Charcoal Agar Base, HiVeg™ / MV344/MV646/MV1053
Charcoal Blood Agar Base, HiVeg™ / Charcoal HiVeg™ Agar Base with Niacin

Charcoal Agar Base, HiVeg with supplements is recommended for the cultivation of *Bordetella pertussis* for vaccine production and also for the maintenance of stock cultures.

Composition** :

Ingredients	MV344 Grams/Litre	MV646 Grams/Litre	MV1053 Grams/Litre
HiVeg infusion	12.00	—	—
HiVeg peptone	10.00	10.00	—
HiVeg peptone No.2	—	—	10.00
HiVeg extract	—	10.00	10.00
Yeast extract	3.50	3.50	—
Sodium chloride	5.00	5.00	5.00
Starch, soluble	10.00	10.00	10.00
Charcoal	4.00	4.00	4.00
Nicotinic acid	—	—	0.001
Agar	18.00	12.00	12.00
Final pH (at 25°C)	7.3 ± 0.2	7.5 ± 0.2	7.4 ± 0.2

** Formula adjusted, standardized to suit performance parameters

Directions :

Suspend 31.25 grams of MV344 in 450 ml or 54.5 grams of MV646 or 51 grams of MV1053 in 1000 ml distilled water. Boil to dissolve the medium with frequent stirring. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Cool to 45-50°C. Aseptically add sterile 50 ml of defibrinated blood and Bordetella Selective Supplement (FD004) in Charcoal HiVeg Agar Base (MV344) or Charcoal HiVeg Agar Base with Niacin (MV1053). Add 10 ml of sterile defibrinated horse blood, 0.3 ml of sterile 100 u/ml Penicillin solution and 0.3 ml of 0.1% solution of 4:4 Diamido-diphenylamine hydrochloride per 100 ml of Charcoal HiVeg Blood Agar Base (MV646).

Principle and Interpretation :

These media are prepared by completely replacing animal based peptones by vegetable peptones. Charcoal Agar Base, HiVeg is the modification of Charcoal Agar Base formulated according to the method devised by Mishulow et al (1) which is recommended for the cultivation of *Bordetella pertussis* and its vaccine production. Necessity of Nicotinic acid as a growth factor was shown by Proom (2). Earlier medium viz. Bordet Gengou, can be replaced by this medium, as the conventional medium for the vaccine production of *Bordetella pertussis* as suggested by Ensminger et al (3) who added charcoal to the medium.
Ingredients like HiVeg infusion, HiVeg peptone, HiVeg peptone No.2, HiVeg extract and yeast extract provide essential nutrients to the organisms. Sodium chloride maintains osmotic balance. Starch servers as carbohydrate source and therefore supports growth of organism. It along with charcoal, neutrilizes toxic substances like fatty acid, which can inhibit growth of *Bordetella*. The difficulty in the isolation of *Bordetella pertussis* from nasopharyngeal secretions is the repression of unwanted flora during the long incubation period on nutritious media. Penicillin can be added to the medium as an antimicrobial agent for restricting other contaminants. However Penicillin resistant floras still cause the contamination which was observed by Lacey (4). Methicillin was found to be superior than Penicillin in suppressing unwanted nasopharyngeal flora as observed by Broome et al (5). Sutcliffe and Abbott found that Cephalexin was still better than Methicillin (6). Therefore this medium with added supplement, Cephalixin and blood is suitable for cultivation of *B.pertussis*. Charcoal Blood Agar Base, HiVeg is used for the cultivation of *Bordetella pertussis* for vaccine production.
The media can also be used for the maintenance of stock cultures of *Bordetella pertussis* on slants with weekly subcultures. Charcoal HiVeg Agar Base or Charcoal HiVeg Agar Base with Niacin can be converted to Chocolate Agar Base for isolation of *Haemophilus* species.

Product Profile :

Vegetable based (Code MV)◉	Animal based (Code M)
MV344/MV646/MV1053 HiVeg peptone HiVeg extract HiVeg peptone No. 2 HiVeg infusion	**M344/M646/M1053** Peptic digest of animal tissue Beef extract Pancreatic digest of gelatin Beef heart, infusion

Recommended for	:	Cultivation of *Bordetella pertussis* for vaccine production
Reconstitution	:	(MV344) : 62.5 g/l
	:	(MV646) : 54.5 g/l
	:	(MV1053) : 51.0 g/l
Quantity on preparation (500g):		(MV344) : 13.15 L
	:	(MV646) : 9.17 L
	:	(MV1053) : 9.80 L
pH (25°C)	:	(MV344) : 7.3 ± 0.2
	:	(MV646) : 7.5 ± 0.2
	:	(MV1053) : 7.4 ± 0.2
Supplement	:	(MV344, MV1053) : Sterile Defibrinated blood, Bordetella Selective Supplement (FD004) (MV646) : Penicillin and Diamidodiphenylamine hydrochloride, sterile defibrinated blood
Sterilization	:	121°C / 15 minutes.

Storage : Dry Medium - Below 30°C, Prepared Medium 2 - 8°C.

Continued ...

◉ Prepared from GMO free Vegetable proteins replacing Animal based peptones. Freedom from BSE/TSE worries.

Charcoal Agar Base, HiVeg™ / MV344/MV646/MV1053
Charcoal Blood Agar Base, HiVeg™ / Charcoal HiVeg™ Agar Base with Niacin

Quality Control:

Appearance of Powder

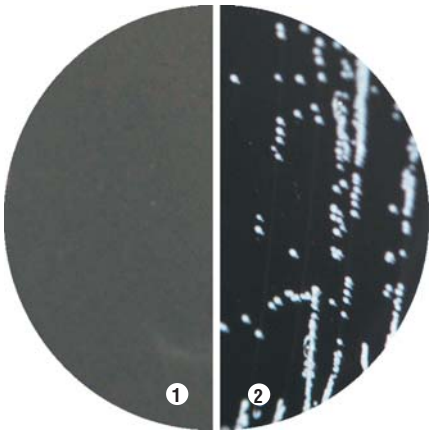
Grey coloured, homogeneous, free flowing powder.

Gelling

Firm, comparable with 1.8% Agar gel of MV344 or 1.2% Agar gel of MV646 or MV1053.

Colour and Clarity

Black coloured,opaque gel forms in petri plates and contains undissolved black particles.



MV344 Charcoal Agar Base, HiVeg

1. Control
2. *Bordetella bronchiseptica*

Reaction

Reaction of 6.25% w/v aqueous solution of MV344 is pH 7.3 ± 0.2 at 25°C.

Reaction of 5.45% w/v aqueous solution of MV646 is pH 7.5 ± 0.2 at 25°C.

Reaction of 5.1% w/v aqueous solution of MV1053 is pH 7.4 ± 0.2 at 25°C.

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 24-48 hours.

Organisms (ATCC)	Inoculum (CFU)	Growth	Recovery
Bordetella bronchiseptica (4617)	10^2-10^3	luxuriant	>50%
Bordetella parapertussis (15237)	10^2-10^3	luxuriant	>50%
Bordetella pertussis (8467)	10^2-10^3	luxuriant	>50%

References :

1. Mishulow L., Sharpe L. S. and Cohen L. L., 1953, J. Pub. Hlth., 43((II) : 1466.
2. Proom H., 1955, J. Gen. Microbiol., 12(I) : 63.
3. Ensminger P. W., Gulberston C. G. and Powell H. M., 1953. J. Infect. Dis., 93(3):266
4. Lacey B.W., 1954, J. Hyg., 59:273.
5. Broome C.V., Fraser D.W. and English J.W., 1979, Internat. Symp. on Pertussis DHEW J., Washington D.C. pp 19-29.
6. Sutcliffe E.M. and Abbott J.D., 1979, B.M.J. II:732-733.