

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MI\MYC\09\04\v01	Page 1 of 2
Section: Mycology Bench Manual	Subject Title: Media / Reagents Esculin Base Medium (EBM) pH 7.1	
Issued by: LABORATORY MANAGER	Original Date: March 22, 2001	
Approved by: Laboratory Director	Revision Date:	

MEDIA / REAGENTS

4. ESCULIN BASE MEDIUM (EBM) pH 7.1

Dist. H ₂ O	1000 ml.
Bacto Agar (Difco)	15 g.
Dextrose (BBL)	5 g.
Bacto-Peptone (Difco)	10 g.
Esculin (Difco/BDH)	0.5 g.
Difco Yeast Extract	1.0 g.

Mix thoroughly to dissolve.
Autoclave at 121°C/ 15 minutes

Cool to 45°-50°C, aseptically remove 5.0-ml. agar, then add:

2.5 ml. Gentamicin sulphate	= 25,000 µg/litre
2.5 ml. Chloramphenicol	= 10,000 µg/litre

Mix well and pour plates.
Store in fridge.

Gentamicin Sulphate Stock Solution (10,000 µg/ml)

Vial contains 2.0 ml. (40 mg/ml) = 80,000 µg

Transfer contents of vial and make up to a volume of 8 ml. using phosphate buffer pH 8.0 (= 10,000 µg/ml). Distribute 3 ml. amounts into bijou bottles. Store at -20°C.

Chloramphenicol Stock Solution (4,000 µg/ml)

TML\MSH Microbiology Department Policy & Procedure Manual	Policy # MI\MYC\09\04\v01	Page 2 of 2
Mycology Bench Manual		

Purpose

Differential medium for isolation of *Cryptococcus neoformans* and also isolation medium for other fungi from contaminated specimens. Also provides presumptive identification of *C. neoformans*.

Principle

C. neoformans produces phenol oxidase enzyme that breaks down the substrate esculin, resulting in the production of a melanin-like pigment and the development of dark brown colonies. It takes about 48-72 hours for colonies to become brown. Other yeast colonies are cream to beige.

Rare strains of *C. neoformans* fail to produce pigmented colonies; also rarely yeasts other than *C. neoformans* produce dark colonies.

Quality Control

<u>Organisms</u>	<u>Incubation Temperature</u>	<u>Results</u>
<i>Cryptococcus neoformans</i>	28°C	Brown pigment
<i>C. laurentii</i> (or <i>T. glabrata</i>)	28°C	No brown pigment

References

S.C. Edberg et al. Esculin - Based Medium for Isolation and Identification of *Cryptococcus neoformans*. J. Clin. Micro. 12:332-335, 1980.