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TECHNICAL PRODUCT INFORMATION

Tryptic Soy Agar w/Lecithin & Polysorbate (Tween™) 80

INTENDED USE:
Tryptic Soy Agar w/Lecithin & Polysorbate 80 is used for the detection and enumeration of microorganisms present on surfaces of sanitary importance. Northeast Laboratory Services offers a wide range of Tryptic Soy Agar w/Lecithin & Polysorbate 80 available in petri dish (standard and contact), tube, and bottle. Consult the NEL Catalog of Products or call for more information.

HISTORY/SUMMARY:
In 1955, Leavitt et al. discovered Tryptic Soy Agar supported excellent growth of aerobic and anaerobic microorganisms.[1] Tryptic Soy Agar is a nutritious base enhanced with a variety of supplements, including Lecithin & Polysorbate (Tween™) 80.

Collection of samples from the same areas before and after use of disinfectant is useful in determining effectiveness of cleaning procedures. Contact plates are used for surface sampling such as establishing and monitoring cleaning techniques and environmental monitoring. After sampling a flat solid surface, the presence and number of microorganisms is determined by the growth of colonies on the surface medium following the incubation period.

TSA with Lecithin and Polysorbate 80 may also be used for air sampling, monitoring fingertips of personnel or sanitary efficiency of containers, equipment, and work area.
The Lecithin & Tween 80 neutralizes some preservatives which may inhibit bacterial growth, thereby reducing the "residual preservative carryover".[2]

PRINCIPLES:
Enzymatic Digest of Casein and Enzymatic Digest of Soybean Meal provide the nitrogen, vitamins, and carbon in Tryptic Soy Agar w/Lecithin & Tween 80. Sodium chloride maintains osmotic balance in the medium. Lecithin & Tween 80 are added to neutralize residual surface disinfectants during sample collection.[2, 3, 4] Lecithin is added to neutralize quaternary ammonium compounds. Tween 80 is incorporated to neutralize phenols, hexachlorophene, formalin, and, in combination with lecithin, ethanol.[5] Agar is the solidifying agent.

FORMULA:

Component per Liter of purified water	Amount
Enzymatic Digest of Casein	**15.0 g**
Enzymatic Digest of Soybean Meal	**5.0 g**
Sodium Chloride	**5.0 g**
Lecithin	**0.7 g**
Polysorbate 80 (Tween™ 80)	**5.0 g**
Agar	**20.5 g**

Final pH: 7.3 ±0.2 @ 25°C

PRECAUTIONS:
Since living organisms used with this material can be infectious to the user, proper handling and disposal methods should be established by the laboratory director. These products are for Laboratory Use Only.

STORAGE:
Store the media at 2-8°C. Do not use if discolored, dehydrated or obviously contaminated.
Protect product from extreme temperatures if product is shipped to contract laboratories.

SPECIMEN COLLECTION:
Refer to appropriate references for test methods and interpretation of results.

The success in recovering microorganisms from a specimen greatly depend on a number of factors such as site chosen for collection, media and environmental conditions used for incubation, temperature, inoculum size used, etc. Standard procedure should be chosen and these procedures should be implemented by suitably trained personnel under the supervision of a microbiologist or other qualified personnel.

PROCEDURE:
15 X 100 mm Monoplate:

1. Allow media to warm to room temperature before use.
2. If culturing using a swab, roll the swab directly on the agar surface
    a. Incubate all samples at 35-37°C for 48 hours and 25°C for 7 days or as required
    b. Upon incubation completion, count the colonies
3. Follow laboratory procedure for isolation and identification of organism growth.

Contact Plate:

1. Samples may be collected in duplicate.
2. Select plate and carefully remove lid.
3. Hold the plate with thumb and second finger:
    a. Use the index finger to gently roll plate edge against sampling site, then gently press plate center to ensure all agar has contacted the sample surface.
    b. Do not move agar plate side to side to avoid spreading contaminants over the agar surface. Moving side to side impedes individual growth of colony formation, by causing confluent growth of colonies.
4. Incubate exposed plates at 35 ± 2°C for 48 hours and 25 ± 2°C for 7 days or as required.
5. After incubation:
    a. Count the colonies with distinct margins
    b. Count all visible colonies
        i. Spreading colonies should be counted as one
        ii. Care should be taken to observe other distinct colonies intermingled in the growth around the periphery or along a hairline
        iii. Count intermingled colony, bicolored colony or halo-type spreaders each as one colony
    c. Generally 200 colonies is the maximum that can be counted on each plate
    d. Record the number of colonies counted
6. Subculture colonies of interest to selective mediums for identification by biochemical testing and/or microscopic examination of smears such as a gram stain.

Contact plate grid method:

1. Subdivide surface (floor or wall) into 36 equal squares per 100 square feet of area by measuring 5 equidistant dividing lines from each of the two adjacent sides.
2. These lines will intersect at twenty-five points.
3. Number intersections consecutively in a circuitous configuration.
4. Use red for odd numbers, black for even numbers.
5. Omit 13 which is located in the center of the total area.
6. For areas smaller than 25 square feet, divide into 36 equal squares (16 intersections). Sample 8 even numbered or odd numbered intersections at each sampling period.
7. For areas 25-100 square feet, divide area as in step 1.
8. Label plates with intersection numbers
9. Incubate exposed plates at 35 ± 2°C for 48 hours and 25 ± 2°C for 7 days or as required.
10. After incubation:
    a. Count the colonies with distinct margins
    b. Count all visible colonies
        i. Spreading colonies should be counted as one
        ii. Care should be taken to observe other distinct colonies intermingled in the growth around the periphery or along a hairline
        iii. Count intermingled colony, bicolored colony or halo-type spreaders each as one colony

c. Generally 200 colonies is the maximum that can be counted on each plate
d. Record the number of colonies counted

11. Subculture colonies of interest to selective mediums for identification by biochemical testing and/or microscopic examination of smears such as a gram stain.

EXPECTED RESULTS:
Each laboratory should establish its own values for what is designated as a clean area.

Colony counts may be recorded as:
1. Individual counts
2. Number of viable particles per square foot (agar area is 3.97 square inches)
3. Means and standard deviations

LIMITATIONS:
The effectiveness of preservative neutralization with this medium depends on both the type and concentration of the preservatives.

PERFORMANCE TEST:
Approval of each lot of Tryptic Soy Agar w/Lecithin & Polysorbate 80 is based on the demonstrated effectiveness in cultivating and/or differentiating the microorganisms listed below. The growth characteristics and reactions listed are typical observations made on challenged samples:

ATCC #	Control Organism	Incubation Temperature	Results
25923	*Staphylococcus aureus*	30-35°C	Growth in 18-24 hrs
25922	*Escherichia coli*	30-35°C	Growth in 18-24 hrs
10231	*Candida albicans*	30-35°C	Growth in 18-24 hrs

QUALITY CONTROL:
The performance characteristics of TSA w/Lecithin & Polysorbate 80 may be confirmed by the Laboratory through a judicious selection of organisms such as those recorded above or additional QC organisms as needed.

REFERENCES:

1. **Leavitt, J.M., I.J. Naidorf and P. Shugaevsky.** 1955. The undetected anaerobe in endodontics: a sensitive medium for detection of both aerobes and anaerobes. The NY J. Dentist. **25:** 377-382.
2. **Orth, D.S. 1993.** Handbook of cosmetic microbiology. Marcel Dekker, Inc., New York, NY.
3. **Quisno, R., I. W. Gibby, and M. J. Foter.** 1946. A neutralizing medium for evaluating the germicidal potency of the quatemary ammonium salts. Am. J. Pharm. **118:**320-323.
4. **Erlandson, A.L. Jr., and C.a. Lawrence.** 1953. Inactivating medium for hexachlorophene (G-11) types of compounds and some substituted phenolic disinfectants. Science **118**: 274-276.
5. **Brummer, B.** 1976. Influence of possible disinfectant transfer on *Staphylococcus aureus* plate counts after contact sampling. App. Environ. Microbiol. **32:**80-84.
6. **Favero (chm.).** Microbiological sampling of surfaces – a state of the art report. Biological Contamination Control Committee, American Association of Contamination Control.
7. **Difco & BBL Manual**, Manual of Microbiological Culture Media, 2003
8. Acumedia Product Information Sheet, 7163, Rev 3, May 2011