## ATCC medium: 482 MPHM medium

McCoy's 5A Medium (modified) (GIBCO 16600). Prepare as directed and aseptically dispense in 4 ml amounts in 16 X 125 mm screw-capped test tubes. Just prior to inoculation, to each tube, aseptically add the following solutions:

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2.5% Proteose Peptone No. 3 (BD 211693)..1.0 ml 0.05% Hemin (see below)......0.1 ml
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Prepare and sterilize the above stock solutions separately. Cap the tubes tightly and slant.

## Hemin Solution:

NaCl0.8 g
KCl0.04 g
NaH <sub>2</sub> PO <sub>4</sub> . H <sub>2</sub> O0.02 g
Na <sub>2</sub> HPO <sub>4</sub> 0.12 g
CaCl <sub>2</sub> 0.011 g
$MgCl_2$ . $6H_2O$ 0.03 g
Hemin (Eastman Organic Chemicals)0.05 g
Distilled water

Dissolve sodium chloride through sodium phosphates in  $50.0\,\mathrm{ml}$  distilled water. Dissolve calcium chloride and magnesium chloride in  $50.0\,\mathrm{ml}$  water. Mix both salts solutions together and add hemin. Adjust to pH  $9.0\,\mathrm{ml}$  with  $10\,\mathrm{N}$  NaOH to dissolve hemin.