APPENDIX M
OHIO DISTRICT MICROBIOLOGY LABORATORY
BUFFER PREPARATION

PB WATER--Phosphate buffered dilution water (purple tape). Use with mTEC, modified mTEC, mEI, and MI agar methods.

STOCK SOLUTIONS

Stock KH_2PO_4 solution

1. Dissolve 34 g of KH_2PO_4 (anhydrous potassium dihydrogen phosphate) in 500 mL deionized water. Adjust to pH 7.2 $\pm$ 0.5 with 1 N NaOH.
2. Dilute to 1 L using deionized water.
3. Autoclave in dilution bottles for 20 minutes.

Stock $MgCl_2$ solution

1. Dissolve 38 g anhydrous $MgCl_2$ (or 81.1 g $MgCl_2 \cdot 6H_20$) in 1 L deinonized water.
2. Autoclave in dilution bottles for 20 minutes.

Store stock solutions at room temperature until opened. Once open, store at 4°C. Buffer is good for 1 year or until it becomes cloudy.
Clearly mark that this is concentrated stock solution and NOT working solution.

WORKING SOLUTION

- Add 1.25 mL stock KH_2PO_4 solution and 5 mL stock $MgCl_2$ solution to 1 L deionized water. For 8 L of working solution, add 10 mL stock KH_2PO_4 solution and 40 mL stock $MgCl_2$ solution to 8 L deionized water.
- Dispense in amounts that will provide 99 mL $\pm$ 2.0 mL in dilution bottles or dispense no more than ¾ full in mason jars.
- Autoclave for at least 30 minutes (larger volumes need longer times).

Liquid volume (liters)	No. of containers	Time to reach 121°C	Total time of cycle
0.5	30	19	29
1	2	34	44
2	10	37	47
4	5	52	62
6	4	60	70

- Store at room temperature until open, then store at 4°C.

FC BUFFER (yellow tape)
Use with mFC and mENDO agar methods

Stock KH_2PO_4 solution
Prepare as described for PB water.

Working solution
- Add 1 g of peptone to 1 L deionized water (final concentration is 0.1% peptone).
- Add 1.25 mL stock KH_2PO_4 solution to the 1 L deionized water containing 0.1% peptone. For 8 L of working solution, add 10 mL stock KH_2PO_4 solution to the 8 L of deionized water containing 0.1% peptone (8 g/8 L).
- Dispense and autoclave as described for PB water.

EC BUFFER (green tape)
Use as an alternative to PB water for mTEC and mEI agar methods

Anhydrous ingredients	**Amount**	
Deionized water	**1 L**	**8 L**
Sodium phosphate monobasic (NaH_2PO_4)	0.58 g	4.64 g
Sodium phosphate dibasic (Na_2HPO_4)	2.50 g	20.0 g
Sodium chloride (NaCl)	8.50 g	68.0 g

OR Hydrated ingredients	**Amount**	
Deionized water	**1 L**	**8 L**
Sodium phosphate monobasic monohydrate ($NaH_2PO_4 \cdot H_20$)	0.67 g	5.30 g
Sodium phosphate dibasic heptahydrate ($Na_2HPO_4 \cdot 7H_20$)	4.72 g	37.7 g
Sodium chloride (NaCl)	8.50 g	68.0 g

- Stir until dissolved. Do not heat.
- Dispense and autoclave as described for PB water.