

# DIFFERENTIATION DISCS

X Factor /V Factor / X + V Factor Discs

## USE

The Diferentiation discs are used for the presumptive identification of Haemophilus species on the basis of their requirements for X or V growth factor or both. Member of the genus Haemophilus require hemin (X factor) and/or nicotinamide-adenine-dinucleotide (V-factor). Together with the X factor and V factor, the need for either one or both factors provides the main means of differentiation of these organisms. Haemophilus species requiring both X and V factors exhibit growth only in the vicinity of the X + V factor disks.

## PRINCIPLE

Traditional identification criteria include haemolysis on horse or rabbit blood and the requirement for X and/or V factors for growth. To establish X and V factor requirements, discs impregnated with each factor are placed on unsupplemented media (Mueller Hinton Agar / Trypticase Soy Agar) that has been inoculated with a light suspension of the organism. After overnight incubation at  $35^{\circ}C$  in ambient air, the plate is examined for growth around each disc. Many X factor requiring organisms are able to carry over in a factor from the primary medium to give false negative results.

### COMPOSITION:

1 pack contains 50 disks.

Sterile filter paper disks:

- impregnated with hemin and nicotinamide adenine dinucleotide (X + V factor disks; ID009).
- impregnated with hemin (X factor disks; ID007).
- impregnated with nicotinamide adenine dinucleotide (V factor disks; ID008).

### PROCEDURE

- 1. Inoculate the surface of the Mueller Hinton Agar / Trypticase Soy Agar plate with the test organisms by surface spreading using sterile swab.
- 2. The inoculums density should be approximately equivalent to McFarland 0.5 Standard.
- 3. Aseptically, place the X, V and X+V factor discs on the plate well apart from each other.
- 4. Incubate the plate at 35-37°C for 24-48 hours.
- 5. Recommended the disc position on the Agar plate:

Disc	Place
X factor disc	12 O'clock
V factor disc	4 O'clock
X + V factor disc	8 Oʻclock

6. Observe the growth in the neighbourhood of the disc. The test organism requiring X factor grows only in the vicinities of the X factor discs, those who require V factor grows only in the vicinities of the V disc and those who require X and V factor grows only in the vicinities of the X + V discs.

### INTERPRETATION OF RESULTS

A clearly defined zone of growth (which may need viewing under magnification), around one or more discs identifies the species as Haemophilus as shown in the Quality Control Table. H influenzae produces discrete zones; about 20mm in size but H. parainfluenzae gives more diffuse zones generally of a larger size.

### STORAGE & STABILITY

Store in the freeze a below -10°C. Allow to equilibrate to the room temperature before opening then return to freezer storage immediately after use.

### PRECAUTIONS

For Invitro Diagnostic use only.





# PRODUCT SPECIFICATION SHEET

Observe approved biohazard precautions and asceptic techniques. Product to be used only by adequately trained and qualified laboratory personnel. All biohazard waste should be sterilized before disposal.

# QUALITY CONTROL

- Check for signs of deterioration.
- Quality control must be performed with at least one organism to demonstrate positive reaction. Use known strains of Haemophilus influenzae to monitor the performance of the differentiation discs and the medium.
- Do not use product if the reactions with control organisms are incorrect.
- Do not use heavy suspension of the test organism as X or V Factor carryover from the primary growth medium may take place.

### Identification of Haemophilus species that require X and / or V Factors:

Organism	Without growth Factor	X Factor	V Factor	X + V Factor
Haemophilus influenzae	-	-	-	+
Haemophilus parainfluenzae	-	-	+	+
Haemophilus ducreyi	-	+	-	+
Bordetella pertussi	+	+	+	+

### LIMITATIONS

It is recommended that biochemical and/or serological tests are performed on colonies from pure culture to confirm identification

### PACKAGING

Product Name : X Factor /V Factor / X + V Factor Differentiation Discs Product Code : ID007 / ID008 / ID009 Available Pack sizes : 50 Discs in a vial or a Box of 20 vials containing 50 discs each.

### REFERENCES

- 1. D.J. Davis, J. Infect. Dis., 21, 392 (1917)
- 2. T. Thjotta, O.T. Avery, J. Exp. Med., 34, 97 (1921)
- A. Lwoff, M. Lwoff, Proc. R. Soc., 122, 352 London (1937)
- 3. R.H. Parker, P.D. Hoeprich, Am. J. Clin. Pathol., 37, 319 (1962)
- 4. E.L. Biberstein, P.D. Mini, M.G. Bills, J. Bacteriol., 86, 814 (1963)
- 5. D.C. White, S. Granick, J. Bacteriol., 85, 842 (1963)
- 6. P.R. Murray, E.J. Baron, M.A. Pfaller, F.C. Tenover, R.H. Yolken, Manual of Clinical Microbiology. 6th ed. ASM, Washington, D.C. (1995)
- 7. A.E. Greenberg, R.R. Trussell, L.S. Clesceri (Eds.), Standard Methods for the Examination of Water and Wastewater, 16th ed., A.P.H.A, Washington, D.C. (1985)
- M. Kilian, Haemophilus. In Manual of Clinical Microbiology, Edited by A. Balows, W.J. Hausler, K.L. Herrmann, H.D. Isenberg, H.J. Shadomy., p 463. Washington, DC: American Society for Microbiology (1991)

### FURTHER INFORMATION

For further information please contact your local MICROMASTER Representative.



MICROMASTER LABORATORIES PRIVATE LIMITED Unit 38/39, Kalpataru Industrial Estate, Off G.B. Road, Near 'R-Mall', Thane (W) - 400607. M.S. INDIA. Ph: +91-9320126789/9833630009/9819991103 Email: <u>sales@micromasterlab.com</u> ID009PSS, QAD/FR/024, Rev.00







Disclaimer :

All Products conform exclusively to the information contained in this and other related Micromaster Publications. Users must ensure that the product(s) is appropriate for their application, prior to use. The information published in this publication is based on research and development work carried out in our laboratory and is to the best of our knowledge true and accurate. Micromaster Laboratories Pvt Ltd reserves the right to make changes to specifications and information related to the products at any time. Products are intended for laboratory, diagnostic, research or further manufacturing use only and not for human or animal or therapeutic use, unless otherwise specified. Statements included herein should not be considered as a warranty of any kind, expressed or implied, and no liability is accepted for infringement of any patents.

