B263	**MUELLER HINTON AGAR**		
Formula			
Ingredients :		**gms/lit.**	
Beef, infusion from		300.00	
Casein acid hydrolysate		17.50	
Starch		1.50	
Agar		17.00	
Final pH (at 25°C) :		7.4 ± 0.1	
Directions :			
Suspend 38 gms in 1000 ml. distilled water. Boil to dissolve the medium completely. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Mix well before pouring.			
Principle :			
Beef heart infusion and casein acid hydrolysate provide nitrogenous compounds, carbon, sulphur and other essential nutrients. Starch is added to absorb any toxic substances present in the medium. Different factors influence the disc diffusion susceptibility tests as, inoculum concentration, agar depth, disc potency, medium pH and beta – lactamase production by test organisms. Agar is the solidifying agent.			
QC Tests – (I)Dehydrated Medium			
	Colour :	Cream to yellow	
	Appearance :	Homogeneous Free Flowing powder	
(II)Rehydrated medium			
	pH (post autoclaving/heating) :	7.4 ± 0.1	
	Colour (post autoclaving/heating) :	Light amber	
	Clarity (post autoclaving/heating) :	Sligthly opalescent	
(III) Q.C. Test Microbiological			
	Cultural characteristics observed after 18- 24 hours at 35-37°C.		
	MICROORGANISM (ATCC)	GROWTH	
	Escherichia coli (25922)	Luxuriant	
	Neisseria gonorrhoeae (49226)	Luxuriant	
	Pseudomonas aeruginosa (77853)	Luxuriant	
	Staphylococcus aureus (25923)	Luxuriant	
	Streptococcus faecalis (19433)	Luxuriant	
	Haemophilus influenzae (49247)	Good – Luxuriant (on chocolate agar)	
Precautions :	1. For Laboratory Use.		
	2. Follow proper, established laboratory procedures in handling and disposing of infectious materials.		

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. Numerous factors can affect results ; inoculum size, rate of growth, medium formulation of pH, length of incubation and incubation environment, disk content and drug diffusion rate, and measurement of endpoints. Therefore, strict adherence to protocol is required to ensure reliable results.				
	3. Disk diffusion susceptibility testing is limited to rapidly growing organisms. Drug inactivation may result from the prolonged incubaction times required by slow growers.				
	4. Media containing excessive amounts of thymidine or thymine can reverse the inhibitory effects of sulfonamides and trimethoprim, causing zones of growth inhibition to be smaller or less distinct.				
	5. Variation in the concentration of divalent cations, primarily calcium and magnesium, affects results of aminoglycoside, tetracycline, and colistin tests with P. aeruginosa isolates. A cation content that is too high reduces zones sizes, whereas a cation content that is too low has the opposite effect.				
	6. When Mueller Hinton Medium is supplemented with blood, the Zone of inhibition for oxacillin and methicillin may be 2 to 3 mm smaller than those obtained with unsupplemented agar. Conversely, sheep blood may markedly increase the zone diameters of some cephalosporins when they are tested against enterococci. Sheep blood may cause indistinct zones or a film of growth within the zones of inhibition around sulfonamide and trimethoprim disks.				
	7. Mueller Hinton Medium deeper than 4 mm may cause false – resistant results, and agar less than 4 mm deep may be associated with a false –suceptibility report.				
	8. A pH outside the range of 7.4 ± 0.1 may adversely affect susceptibility test results. If the pH is too low, aminoglycosides and macrolides will appear to lose potency; others may appear to have excessive activity. The opposite effects are possible if the pH is too high.				
	9. When Mueller Hinton Medium is inoculated, no droplets of moisture should be visible on the surface or on the petri dish cover.				
	10. Mueller Hinton Medium should be inoculated within 15 minutes after the inoculum suspension has been adjusted.				
	11. The zone of inhibition diameters of some drugs, such as the aminoglycosides, macrolides, and tetracyclines, are significantly altered by CO_2 Plates should not be incubated in increased CO_2.				
	12. This medium is recommended for susceptibility testing of pure cultures only.				
Use:	For cultivation of Neisseria and for determination of susceptibility of microorganisms to antimicrobial agents.				
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
B263	38g/l	13.157L	7.4 ± 0.1	NIL	121^0C / 15 minutes