**ATCC medium: 947 L diphasic blood agar with Locke's solution overlay reduced from 3.0 ml to 1.0 ml**

ATCC Medium 1011 (see below) with Locke's solution overlay reduced from 3.0 ml to 1.0 ml

*ATCC Medium 1011:*
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 121C.

*Locke's Solution:*
NaCl.....8.0 g
KCl.....0.2 g
$CaCl_2$.....0.2 g
$KH_2PO_4$.....0.3 g
Glucose.....2.5 g
Distilled water.....1.0 L