently isolated species are *Candida albicans, Cryptococcus neoformans* and *Aspergillus fumigatus*. Additionally, numerous other species are responsible for fungal diseases of the skin and skin appendages, as well as mucous membranes of the digestive system and the genital tract.
Considering the diversity of at-risk patients groups and the variety of pathogenic fungi, opportunistic fungal infections present a major diagnostic and therapeutic challenge.

Cat. no:	**Medium type:**	**Packaging:**
1231PD90; 201231	Solid medium on a plate	1x10 pcs (90 mm)

2. Principle of the procedure

Enzymatic digest of casein and enzymatic digest of animal tissue provide necessary nutrients. Highly concentrated glucose is a source of carbon and energy, which promotes fungal growth and inhibits most bacteria. Low pH (5,6) of the medium also supports the growth of fungi while inhibiting contaminating bacteria present in test specimens. Chloramphenicol is a selective agent, significantly inhibitory to a wide range of gram-negative and gram-positive bacteria.

3. Medium composition

w g/l distilled water	
Enzymatic digest of casein	5,0 g
Enzymatic digest of animal tissue	5,0 g
Glucose	40,0 g
Chloramphenicol	0,05 g
Agar	15,0 g

pH 5,6 ± 0,2 in 25°C.

Appearance of the medium - clear, straw

4. Medium preparation

The medium is ready to use. Bring the medium to room temperature immediately before use.

5. Equipment required, not provided

Standard laboratory equipment necessary to perform microbiological tests, including an incubator.

6. Precaution

- The product is for professional use only.
- Non-automated product.
- The medium contains components of animal origin, which may be associated with the presence of biological pathogens, therefore must be handled in accordance with principles of handling potentially infectious biological material.
- Do not use plates if the medium shows signs of microbial contamination, discoloration, drying, cracking, or other signs of deterioration.
- Do not use damaged plates.
- Do not use plates after the expiration date.
- Re-incubation of previously inoculated plates is not allowed.
- To ensure correct test results, follow these instructions.
- If the handling of the medium differs from that described in this manual, the laboratory is obliged to validate the procedure adopted.

7. Storage

Store plates at 2-12ºC until the expiration date. Store plates in their original packaging, in an inverted position (agar side up), away from direct light sources. To avoid freezing of agar, do not store plates close to the refrigerator walls. To avoid the appearance of water condensation on the plate lid, do not open the refrigerator ~~too often~~ more often than necessary and do not store plates in an overfilled refrigerator.

8. Expiration date

The medium stored at 2-12ºC retains its properties up to 3 months from the date of production.

9. Specimen types

Human clinical specimens and other specimens.

Specimens tested for presence of dermatophytes are human hair, fingernail and skin scrapings that should be colllected in sterile Petri plates. Specimens can be stored at room temperature for up to 72 hours.

Other kinds of clinical specimens to be tested for presence of other pathogenic fungi should be collected in, sterile containers, in accordance with the guidelines for handling the specimen in question. Specimens should be delivered to the laboratory as soon as possible, preferably within 2 hours from collection. If not possible, store samples at 4°C for up to 24 hours or until they can be delivered to the laboratory.

10. Test procedure

1. Allow the medium to warm to room temperature before inoculation.
2. Inoculate the specimen by spreading it directly on the agar surface.
3. If the specimen was taken on a swab - gently rotate the tip of the swab on a small area of agar just around the edges of the plate, and then inoculate specimen using streak plate method with using a sterile loop.
4. Incubate the inoculated plates at 25-30˚C.
5. Examine for growth results after 18-168 hours of incubation
6. For dermatophyte cultures, extend incubation to 20 days, examine for growth every 4-6 days.

11. Reading and interpretation

After incubation period, observe:

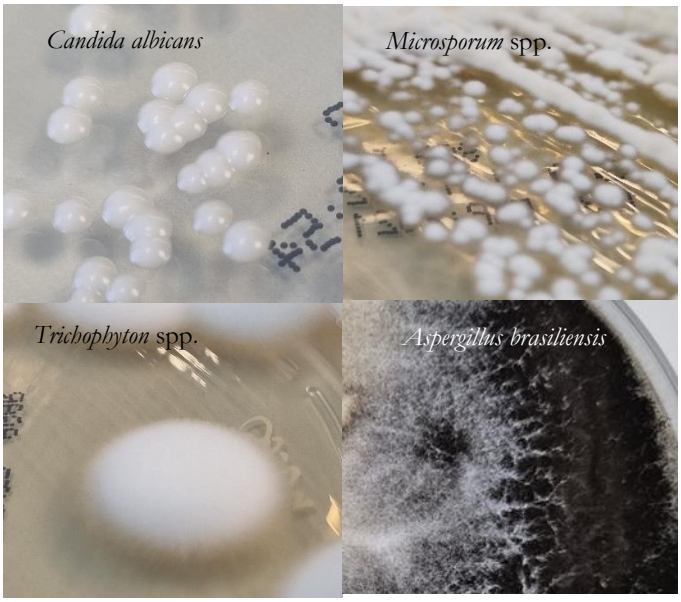
- the presence of bacterial colony growth,
- colony morphology,

Typical morphology of colonies grown on Sabouraud Dextrose Agar + Chloramphenicol:

Microorganism	**Typical colony morphology**
Candida	Regular shape, large, convex, smooth, circular, cream coloured
Sacharomyces	Regular shape, large, convex, smooth, circular, cream coloured

Trichophyton	White to cream coloured
Microsporum	Flat, white to cream colored, fuzzy upper surface and white to brown colored lower surface
Aspergillus	White mycelium with black spores

For the final identification of cultured microorganisms, additional tests and/or confirmatory tests should be carried out using other methods used in the laboratory.



Colony morphology and growth pattern of on Saboraud Dextrose Agar + Chloramphenicol medium

12. Quality control

The nutritional properties of the medium should be checked using reference strains giving the expected positive and negative reactions. The test should be performed using clean, fresh cultures of reference strains giving the desired reactions. Use the following reference strains to perform the medium quality control:

Reference strain:	**Growth intensity:**	**Colony morphology:**
Candida albicans ATCC 10231	Good growth	Cream coloured, circular, smooth, full-edged, convex
Escherichia coli ATCC 25922	No growth	–

Other reference strains may be used in accordance with the laboratory's quality control procedures and instructions. Quality control procedures should meet the requirements of applicable regulations and guidelines/recommendations.

13. Limitations of the method

- Due to variability in nutritional requirements, some strains may grow poorly or not at all on Sabouraud Dextrose Agar + Chloramphenicol.
- The medium provides good fungal growth but may not be sufficient in inducing spores.
- Chloramphenicol, when used to inhibit bacterial growth, may have an inhibitory effect against certain pathogenic fungi, e.g. opportunistic fungi that cause dermatophytosis but are sensitive to chloramphenicol
- test specimens should be taken before starting antibiotic therapy.
- Do not use the medium to culture blood samples

14. Characteristics of the method

Sabouraud Dextrose Agar (SDA) was developed for culture and detection of dermatophytes. The factors that support fungal growth are low pH and high concentration of glucose (dextrose). In order to increase selectivity, the composition of SDA

medium is modified by the addition of various antimicrobics e.g. chloramphenicol, which is a wide spectrum antibiotic that inhibits the growth of both Gram-positive and Gram-negative bacteria.

The presence of chloramphenicol in the medium increases the effectiveness of fungal growth in samples containing mixed bacterial flora. Chloramphenicol at 35°C can also inhibit the growth of fungi such as *Histoplasma capsulatum, Blastomyces dermatitidis*, which will not be inhibited when cultivated at 25-30°C. Chloramphenicol can also be an inhibiting factor for some of the other species of pathogenic fungi.

15. Disposal of used material

Used and unused materials should be disposed of in accordance with current medical waste handling regulations and laboratory procedures for the disposal of infectious and potentially infectious materials.

16. Reporting of adverse events

According to current regulations, adverse events and incidents that can be directly linked to the described medium must be reported to the manufacturer and to the competent authorities.

17. References

1. 1.Sabouraud, R. 1892. Contribution a l'etude de la trichophytie humaine. Etude clinique, microscopique et bacterioloqique sur la pluralite des trichophytons de l'homme. Ann. Dermatol. Syphil. 3:1061-1087.
2. Haley, L.D., J. Trandel, and M.B. Coyle. 1980. Cumitech 11, Practical methods for culture and identification in the clinical microbiology laboratory. Coordinating ed., J.C. Sherris. American Society for Microbiology, Washington, D.C.
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. Kane, J., and R.C. Summerbell. 1999. Trichophyton, Microsporum, Epidermophyton, and agents of superficial mycoses, . 1275-1294. In P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, and R.H. Yolken (ed.), Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.
5. Lorian, V. (ed.). 1996. Antibiotics in laboratory medicine, 4th ed. Williams & Wilkins, Baltimore.
6. Clinical and Laboratory Standards Institute. 2005. Approved Guideline M29-A3. Protection of laboratory workers from occupationally acquired infections, 3rd ed. CLSI, Wayne, PA.
7. Garner, J.S. 1996. Hospital Infection Control Practices Advisory Committee, U.S. Department of Health and Human Services, Centers for Disease Control and Prevention. Guideline for isolation precautions in hospitals. Infect. Control Hospital Epidemiol. 17:53-80.
8. U.S. Department of Health and Human Services. 2007. Biosafety in microbiological and biomedical laboratories, HHS Publication (CDC), 5th ed. U.S. Government Printing Office, Washington, D.C.
9. Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC). Official Journal L262, 17/10/2000, p. 0021-0045.
10. Murray, P.R., E.J. Baron, J.H. Jorgensen, M.A. Pfaller, and R. H. Yolken (ed.). 2003. Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
11. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 2002. Bailey and Scott's diagnostic microbiology, 11th ed. Mosby, Inc., St. Louis.
12. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore.
13. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1997. Color atlas and textbook of diagnostic microbiology, 5th ed. Lippincott-Raven, Philadelphia.
14. red A.Przondo-Mordarska Procedury diagnostyki mikrobiologicznej w wybranych zakażeniach układowych, Continuo, Warszawa 2004
15. Z.Adamski, H. Batura-Gabryel , Mikologia lekarska, UM w Poznaniu, Poznań 2007
16. P.Krzyściak, M.Skóra, A.B. Macura, Atlas grzybów chorobotwórczych człowieka, MedPharma, Wrocław 2011
17. MacFaddin J.F, Media for isolation-cultivation-identification-maintenance of medical bateria, Williams&Wilkins, Baltimore/London, 1985

History of document changes

Date of change	Section	Description of the change
2023/02/08	Entire document	Adaptation to the requirements of EU Regulation 2017/746

NOTE

The revision history of the document does not include editorial changes.

SYMBOL	NAME OF SYMBOL	DESCRIPTION	REF.
	Manufacturer	Indicates the medical device manufacturer.	5.1.1
	Date of manufacture	Indicates the date after which the medical device is not to be useed.	5.1.3
	Catalogue number	Indicates the manufacturer's catalogue number so that the medical device can be used..	5.1.6
	Batch code	Indicates the manufacturer's batch code so that the batch or lot can be identified.	5.1.5
	In vitro diagnostic medical device	Indicates a medical device that is intended to be used as an invitro diagnostic medical device.	5.5.1
	Do not re-use	Indicates a medical device that is intended for one single use only.	5.4.2
	Contains sufficient for <n> tests	Indicates the total number of tests that can be performed with the medical device.	5.5.5
	Use –by date	Indicates the date after which the medical device is not to be used	5.1.4
	Temperature limit	Indicates the temperature limits of temperature shall be indicates adjacent to the upper and lower horizontal lines.	5.3.7

CE	Safety symbol (Compliance with EU requirements)	The CE marking on a product is a manufacturer's declaration that the product complies with the essential requirements of the relevant European Union health, safety and environmental regulations.	nd.
	Consult instructions for use or consult electronic instructions for use	Indicates the need for the user to consult the instructions for use.	5.4.3
	Sterilized using aseptic processing techniques	Indicates a medical device that has been manufactured using accepted aseptic techniques.	5.2.2
	Do not use if package is damaged and consult instructions for use	Indicates that a medical device that should not be used if the package has been damaged or opened and that the user should consult the instructions for use for addictional information.	5.2.8
	Contains biological material of animal origin	Indicates a medical device that contains biological tissue, cells, or their derivatives, of animal origin	5.4.8

GRASO®

Graso Zenon Sobiecki
Krąg 4A; 83-200 Starogard Gdański
www.grasobiotech.pl
tel. + 48 (58) 562 30 21

Production Department
Leśna 1, Owidz
83-211 Jablowo

IVD CE