

APPENDIX: PREPARATION of MRA-154 MEDIUM

1. **Incomplete Medium:** Used for many applications involving wash steps during preparation of parasites for culture or assay. The incomplete medium consists of RPMI 1640 medium supplemented with the following components¹:

Incomplete MediumRPMI 1640 medium^{2,3}

Sodium bicarbonate (NaHCO ₃) ⁴	2.4 g/L
L-Glutamine	2 mM
HEPES [4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid]	25 mM
D-Glucose ⁵	2 g/L
Hypoxanthine	27 µg/mL
Gentamicin (optional)	5 µg/mL

¹Prepare sterile stock solutions at concentrations that are easily diluted into the liquid medium to obtain the appropriate user concentrations and add aseptically. Ready-made stock solutions for many of the components are available from numerous manufacturers.

²RPMI 1640 medium is available from numerous manufacturers as both a powder and a sterile, prepared liquid, with or without L-glutamine and HEPES. If using powdered RPMI 1640 medium, prepare the medium following manufacturer instructions, sterile-filter using a 0.22 µm filter, then aseptically add the necessary components in the appropriate concentrations.

³If stock solutions were not sterile or aseptic techniques were not followed, sterile-filter the medium using a 0.22 µm filter after the addition of all components. Store at 4°C.

⁴Prepared, liquid medium typically contains sodium bicarbonate while powdered medium does not. A typical concentration of sodium bicarbonate in RPMI 1640 medium is 2 g/L, though some formulations contain different amounts.

⁵A typical concentration of D-glucose in RPMI 1640 medium is 2 g/L. The option to supplement with an additional 2 g/L yields a final concentration of 4 g/L D-glucose.

2. **Complete Medium:** Consists of incomplete medium (above) supplemented with 10% heat-inactivated human serum. If necessary, filter the complete medium with a 0.22 µm filter. Since serum tends to clog sterilizing filters, a serum pre-filter may be used first, followed by a 0.22 µm sterilizing filter.

Note: Human serum type A is used with washed type O blood. Serum substitutes may be used; however, they may not be acceptable for all parasite strains.