# Fermentation HiVeg™ Medium Base for C. perfringens MV919

Fermentation HiVeg Medium Base for C. perfringens is recommended for determination of fermentation reaction of *Clostridium perfringens* with added carbohydrates.

## Composition ** :

| Ingredients | Grams/Litre |
|---|---|
| HiVeg hydrolysate | 10.0 |
| HiVeg special peptone | 10.0 |
| Sodium thioglycollate | 0.25 |
| Agar | 2.0 |

Final pH (at 25°C ) 7.4 ± 0.2
** Formula adjusted, standardized to suit performance parameters.

## Directions :

Suspend 22.25 grams in 1000 ml distilled water. Heat to boiling to dissolve the medium completely. Dispense 9 ml amounts in test tubes containing inverted Durham's tubes. Sterilize by autoclaving at 15 lbs pressure (121°C) for 15 minutes. Just before use heat in boiling water or free flowing steam for 10 minutes to remove dissolved oxygen and add 1 ml of 1% sterile Salicin and Raffinose solutions in separate tubes.

## Principle and Interpretation :

Fermentation HiVeg Medium Base is prepared by using HiVeg special peptone and HiVeg hydrolysate which are free from the BSE/TSE associated risks. This medium is the modification of Fermentation Medium Base which was formulated by Spray (1) and is recommended by APHA (2) for studying fermentation reaction of *Clostridium perfringens*.
HiVeg hydrolysate and HiVeg special peptone provide growth nutrients. Sodium thioglycollate creates low oxygen tension required in the medium to facilitate the growth of anaerobic organisms.
Pure isolate is inoculated into fermentation medium containing 1% Salicin and 1% Raffinose to differentiate *Clostridium perfringens* from other *Clostridia* on the basis of acid production. The inoculated media is incubated at 35°C to 37°C for 24 hr and checked for production of acid. To test for acid, 1ml of culture is transferred to a test tube and 2 drops of 0.04% bromo thymol blue is added. Acid production is indicated by yellow colour. Salicin is rapidly fermented by *Clostridia* other than *Clostridium perfringens* while *Clostridium perfringens* produces acid from raffinose within 3 days but not by other species.

**Product Profile :**

| Vegetable based (Code MV)◉ | Animal based (Code M) |
|---|---|
| **MV919**<br>HiVeg hydrolysate<br>HiVeg special peptone | **M919**<br>Casein enzymic hydrolysate<br>Peptone special |

| | | |
|---|---|---|
| **Recommended for** | : | Determination of fermentation reaction of *Clostridium perfringens* with added carbohydrates. |
| **Reconstitution** | : | 22.25 g/l |
| **Quantity on preparation (500g)** | : | 22.47 L |
| **pH (25°C)** | : | 7.4 ± 0.2 |
| **Supplement** | : | 1% Salicin and Raffinose |
| **Sterilization** | : | 121°C / 15 minutes. |

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

## Quality Control :

**Appearance of powder**
Light yellow coloured, may have slightly greenish tinge, homogeneous, free flowing powder.

**Colour and Clarity**
Light amber coloured, clear solution without any precipitate.

**Reaction**
Reaction of 2.22% w/v aqueous solution is pH 7.4 ± 0.2 at 25°C.

**Cultural Response**
Cultural characteristics observed after an incubation at 35-37°C for 24-72 hours under anaerobic condition with added 1% Salicin and Raffinose solutions in 2 separate tubes containing media.

| Organisms (ATCC) | Growth | Salicin (24 hours) | Raffinose (72 hours) |
|---|---|---|---|
| *Clostridium paraperfringens* | luxuriant | AG | - |
| *Clostridium perfringens*(12924) | luxuriant | - | A |

Key : A = Acid production
AG = Acid and Gas production

## References :

1. Spray R.S., 1936, J. Bacteriol., 32:135.
2. Frances Pouch Downes and Keith Ito (Eds.), 2001, Compendium of Methods For the Microbiological Examination of Foods, 4th ed., APHA, Washington, D.C.

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

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