ALKEN-MURRAY CORPORATION

P. O. Box 400, New Hyde Park, NY 11040
TELEPHONE 540-636-1236 - Fax 540-636-1770

QUALITY CONTROL METHOD - 16

Preparation of Standard Methods (SMA) Agar

Description:

This quality control procedure is designed to verify that SMA agar is obtained and stored properly. This agar is used to grow a large culture of bacteria for quality control procedure QC-1 and QC-3. This procedure should be performed by a trained laboratory technician.

Equipment & Supplies:

Incubator
Sterilizer or autoclave
Orbital incubator-shaker
Chemical balance (≥ 200 g capacity; 0.01 gram precision)
Heated stir plate and magnetic stir bar
pH meter with dual probe for temperature and pH
Eppendorf Adjustable Air Displacement Pipetter

1 Erlenmeyer flask or beaker (1000 ml - or double the volume of media being prepared)
2 Weighing dishes (not necessary to sterilize for media preparation)
1 500 ml glass measuring cylinder
1 sterile weighing dish for each bacterial sample
1 Calibrated pH solution to verify pH meter reading
1 Sterile petri dish per sample tested
1 Sterile bacterial spreader per sample tested
1 pipet tip for each sample to be inoculated
1 Sterile Erlenmeyer flask (250 ml) for each sample that must be rehydrated for testing
1 Sterile weighing dish per sample to be tested
1 Sterile funnel to filter bran from mixture (for each dry sample)
1 *Weber* Phosphate Dilution bottle (99 ml - #3026-71) for each product to be tested
1 Wash bottle with 500 ml of distilled water
1 Steri-Wrap II (green)
1 Rapid-Flo double gauze Milk-filter or Bellco silicone sponge stopper (to give body to the closure)
1 rubber band to secure closure

Ingredients:
Standard Methods agar (Weber #3080-04)
500 ml distilled water

Procedure:

1. Purchase SMA Agar from Weber Scientific.

2. Place a label on package with expiration date marked in bold RED letters

3. Store powdered agar between 2° and 30° C

4. Discard powdered agar after expiration date

5. Suspend 11.75 grams of powder SMA agar in 500 ml distilled water

6. If medium is to be used with rolled tube method, add 1% pure agar.

7. Heat with frequent agitation to boil. Boil for 1 minute to completely dissolve the powder.

8. Apply closure.
   - 8.1 A 250 ml flask closure consists of a Bellco silicone sponge stopper (or equivalent) covered with double layer of green Steri-Wrap II (or equivalent) and secured with rubber bands.
   - 8.2 A 2 liter flask closure consists of a double layer of Steri-Wrap (or equivalent) with a single gauze milk filter (or equivalent) sandwiched in between secured with rubber bands

9. Autoclave for 15 minutes at 121°C (13 lbs pressure) before use.

10. Cool to 45°C and dispense 15-20 ml of SMA agar per sterile, plastic disposable petri dish (enough to just coat the bottom of the dish).

11. Store prepared agar in the refrigerator, in the dark, until used. Use prepared agar within one week.

12. Prepare bacteria according to instructions in QC-1 or the QC procedure that calls for this agar.

13. When media has cooled to room temperature, it can be inoculated with prepared sample as in item 17 below.

14. Add 2.0 grams of ***Alken Clear-Flo*** dry product to the sterile phosphate buffer solution in the buffer bottle. Shake by hand to mix. Place the bottle on an orbital shaker for 30 minutes at 150 cycles per minute at 35°C.

15. Place one sterile gauze sponge in the funnel, supported on a ring stand, above an Erlenmeyer flask. Pour the buffer bottle contents into the gauze pad, to filter it. Use the wash bottle to direct a fine stream of water into the buffer bottle, to rinse out all the remaining bran into the gauze pad. Use as little water as possible to do this. Then partly fill the bottle with distilled water, shake it and discard the rinse water.

16. With sterile gloves on your hands, squeeze the gauze pad, to gather any further bacterial culture into the collection flask. Return the filtrate to the buffer bottle.

17. When testing liquid products, dilute by 20% with distilled water or phosphate buffer and shake in incubator as mentioned in item 14. above, but filtration is not necessary, for liquids.

18. Use *Eppendorf* pipetter to dispense 0.1 ml onto the surface of each petri dish. See QC-1 for techniques to use air displacement pipetter.

19. Use sterile cell spreader to lightly and evenly spread bacteria thinly around the petri dish.

20. Incubate plates inverted at 32° to 35°C (90° to 95°F) for 24 hours.