ATCC Medium: 5608 PY Medium (M2-VPI) With D-Glucose w/ Durham Tube

D-Glucose……10.0g
Peptone……..5.0 g
Trypticase……...5.0 g
Yeast Extract……10.0 g
0.025% Resazurin……4.0 ml
Salt Solution (see below)……40.0 ml
Hemin Solution (see below)……10.0 ml
Vitamin K_1 Solution (see below)…….0.2 ml
Cysteine HCl x H_2O.....0.5 g
DI Water……..950.0 ml

Adjust medium for final pH 7.0 +/- 0.2. Prepare medium anaerobically using a gas phase of 80% N_2, 10% CO_2 and 10% H_2.

Add durham tubes and purge air by autoclaving at 110ºC for 1-3 minutes.
***** For stabs, do not use durham tubes.

Hemin Solution
Hemin……..50.0 mg
1N NaOH……1.0 ml
DI Water……...100.0 ml

Dissolve hemin in NaOH. Add DI Water. Sterilize by autoclaving at 121ºC.

Vitamin K_1 Solution
Vitamin K_1 (2-Methyl-3-phytl-1,4-naphthoquinine)…0.15 ml
95% Ethanol……30.0 ml

Refrigerate Vitamin K_1 in a brown bottle. Discard after one month.

Salt Solution
$CaCl_2$……0.2 g
$MgSO_4$……..0.2 g
K_2HPO_4……1.0 g
KH_2PO_4……1.0 g
$NaHCO_3$……...10.0 g
NaCl……2.0 g
DI Water……1000 ml

Mix calcium chloride, magnesium sulfate and sodium chloride in 300 ml of distilled water. Mix di- and monopotassium phosphates in 200 ml of distilled water. Dissolve sodium bicarbonate in 500 ml of distilled water. Combine the 3 solutions. Store at 4ºC.