



PRODUCT SPECIFICATION SHEET

Lactose Sulfite Broth Base (DM916)

Intended Use

Lactose Sulfite Broth Base (DM916) is recommended for detection and enumeration of *Clostridium perfringens* in pharmaceutical products.

Product Summary and Explanation

Lactose Sulfite Broth Base is a selective medium used to detect and enumerate *Clostridium perfringens* based on lactose fermentation and production of hydrogen sulfide. 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⁾.

Principles of the Procedure

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 nutrient base provides optimal conditions for the development of *Clostridia*. Casein peptone and Yeast extract provide the basic nutrients of the medium: nitrogen, vitamins, minerals and amino acids. Lactose is a complex carbohydrate energy source. Cysteine hydrochloride is the reducing agent.

The European Pharmacopoeia recommends to prepare samples using 1:100 and 1:1000 dilutions with Buffered Peptone Water. Mix sample with medium with minimum shaking and incubate at 45.5 - 46.5°C for 24 - 48 hours. Colonies producing hydrogen sulfide are characterized by blackening due to the reaction of Sodium bisulfite and the Ferric ammonium citrate salt. The containers showing a blackening and abundant formation of gas in the Durham tube (at least 1/10 of the volume) indicate the presence of *C. perfringens*. Estimate the most probable number using the appropriate table (MPN Table).

Formula / Liter

| Ingredients | Gms / Litre |
|--|-------------|
| Casein enzymic hydrolysate | 5.00 |
| Yeast extract | 2.50 |
| Sodium chloride | 2.50 |
| Lactose | 10.00 |
| Cysteine hydrochloride | 0.30 |
| Final pH: 7.1 ± 0.2 at 25°C | |
| Formula may be adjusted and/or supplemented as required to meet performance specifications | |

Precautions

1. For Laboratory Use only.
2. IRRITANT. Irritating to eyes, respiratory system, and skin.

Directions

1. Suspend 20.3 grams of the medium in one liter of distilled water.
2. Heat with frequent agitation for one minute until completely dissolved.
3. Dispense 8 ml in test tubes with gas collection Durham tubes.
4. Sterilize at 121°C for 15 minutes. Store at 4°C.



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- Before using, add to each tube 0.5 ml of a 12 g/liter solution of Sodium Metabisulfite and 0.5 ml of a solution of 10 g/liter of Ferric Ammonium Citrate.
- Both solutions have to be freshly prepared and filter sterilized.

Quality Control Specifications

| | |
|-----------------------------------|--|
| Dehydrated Appearance | Yellow colored, homogeneous, free flowing powder |
| Solution | 2.03% Solution in Distilled or deionized water is soluble on boiling, Light to medium Amber colored, and very slightly to slightly opalescent. |
| Prepared Medium | Light to medium Amber colored, and very slightly to slightly opalescent. |
| Reaction of 2.03% Solution | pH 7.1 ± 0.2 at 25°C |
| Gel Strength | Not Applicable |

Expected Cultural Response: Cultural response on Lactose Sulfite Broth Base after an incubation at 46 ± 0.5°C for 24-48 hours.

| Sr. No. | Organisms | Results to be achieved | | | |
|---------|---|------------------------|-----------|---|-------------------|
| | | Inoculum (CFU) | Growth | H ₂ S | Gas |
| 1. | <i>Clostridium perfringens</i> ATCC 12924 | 50-100 | luxuriant | Positive reaction, blackening of medium | positive reaction |
| 2. | <i>Clostridium perfringens</i> ATCC 13124 | 50-100 | luxuriant | Positive reaction, blackening of medium | positive reaction |
| 3. | <i>Clostridium sporogenes</i> ATCC 19404 | 50-100 | luxuriant | Negative reaction | positive reaction |
| 4. | <i>Clostridium sporogenes</i> ATCC 11437 | 50-100 | luxuriant | Negative reaction | positive reaction |

The organisms listed are the minimum that should be used for quality control testing.

Test Procedure

Refer to appropriate references for specific procedures.



Uninoculated Tube *Clostridium perfringens* ATCC 12924

Results



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The fermentation of lactose is indicated by the presence of gas in the Durham tubes (minimum volume equal to $\frac{1}{4}$ of the volume of the Durham tube itself) in 24 hours, as well as simultaneous appearance of a black iron sulfide precipitate in the culture tubes indicating the presence of *Clostridium perfringens*.

Storage

Store the sealed bottle containing the dehydrated medium at 10 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

Expiration

Refer to the expiration date stamped on the container. The dehydrated medium should be discarded if not free flowing, or if the appearance has changed from the original color. Expiry applies to medium in its intact container when stored as directed.

Limitations of the Procedure

Due to nutritional variation, some strains may be encountered that grow poorly or fail to grow on this medium

Packaging

Product Name : Lactose Sulfite Broth Base

Product Code : DM916

Available Pack sizes : 100gm / 500gm

- References**
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.

Further Information

For further information please contact your local MICROMASTER Representative.



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