BD™ Sabouraud Glucose Agar

INTENDED USE

BD Sabouraud Glucose Agar, supplied in bottles, is a partially completed medium used for the isolation and cultivation of fungi (yeasts, molds, and dermatophytes) from clinical and nonclinical materials.

PRINCIPLES AND EXPLANATION OF THE PROCEDURE

Microbiological method.
Sabouraud Glucose Agar was devised by Sabouraud for the cultivation of dermatophytes.[1,2] Today it is used for the isolation and cultivation of all fungi, including those from clinical specimens.[3,4] The medium is slightly inhibitory to contaminating bacteria due to its low pH and the high glucose concentration. It is mentioned in the USP in the microbial limit tests.[5] When supplemented with 100 mg of tetracycline or 100 mg of benzylpenicillin sodium per liter medium, the medium meets the specification of the EP.[6]

In **BD Sabouraud Glucose Agar**, Neopeptone is a source of nitrogenous growth factors. Glucose (=dextrose) provides a carbon and energy source for the growth of microorganisms. The high glucose concentration and the relatively low pH provide are advantageous for the growth of fungi while many bacteria do not tolerate the high sugar concentration and are partially inhibited by the low pH. However, without supplementation of antibacterial antimicrobials, the medium has only a weak selectivity.

BD Sabouraud Glucose Agar are partially completed (=semi-finished) media supplied in bottles from which the user can prepare plated or tubed media. Antimicrobial agents, such as gentamicin (0.04 g/l) and chloramphenicol (0.4 g/l), tetracycline (0.1 g/l), or benzylpenicillin (0.1 g/l), may be added before completion to increase selectivity.

REAGENTS

BD Sabouraud Glucose Agar
Formula* Per Liter Purified Water

Bacto™ Neopeptone	5.0 g
Glucose (=Dextrose)	40.0
Agar	19.0

pH 5.6 +/- 0.2
*Adjusted and/or supplemented as required to meet performance criteria.

PRECAUTIONS

IVD . For professional use only.
Do not use bottles if they show evidence of microbial contamination, discoloration, drying, cracking or other signs of deterioration. For completion of this partially completed bottled medium, follow the methods and observe the warnings described under **PROCEDURE** - **Reagent Preparation**. Consult **GENERAL INSTRUCTIONS FOR USE** document for aseptic handling procedures, biohazards, and disposal of used product.

STORAGE AND SHELF LIFE

On receipt, store bottles in the dark at 5 to 25° C. Avoid freezing and overheating. The medium may be used up to the expiration date and incubated for the recommended incubation times. Bottles from opened packages can be used up to the expiration date. Opened bottles must be used immediately. To prepare plates or tubes from the bottled medium, it must first be liquefied. Do not liquefy the medium more than once after solidification.

USER QUALITY CONTROL

Plates prepared from the semi-finished medium are inoculated with 10 to 100 cfu per plate of the *C. albicans* and *A. niger* strains. For *S. cerevisiae* and *T. mentagrophytes*, use a tenfold dilution of a suspension adjusted to MacFarland 0.5 standard and inoculate 10 µl (approximately 10^3 CFU) per plate. For further details, see **GENERAL INSTRUCTIONS FOR USE** document. Incubate plates as indicated in the footnote of the table.

**Candida albicans* ATCC™ 10231	Growth good to excellent
**Saccharomyces cerevisiae* NCPF 1211or DSM 1333	Growth good to excellent
*** Aspergillus niger* ATCC16404	Growth good to excellent
****Trichophyton mentagrophytes* ATCC 9533	Growth good to excellent
Uninoculated	Amber; bottled medium may be opaque

Incubation: *48 h / **3 to 4 days / ***5 to 7 days, 25°C - 30°C, aerobically

PROCEDURE

Materials Provided

BD Sabouraud Glucose Agar (partially completed bottled media). See **AVAILABILITY** for fill volumes and package sizes.

STERILE

Materials Not Provided

Autoclave (set at 100 +/- 2° C), steam cooker, or hot plate; waterbath (48-50°C); sterile glassware and sterile plastic Petri dishes or tubes.
Ancillary reagents and laboratory equipment as required.

Reagent Preparation

Liquefy **BD Sabouraud Glucose Agar** (Bottled Media) by heating in an autoclave or steam cooker. Alternatively, the bottle may be placed into a suitable vessel containing water, which is placed on a hot plate and brought to boiling. **Slightly loosen the cap before heating to allow pressure exchange.**

Warning: it is not recommended to use microwave ovens for liquefaction of the medium. Do not place media bottles with metal closures into a microwave oven.

When using an autoclave, set the temperature to not more than 100 +/- 2° C as excessive heating may deteriorate the ingredients, possibly leading to unsatisfactory microbiological performance. When using a hot plate and/or a waterbath, boil sufficiently long to dissolve the whole medium. The time needed for complete liquefaction of the medium may vary considerably and depends on the actual temperature of the heating device before use, its wattage, size, and the volume and temperature of the medium in the container. It is recommended to test and record the time needed for liquefaction after the first use.
After complete liquefaction, remove the container from the heating device and place into a waterbath set at 48 to 50° C.
Warning: Wear heat-protective gloves! Do not place the hot container into an icebath or in cold water to accelerate cooling as this might cause cracks in the glass. Risk of severe scald!
Leave the container in the waterbath sufficiently long to allow cooling of the complete medium to the set temperature.
If heat-sensitive supplements are added for the preparation of a plated medium, the temperature of the medium must not be higher than 50° C. Antimicrobial agents such as gentamicin (0.04 g/l) and chloramphenicol (0.4 g/l), tetracycline (0.1 g/l), or benzylpenicillin (0.1 g/l) can be added to increase the selectivity. Completely dissolve the antimicrobials in a small volume (10 to 15 ml) of water, filter sterilize the solution, and add to the medium. Apply aseptic conditions during addition of the supplement and during pouring of the plates! Use sterile dishes and tubes. Mix the medium gently after addition of the supplement, but avoid formation of foam and bubbles.

Pour the medium into the dishes if surface inoculation is desired. For a normal 90 to 100 mm dish, 19 to 21 ml is an appropriate volume. Allow the completed medium to solidify, invert the plates and allow to dry at room temperature for an adequate time (for complete solidification, store overnight at 18 to 23° C). Wrap in fresh plastic bags and store at 2 to 8° C. Prepared plates of this medium may be used for 5 to 7 days.
If the pour plate method shall be applied, add the material to be tested or its dilution into the empty dish, overlay with the medium, rotate the dish gently to mix, and allow to solidify completely.

For the preparation of slants in tubes, add the appropriate amount of liquefied medium (without antibacterial supplements), cooled to 48 to 50° C, to the tubes and allow to solidify in the desired slanted position. Tubes, when closed tightly with screw caps, can be used for 2 to 3 weeks when stored in the dark at room temperature or at 2 to 8° C.
Before use, the medium surfaces must not be excessively wet.

Liquefy the bottled medium only once. Do not allow any leftovers to solidify and liquefy thereafter for a second time as repeated heating will damage the ingredients of the medium, leading to unsatisfactory microbiological performance.

Specimen Types
The unsupplemented medium, poured into Petri dishes or tubes is used in a variety of procedures, e.g., for pharmaceutical tests. In clinical microbiology, both unsupplemented medium and medium supplemented with antibacterial agents in Petri dishes can used for the isolation of fungi from all types of clinical specimens. The plated medium supplemented with gentamicin and chloramphenicol should be used if the specimens contain a large number of bacterial contaminants. For collection and processing of specimens, consult the references.[7,8]
Medium filled in tubes must not be used for the isolation of fungi directly form clinical specimens but is used for the growth and maintenance of fungal cultures only.

Test Procedure
Agar surfaces should be smooth and moist, but without excessive moisture which could cause confluent growth.
Streak the specimen as soon as possible after it is received in the laboratory. The streak plate is used primarily to isolate pure cultures from specimens containing mixed flora. Alternatively, if material is being cultured directly from a swab, roll the swab over a small area of the surface at the edge; then streak from this inoculated area. Incubate the plates or tubes under the conditions chosen. Consult appropriate references for further information on processing and incubation of specimens.[2-8]

If used for the detection of yeasts (e.g., *Candida* species) in clinical specimens, incubate for 48 hours at 30 to 35° C. If filamentous fungi, including dermatophytes are suspected, incubate for one week at 25 to 30° C or longer. Dermatophytes occasionally need 3 weeks or longer to produce growth. If used for hygiene monitoring, incubate for up to 7 days at 20 to 25° C. For the isolation o dermatophytes, **BD Dermatophyte Agar** should also be used. If incubated longer than 3 days, provide adequate moisture. Plates may be sealed with adhesive plastic tape to avoid desiccation. For details on growth temperature and incubation, consult the references.[2-8]

The slanted medium in tubes is used for the cultivation and maintenance of fungal cultures. Streak the strain directly of after suspension in sterile water or saline onto the whole slanted surface. Incubate as appropriate for the isolate. During incubation, caps should be slightly loosened to allow aeration. After incubation and during storage, close completely.

Results
After incubation, plates may show an area of confluent growth. Because the streaking procedure is, in effect, a "dilution" technique, diminishing numbers of microorganisms are deposited on the streaked areas. Consequently, one or more of these areas should exhibit isolated colonies of the organisms contained in the specimen. In addition, growth of each

organism may be semi-quantitatively scored on the basis of growth in each of the streaked areas.

If inoculated with an appropriate inoculum, slants will show growth on the whole surface. Inoculated tubes may be stored refrigerated for several months without loss of viability of the culture. The survival time depends on the individual strains.

The number and types of fungi growing on the completed media prepared from **BD Sabouraud Glucose Agar** (Bottled Media) is very large. Therefore, no specific details on their appearance can be given here. Consult the references.[2,4,8-10]

From the isolates obtained on the plated medium, appropriate subcultures should be set up to allow a further differentiation and identification of the fungi isolated.

PERFORMANCE CHARACTERISTICS AND LIMITATIONS OF THE PROCEDURE

BD Sabouraud Glucose Agar (Bottled Media) is used in a variety of nonclinical procedures.[5,6]
In clinical microbiology, the plated medium can be used for the isolation of fungi from all types of clinical specimens.[2-4,8-10]
The slanted medium in tubes is not used directly with clinical specimens but is used for maintenance and growth of pure fungal cultures only.
The unsupplemented medium is only weakly selective; therefore, bacteria may grow, especially after extended incubation. If bacterial contamination of the specimens, materials, or areas under investigation is suspected, the medium should be supplemented before use with antibacterial antimicrobials, e.g., gentamicin and chloramphenicol, tetracycline, benzylpenicillin, or others as appropriate (see **Reagent Preparation**).

Due to the wide growth temperature range of fungi occurring as infectious agents, it may be necessary to inoculate several plates of the same medium and incubate them at different temperatures. Consult the **Test Procedure** section and appropriate references.[2,4,8,10]

REFERENCES

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PACKAGING/AVAILABILITY

BD Sabouraud Glucose Agar (partially completed bottled media)

Cat. no. 257104	cpu 12	250 ml fill volume; in 300 ml flat bottle
Cat. no. 257153	cpu 25	100 ml fill volume; in 150 ml sirup bottle
Cat. no. 257261	cpu 4	400 ml fill volume; in 500 ml laboratory bottle

FURTHER INFORMATION

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