ATCC medium: 1012 Diphasic blood agar medium

Cool ATCC Medium 449 (see below) until it may be held comfortably in the hand. Aseptically add 10% sterile, defibrinated rabbit blood; dispense 5 ml per 16 X 125 mm screw-capped test tube, slant and cool; add 3.0 ml sterile Locke's solution overlay (see below).

**ATCC Medium 449:**
- Beef (BD 213110) ..................25.0 g
- Distilled water ..................250.0 ml

Infuse beef in water by bringing to a rapid boil for 2-3 minutes, stirring constantly. Filter through Whatman #2V filter paper and add:

- Neopeptone, DIFCO (BD 211681).....10.0 g
- NaCl ...............................2.5 g
- Distilled water to ..........500.0 ml

Heat to boiling and filter through Whatman #2V filter paper. Add:

- Agar............................10.0 g

Make up volume to 500 ml; adjust pH to 7.2-7.4. Autoclave for 20 minutes at 121°C.

**Locke's solution:**
- NaCl...............................8.0 g
- KCl................................0.2 g
- CaCl₂..............................0.2 g
- KH₂PO₄ .............................0.3 g
- Glucose............................2.5 g
- Distilled water .................1.0 L