



Dey-Engley Neutralizing Agar (D/E Agar Disinfectant Testing) M186

Intended use

Dey-Engley Neutralizing Agar is used in disinfectant testing, where neutralization of the chemical is important for determining its bactericidal activity.

Composition**

Ingredients	Gms / Litre
Tryptone	5.000
Yeast extract	2.500
Dextrose(Glucose)	10.000
Sodium thiosulphate	6.000
Sodium thioglycollate	1.000
Sodium bisulphite	2.500
Lecithin	7.000
Polysorbate 80	5.000
Bromocresol purple	0.020
Agar	15.000
Final pH (at 25°C)	7.6±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 54.02 grams in 1000 ml purified / distilled water. Heat to boiling to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Mix well and pour into sterile Petri plates.

Principle And Interpretation

Dey-Engley Neutralizing Agar is formulated as per the procedure described by Engley and Dey (3). A strongly bacteriostatic substance inhibits the growth and reproduction of bacteria without killing them. These bacteria hold the ability to cause infection under favorable conditions. Dey-Engley Neutralizing Agar neutralizes a broad spectrum of antiseptics and disinfectants including quaternary ammonium compounds, phenolic, iodine and chlorine preparations, mercurial, formaldehyde and glutaraldehyde. (3).

Tryptone provide nitrogen and carbon source, long chain amino acids, vitamins and other essential nutrients. Dextrose is an energy source. Yeast extract is also a rich source of vitamin B-complex. The present formulation incorporate neutralizing substances for almost all the active products used as antiseptics and disinfectants. Sodium bisulfite neutralizes aldehydes; sodium thioglycollate neutralizes mercurial; sodium thiosulfate neutralizes iodine and chlorine (3); lecithin neutralizes quaternary ammonium compounds; and polysorbate 80, a non-ionic surface-active agent, neutralizes substituted phenolic (2,4,7,8). Bromocresol purple is an indicator for dextrose utilization. Due to the high concentration of lecithin in the broth medium, turbidity cannot be used to detect growth. Therefore, bromocresol purple and dextrose are added to the medium. Those organisms that ferment dextrose will turn the medium from purple to yellow. (3). For Agar Medium: Dey -Engley Neutralizing 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. Incubate the plates, by covering the lids, at an appropriate temperature. The presence of microorganism is determined by the appearance of colonies on the surface of agar medium. Neutralization Test: Growth in Neutralizing Broth and no growth in Neutralizing Broth Base indicate neutralization of disinfectant. To check bactericidal activity, both broth tubes are inoculated on D/E Neutralizing Agar. Positive growth from negative tubes of Neutralizing Broth Base indicates bacteriostatic substance while negative growth indicates a bactericidal disinfectant. All positive tubes should show growth on Dey-Engley Neutralizing Agar. The control disinfectants used in test procedure are 2% chlorine, 2% formaldehyde, 1% glutaraldehyde, 2% iodine, 2% phenol, 1/750 quaternary ammonium compounds, 1/1000 mercurial etc.

Type of specimen

Food and dairy samples; Environmental samples, cosmetic, pharmaceutical .

Specimen Collection and Handling

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (1,8,9). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

Read the label before opening the container. Wear protective gloves/protective clothing/eye protection/ face protection. Follow good microbiological lab practices while handling specimens and culture. Standard precautions as per established guidelines should be followed while handling specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Due to nutritional variations, some strains may show poor growth

Performance and Evaluation

Performance of the medium is expected when used as per the direction on the label within the expiry period when stored at recommended temperature.

Quality Control

Appearance

Light yellow to bluish grey homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Purple to reddish purple coloured, opalescent gel (may have particulate precipitate) forms in Petri plates.

Reaction

Reaction of 5.4% w/v aqueous solution at 25°C. pH : 7.6±0.2

pH

7.40-7.80

Cultural Response

Cultural characteristics observed after an incubation at 35 - 37°C for 40 - 48 hours.

Organism	Inoculum (CFU)	Growth	Recovery
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	≥70%
<i>Pseudomonas aeruginosa</i> ATCC 27853 (00025*)	50-100	luxuriant	≥70%
<i>Salmonella</i> Typhimurium ATCC 14028 (00031*)	50-100	luxuriant	≥70%
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923 (00034*)	50-100	luxuriant	≥70%
<i>Bacillus subtilis</i> subsp. <i>spizizenii</i> ATCC 6633 (00003*)	50-100	luxuriant	≥70%
<i>Candida albicans</i> ATCC 10231 (00054*)	50-100	luxuriant	≥70%
<i>Aspergillus brasiliensis</i> ATCC 16404 (00053*)	50-100	luxuriant	≥50%

* - Corresponding WDCM numbers

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 2-8°C. Use before expiry date on the label. On opening, product should be properly stored dry, after tightly capping the bottle in order to prevent lump formation due to the hygroscopic nature of the product. Improper storage of the product may lead to lump formation. Store in dry ventilated area protected from extremes of temperature and sources of ignition Seal the container tightly after use. Use before expiry date on the label.

Product performance is best if used within stated expiry period.

Disposal

User must ensure safe disposal by autoclaving and/or incineration of used or unusable preparations of this product. Follow established laboratory procedures in disposing of infectious materials and material that comes into contact with clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (5,6).

Reference

1. American Public Health Association, Standard Methods for the Examination of Dairy Products, 1978, 14th Ed., Washington D.C.
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4. Erlandson A. L., and Lawrence C. A., 1953, Science 118:274.
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8. Salfinger Y., and Tortorello M.L. Fifth (Ed.), 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
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