Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar) • Trypticase™ Soy Agar with Lecithin and Polysorbate 80 • Trypticase™ Soy Agar with Penicillinase • Trypticase™ Soy Agar with Lecithin, Polysorbate 80 and Penicillinase

Intended Use

These media are recommended for the detection and enumeration of microorganisms present on surfaces of sanitary importance. Prepared plates are provided for environmental monitoring. Sterile Pack and Isolator Pack **RODAC**™ prepared plates are particularly useful for monitoring surfaces in clean rooms, Isolator Systems and other environmentally-controlled areas and are also recommended for use in air sampling equipment such as the Surface Air System. **Finger Dab**™ Sterile Pack and Isolator Pack plates are intended for sampling gloved hands.

Summary and Explanation

These media may be employed to establish and monitor cleaning techniques and schedules.[1-4] Collection of "samples" from identical areas before and after treatment with disinfectant yields data useful in evaluating cleaning procedures in environmental sanitation. Tryptic (**Trypticase**) Soy Agar with Lecithin and Polysorbate 80 is recommended for the Aerobic Plate Count (Microbial Limit Test) for water-miscible cosmetic products containing preservatives.[5]

RODAC (Replicate Organism Detection and Counting) and contact plates are used in a wide variety of surface sampling programs and may be employed to establish and monitor cleaning techniques and schedules.[1-4,6] The presence and number of microorganisms on a flat impervious surface is determined by the appearance of colonies on the surface of the medium following application to the test surface and incubation.[7,8] The **RODAC** plate has a marked grid to facilitate counting organisms. The **RODAC** SL (Secure Lid) has three lugs on the base, providing a tight fit between lid and base to reduce accidental contamination.

The 100 × 15 mm and the 150 × 15 mm style plates can be used for active and passive air sampling. These plates are also designed for personnel monitoring of finger tips (**Finger Dab**).

Principles of the Procedure

Casein and soy peptones are a source of nutrients required for the replication of microorganisms. Sodium chloride maintains osmotic equilibrium. Lecithin and polysorbate 80, two commonly used neutralizers, are reported to inactivate residual disinfectants when the sample is being collected.[7] Lecithin is incorporated to neutralize quaternary ammonium compounds and polysorbate 80 is used to neutralize substituted phenolic disinfectants.[9-12] Agar is the solidifying agent.

Trypticase Soy Agar with Penicillinase and **Trypticase** Soy Agar with Lecithin, Polysorbate 80 and Penicillinase contain 50 mL/L of penicillinase, which inactivates antibiotics such as penicillins and cephalosporins.

With the Sterile Pack and Isolator Pack plates, the entire double-wrapped (Sterile Pack) or triple-wrapped (Isolator Pack) product is subjected to a sterilizing dose of gamma radiation, so that the contents inside the outer package(s) are sterile.[13] This allows the inner package to be aseptically removed 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 true recovery and not pre-existing medium contaminants. A third rolled sterile bag is included as a transport device. Isolator Pack plates have been validated to protect the medium from vaporized hydrogen peroxide when used in an Isolator System.

Formulae

Difco™ Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar)

Approximate Formula* Per Liter

Pancreatic Digest of Casein	15.0	g
Soy Peptone	5.0	g
Sodium Chloride	5.0	g
Lecithin	0.7	g
Polysorbate 80	5.0	g
Agar	15.0	g

BBL™ Trypticase™ Soy Agar with Lecithin and Polysorbate 80

Approximate Formula* Per Liter

Pancreatic Digest of Casein	15.0	g
Papaic Digest of Soybean Meal	5.0	g
Sodium Chloride	5.0	g
Lecithin	0.7	g
Polysorbate 80	5.0	g
Agar	15.0	g

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

Directions for Preparation from Dehydrated Product

1. Suspend 45.7 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Cool to approximately 45°C.
4. In **RODAC** plates, use 16.5-17.5 mL per plate.
5. Test samples of the finished product for performance using stable, typical control cultures.

User Quality Control

NOTE: Differences in the Identity Specifications and Cultural Response testing for media offered as both **Difco**™ and **BBL**™ brands may reflect differences in the development and testing of media for industrial and clinical applications, per the referenced publications.

Identity Specifications

Difco™ Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar)

Dehydrated Appearance:	Beige, free-flowing, homogeneous, may appear moist.
Solution:	4.57% solution, soluble in purified water upon boiling with frequent gentle swirling. When hot, solution is medium amber, slightly opalescent with a resuspendable precipitate.
Prepared Appearance:	Light to medium amber, slightly opalescent, may have a precipitate.
Reaction of 4.57% Solution at 25°C:	pH 7.3 ± 0.2

Cultural Response

Difco™ Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar)

Prepare the medium per label directions. Test the medium in parallel with Plate Count Agar, using the pour plate method. Apply disks impregnated with varying dilutions of a quaternary ammonium compound to the medium surface. Incubate plates at 35 ± 2°C for 40-48 hours and inspect for zones of inhibition.

ORGANISM	ATCC™	INOCULUM CFU	GROWTH*
Escherichia coli	11229	10^2-10^3	Smaller zone of inhibition of growth compared to Plate Count Agar
Staphylococcus aureus	6538P	10^2-10^3	Smaller zone of inhibition of growth compared to Plate Count Agar

**Interpretation: The smaller zones of inhibition indicate neutralization of the quaternary ammonium compound by the medium.*

Identity Specifications

BBL™ Trypticase™ Soy Agar with Lecithin and Polysorbate 80

Dehydrated Appearance:	Medium fine, softly lumped powder, free of extraneous material. NOTE: The dehydrated medium has a characteristic "brown sugar" appearance and may seem moist.
Solution:	4.57% solution, soluble in purified water upon boiling. Solution is light to medium, yellow to tan, slightly to moderately hazy.
Prepared Appearance:	Light to medium, yellow to tan, slightly to moderately hazy.
Reaction of 4.57% Solution at 25°C:	pH 7.3 ± 0.2

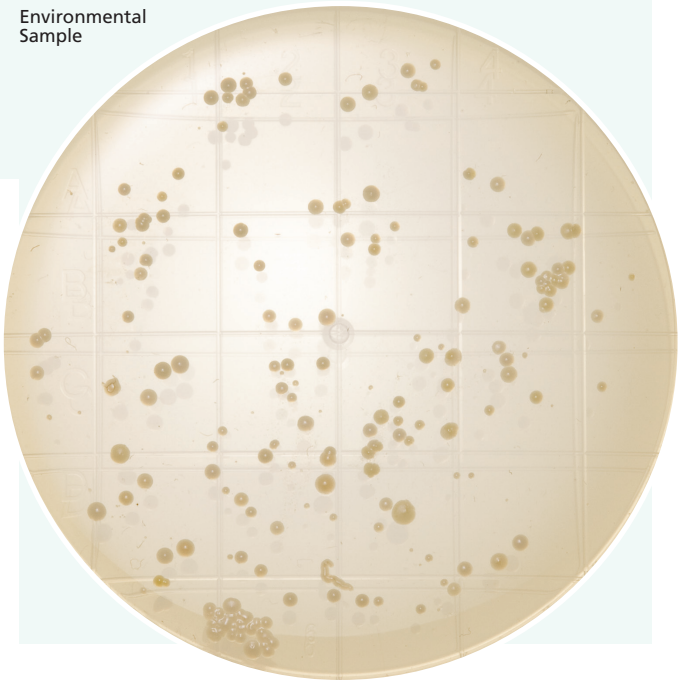
Cultural Response

BBL™ Trypticase™ Soy Agar with Lecithin and Polysorbate 80

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 2 days.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Pseudomonas aeruginosa	10145	10^3-10^4	Good
Staphylococcus aureus	25923	10^3-10^4	Good

Environmental Sample



Procedure

100 × 15 mm and 150 × 15 mm-Style Plates

1. If specimen is being cultured from a swab, roll the swab directly on the medium surface.
2. Incubate all plates at 35-37°C for 48 hours, and 25°C for 7 days or as required.
3. When incubation has been completed, count the colonies.

RODAC™/Contact Plates

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 the 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 35-37°C for 48 hours, and 25°C for 7 days or as required.

Expected Results

Because interpretations are relative, each laboratory should establish its own values for what constitutes a clean area.

Count all developing 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 contact 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 and/or serological testing.

Limitation of the Procedure

The effectiveness of preservative neutralization with this medium depends on both the type and concentration of the preservative(s).

References

1. Vesley and Michaelson. 1964. Health Lab. Sci. *1*:107.
2. Pryor and McDuff. 1969. Exec. Housekeeper, March.
3. Dell, L. A. 1979. Pharm. Technol. *3*:47.
4. Hickey, Beckelheimer and Parrow. 1993. *In* Marshall (ed.), Standard methods for the examination of dairy products, 16th ed. American Public Health Association, Washington, D.C.
5. Orth. 1993. Handbook of cosmetic microbiology. Marcel Dekker, Inc., New York, N.Y.
6. Hall and Hartnett. 1964. Public Health Rep. 79:1021.
7. McGowan. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
8. Bryan. 1995. *In* Murray, Baron, Pfaller, Tenover and Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
9. Favero, Gabis and Vesley. 1984. *In* Speck (ed.), Compendium of methods for the microbiological examination of foods, 2nd ed. American Public Health Association, Washington, D.C.
10. Quisno, Gibby and Foter. 1946. Am. J. Pharm. *118*:320.
11. Erlandson and Lawrence. 1953. Science *118*:274.
12. Sveum, Moberg, Rude and Frank. 1992. *In* Vanderzant and Splittstoesser (ed.), Compendium of methods for the examination of foods, 3rd ed. American Public Health Association, Washington, D.C.
13. Association for the Advancement of Medical Instrumentation. 1984. Process control guidelines for gamma radiation sterilization of medical devices. AAMI, Arlington, Va.

Availability

Difco™ Tryptic Soy Agar with Lecithin and Polysorbate 80 (Microbial Content Test Agar)

CCAM

Cat. No.	255320	Dehydrated – 500 g*
	255310	Dehydrated – 2 kg*

BBL™ Trypticase™ Soy Agar with Lecithin and Polysorbate 80

CCAM

Cat. No.	211764	Dehydrated – 500 g*
	212263	Dehydrated – 5 lb (2.3 kg)*

United States and Canada

Cat. No.	221943	Prepared Plates (Double Bag) – Ctn. of 100*
	221945	Contact Plates (Double Bag) – Pkg. of 20*
	221288	Prepared **RODAC**™ Plates – Pkg. of 10*
	221287	Prepared **RODAC**™ Plates – Ctn. of 100*
	222242	Prepared **RODAC**™ SL Plates – Pkg. of 20*
	222249	Prepared **RODAC**™ SL Plates – Ctn. of 100*
	221961	Sterile Pack Contact Plates – Pkg. of 10*
	222208	Sterile Pack Contact Plates – Ctn. of 100*
	221238	Sterile Pack **RODAC**™ Plates – Pkg. of 10*
	222207	Sterile Pack **RODAC**™ Plates – Ctn. of 100*
	222248	Sterile Pack **RODAC**™ SL Plates – Pkg. of 10*
	222247	Sterile Pack **RODAC**™ SL Plates – Ctn. of 100*
	292335	Isolator Pack **RODAC**™ Plates – Ctn. of 100*
	222252	Isolator Pack **RODAC**™ SL Plates – Pkg. of 10*
	222253	Isolator Pack **RODAC**™ SL Plates – Ctn. of 100*
	292271	Sterile Pack **Finger Dab**™ Plates – Ctn. of 100*
	292648	Isolator Pack **Finger Dab**™ Plates – Pkg. of 10*
	292649	Isolator Pack **Finger Dab**™ Plates – Ctn. of 100*
	292650	Isolator Pack **Finger Dab**™ Plates (150 × 15 mm-style) – Pkg. of 5*

Europe

Cat. No.	254038	Contact Plates – Pkg. of 33*
	254542	Contact Plates – Pkg. of 220*
	257383	Isolator Pack Plates – Pkg. of 10*
	257384	Isolator Pack Plates – Ctn. of 100*
	257379	Isolator Pack Plates (HF) – Ctn. of 100*
	257380	Isolator Pack **RODAC**™ Plates – Pkg. of 10*
	257381	Isolator Pack **RODAC**™ Plates – Ctn. of 100*
	257378	Isolator Pack **RODAC**™ SL Plates – Pkg. of 10*
	257382	Isolator Pack **RODAC**™ SL Plates – Ctn. of 100*

BBL™ Trypticase™ Soy Agar with Penicillinase

Cat. No.	221839	Sterile Pack Plates – Pkg. of 10*
	221837	Sterile Pack Plates (150 × 15 mm-style) – Pkg. of 5*

BBL™ Trypticase™ Soy Agar with Lecithin, Polysorbate 80 and Pencillinase

United States and Canada

Cat. No.	221987	Contact Plates – Pkg. of 10*
	221234	Sterile Pack **RODAC**™ Plates – Pkg. of 10*
	222246	Sterile Pack **RODAC**™ SL Plates – Pkg. of 10*

Europe

Cat. No.	257400	Sterile Pack **RODAC**™ Plates – Ctn. of 100*
	257421	Isolator Pack **RODAC**™ SL Plates – Pkg. of 10*
	257455	Sterile Pack Plates – Ctn. of 100*
	257403	Isolator Pack Plates – Ctn. of 100*

**Store at 2-8°C.*