Charcoal Agar Base, HiVeg™ / 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** :

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>MV344 Grams/Litre</th>
<th>MV646 Grams/Litre</th>
<th>MV1053 Grams/Litre</th>
</tr>
</thead>
<tbody>
<tr>
<td>HiVeg infusion</td>
<td>12.00</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>HiVeg peptone</td>
<td>10.00</td>
<td>10.00</td>
<td>—</td>
</tr>
<tr>
<td>HiVeg peptone No. 2</td>
<td>—</td>
<td>—</td>
<td>10.00</td>
</tr>
<tr>
<td>HiVeg extract</td>
<td>—</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>3.50</td>
<td>3.50</td>
<td>—</td>
</tr>
<tr>
<td>Sodium chloride</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Starch, soluble</td>
<td>10.00</td>
<td>10.00</td>
<td>10.00</td>
</tr>
<tr>
<td>Charcoal</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Nicotinic acid</td>
<td>—</td>
<td>—</td>
<td>0.001</td>
</tr>
<tr>
<td>Agar</td>
<td>18.00</td>
<td>12.00</td>
<td>12.00</td>
</tr>
</tbody>
</table>

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 serves as carbohydrate source and therefore supports growth of organism. It along with charcoal, neutralizes 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, Cephalexin 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 :

<table>
<thead>
<tr>
<th>Vegetable based (Code MV)</th>
<th>Animal based (Code M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MV344/MV646/MV1053</td>
<td>M344/M646/M1053</td>
</tr>
<tr>
<td>HiVeg peptone</td>
<td>Peptic digest of animal tissue</td>
</tr>
<tr>
<td>HiVeg extract</td>
<td>Beef extract</td>
</tr>
<tr>
<td>HiVeg peptone No. 2</td>
<td>Pancreatic digest of gelatin</td>
</tr>
<tr>
<td>HiVeg infusion</td>
<td>Beef heart, infusion</td>
</tr>
</tbody>
</table>

### 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) (MF646) : Penicillin and Diamidodiphenylamine hydrochloride, sterile defibrinated blood

### Sterilization :

- 121°C / 15 minutes.

### Storage : Dry Medium - Below 30°C, Prepared Medium 2 - 8°C.
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 undisolved 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) 10^1-10^3 luxuriant >50%
Bordetella parapertussis (15237) 10^1-10^3 luxuriant >50%
Bordetella pertussis (8467) 10^1-10^3 luxuriant >50%

References: