Saline Agar

M942

Intended Use:

For alpha-toxin detection in *Clostridium perfringens.*

Composition**

Ingredients	g/ L
Sodium chloride	8.500
Agar	15.000
Final pH (at 25°C)	7.0±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 23.5 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. After cooling to 45-50°C, add blood to give final concentration of 5% v/v. Mix well and pour into sterile Petri plates.

Principle And Interpretation

A heat-labile enterotoxin produced only by sporulating cells (1) induces the major symptoms of diarrhea in perfringens poisoning. The enterotoxin appears to be released in vivo in the intestine by the sporulating organisms (2). Hence alpha toxin can be used as an index for detecting the presence of *Clostridium perfringens* in food (3). However, the viability of *C. perfringens* cells are lost if the suspected food samples are frozen (4). Saline Agar Base with blood is used to measure the haemolytic activity of alpha toxin (5,6,7).

Sodium chloride provides essential ions. Red blood cells are added in the medium to examine haemolytic reactions, which indirectly helps in detection of alpha toxin.

Additional Test:

The plates containing 7 ml of medium is dried overnight at room temperature and stored at 4°C till use. Just prior to use, test wells are cut in the agar using a template space of test wells, 3 cm apart and 2 cm from the edge of the plate. Make 2 additional wells 3 cm apart near the centre of the plate. Peripheral wells of duplicate plates are filled with the undiluted extract (alpha toxin extraction) and eight twofold dilutions of extract. To determine whether the haemolysis caused by the extract is due to alpha toxin, a portion of the 1:2 dilution of the extract is mixed with *C. perfringens* alpha toxin and with *C. perfringens* type A diagnostic antiserum containing alpha toxin and placed in the two center wells.The plates are incubated for 24 hours at 35°C and examined for haemolytic zones surrounding the wells. A 1 mm zone of haemolysis is considered as significant (5).

Type of specimen

Clinical samples - faeces; Food and dairy samples.

Specimen Collection and Handling:

For clinical samples follow appropriate techniques for handling specimens as per established guidelines (8,9).

For food and dairy samples, follow appropriate techniques for sample collection and processing as per guidelines (2,7,10,11). After use, contaminated materials must be sterilized by autoclaving before discarding.

Warning and Precautions :

In Vitro diagnostic Use. For professional use only. 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 clinical specimens. Safety guidelines may be referred in individual safety data sheets.

Limitations :

1. Further biochemical and serological tests must be carried out for further identification.

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

White to light yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity of prepared medium

Basal Medium yields light yellow coloured, clear gel. On addition of red blood cells, red coloured opaque gel forms in Petri plates

Reaction

Reaction of 2.35% w/v aqueous solution at 25°C. pH : 7.0±0.2

pH

6.80-7.20

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours with added red blood cells.

Organism	Inoculum (CFU)	Haemolysis
Clostridium perfringens ATCC 12924	50-100	positive reaction

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 clinical sample must be decontaminated and disposed of in accordance with current laboratory techniques (8,9).

Reference

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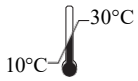
IVD ***In vitro*** **diagnostic medical device**



Storage temperature

EC REP CEpartner4U, Esdoornlaan 13,
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CE Marking

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