

UNI 11731: 1998

Scheda 31 : Legionella GVPC

6.2.3 Selective medium:

Buffered Charcoal Yeast Extract medium with selective supplements (GVPC medium)

NOTE - This medium is identical to BCYE except that three antibiotic supplements and glycine are added to the BCYE medium

6.2.3.1 Selective supplements

The final concentrations in the GVPC medium shall be:

Ammonium-free glycine	3 g/l
Polymyxin B sulfate	80 000 iu/l
Vancomycin hydrochloride	0,001 g/l
Cycloheximide	0,08 g/l

6.2.3.2 Preparation of antibiotic supplements

Add the appropriate amount (usually 200 mg) of polymyxin B sulfate to 100 ml of distilled water to achieve a concentration of 14545 iu/ml. Mix and decontaminate by membrane filtration as described in 6.2.1.2. Dispense ...ml volumes into sterile containers and store at $-(20 \pm 3) ^\circ\text{C}$. For use, thaw at room temperature.

Add 20 mg of vancomycin hydrochloride to 20 ml of distilled water, mix and decontaminate by membrane filtration. Dispense in 1 ml volumes in sterile containers and store at $-(20 \pm 3) ^\circ\text{C}$. For use, thaw at room temperature.

Add 2 g of cycloheximide to 100 ml of distilled water and decontaminate by membrane filtration as described in 6.2.1.2. Dispense in 4 ml volumes in sterile containers and store at $-(20 \pm 3) ^\circ\text{C}$. For use, thaw at room temperature.

NOTE - Antibiotic supplements may be stored for up to 6 months when frozen.

WARNING - Cycloheximide is hepatotoxic. Wear gloves and dust mask when handling this powder form.

6.2.3.3 Preparation of GVPC medium

Follow the instructions for preparation of BCYE medium given in 6.2.1.2, but add 3 g of ammonium-free glycine after the addition of the α -ketoglutarate and then adjust the pH to $6,9 \pm 0,4$.

After the addition of the L-cysteine and iron, add one volume of each of the above three antibiotic supplements (6.2.3.2) to the final medium. Mix well.

6.2.4 Quality control of media

Prolonged heating during sterilization or heating at too high a temperature shall be avoided, as it can affect the nutritional qualities of BCYE medium. Batch-to-batch variation of the ingredients of the medium (particular α -ketoglutarate) can also affect its performance. Therefore it is essential to check the quality of each new prepared batch of media for its ability to support the growth of *L. pneumophila* serogroup 1 within three days incubation.

For most bacteria, it is usual to assess the suitability of culture media to support their growth by using cultures previously isolated organisms, maintained in the laboratory. For Legionellas this method may be misleading, as they can easily adapt to grow on culture media that would not support the primary isolation of 'wild' strains. The following procedure is therefore recommended for assessing the suitability of GVPC selective agar medium for Legionellas organisms.

Either

a) use plates of a previous batch of GVPC medium known to support the growth of Legionella together with plates

from the new batch of medium and inoculate them with a water sample known to contain Legionella organism

b) from a nationally recognized source of reference cultures, obtain a lyophilized strain of Leg serogroup 1. Reconstitute and recover as recommended, and subculture onto BCYE (6.2.

culture is not available, use a freshly isolated and confirmed strain of *L. pneumophila* serogroup *L. pneumophila* shall be replaced after not more than 10 subcultures. After incubation, make

the resulting growth just visible to the naked eye and dispense in 1 ml volumes in sterile glycerol for storage at $-20 \pm 3^{\circ}\text{C}$, or alternatively in Page's Saline (6.3.2.1) or distilled water for storage. Plate out one suspension of each isolate onto BCYE medium for subsequent identification and Legionella species and serogroup (see 9.3). For use, allow a stock suspension of one (or more) to reach room temperature. Shake thoroughly, wait 5 min to 10 min to allow aerosols to settle, and inoculate a volume (e.g. 0.1 ml) onto each of two plates of GVPC medium from the batch to be tested. After incubation, record and compare the results to ensure that the colonial morphology (9.2.6) and appearance are similar.