



PRODUCT SPECIFICATION SHEET

Differential Reinforced Clostridial Broth Base (DM519)

Intended Use

Differential Reinforced Clostridial Broth Base (DM519) is recommended for cultivation of *Clostridia* species from water.

Product Summary and Explanation

Hirsch and Grinstead⁽¹⁾ originally described Differential Reinforced Clostridial Medium for the cultivation and enumeration of clostridia. They showed that the medium was more fertile and enabled growth to be initiated from small inocula and gave a higher Clostridial count. Later, Barnes and Ingram⁽²⁾ used the medium to develop vegetative cells in assays of *Clostridium perfringens*. This medium is developed for the isolation of sulphite-reducing Clostridia from food and for their enumeration in water by multiple tube method. Differential Reinforced Clostridial Broth is used to determine the count of sulphite-reducing clostridia and *Cl. perfringens* in drinking water by MPN technique.⁽³⁾

Principles of the Procedure

Differential Reinforced Clostridial Broth Base contains peptic digest of animal tissue, beef extract, yeast extract, starch which provide essential nutrients and co-factors required for good growth of clostridia. Glucose is the fermentable carbohydrate and serves as carbon and energy source. Partial selectivity of the medium is achieved through the addition of sodium acetate. L-cysteine hydrochloride acts as reducing agent. Sodium sulphite and ferric citrate are added as indicators. The addition of ferric ammonium citrate to the medium is used to detect sulfite reduction. Blackening of the medium is due to the formation of iron sulfide.

Formula / Liter

Ingredients	Gms / Liter
Peptic digest of animal tissue	10.00
Beef extract	10.00
Yeast extract	1.50
Starch	1.00
Sodium acetate, hydrated	5.00
Glucose	1.00
L-Cysteine hydrochloride	0.50
Final pH: 7.2 ± 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 29 grams of the medium in one litre of distilled water.
2. Heat to boiling to dissolve the media completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. Just before use add 0.5 ml filter sterilized solution, prepared by mixing equal volumes of 4% w/v solution of sodium sulphite and 7% w/v ferric citrate, to 25 ml of single strength medium or 0.4 ml and 2 ml to 10 ml and 50 ml of double strength medium respectively. Mix well.





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Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light yellow coloured, clear solution without any precipitate
Reaction of 2.9% solution	pH 7.2 ± 0.2 at 25°C
Gel Strength	Not Applicable

Expected Cultural Response: Cultural characteristics observed in an anaerobic atmosphere, with added 4% w/v solution of Sodium sulphite and 7% w/v Ferric citrate after an incubation at 30-35°C within 1 week.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	H ₂ S Production
1.	<i>Clostridium perfringens ATCC 13124</i>	50-100	good-luxuriant	positive reaction, blackening of medium
2.	<i>Clostridium sporogenes ATCC 11437</i>	50-100	good-luxuriant	positive reaction, blackening of medium

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

Test Procedure

Refer appropriate references for standard test procedures.

Results

Refer appropriate references and procedures for interpretation of results.

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

1. For identification, organisms must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification.
2. Consult appropriate texts for detailed information and recommended procedures.

Packaging

Product Name : Differential Reinforced Clostridial Broth Base

Product Code : DM519

Available Pack sizes : 100gm / 500gm

References

1. Hirsch A. and Grinstead E., 1954, J. Dairy Res. 21:101
2. Barnes E. M. and Ingram M., 1956, J. Appl. Bacteriol., 19(1):117.
3. Collee J. G., Fraser A. G., Marmion B. P., Simmons A., (Eds.), Mackie and McCartney, Practical Medical Microbiology, 1996, 14th edition, Churchill Livingstone.





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Further Information

For further information please contact your local MICROMASTER Representative.



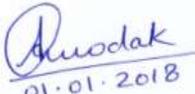
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