

**APPENDIX C**  
**OHIO WATER MICROBIOLOGY LABORATORY**  
**MI AGAR PREPARATION**

---

**BASAL MEDIUM**

Ingredients	Amounts (in grams, unless specified)		
	1000 mL	500 mL	300 mL
Distilled or deionized water			
Proteose Peptone #3	5.0	2.5	1.5
Yeast Extract	3.0	1.5	0.9
$\beta$ -D-lactose	1.0	0.5	0.3
4-Methylumbelliferyl- $\beta$ -D-galactopyranoside (MUGal) (final concentration 100 $\mu$ g/mL)	0.1	0.05	0.03
Indoxyl- $\beta$ -D-glucuronide (IBDG) (final concentration 320 $\mu$ g/mL)	0.32	0.16	0.096
NaCl	7.5	3.75	2.25
K <sub>2</sub> HPO <sub>4</sub> (anhydrous)	3.3	1.65	0.99
KH <sub>2</sub> PO <sub>4</sub> (anhydrous)	1.0	0.5	0.3
Sodium lauryl sulfate	0.2	0.1	0.06
Sodium desoxycholate	0.1	0.05	0.03
Bacto Agar	15	7.5	4.5

**PREPARATION OF BASAL MEDIUM**

- Prepare mixture according to above ingredients or according to directions on bottle (for dehydrated agar purchased from a commercial supplier)
- Heat to boiling until the ingredients dissolve with a stirring rod on a hot plate.
- Add exactly 100 mL to each dilution bottle.
- Autoclave for 15 minutes.
- Store dilution bottles at 4°C for up to six months.

**CEFSULODIN SOLUTION (1 mg/1 mL)**

- Add 0.02 g of cefsulodin to 20 mL distilled or deionized water (this can be done in a test tube).
- Sterilize the solution into a labeled sterile test tube using a 0.22- $\mu$ m filter and a 5- or 10-cc syringe.
- Use immediately or store at 4°C for a short period of time (less than 4 hours).
- Do not save the unused portion.

**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.
- Add 0.5 mL of freshly prepared cefsulodin solution to 100 mL of tempered agar medium (50-60°C). The final concentration of cefsulodin in the agar is 5  $\mu$ g/100 mL.
- Gently mix the agar and pour plates.
- Store the plates at 4°C for up to 2 weeks in a tightly sealed container.