Glucose Starch Agar

M989

Glucose Starch Agar is used as a basal medium with the addition of salicin, raffinose and phenol red for detection of *Clostridium perfringens* .

Composition**

Ingredients	Gms / Litre
Proteose peptone	15.000
Dextrose	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 grams in warm 1000 ml 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 (3).

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.

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

Cultural Response

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

Storage and Shelf Life

Store below 30°C in tightly closed container and prepared medium at 2-8°C. Use before expiry period on the label.

Reference

1. Czeczulin 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. Speck M. L., (Eds.), 1984, Compendium of Methods For The Microbiological Examination of Foods, 2nd Ed., APHA, Washington, D.C.

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