

BD BBL™ Prepared Sterile Pack Plates for Environmental Monitoring Sabouraud Dextrose Agar, Sterile Pack

8809191
Revised: February 2000

INTENDED USE

Sabouraud Dextrose Agar, Sterile Pack environmental sampling plates are used for the detection and enumeration of microorganisms present on surfaces of sanitary importance. The Sterile Pack plates are particularly useful for monitoring surfaces in clean rooms and other environmentally-controlled areas.

The contact-style plates are also recommended for use in air sampling equipment such as the Surface Air System.

SUMMARY AND EXPLANATION

Environmental sampling plates are specially constructed so that an agar medium can be over-filled, producing a meniscus or dome-shaped surface that can be pressed onto a surface for sampling its microbial burden. These plates are used in a variety of programs to establish and monitor cleaning techniques and schedules.¹⁻⁵

After touching the surface to be sampled with the medium, the dish is covered and incubated at an appropriate temperature. The presence and number of microorganisms is determined by the appearance of colonies on the surface of the agar medium.⁶ Collection of samples from the same area before and after cleaning and treatment with a disinfectant permits the evaluation of the efficacy of sanitary procedures.

Sabouraud Dextrose Agar was devised by Sabouraud for the cultivation of dermatophytes.⁷ The low pH of approximately 5.6 is favorable for the growth of fungi, especially dermatophytes, and inhibitory to contaminating bacteria in clinical specimens.⁸⁻¹⁰

PRINCIPLES OF THE PROCEDURE

Sabouraud Dextrose Agar is a peptone medium supplement with dextrose to support the growth of fungi. The peptones are sources of nitrogenous growth factors. Dextrose provides an energy source for the growth of microorganisms.

Because the entire double-bagged product is subjected to a sterilizing dose of gamma radiation, the contents inside the outer bag are sterile.¹¹ This allows the inner bag to be aseptically removed and brought into an environmentally-controlled area without introducing contaminants.

Since the agar medium has been sterilized after packaging, the presence of microbial growth after sampling and incubation can be relied upon to represent the presence of environmental contaminants and not pre-existing microorganisms in the medium that may have been introduced during manufacture.

REAGENTS

Formula:

Sabouraud Dextrose Agar

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein5.0 g
Peptic Digest of Animal Tissue5.0
Dextrose40.0
Agar15.0

* Adjusted and/or supplemented as required to meet performance criteria and additionally, to compensate for radiation effects.

Precautions: For Laboratory Use

Observe aseptic techniques and established precautions against microbiological hazards throughout all procedures. After use, prepared plates, specimen containers and other contaminated materials should be sterilized by autoclaving.

Storage Instructions: On receipt, store plates in the dark with top side up (agar bed at bottom) at 2 to 8°C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light.

Prepared plates stored in their original wrapping at 2 to 8°C should be warmed to room temperature prior to use.

Packages may be stored at room temperature (not exceeding 30°C) for up to 168 hours. Plates may be inoculated up to their expiration date and incubated for recommended incubation times. Discard the unused portion of all packages.

Product Deterioration: The contents of unopened or undamaged packages are sterile. Do not use packages if they show evidence of damage, microbial contamination, drying or other signs of deterioration.

SPECIMEN COLLECTION AND HANDLING

This product is not for use directly with clinical specimens.

PROCEDURE

Material Provided: Depending upon which product is ordered, one of the prepared plates listed above is provided.

Materials Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required for this procedure.

Instructions: The bags may be opened by either peeling apart the two films, or by cutting with sterile scissors. To peel open, grasp and hold the edge of the clear plastic and pull corner of the white opaque outer layer away from the plastic. Open the outer bag using aseptic technique. Once the outer bag is opened, appropriate measures should be used to maintain the sterility of the inner bag and its contents.

Selected surfaces are sampled by firmly pressing the agar medium against the test area. Hold the plate with thumb and second finger and use index finger to press plate bottom firmly against surface. Pressure should be the same for every sample. Do not move plate laterally; this spreads contaminants over the agar surface making resolution of colonies difficult. Slightly curved surfaces may be sampled with a rolling motion.

Areas (walls, floors, etc.) to be assayed may be divided into sections or grids and samples taken from specific points within the grid.

Grid method:

1. Subdivide surface (floor or wall) into 36 equal squares per 100 square feet of area by striking five equidistant dividing lines from each of two adjacent sides.
2. These dividing lines intersect at twenty-five points.
3. Number these intersections consecutively in a serpentine configuration.
4. Use red numerals for odd numbers, black numerals for even numbers.
5. Omit number 13 which falls in the center of the total area.
6. Sample odd points at one sampling period, even points at the next sampling period.
7. For areas greater than 100 square feet, extend grid to include entire area.
8. For areas smaller than 25 square feet, divide the areas into twenty-five equal squares (sixteen intersections). Sample eight even-numbered or odd-numbered intersections at each sampling period.
9. For areas between 25 and 100 square feet, divide into 36 equal squares as in #1.
10. Mark plates with intersection numbers.

Incubate exposed plates at 35 to 37°C for 48 h, and 25°C for 7 days or as required.

User Quality Control:

1. Examine the plates for signs of deterioration as described under "Product Deterioration."
2. Check performance by inoculating a representative sample of plates with pure cultures of stable control organisms that give known, desired reactions. The following test strains are recommended:

TEST STRAIN	EXPECTED RESULTS
<i>Candida albicans</i> ATCC™ 60193	Growth
<i>Trichophyton mentagrophytes</i> ATCC 9533	Growth
<i>Aspergillus fumigatus</i> ATCC 26934	Growth

RESULTS

After incubation, count the colonies.

Counting of plates containing a profusion of growth can lead to considerable error. A basic decision to be made is whether distinct colony margins can be observed.

Count all visible colonies. Spreading colonies should be counted as one but care should be taken to observe other distinct colonies intermingled in the growth around the plate periphery or along a hair line. These should also be counted as one colony, as should bi-colored colonies and halo-type spreaders.

It is generally agreed that 200 colonies is the approximate maximum that can be counted on these plates.

Colony counts may be recorded by:

1. Simply keeping individual counts.
2. Number of viable particles per square foot (agar area is 3.97 square inches).
3. Means and standard deviations.

Subculture colonies of interest so that positive identification can be made by means of biochemical testing and/or microscopic examination of organism smears.

LIMITATIONS OF THE PROCEDURE

This prepared plated medium is intended for the enumeration of microorganisms on surfaces of sanitary importance. For identification, the organisms must be in pure culture. Biochemical tests may be performed for complete identification. Appropriate texts should be consulted for further information.^{9,10,12-15}

AVAILABILITY

Cat. No. Description

- 221988 Sabouraud Dextrose Agar, Sterile Pack, Pkg. of 10 contact plates.
221235 Sabouraud Dextrose Agar, Sterile Pack, Pkg. of 10 RODAC™ plates.

REFERENCES

1. Hall, L.B., and M.J. Hartnett. 1964. Measurement of the bacterial contamination on surfaces in hospitals. *Public Health Rep.* 79:1021-1024.
2. Vesley, D., and G.S. Michaelson. 1964. Application of a surface sampling technic to the evaluation of bacteriological effectiveness of certain hospital housekeeping procedures. *Health Lab. Sci.* 1:107-113.
3. Pryor, A.K., and C.R. McDuff. 1969. A practical microbial surveillance system. *Exec. Housekeeper*, March.
4. Dell, L.A. 1979. Aspects of microbiological monitoring for nonsterile and sterile manufacturing environments. *Pharm. Technol.* 3:47-51.
5. Marshall, R.T. (ed.). 1993. *Standard methods for the examination of dairy products*, 16th ed. American Public Health Association, Washington, D.C.
6. McGowan, J.E., Jr. 1985. Role of the microbiology laboratory in prevention and control of nosocomial infections, p. 110-122. *In* E.H. Lennette, A. Balows, W.J. Hausler, Jr., and H.J. Shadomy (ed.), *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, Washington, D.C.
7. Sabouraud, R. 1892. Contribution a l'etude de la trichophytie humaine. Etude clinique, microscopique et bacterologique sur la pluralite des trichophytions de l'homme. *Ann. Dermatol. Syphil.* 3:1061-1087.
8. 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.
9. 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.
10. Kwon-Chung, K.J., and J.E. Bennett. 1992. *Medical mycology*. Lea & Febiger, Philadelphia.
11. Association for the Advancement of Medical Instrumentation. 1984. *Process control guidelines for gamma radiation sterilization of medical devices*. Association for the Advancement of Medical Instrumentation, Arlington, Va.
12. Haley, L.D., H. Trandel, and M.B. Coyle. 1980. *Cumitech 11, Practical methods for culture and identification of fungi in the clinical mycology laboratory*. Coordinating ed., J.C. Sherris. American Society for Microbiology, Washington, D.C.
13. Larone, D.H. 1993. *Medically important fungi: a guide to identification*, 2nd ed. American Society for Microbiology, Washington, D.C.
14. Forbes, B.A., D.F. Sahm, and A.S. Weissfeld. 1998. *Bailey & Scott's diagnostic microbiology*, 10th ed. Mosby, Inc., St. Louis.
15. Koneman, E.W., S.D. Allen, W.M. Janda, P.C. Schreckenberger, and W.C. Winn, Jr. 1992. *Mycology*, p. 791-877. *Color atlas and textbook of diagnostic microbiology*, 4th ed., J.P. Lippincott Co., Philadelphia.

TECHNICAL INFORMATION: In the United States, telephone Technical Services, toll free (800) 638-8663.

2000 Becton Dickinson and Company.

BD, BBL and RODAC are trademarks of Becton Dickinson and Company.

ATCC is a trademark of the American Type Culture Collection.

BD Biosciences

7 Loveton Circle

Sparks, MD 21152 USA

