

UNI 11731: 1998

**Scheda 30 : Legionella BCYE agar + BCYE agar senza L-cysteine**

**6.2.1 Buffered Charcoal Yeast Extract agar medium (BCYE)**

**Composition**

Yeast extract (bacteriological grade)	10,0	g
Agar	12,0	g
Activated charcoal	2,0	g
Alpha-ketoglutarate, monopotassium salt	1,0	g
ACES buffer (N-2-acetamido-2-aminoethanesulfonic acid)	10,0	g
Potassium hydroxide (KOH) (pellets)	2,8	g
L-cysteine hydrochloride monohydrate	0,4	g
Iron (III) pyrophosphate [ $\text{Fe}_4(\text{P}_2\text{O}_7)_3$ ]	0,25	g
Distilled water	to 1000	ml

**Preparation**

a) Cysteine and iron solutions.

Prepare fresh solutions of L-cysteine hydrochloride and iron(III) pyrophosphate by adding 0.4 g and 0.25 g respectively to 10 ml volumes of distilled water. Decontaminate each solution by filtration through a membrane filter with an average pore size of 0,22  $\mu\text{m}$ . Store in clean sterile containers at  $-(20 \pm 3)^\circ\text{C}$  for not more than 3 months.

b) ACES buffer

Add the ACES granules to 500 ml of distilled water and dissolve by standing in a water bath at (45 to 60)  $^\circ\text{C}$ . To a separate 480 ml of distilled water, add all the potassium hydroxide pellets and dissolve with gentle shaking. To

prepare the ACES buffer, mix the two solutions.

**NOTE** – ACES buffer can cause denaturation of the yeast extract if the following sequence is not followed.

c) Final medium.

Add sequentially to the 980 ml of ACES buffer, the charcoal, yeast extract and  $\alpha$ -ketoglutarate. Prepare a 0,1 mol/l solution of potassium hydroxide (KOH) by dissolving 5,6 g in 1 litre of distilled water. Prepare a 0,1 mol/l solution of sulfuric acid ( $\text{H}_2\text{SO}_4$ ) by carefully adding 5,3 ml of  $\text{H}_2\text{SO}_4$  to 1 litre of distilled water. Use the solutions of 0,1 mol/l potassium hydroxide or 0,1 mol/l sulfuric acid as appropriate to adjust the pH to  $6,9 \pm 0,2$ . Add the agar, mix and autoclave at  $(121 \pm 1)^\circ\text{C}$  for  $(15 \pm 1)$  min (see 6.2.4. first paragraph). After autoclaving, allow to cool to  $(50 \pm 2)^\circ\text{C}$  in a water bath.

Add the L-cysteine and the iron (III) pyrophosphate solutions aseptically, mixing well between additions.

Dispense in 20 ml volumes into Petri dishes of 90 mm to 100 mm diameter. The pH of the final medium is  $6,9 \pm 0,4$

at  $25^\circ\text{C}$ . Allow excess moisture on the plates to dry and store at  $(4 \pm 2)^\circ\text{C}$  in airtight containers in the dark for up to 4 weeks.

**Buffered Charcoal Yeast Extract agar medium (BCYE) senza L-cysteine**

Prepare this medium in the identical manner to BCYE but omit the L-cysteine.