Detection and Determination of Anaerobic Mesophilic Spore \Formers in Foods (Clostridium perfringens)

Food poisoning caused by *Clostridium perfringens* may occur when foods such as meat or poultry are cooked and held without maintaining adequate heating or refrigeration before serving. The presence of small numbers of *C. perfringens* is not uncommon in raw meats, poultry, dehydrated soups and sauces, raw vegetables, and spices. Because the spores of some strains are resistant to temperatures as high as 100°C for more than l h, their presence in foods

may be unavoidable. Furthermore, the oxygen level may be sufficiently reduced during cooking to permit growth of the clostridia. Spores that survive cooking may germinate and grow rapidly in foods that are inadequately refrigerated after cooking. Thus, when clinical and epidemiological evidence suggests that *C. perfringens* is the cause of a food poisoning outbreak, the presence of hundreds of thousands or more of these organisms per gram of food substantiates the diagnosis.

Illness typically occurs 8-15 h after ingestion of the contaminated food. The symptoms, which include intense abdominal cramps, gas, and diarrhea (nausea and vomiting are rare), have been attributed to a protein enterotoxin produced during sporulation of the organism in the intestine.

The enterotoxin can be detected in sporulating cultures, and a method for this purpose is included. A high correlation has been established

between the ability of ***C. perfringens*** strains to produce enterotoxin and their ability to cause food poisoning. However, it is difficult to obtain consistent sporulation with some strains.

C . perfringens cells lose their viability when foods are frozen or held under prolonged refrigeration unless special precautions are taken. Such losses may make it difficult to establish

C . perfringens as the specific cause of a food poisoning outbreak. It is recommended that samples which cannot be examined immediately be treated with buffered glycerin-salt solution and stored or shipped frozen to the laboratory as described below.

Procedure

Inoculate 2 g of food sample into 15 to 20 ml of **Cooked Meat Medium(CMM)** in duplicates. Incubate at 35 for 24 h.

Positive tubes showing turbidity and gas production are streaked on to **TSC agar (Tryptone Sulfite Cycloserine)** plates. Overlay with TSC agar. Incubate plates upright, anaerobically for 18 to 20 h at 35 C.

Count all colonies that are black in color surrounded by a zone of precipitate. agar, and they are positive for reduction of nitrate to nitrite which is indicated by the development of red or orange color of the medium.

On **Lactose Gelatin Medium (LGM),** the culture shows positive reaction for fermentation of lactose as indicated by gas bubbles and change in color of medium from red to yellow.

Gelatin is liquified by *C.perfringens.*

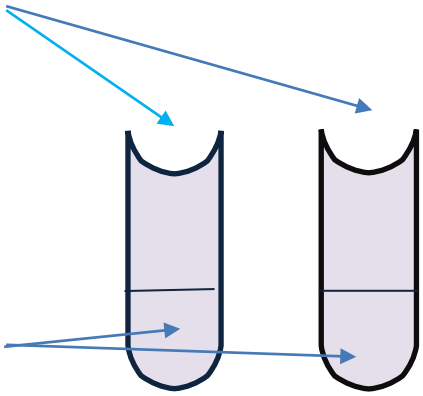
Calculation:- NA

Expression of Result

Clostridium perfringens = present/absent

Chart of clostridium perfringens

2g of food sample



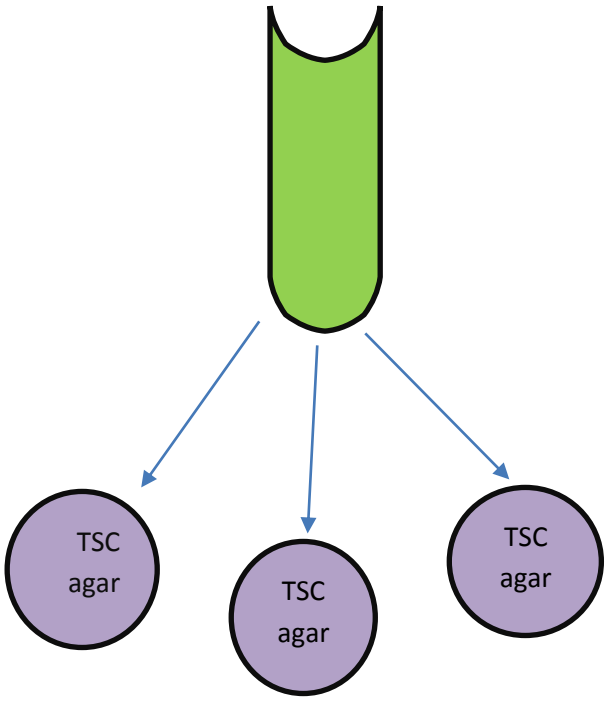
15-20ml of

Cooked meat

ediaM

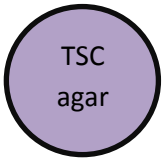
incubated at 35c for 24 h

Positive tube (turbidity and gas production)



Incubated upright 18-20 h

anaerobically


TSC
agar