PRODUCT SPECIFICATION SHEET

Anaerobic Agar (DM011)

Intended Use
Anaerobic Agar (DM011) a general purpose anaerobic medium recommended for cultivation of anaerobes, especially *Clostridium* species.

Product Summary and Explanation
Anaerobic bacteria cause a variety of infections in humans, including otitis media, oral infections, endocarditis, meningitis, wound infections following bowel surgery or trauma, and bacteremia.[1,2] Anaerobic bacteria are the predominant flora colonizing the skin and mucous membranes of the body.[3] Anaerobic bacteria vary in their sensitivity to oxygen and nutritional requirements.[4] Anaerobic bacteria lack cytochromes and thus are unable to use oxygen as a terminal electron acceptor.[5] Anaerobic Agar was originally formulated for surface cultivation of members of the genus *Clostridium* and other anaerobic organisms on plates. Brewer[6] described a special Petri dish cover that allowed surface growth of anaerobes and microaerophiles without anaerobic equipment. The microorganisms were grown on an agar-based medium having a low oxidation-reduction potential. Anaerobic Agar is a modification of Brewer's original formula. This medium is suitable for isolation of facultative and obligate anaerobes and for the study of colonial morphology as colonies can be readily seen on the light coloured agar and are easily accessible.[4,7]

Principles of the Procedure
Anaerobic Agar contains casein enzymic hydrolysate provides the nitrogen, vitamins and amino acids essential for growth. Dextose is a carbon source. Sodium chloride maintains osmotic equilibrium. Sodium thioglycollate and sodium formaldehyde sulphoxylate provide adequate anaerobiosis which is indicated by methylene blue present in the medium which yields blue colour to medium in presence of oxygen.

Formula / Liter

Ingredients	Gms / Liter
Casein enzymic hydrolysate	20.00
Dextrose	10.00
Sodium chloride	5.00
Sodium thioglycollate	2.00
Sodium formaldehyde sulphoxylate	1.00
Methylene blue	0.002
Agar	20.00
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 58 grams of the medium in 1000 ml of 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. Mix well and pour into sterile petri plates.

Quality Control Specifications

Dehydrated Appearance	Cream to yellow homogeneous free flowing powder
Prepared Medium	Light amber coloured, clear to slightly opalescent gel forms in Petri plates that becomes greenish due to aeration on standing
Reaction of 5.8% solution	pH 7.2 ± 0.2 at 25°C

Gel Strength	Firm, comparable with 2.0% agar gel

Expected Cultural Response: Cultural characteristics observed under anaerobic condition after an incubation at 35-37°C for 48-72 hours.

Sr. No.	Organisms	Results to be achieved		
		Inoculum (CFU)	Growth	Recovery
1.	*Clostridium butyricum ATCC 13732*	50 - 100	good-luxuriant	>=50%
2.	*Clostridium perfringens ATCC 12924*	50 - 100	good-luxuriant	>=50%
3.	*Clostridium sporogenes ATCC 11437*	50 - 100	good-luxuriant	>=50%

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

Test Procedure

1. When standard 90-100mm plates are used, dispense 0.1 to 1.0 ml of inoculum into plates and mix with 20 - 25 ml of sterile medium.
2. After solidification, incubate anaerobically as required by particular organism under study.
3. Extended incubation may be necessary to recover some anaerobes.

Results

Refer appropriate references and test 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. Clinical specimens must be obtained properly and transported to the laboratory in a suitable anaerobic transport container.
2. The microbiologist must be able to verify quality control of the medium and determine whether the environment is anaerobic.
3. The microbiologist must perform aero-tolerance testing on each isolate recovered to ensure that the organism is an anaerobe.
4. Methylene blue is toxic to some anaerobic bacteria.

Packaging

Product Name : Anaerobic Agar
Product Code : DM011
Available Pack sizes : 100gm / 500gm

References

1. Allen, Siders and Marler. 1985. *In* Lennette, Balows, Hausler and Shadomy (ed.). Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
2. Smith. 1975. The pathogenic anaerobic bacteria, 2nd ed. Charles C. Thomas, Springfield, Ill.
3. Baron, Peterson and Finegold. 1994. Bailey & Scott's diagnostic microbiology, 9th ed. Mosby-Year Book, Inc. St. Louis, Mo.

4. Isenberg (Ed.), 1992, Clinical Microbiology Procedures Handbook, American Society for Microbiology, Washington, D.C.
5. Baron E. J., Peterson and Finegold S. M., Bailey & Scotts Diagnostic Microbiology, 9th Ed., 1994, Mosby-Year Book, Inc., St. Louis,Mo.
6. Brewer J. H., 1942, Science, 95:587.
7. Vera J., 1942, J. Bacteriol., 44:497.

Further Information

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