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QUALITY CONTROL METHOD - 8

Detection of *Staphylococcus*

PURPOSE

This procedure is designed to verify that all Alken Clear-Flo® products are free of *Staphylococcus aureus.* The primary presumptive European test for *Staphylococcus* is "Chapman's Agar", the equivalent of our "Mannitol Salt Agar" (QC-21), which does not distinguish between *Staphylococcus* and many of Alken-Murray's gram-positive, salt loving, Mannitol utilizing *Bacillus* strains. It is therefore important to thoroughly follow each step to assure accuracy. *Staphylococus* is an opportunistic pathogen, attacking those with compromised immune systems, such as IV drug users, newborns, elderly, and those using catheters or other artificial appliances. This test should be performed by a trained laboratory technician.

EQUIPMENT

1. Bunsen burner or Oxford Bacti-cinerator III
2. Microscope with 100X oil-immersion lens
3. Refrigerator
4. Timer
5. Petri dishes
6. Test tubes
7. Timer or stopwatch
8. Microbiological loop (3 mm platinum loop or sterile blue loop-needle combinations from Weber)
9. Green Steri-Wrap II
10. Rubber bands for closures on tubes and flasks
11. 4-ply Mirasorb gauze sponges (test tube closures)
12. Rapid-Flo double gauze milk filter (flask closure)
13. Thermometer
14. Weighing dishes
15. Laboratory balance sensitive to 0.001 g
16. Autoclave
17. Incubator
18. Erlenmeyer flasks to prepare media
19. Heated magnetic stirrer
20. BBL SMA Agar (see QC-16) http://www.alken-murray.com/QC16.pdf
    or Tryptic Soy Agar (QC-22) http://www.alken-murray.com/QC22.pdf
21. Mannitol Salts Agar (see QC-21) http://www.alken-murray.com/QC21.pdf
22. BBL or Hardy Diagnostic Dextrose Agar (see QC-25) http://www.hardydiagnostics.com
23. Hardy Diagnostic Rose Agar prepared media or PEA (QC-84)
24. MacConkey Agar (see QC-6) http://www.alken-murray.com/QC6.pdf
25. CHROMagar Staphylococcus from Hardy Diagnostic Lab. http://www.hardydiagnostics.com

26. CHROMagar Aureole from Hardy Diagnostic Laboratories
27. Hydrogen peroxide
28. Catalase Agar (see QC-26)
29. Disinfectant
30. Gram stain reagents - (see QC -10)
31. Sample of *Staphylococcus aureus*, such as ATCC 25923

PROCEDURE

All samples handled in this procedure should be autoclaved before disposal.

1. Culture & Gram Stain

1.1 Grow sample cultures on SMA or TSA Agar, using the technique outlined for culturing in QC-1, and perform the Gram stain procedure as outlined in QC-16.

1.2 If culture is Gram-negative, it cannot be *Staphylococcus aureus*. If the culture is positive, and you are not sure whether you are seeing cocci or small, young rods, further tests are required to isolate the particular gram-positive strain(s) present in the sample.

1.3 If it is more convenient to culture for gram separation, streak culture onto MacConkey Agar (QC-6) to rule out gram negative strains, and Hardy Diagnostic Rose Agar to identify gram positive strains, using a 3 mm sterile microbiological loop.

2. Catalase test (QC-26) Some bacteria possess the defense mechanism of producing catalase to minimize the harmful effects of exposure to hydrogen peroxide and superoxide.

2.1 Completely cover entire petri dish with hydrogen peroxide.

2.2 Observe the dish for reactions.

2.3 If the bacteria in question produce catalase, they will convert the hydrogen peroxide into oxygen gas, causing bubbles to form on the dish. If bubbles form, this test result is considered positive. *Bacillus licheniformis, Bacillus subtilis, Bacillus amyloliquefaciens, Bacillus mojavensis, Bacillus pumilus* are gram-positive strains that are also catalase positive, so additional testing is required if the result of this test is positive.

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3. CHROMagar Tests

3.1 Specimen Collection and Preparation

3.1.1 Test only fresh cultures (18 to 36 hours incubation)

3.2 Test Procedure

3.2.1 Using a 3 mm sterile platinum-iridium microbiological loop, streak both CHROMagar Aureole and CHROMagar Staphylococcus agars

3.2.2 Incubate at 37° C for 24 ± 2 hours

3.3 Interpretation of plates for typical *Staphylococcus* colonies.

3.3.1 Examine CHROMagar Staphylococcus plate at 24 ± 2 hours. Reincubate negative plates and re-examine the following day.

3.3.2 *Staphylococcus* will have mauve colonies, while other strains will be blue, colorless or inhibited. If no typical colonies are observed, the sample(s) are assumed to be *Staphylococcus* free.

3.3.4 Examine CHROMagar Aureole plate at 24 ± 2 hours. Reincubate negative plates and re-examine the following day.

3.3.5 *Staphylococcus* will have black colonies with white halos, while other strains will be colorless or inhibited. If no typical colonies are observed, the sample(s) are assumed to be *Staphylococcus* free.

4. Microscope

4.1 Pick a suspect colony and observe for cocci pairs, tetrads or clusters indicative of *Staphylococcus aureus*, microscopically under phase contrast using the 100X oil immersion lens. If cocci pairs are observed and all other tests are indicative of *Staphylococcus aureus*, then the sample can be declared positive.

4.2 Record visual interpretation in the appropriate QC database log.

4.3 If the person conducting the test is a distributor or client of Alken-Murray, contact Valerie or Ken for verification of retained sample of lot (to rule out malicious contamination by vandals). If our test is also positive, Alken-Murray will replace all product that was purchased from that lot by all clients.

5. Quality Control

5.1 Once a month (Alken-Murray lab) run a positive *Staphylococcus aureus* control, using ATCC 25923

5.2 From a cryovial, inoculate one test tube containing sterile Luria Broth or Nutrient Broth and incubate overnight at 42°C until turbidity develops.

5.3 Prepare a 1:10 dilution in phosphate buffer and measure the OD at 580 nm.

5.5 Calculate the estimated cell concentration using undiluted OD constant of 0.66 = 4.8 x 10^8 cfu/ml.

5.5 Dilute accordingly in sterile phosphate buffer to obtain a dilution containing 1-10 *Staphylococcus aureus* cells/ml.

5.6 Add an estimated 25 grams of a known *Staphylococcus* negative dry product to 225 ml of sterile lactose broth.

5.7 Asceptically inoculate with 1 ml of the diluted *Staphylococcus* to achieve a starting

concentration of 1-10 *Staphlylococcus* per 25 grams (flask).

5.8 Perform plate count on SMA, QC-16 (final dilutions usually 10^6 and 10^7) to determine the actual starting *Staphylococcus* concentration.

5.9 Complete the rest of the *Staphylococcus* test.

6. References

6.1 Kloos, W.E. and T.L. Bannerman. 1995. *Staphylococcus and Micrococcus*, p. 282-298. In P.R. Murray, E. J. Baron, M. A. Pfaller, F.C. Tenover and R. H. Yolken (ed) Manual of Clinical Microbiology, 6th edition American Society for Microbiology, Washington D.C.

6.2 Taussig, M. J. 1984. Processes in Pathology and Microbiology, 2nd edition. Oxford; Boston. Blackwell Scientific Publ. St. Louis, Mo. Blackwell Mosby Book Distributors. 520-530.

6.3 Roberts, J. I. S., and M. A. Gaston. 1987. Protein A and Coagulase expression in Epidemic and Non-Epidemic *Staphylococcus aureus*, J. Clin Pathol. 40:837-840.

6.4 http://www.chromagar.com and http://www.hardydiagnostics.com websites

7. Results of Tests of Bacillus strains in various Alken-Murray microbial blends

7.1 *Bacillus licheniformis*, 634 & 406:
- 7.1.1 Gram-stain - positive
- 7.1.2 Catalase positive
- 7.1.3 CHROMagar tests **- negative**
- 7.1.4 Microscope - **negative** for grape-like clusters

7.2 *Bacillus subtilis*, 003, 405, 505
- 7.2.1 Gram-stain - positive
- 7.2.2 Catalase positive
- 7.2.3 CHROMagar tests - **negative**
- 7.2.4 Microscope - **negative** for grape-like clusters

7.3 *Bacillus amyloliquefaciens* 842 through 845, AMH 102, AMH 104 & AMH 107
- 7.3.1 Gram-stain positive
- 7.3.2 Catalase positive
- 7.3.3 CHROMagar tests - **negative**
- 7.3.4 Microscope - **negative** for grape-like clusters

7.4 *Sporosarcina pasteurii*, 453
- 7.4.1 Gram stain positive
- 7.4.2 Catalase positive
- 7.4.3 CHROMagar tests - **negative**
- 7.4.4 Microscope - **negative** for grape-like clusters

7.5 *Bacillus laevolacticus*, 494
- 7.5.1 Gram-stain positive
- 7.5.2 Catalase positive
- 7.5.3 CHROMagar tests - **negative**
- 7.5.4 Microscope - **negative** for grape-like clusters

7.6 *Bacillus megaterium*, 112
- 7.6.1 Gram-stain positive
- 7.6.2 Catalase positive
- 7.6.3 CHROMagar tests - **negative**
- 7.6.4 Microscope - **negative** for grape-like clusters

7.7 *Bacillus thuringiensis*, 866
- 7.7.1 Gram stain positive
- 7.7.2 Catalase positive
- 7.7.3 CHROMagar tests - **negative**
- 7.7.4 Microscope - **negative** for grape-like clusters

7.8 *Paenebacillus polymyxa*, 525, 407
- 7.8.1 Gram -stain positive
- 7.8.2 Catalase positive
- 7.8. CHROMagar tests - **negative**
- 7.8.4 Microscope - **negative** for grape-like clusters

7.9 *Bacillus mojavensis* , AMH 100, AMH 105, AMH 108 & AMH 118
- 7.8.1 Gram -stain positive
- 7 8.2 Catalase positive
- 7.8.3 CHROMagar tests - **negative**
- 7.8.4 Microscope - **negative** for grape-like clusters