PRODUCT SPECIFICATION SHEET

Cystine Heart Agar Base (DM067)

Intended Use
Cystine Heart Agar Base (DM067) is recommended for excellent growth of gram-negative cocci and other pathogenic organisms. With added haemoglobin it is used for cultivation of *Francisella tularensis*.

Product Summary and Explanation
Francisella tularensis is a gram negative, fastidious organism and is the causative agent of tularaemia, a plague-like disease of rodents and other small organisms. It was first described in humans in 1907.[1] The organisms are strict aerobes; fresh isolates cannot be cultured on ordinary medium but require a complex medium containing blood, or tissue extracts and cystine. Several media formulations were employed to isolate this microorganism. Initial formulations contained egg or serum and were difficult to prepare. Blood Dextrose Cystine Agar, described by Francis[2] was found to be satisfactory for cultivating *F. tularensis*. Shaw[3] added 0.05% cystine and 1% dextrose to Heart Infusion Agar for the cultivation of *F. tularensis*. Subsequently haemoglobin was added to Cystine Heart Agar Base, by Rhamy[4] to develop a satisfactory cultivation medium for *F. tularensis*. This medium is also known as Cystine Glucose Blood Agar and is the most suitable medium for isolating *F. tularensis*.[2] Enrichment with 2% hemoglobin provides additional growth factors. Without enrichment, Cystine Heart Agar supports excellent growth of gram-negative cocci and other pathogenic microorganisms.[5] This medium is a nutritionally rich medium, which may also be used for cultivating many other organisms generally difficult to grow.

Principles of the Procedure
Cystine Heart Agar Base contains beef heart infusion and proteose peptone which are sources of carbon, nitrogen, vitamins and minerals. Dextrose is an energy and carbon source. L-Cystine is the source of amino acid. Sodium chloride provides the essential ions and helps to maintain the osmotic balance of the medium. Overgrowth by contaminating organisms can be reduced by incorporating 100-500 units penicillin per ml into the medium.

Formula / Liter

Ingredients	Gms / Liter
Beef heart, infusion from	500.00
Proteose peptone	10.00
Dextrose	10.00
Sodium chloride	5.00
L-Cystine	1.00
Agar	15.00
Final pH: 6.8 ± 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.

PRODUCT SPECIFICATION SHEET

3. *Francisella tularensis* is a Biosafety Level 2 pathogen that can be transmitted by aerosols or by penetration of unbroken skin. Wearing of gowns, gloves and masks is advocated for laboratory staff handling suspected infectious material.

Directions

1. Suspend 51 grams in one liter distilled water.
2. Heat to boiling to dissolve the medium completely.
3. Autoclave at 121°C, 15 psi pressure, for 15 minutes / validated cycle.
4. When to be enriched with haemoglobin (2%), suspend 10.2 grams of medium in 100 ml distilled water. Sterilize as above.
5. Cool medium to 50°C and aseptically add 100 ml of 2% sterile haemoglobin solution.
6. Mix well and pour into sterile Petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Basal medium :Amber coloured clear to slightly opalescent gel After addition of 2% haemoglobin solution: Chocolate brown coloured opaque gel forms in Petri plates
Reaction of 5.1% Solution	pH : 6.8 ± 0.2 at 25°C
Gel Strength	Firm, comparable with 1.5% Agar gel

Expected Cultural Response: Cultural characteristics observed with added 2% Haemoglobin after an incubation at 35-37°C for 40-48 hours.

Sr. No.	Organisms	Results to be achieved
		Growth
1.	*Francisella tularensis ATCC 29684*	good-luxuriant
2.	*Neisseria meningitidis ATCC 13090*	good-luxuriant
3.	*Streptococcus pneumonia ATCC 6303*	good-luxuriant
4.	*Streptococcus pyogenes ATCC 19615*	good-luxuriant

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

Test Procedure

1. Inoculate and streak specimens as soon as possible. Refer to appropriate references for a complete discussion on the inoculation and identification of *Francisella*.
2. Overgrowth by contaminating organisms can be reduced by incorporating 100-500 units penicillin per mL into the medium.

Results

Refer appropriate references and procedures for interpretation of results.

Storage

Store the sealed bottle containing the dehydrated medium at 2 - 30°C. Once opened and recapped, place container in a low humidity environment at the same storage temperature. Protect from moisture and light.

PRODUCT SPECIFICATION SHEET

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 : Cystine Heart Agar Base
Product Code : DM067
Available Pack sizes : 100gm/ 500gm

References

1. Murray P. R., Baron J. H., Pfaller M. A., Jorgensen J. H. and Tenover F. C., Yolken R. H., (Ed.), 1999, Manual of Clinical Microbiology, 7th Ed. American Society for Microbiology, Washington, D.C.
2. Francis, 1928, JAMA, 91:1155.
3. Shaw, 1930, Zentr. Bakt. I. Abt. Orig., 118:216.
4. Rhamy, 1933, Am. J. Clin. Pathol., 3:121.
5. Isenberg, (Ed.), 1992, Clinical Microbiology Procedures Handbook, Vol. 1. American Society for Microbiology, Washington, D.C.

Further Information
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PRODUCT SPECIFICATION SHEET

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