

**APPENDIX F**  
**OHIO DISTRICT MICROBIOLOGY LABORATORY**  
**MTEC AGAR AND UREA-PHENOL SOLUTION PREPARATION**

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**BASAL MEDIUM**

Ingredients	Amounts (in grams, unless specified)	
Reagent water	<b>1 L</b>	<b>1.5 L</b>
Proteose Peptone #3	5.0	7.5
Yeast Extract	3.0	4.5
Lactose	10	15
NaCl	7.5	11.25
K <sub>2</sub> HPO <sub>4</sub> (anhydrous)	3.3	4.95
KH <sub>2</sub> PO <sub>4</sub> (anhydrous)	1.0	1.5
Sodium lauryl sulfate	0.2	0.3
Sodium desoxycholate	0.1	0.15
Brom cresol purple	0.08	0.12
Brom phenol red	0.08	0.12
Bacto Agar	15	22.5

**PREPARATION OF BASAL MEDIUM**

- Heat to boiling with a stirring rod on a hot plate until dissolved.
- Pour into 100-mL dilution bottles.
- Autoclave for 15 minutes.
- Store dilution bottles at 4°C for up to 6 months.

**Start if purchasing the agar medium**

**PREPARATION OF AGAR PLATES**

- Melt the basal medium using a beaker with water on a hot plate or by placing in the autoclave for a 5-minute cycle.
- Pour the plates after the agar is tempered (50-60°C).
- Store the plates at 4°C for up to 2 weeks in a tightly sealed container.

**UREA-PHENOL SOLUTION**

Ingredients	Amounts (in grams, unless specified)	
Deionized water	<b>100 mL</b>	<b>500 mL</b>
Urea	2.0	10.0
Phenol red	0.01	0.05

- Combine ingredients into an Erlenmeyer flask and mix for several minutes with a stir bar. Not all the red particles will dissolve.
- The solution should be a straw-yellow color.  
If the color is too red, adjust the pH to 5 with 1N HCl.
- Pour into a sealed, labeled bottle.
- Store at 4°C for up to 6 months. If the solution changes color, readjust the pH.