Lactose Sulphite Broth Base

M1287

Lactose Sulphite Broth Base is recommended for detection and enumeration of *Clostridium perfringens* in pharmaceutical products.

Composition**

Ingredients	Gms / Litre
Enzymatic digest of casein	5.000
Yeast extract	2.500
Sodium chloride	2.500
Lactose	10.000
L-Cysteine hydrochloride	0.300
Final pH (at 25°C)	7.1±0.2

**Formula adjusted, standardized to suit performance parameters

Directions

Suspend 20.3 grams in 1000 ml distilled water. Heat if necessary to dissolve the medium completely. Dispense in tubes containing inverted Durham s tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C and add filter-sterilized solution of 1.2% sodium metabisulphite (0.5ml) and 1.0% ferric ammonium citrate (0.5ml) to each tube.

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 soil (1). *Clostridium perfringens* are 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 (3).

Lactose Sulphite Broth Base is formulated as per the European Pharmacopoeia (4th Edition) (1). This medium is useful in semi-quantitative test for presence of *C. perfringens* in pharmaceutical products where the level of this species is a criterion of quality (2).

The medium contains enzymatic digest of casein and yeast extract, which provide essential nitrogenous compounds for *Clostridia* . Lactose serves as a carbon or fermentable carbohydrate source. Gas production formed due to fermentation gets trapped in the inverted Durhams tubes. Cysteine hydrochloride provides reduced conditions. Sodium metabisulphite and ferric ammonium citrate act as indicators of sulphite reduction, indicated by blackening of the medium. Refer appropriate references for standard procedures (1).

Quality Control

Appearance
Cream to yellow homogeneous free flowing powder

Colour and Clarity of prepared medium
Light amber coloured, clear solution without any precipitate

Reaction
Reaction of 2.03% w/v aqueous solution at 25°C. pH : 7.1±0.2

pH
6.90-7.30

Cultural Response
M1287: Cultural characteristics after an incubation at 46±0.5°C for 24-48 hours.

Organism	Inoculum (CFU)	Growth	H2S	Gas

Clostridium perfringens ATCC 12924	50-100	luxuriant	positive reaction, blackening of medium	positive reaction
Clostridium perfringens ATCC 13124	50-100	luxuriant	positive reaction, blackening of medium	positive reaction
Clostridium sporogenes ATCC 19404	50-100	luxuriant	negative reaction	positive reaction
Clostridium sporogenes ATCC 11437	50-100	luxuriant	negative reaction	positive reaction

Storage and Shelf Life

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

Reference

1. European Pharmacopoeia, 2002, Suppl.4.2. (2001). Chp. 2.6.13, 4th Ed., Council of Europe, Strasbourg
2. British Pharmacopoeia, 2004, The Stationery office British Pharmacopoeia.
3. Czeczulin J. R., Hanna P. C., Mcclane B. A., 1993, Infect. Immun., 61: 3429-3439.
4. International Organization for Standardization (ISO), 1997, Draft ISO/DIS 7937:1997.

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