# Medium Preparation

## R & F® *Campylobacter jejuni/C. coli* Chromogenic Medium

### Plating Medium Powder

Add 49.5 grams of powder to l liter of distilled or deionized water. Gently, but thoroughly, swirl to disperse the powder to form a chalky white solution. Adjust pH to 6.80-6.90 at 25ºC using either 1N NaOH or HCL. Warm slightly. Stir to dissolve any clumps of powder from the surface of the hydrated media. Boil to dissolve completely. IMPORTANT: AVOID OVERHEATING AND DO NOT AUTOCLAVE. Allow to cool in a waterbath at 50-55ºC.

Prepare R & F® *Campylobacter jejuni/C. coli* supplement for the chromogenic plating medium following instructions. After the solution in vial M-9A and M-9E are filter sterilized, aseptically add 2 ml from each vial to the cooled chromogenic plating medium. After the powder in vial M-9B is completely dissolved, add 6 ml (total contents) to the cooled chromogenic plating medium. Gently swirl the medium to dispense the solution in the medium. Make sure the white precipitate is thoroughly dispensed in the medium. Pour the completed medium into Petri plates. Gently swirl intermittently during pouring. Allow the agar surface to dry by keeping the plates at room temperature 2 days in the dark. Store the plates in Petri plate vented sleeves in the dark at 2-8ºC for up to 60 days. Final pH 6.80 to 7.20 at 25ºC

### Supplement Powders

The contents contain seventeen (17) labeled vials, including 6 vials labeled M-9A (3 vials labeled cefsulodin/cefoperazone and 3 sterile vials for cefsulodin/cefoperazone solution), 5 vials labeled M-9B (ALDOL™ acetate), and 6 vials labeled M-9E (3 vials labeled vancomycin and 3 vials labeled vancomycin solution vials). Pipette 5 mL of sterile deionized or distilled water into one M-9A vial (cefsulodin/cefoperazone) and 5 mL into one M-9E vial (vancomycin). Secure the cover and shake the contents of each vial until the powder is completely dissolved. Do not use heat. Filter sterilize the solution in the vial using a 0.45 or 0.20 µm pore size filter and add the filtrate directly into the corresponding solution vials labeled cefsulodin/cefoperazone solution vial and vancomycin solution vial, respectively. Pipette 6 ml of 95% ethanol into one of the M-9B vials labeled ALDOL™ acetate. Secure the cover tightly. Swirl the vial in a water bath at a temperature ranging from 50-55ºC until the powder is completely dissolved. This should take several minutes. PLEASE NOTE: ENSURE THAT WATER FROM THE WATER BATH DOES NOT SEEP INTO VIAL M-9B. A COLOR CHANGE FROM YELLOW TO ORANGE-RED WILL OCCUR, WHICH INDICATES THAT THE VIAL WAS PREPARED INCORRECTLY. DO NOT USE HEAT FROM A HOT PLATE.

CAUTION: BE SURE NOT TO MIX SOLUTIONS OR POUR INTO THE WRONG VIAL. Date the vial with the date of preparation and 3 months from preparation as the expiration date. Store vials containing the solution at 2-8ºC until use. The powder in vial M-9B (ALDOL™ acetate) will go out of solution at 2-8ºC storage. When ready to use at a later date swirl the contents of vial M-9B in the water bath (50-55ºC) until the powder is completely dissolved. Follow the directions on the R & F® *Campylobacter jejuni/ C. coli* Chromogenic Plating Medium bottle label to make 1 liter of medium. For less than l liter adjust the solutions to the medium accordingly.