Technical Information

Saline Agar

Product Code: DM 1942

Application: - Saline Agar Base is recommended for the detection of alpha-toxin in *Clostridium perfringens*.

Composition**

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

**Formula adjusted, standardized to suit performance parameters

Principle & Interpretation

Saline Agar Base with blood is recommended to measure the haemolytic activity of alpha toxin (5, 6, 7). 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).

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.

Methodology

Suspend 23.5 grams of dehydrated powder media in 1000 ml distilled water. Mix thoroughly & heat to boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. After cooling to 50°C, add blood to give final concentration of 5 % v/v. Shake well before pour into sterile Petri plates.

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 (7).

Quality Control

Appearance

White to light yellow homogeneous free flowing powder

Gelling

Firm, comparable with 1.5% Agar gel

Colour and Clarity

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 Range

6.80-7.20

Cultural Response

DM 1942: 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

Dried Media: Store below 30°C in tightly closed container and the prepared medium below 2-8°C. Use before expiry date on the label.
Prepared Media: 2-8° in sealable plastic bags for 2-5 days.

Further Reading

1. Duncan C. L., 1973, J. Bacteriol.,113:932.
2. Vanderzant C. and Splittstoesser D. F., (Eds.), 1992, Compendium of Methods for the Microbiological Examination of Foods, 3rd Ed., APHA, Washington, D.C.
3. Harmon S. M., Kantler D. A., 1970, Method for Estimating the presence of Clostridium perferingens in Food.
4. Hall H. E., 1968, J. Asst office Agar. Chem: 51: 1338-134.
5. Noyes N. E. and Easterling R., 1967, J. Bacteriol., 93:1254-1261.
6. Sheldon D. R., Moskowitz M. and Daercerell M. W., 1958, J. Bacteriol., 77:375 - 382.
7. Dr. Williams Horwitz, (Ed.), 2000, Official Methods of Analysis of AOAC International, Maryland.

Disclaimer :