

## ATCC Medium: 1671      Blastocystis Egg Medium

Whole emulsified egg	783	ml
Stone's Modification of Locke's Solution (see below)	217	ml

**Use only fresh fertile eggs** (one source for such eggs is B&E eggs in York Spring Pa. Ship at Day 0)

Filter through gauze into a filtration flask.

Place under vacuum to draw out air bubbles.

Dispense in 4.0 ml amounts to a 16 x 125 mm screw-cap tube.

Inspissate (80 °C) at a 30 degree slant .Generally it takes approximately 1 hour to set. Remove when eggs have solidified. Be careful not to overcook.

Allow to cool and add a 4.5 ml overlay of Stone's Modification of Locke's Solution.

Cap with rubber stoppers then autoclave for 20 minutes at 121°C.

Remove rubber stoppers after the medium is cooled the aseptically replace with screw-caps.

### Stone's Modification of Locke's Solution\*:

NaCl	8.0	g
CaCl <sub>2</sub>	0.2	g
KCl	0.2	g
MgCl <sub>2</sub> x 6H <sub>2</sub> O	0.01	g
Na <sub>2</sub> HPO <sub>4</sub>	2.0	g
NaHCO <sub>3</sub>	0.4	g
KH <sub>2</sub> PO <sub>4</sub>	0.3	g
Distilled water	1000.0	ml

\*Reference: *Am. J. Trop. Med.*, 15, 681- , 1935.

Note: Solution will precipitate when Na<sub>2</sub>HPO<sub>4</sub> is added. Dispense while stirring to evenly distribute salts.

Protistology will add 25% (v/v) heat-inactivated (56C for 30 minutes) horse serum (~1.5ml) to each tube just before use (before placing the tubes in the anaerobic jar 48 hours prior to inoculation with Blastocystis).