ATCC medium: 2124 Modified chopped meat medium (with clindamycin 5 mcg/ml)

NOTE: The preparation of this medium is complex owing to the anaerobic and aseptic dispensing required. A simpler alternative to this formulation is to prepare ATCC medium 1490 (Modified chopped meat medium) and a separate solution of clindamycin. The technologist can aseptically add a predetermined amount of the drug solution to a tube of the medium prior to use. Medium 1490 is usually dispensed in 8.0 ml quantities. For this quantity, a filter-sterilized solution of clindamycin containing 160 micrograms of drug per ml should be prepared. The technologist should aseptically add 0.25 ml of the stock solution to each tube of ATCC Medium 1490 to achieve a concentration of 5.0 micrograms per ml. The stock solution should be prepared fresh and not kept for longer than one week at 2-4C.

Ground beef (fat-free).....500.0 g
Distilled water.....1.0 L
N NaOH.....25.0 ml

Mix ingredients well. Bring to boil for 5 minutes with stirring. Allow to cool to room temperature and skim the fat. Alternatively, you may cool the solution in a refrigerator and remove the hardened fat from the surface. Filter through cheese cloth and retain both meat particles and the filtrate. To the filtrate, add sufficient water to restore the volume to 1.0 L.

To the retained filtrate, add:
Trypticase.....30.0 g
Yeast extract.....5.0 g
K_2HPO_4 .....5.0 g
Resazurin (0.025%).....4.0 ml
Agar (if necessary).....20.0 g

Boil and cool medium under 80% N_2, 10% H_2, 10% CO_2. When cool, add:

L-Cysteine . HCl.....0.5 g
Hemin Solution (see below).....10.0 ml
Vitamin K1 Solution (see below)....0.2 ml

Autoclave at 121C for 15 minutes. Cool under gas and adjust pH to 7.0.

Add to the cooled filtrate:
Clindamycin Solution (1 mg/ml).....5.0 ml

Filter-sterilize.

Place about 2.0 cc of meat particles in each test tube. Seal the test tubes and autoclave at 121C for 15 minutes.

Anaerobically and aseptically dispense 7.0 ml of the completed and sterilized broth into tubes containing sterile meat particles. Use the gas mixture stated above.

Hemin Solution:
Hemin.....50.0 mg
N NaOH.....1.0 ml
Distilled water.....100.0 ml

Vitamin K1 Solution:
Vitamin K1.....0.15 ml
Ethanol.....30.0 ml

Store in a dark bottle. Refrigerate. Discard after 10 days.