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# TMV 625 – DIFFERENTIAL REINFORCED CLOSTRIDIAL BROTH BASE (VEG.)

### **INTENDED USE**

For cultivation of *Clostridia* from water.

#### **PRODUCT SUMMARY AND EXPLANATION**

This medium is prepared by using Veg peptone and Veg extract which is free from BSE/TSE risks. Differential Reinforced Clostridial Veg Broth is the modification of Differential Reinforced Clostridial Medium which is based on the formulation described by Barnes and Ingram and Gibbs and Freame for the sulphite reducing Clostridia from food and for enumeration in water by multiple tube method. Differentiation is based on the ability to reduce sulphite to sulphide to form iron sulphide resulting in black colour.

### COMPOSITION

Ingredients	Gms / Ltr	
Veg Peptone	10.000	
Veg extract	10.000	
Yeast extract	1.500	
Starch	1.000	
Sodium acetate, hydrated	5.000	
Glucose	1.000	
L-Cysteine hydrochloride	0.500	

# PRINCIPLE

The medium consists of Veg Peptone, veg extract, yeast extract, starch, sodium acetate which provide essential nutrients for bacterial metabolism. Glucose is the fermentable carbohydrate and serves as carbon and energy source. L-cysteine hydrochloride acts as reducing agent. Sodium sulphite and ferric citrate are added as indicators. Sulphite reducing clostridia produce sulphide from sulphite, which results in the formation of black coloured medium.

#### **INSTRUCTION FOR USE**

- Dissolve 29 grams in 1000 ml purified/distilled water.
- Heat to boiling to dissolve the medium completely.
- Sterilize by autoclaving at 15 psi pressure (121°C) for 15 minutes.
- Cool to 45-50°C. Just before use add 0.5 ml filter sterilized solution, prepared by mixing equal volumes of 4% w/v solution of sodium sulphite and 7% w/v ferric citrate, to 25 ml of single strength medium or 0.4 ml and 2 ml to 10 ml and 50 ml of double strength medium respectively. Mix well.

# QUALITY CONTROL SPECIFICATIONS

Appearance of Powder	: Light yellow coloured, may have slightly greenish tinge, homogeneous, free		
	flowing powder.		
Appearance of prepared medium	: Light yellow coloured, clear solution without any precipitate.		
pH (at 25°C)	: 7.2 ± 0.2		

# **INTERPRETATION**

A- 902A, RIICO Industrial Area, Phase III, Bhiwadi-301019.





Cultural characteristics observed with added 4% w/v solution of Sodium sulphite and 7% w/v Ferric citrate after incubation.

Microorganism	ATCC	Inoculum (CFU/ml)	Growth	H <sub>2</sub> S production	Incubation Temperature	Incubation Period
Clostridium perfringens	13124	50-100	Good- luxuriant	Positive reaction, blackening of medium	30°C	1 Week
Clostridium sporogenes	11437	50-100	Good- luxuriant	Positive reaction, blackening of medium	30°C	1 Week

# PACKAGING:

In pack size of 100 gm and 500 gm bottles.

#### STORAGE

Dehydrated powder, hygroscopic in nature, store in a dry place, in tightly-sealed containers between 25-30°C and protect from direct sunlight. Under optimal conditions, the medium has a shelf life of 4 years. When the container is opened for the first time, note the time and date on the label space provided on the container. After the desired amount of medium has been taken out replace the cap tightly to protect from hydration.

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

# DISPOSAL

After use, prepared plates, specimen/sample containers and other contaminated materials must be sterilized before discarding.

#### REFERENCES

1 Barnes E.M. and Ingram M., 1956, J. Appl. Bacteriol., 19(1):117.

2. Gibbs B.M. and Freame B., 1965, J. Appl. Bacteriol., 28(1):95.



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