

APPENDIX: REVISED BARBOUR-STOENNER-KELLY (BSK) BROTH

1. Prepare the Revised BSK medium directly before each use following the recipe below by dissolving each component one at a time in distilled water:

HEPES	5.64 g
Neopeptone	4.7 g
Sodium citrate	0.7 g
Glucose	5.64 g
NaHCO ₃	2 g
TC-Yeastolate	2 g
Sodium pyruvate	0.75 g
N-acetylglucosamine	0.37 g
Bovine serum albumin, fraction V	47 g
Distilled water	840 mL

2. Adjust the pH of the base medium to 7.5 using 1 N HCl or 1 N NaOH and filter-sterilize using a 0.22 µm filter.
3. Aseptically add the next two components to the base medium:

CMRL 1066 Medium, 10× (w/o Glutamine and NaHCO ₃)	100 mL
Heat-inactivated rabbit serum	60 mL

4. Mix well and aseptically dispense into appropriate vessels. The medium may be stored in aliquots of 50 mL in freezer-safe vessels and stored frozen at -20°C until use. Once thawed, each aliquot should be kept at 2°C to 8°C and used within one month.
5. Adjust the pH of the complete medium to 7.5 to 7.6, as needed, using sterile solutions of 1 N HCl or 1 N NaOH before use.

Note: Medium should be prepared fresh directly before each use or immediately aliquoted and frozen at -20°C until needed. Once thawed, each aliquot should be kept at 2°C to 8°C and used within one month.