



Technical Data

Glucose Starch Agar

M989

Intended Use:

Recommended for detection of *Clostridium perfringens* with addition of salicin, raffinose and phenol red.

Composition**

Ingredients	Gms / Litre
Proteose peptone	15.000
Dextrose (Glucose)	10.000
Starch, soluble	5.000
Sodium chloride	5.000
Disodium hydrogen phosphate	3.000
Gelatin	20.000
Agar	10.000
Final pH (at 25°C)	7.2±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 68.0 grams in warm 1000 ml purified/distilled water. Heat to boiling to dissolve the medium completely. Dispense in tubes and sterilize by autoclaving at 15 lbs pressure (121°C) for 30 minutes. Allow the tubed medium to cool in an upright position.

Principle And Interpretation

Clostridial species are one of the major causes of food poisoning/ gastro-intestinal illnesses. They are gram-positive, spore-forming rods that occur naturally in the soil (1). Among the family are: *Clostridium botulinum*, which produces one of the most potent toxins in existence; *Clostridium tetani*, causative agent of tetanus; and *Clostridium perfringens*, commonly found in wound infections and diarrhoea cases. The use of toxins to damage the host is a method deployed by many bacterial pathogens. The major virulence factor of *C.perfringens* is the CPE enterotoxin, which is secreted upon invasion of the host gut, and contributes to food poisoning and other gastrointestinal illnesses (1). Glucose Starch Agar is used as a basal medium, which with the addition of raffinose, salicin and phenol red indicator is used for detecting *C. perfringens* (2). This medium is also recommended by APHA (6).

The medium contains proteose peptone, which supplies the nitrogenous nutrients for *C.perfringens*. Dextrose is the fermentable carbohydrate source and is fermented by most Clostridia. However, raffinose and salicin are fermented with acid and gas production by only some strains of *C.perfringens*. Dispense the medium in different tubes and add a few drops of phenol red, the pH indicator, which turns yellow at acidic pH. Gas production is indicated by bubble formation. Gelatin is liquefied by *C. perfringens* within 48 hours. Sodium chloride maintains the osmotic balance of the medium.

Type of specimen

Food samples.

Specimen Collection and Handling

For food samples, follow appropriate techniques for sample collection and processing as per guidelines (5). 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. NA

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

Cream to beige homogeneous free flowing powder

Gelling

Firm, comparable with 1.0% Agar gel and 2.0% Gelatin.

Colour and Clarity of prepared medium

Light amber coloured, clear to slightly opalescent gel forms in tubes as butts

Reaction

Reaction of 6.8% w/v aqueous solution at 25°C. pH : 7.2±0.2

pH

7.00-7.40

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-72 hours. Dextrose fermentation is detected using phenol red indicator

Organism	Inoculum (CFU)	Growth	Raffinose (72 hours)	Salicin (24 hours)
<i>Clostridium perfringens</i> ATCC 12924	50-100	luxuriant	acid production, yellow colour	negative reaction, no colour change or red
<i>Clostridium paraperfringens</i>	50-100	luxuriant	negative reaction, no colour change or red	acid and gas production, yellow colour and bubble formation
<i>Escherichia coli</i> ATCC 25922 (00013*)	50-100	luxuriant	negative reaction, no colour change or red	negative reaction, no colour change or red

Key : *Corresponding WDCM numbers.

Storage and Shelf Life

Store between 10-30°C in a tightly closed container and the prepared medium at 20-30°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. 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 sample must be decontaminated and disposed of in accordance with current laboratory techniques (3,4).

Reference

1. Czeaczulin J.R., Hanna P.C., Mcclane B.A., Cloning, nucleotide sequencing, and expression of the *Clostridium perfringens* enterotoxin gene in *Escherichia coli*. Infect. Immun. 61: 3429-3439 (1993).
2. Hauschild A. H. W. and Hilsheimer R., 1974, Appl. Microbiol., 27:78.
3. Isenberg, H.D. Clinical Microbiology Procedures Handbook 2nd Edition
4. Jorgensen, J.H., Pfaller, M.A., Carroll, K.C., Funke, G., Landry, M.L., Richter, S.S and Warnock., D.W. (2015) Manual of Clinical Microbiology, 11th Edition. Vol. 1.
5. Salfinger Y., and Tortorello M.L., 2015, Compendium of Methods for the Microbiological Examination of Foods, 5th Ed., American Public Health Association, Washington, D.C.
6. Speck M. L., (Eds.), 1984, Compendium of Methods For The Microbiological Examination of Foods, 2nd Ed., APHA, Washington, D.C.

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