QUALITY CONTROL PROCEDURES (Optional)

I INTRODUCTION

Egg Yolk Agar, Modified is a differential medium used in the isolation and presumptive differentiation of *Clostridium* species and other obligately anaerobic bacilli.

II PERFORMANCE TEST PROCEDURE

1. Reduce plates at room temperature for 18–24 h prior to use in a **BD GasPak™** EZ anaerobic system.
2. Inoculate representative samples with the cultures listed below.
   a. Streak inoculate with 0.01 mL of dilution containing 10^3–10^4 CFUs.
   b. Incubate plates at 35 ± 2 °C in an anaerobic atmosphere.
   c. Include plates of a previously tested lot of TSA with 5% Sheep Blood as controls.
3. Examine plates after 48 h and 96 h for growth, lecithinase production, lipase production and proteolytic activity (zone of clearing).
4. Expected Results

Organisms	ATCC®	Recovery	Lecithinase production	Lipase production	Zone of clearing
**Clostridium perfringens*	13124	Fair to heavy growth	+	–	–
**Clostridium sporogenes*	11437	Fair to heavy growth	–	+	+
**Fusobacterium necrophorum*	25286	Fair to heavy growth	–	+	–

*Recommended organism strain for User Quality Control.

NOTE: This medium is exempt from User Quality Control testing according to CLSI M22-A3 Table 1B.

III ADDITIONAL QUALITY CONTROL

1. Examine plates as described under "Product Deterioration."
2. Visually examine representative plates to assure that any existing physical defects will not interfere with use.
3. Determine the pH potentiometrically at room temperature for adherence to the specification of 7.3 ± 0.2.
4. Note the firmness of the agar beds during the inoculation procedure.
5. Incubate uninoculated representative plates at 33–37 °C for 72 h and examine for microbial contamination.

PRODUCT INFORMATION

IV INTENDED USE

Egg Yolk Agar, Modified is a differential medium used in the isolation and presumptive differentiation of *Clostridium* species and other obligately anaerobic bacilli.

V SUMMARY AND EXPLANATION

Egg Yolk Agar, Modified, is based on an egg yolk medium developed by McClung and Toabe for the isolation and presumptive differentiation of clostridia based on lecithinase and lipase production and proteolytic activity.[1]

In this modification, CDC Anaerobe Agar (without vitamin K_1, which is subsequently added by egg yolks) is used as the basal medium instead of the McClung and Toabe formulation. CDC Anaerobe Agar is an enriched, nonselective medium that was developed at the Centers for Disease Control for use in the cultivation of obligately anaerobic microorganisms, particularly those found in clinical materials.[2]

CDC Anaerobe Agar is supplemented with egg yolk suspension for demonstration of lecithinase and lipase production and proteolytic activity.[1-7]

VI PRINCIPLES OF THE PROCEDURE

Enzymatic digests of casein and soybean meal supply amino acids and other complex nitrogenous substances. Yeast extract primarily provides the B-complex vitamins. Hemin improves the growth of anaerobic microorganisms.[5] L-cystine is a reducing agent and an essential amino acid.

An egg yolk suspension is incorporated to detect the production of lecithinase and lipase and proteolytic activity. Lecithinase degrades the lecithin present in the egg yolks, producing an insoluble, opaque precipitate in the medium surrounding growth.

Lipase breaks down free fats present in the egg yolks, causing an iridescent, “oil on water” sheen to form on the surface of the colonies. Since the lipase reaction may be delayed, plates should be kept up to 7 days before regarding them as negative for lipase production.

Proteolysis is indicated by clear zones in the medium surrounding growth.

VII REAGENTS

Egg Yolk Agar, Modified

Approximate Formula* Per Liter Purified Water

Pancreatic Digest of Casein	15.0 g	Agar	20.0 g
Papaic Digest of Soybean Meal	5.0 g	L-Cystine	0.4 g
Yeast Extract	5.0 g	Hemin	5.0 mg
Sodium Chloride	5.0 g	Egg Yolk Suspension	100.0 mL

*Adjusted and/or supplemented as required to meet performance criteria.

Warnings and Precautions: For *in vitro* Diagnostic Use.

If excessive moisture is observed, invert the bottom over an off-set lid and allow to air dry in order to prevent formation of a seal between the top and bottom of the plate during incubation.

Pathogenic microorganisms, including hepatitis viruses and Human Immunodeficiency Virus, may be present in clinical specimens. "Standard Precautions"[8-11] and institutional guidelines should be followed in handling all items contaminated with blood and other body fluids. After use, prepared plates, specimen containers and other contaminated materials must be sterilized by autoclaving before discarding.

Storage Instructions: On receipt, store plates in the dark at 2–8 °C. Avoid freezing and overheating. Do not open until ready to use. Minimize exposure to light. Prepared plates stored in their original sleeve wrapping at 2–8 °C until just prior to use may be inoculated up to the expiration date and incubated for recommended incubation times. Allow the medium to warm to room temperature before inoculation.

Product Deterioration: Do not use plates if they show evidence of microbial contamination, discoloration, drying, cracking, or other signs of deterioration.

VIII SPECIMEN COLLECTION AND HANDLING

Refer to appropriate texts for details of specimen collection and handling procedures.[4-7,12-14]

Specimens should be obtained before antimicrobial agents have been administered. Provision must be made for prompt delivery to the laboratory.

IX PROCEDURE

Material Provided: Egg Yolk Agar, Modified

Materials Required But Not Provided: Ancillary culture media, reagents, quality control organisms and laboratory equipment as required.

Test Procedure: Observe aseptic techniques.

The agar surface should be smooth and moist, but without excessive moisture.

This medium should be reduced immediately prior to inoculation by placing it under anaerobic conditions for 18–24 h.[3] An efficient and convenient method for obtaining suitable anaerobic conditions is through the use of the **BD GasPak** EZ anaerobic system.[15]

Inoculate the medium as soon as possible after the specimen arrives at the laboratory. To culture a specimen from a swab, inoculate the medium by rolling the swab over a third of the agar surface, and streak the remainder of the surface to obtain isolated colonies. Material not being cultured from swabs should be streaked onto the medium with a sterilized inoculating loop. The streak plate technique is used primarily to obtain isolated colonies from specimens containing mixed flora.

Inoculate an enrichment broth, such as Enriched Thioglycollate medium, at the same time as the primary plates to detect small numbers of anaerobes.

Incubate plates and tubes immediately after inoculation, with plates in an inverted position (agar side up), under anaerobic conditions at 35 °C, or place the media in a holding jar flushed with oxygen-free gas(es) until a sufficient number of plates and tubes is accumulated (no longer than 3 h).[16] Incubate for at least 48 h, and, if no growth occurs, continue incubation for up to 7 days. It is recommended that an indicator of anaerobiosis be used.

Examine for growth after 48 h of incubation. Cultures should not be regarded as negative until after 7 days of incubation.

User Quality Control: See “Quality Control Procedures.”

Each lot of media has been tested using appropriate quality control organisms and this testing meets product specifications and CLSI standards, where relevant. As always, QC testing should be performed in accordance with applicable local, state, federal or country regulations, accreditation requirements, and/or your laboratory's standard quality control procedures.

X RESULTS

After a minimum of 48 h of incubation, the plates should show isolated colonies in streaked areas and confluent growth in areas of heavy inoculation.

Examine for lecithinase and lipase production and proteolytic activity. Plates with isolates negative for lipase should be held up to 7 days. Examine colonial morphology and a Gram stain of the organism to confirm presumptive identification.

XI LIMITATIONS OF THE PROCEDURE

For identification, the organism must be in pure culture. Morphological, biochemical and/or serological tests should be performed for final identification. Consult appropriate texts for detailed information and recommended procedures.[3,4,6,7,17,18]

XII AVAILABILITY

Cat. No.	Description
297873	**BD BBL™** Egg Yolk Agar, Modified, Pkg. of 10 plates

XIII REFERENCES

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