EGG YOLK AGAR

-For in vitro use only- Catalogue No. PE30

Our Egg Yolk Agar is used for the isolation and differentiation of *Clostridium* species and other relevant anaerobic organisms based on lecithinase and lipase activity.

Hayward first recognized the value of egg yolk opacity in the identification of *Clostridium perfringens* and referred to this result as the Nagler reaction. The alpha toxin of *C. perfringens* has phospholipase activity and hence, when grown on a medium containing egg yolk phospholipid (lecithin), results in the release of diglycerides that is seen as an area of opacity around the bacterial colonies.

Pancreatic digest of casein and beef extract supply amino acids, and other complex nitrogenous components. Yeast extract also serves as a source amino acids but, more importantly it is rich in B-complex vitamins that are essential for growth. The differential component of this medium is the egg yolk emulsion that contains lecithin, proteins, and free fats that will allow differentiation between the *Clostridium* species based on their enzymatic activity on these three components.

Formula per Litre of Medium

Pancreatic Digest of Casein	10.0 g
Beef Extract	3.0 g
Yeast Extract	1.0 g
Sodium Chloride	5.0 g
Agar	15.0 g
Egg Yolk Emulsion	100.0 mL

pH 7.4 ± 0.2

Recommended Procedure

1. Allow medium to reach room temperature.
2. Using a direct inoculum from an appropriate organism, perform a four-quadrant streak to obtain well-isolated colonies.
3. Incubate anaerobically at 35°C.
4. Examine after 48-72 hours.
5. Plates should be kept up to 7 days before regarding them as lipase negative.

Interpretation of Results

The egg yolk emulsion contains lecithin and free fats that can be broken down by the bacterial enzymes lecithinase and lipase, respectively. Lecithinase breaks down lecithin and produces an insoluble precipitate resulting in an opaque zone in the medium surrounding the colonies, which is characterized as a positive lecithinase reaction. The bacterial enzyme lipase acts on free fats resulting in free fatty acid formation, which can be observed on the surface of the bacterial colonies as a reflective, iridescent sheen when held at an angle to a light source; this is characterized as a positive lipase reaction. Proteolytic activity can also be observed on this medium as clear zones surrounding the colonies.

Additional biochemical and/or serological tests should be performed on isolated colonies from pure culture to complete identification.

- *To detect Lipase production for some bacterial species it may be necessary to re-incubate the plates for up to 7 days*
- *Most strains of Clostridium sporogenes are lecithinase negative*
- *This medium is suitable for the Nagler's test by application of Clostridium perfringens type A antitoxin to the surface of the plate*

Quality Control

After checking the medium for correct pH, colour, depth, and sterility, the following organisms are used to determine the growth and differential performance of the completed medium.

Organism	Expected Results	
	Lec	Lip
Clostridium perfringens ATCC 13124	+	-
Clostridium sporogenes ATCC 19404	-	+
Bacteroides fragilis ATCC 25285	-	-

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Storage and Shelf Life

Our Egg Yolk Agar should be stored away from direct light at 4 to 8°C. The medium side should be uppermost to prevent excessive accumulation of moisture on the surface of the agar. Under these conditions this medium has a shelf life of 4 weeks from the date of manufacture.

References

1. Nagler FP. Observations on a reaction between the lethal toxin of *Cl. welchii* (type A) and human serum. Br J Exp Pathol 1939; 20:473-85.
2. Hayward NJ. Rapid identification of *Cl. welchii* by the Nagler reaction. Br Med J 1941; 1:811-4.
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4. McClung LS, Toabe R. The egg yolk plate reaction for the presumptive diagnosis of *Clostridium sporogenes* and certain species of the gangrene and botulinum groups. J. Bacteriol 1947, 53:139-47.
5. Dowell VR Jr, Lombard GL, Thompson FS, Armfield AY. Media for isolation, characterization
6. Balows A, Hausler WJ, Herrmann KL et al. Manual of clinical microbiology. 5th ed. Washington: ASM, 1991.
7. MacFaddin JF. Biochemical tests for identification of medical bacteria. 3rd ed. Philadelphia: Lippincott Williams & Wilkins, 2000.