B422	BILE ESCULIN AGAR
Formula	
Ingredients :	**gms/lit.**
Peptone	5.00
Beef extract	3.00
Bile	40.00
Esculin	1.00
Ferric citrate	0.50
Agar	15.00
Final pH (at 25°C) : 6.6 ± 0.2	

Directions :

Suspend 64.5 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Mix and dispense into tubes or flasks as desired. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Allow the tubed medium to solidify in slanted position.

Principle :

The medium is highly nutritious. Peptone and Beef extract serves as sources of carbon, nitrogen, amino acids, vitamins and essential growth nutrients. Bile inhibits most of the other accompanying bacteria. Esculin in the medium is hydrolyzed to esculetin and dextrose. Esculetin reacts with ferric citrate to form a dark brown or black complex, visualized as a zone of black precipitate around the colonies. If the media is dispensed in tubes in the form of slants, a positive reaction is indicated by blackening of more than half of the slant within 24-48 hours.

QC Tests – (I)Dehydrated Medium			
	Colour :	Light yellow to brownish yellow	
	Appearance :	Homogeneous Free Flowing powder	
(II)Rehydrated medium			
	pH (post autoclaving/heating) :	6.6 ± 0.2	
	Colour (post autoclaving/heating) :	Yellow to medium amber	
	Clarity (post autoclaving/heating) :	Clear to Slightly opalescent	
(III)Q.C. Test Microbiological			
	Cultural characteristics observed in an increased atmosphere of Carbon dioxide after an incubation at 35-37°C for 18-24 hours.		
	MICROORGANISM (ATCC)	GROWTH	ESCULIN HYDROLYSIS
	Enterococcus faecalis (29212)	Luxuriant	positive reaction,blackening of medium around the colony
	Streptococcus pyogenes (19615)	Luxuriant	negative reaction
	Proteus mirabilis (25933)	Luxuriant	negative reaction

Precautions :	1. For Laboratory Use.				
	2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.				
	3. IRRITANT. Irritating to eyes, respiratory system and skin. Avoid contact with skin and eyes. Do not breathe dust. Wear suitable protective clothing. Keep container tightly closed. Target organ(s) : Lungs.				
Limitations :	1. Since the nutritional requirements of organisms vary, some strains may be encountered that fail to grow or grow poorly on this medium.				
	2. The bile esculin test was originally formulated to identify enterococci. However, the properties of growth on 40% bile media and esculin hydrolysis are characterstics shared by most strains of Group D streptococci. The bile esculin test should be used in combination with other tests to make a positive identification. Facklam and Facklam et al. recommend a combination of the bile esculin test and salt tolerance (growth in 6.5 % NaCl). Streptococcus bovis will give a positive reaction on Bile Esculin Agar, but unlike Enterococcus spp., it cannot grow on 6.5% NaCl or at 10°C.				
	3. Bile Esculin Agar should be considered a differential medium, but with the addition of sodium azide (which inhibits gram –negative bacteria) the medium can be made more selective (see Bile Esculin Azide Agar).				
	4. Occasional viridans strains will be positive on Bile Esculin Agar or will display reactions that are difficult to interpret. Of the viridans group, 5 to 10% may be able to hydrolyze esculin in the presence of bile.				
	5. Use a light inoculum when testing E. Coli on Bile esculin agar. Wasilauskas suggests that the time required for an isolate to hydrolyze esculin is directly proportional to the size of the inoculum. For a tabulation of those Enterobacteriaceae that can hydrolyze esculin, refer to Farmer.				
Use :	For differential isolation and presumptive identification of group D Streptococci in food and pharmaceutical products.				
Storage :	Dehydrated medium-below 30°C Prepared medium- Between 2 to 8°C.				
Packing :	500 gm. bottle				
Product profile:	Reconstitution	Quantity on Preparation (500g)	pH (25°C)	Supplement	Sterilization
B422	64.5g/l	7.751L	6.6 $\pm$ 0.2	NIL	121^{0}C / 15 minutes