CONFIDENTIAL

Biofilm Laboratory Process

A. Biofilm Analytical Process

1. The process begins with a nares culture (deep nasal culture) for MARCoNS; Multiple Antibiotic Resistant Coagulase Negative Staphylococcus.
2. The nares swab is planted on the appropriate agar media and incubated at 37°C for 24 to 48 hours depending on the growth characteristics of the organism.
3. After incubation, colonies that are characteristic of Coagulase Negative Staphylococcus (CNS) are tested for identification.
4. Once identified as CNS, a susceptibility tests is set up and interpreted 24 hours later.
5. If the susceptibility test shows 2 or more resistant or intermediate antibiotics then the CNS is characterized as MARCoNS.
6. MARCoNS reside in the deep nasal passage, is common in biotoxin illness, is a marker of low MSH and can produce biofilm which form a barrier to immune defenses and anti-infection therapy. MARCoNS release exotoxins which lead to increase inflammation and hemolysin which disrupt RBCs and endothelial cells. It may be colonized or cause infection.
7. The culture plate may now be forwarded to test MARCoNS for biofilm production.

B. Biofilm Testing Procedure

Bioflim testing is performed on CNS characterized as MARCoNS.
The materials required are the following: MARCoNS culture plate, TSB broth, 12x75mm polystyrene tubes (PS), McFarland Standard (MF) of 0.5 to 1.0 for organisms and controls, Crystal Violet stain, PBS buffer, Acetic Acid, Deionized water and worksheet listing each patient ID number.

1. Set up and label two 12x75mm PS tubes for each patient sample.
2. Set up and label two PS tubes for the positive control (ATCC 35984 Staph epidermidis) and 2 tubes for the negative control (ATCC 12228 Staph epidermidis).
3. Prepare a MF of 0.5 to 1.0 for each patient and controls in 2mls PBS buffer.
4. Pipette 2mls of TSB broth into each patient and control tube.
5. Pipette 50µl of MF prepared for each patient and control into the appropriately labeled 12x75 tubes containing the TSB broth.
6. Cap and invert each tube twice and then place the test tubes into a rack and incubate at 37°C.
7. Incubate for 48 to 72 hours. Remove rack from incubator and invert each tube 2 times daily.
8. After 72 hours, remove the test tube rack from the incubator and invert each tube 2 times.
9. Decant each tube and observe for a film coating the tubes. Start with the positive control (ATCC 35984 SE strong biofilm producer) and then observe the negative control (ATCC 12228 SE non-biofilm producer). Proceed with decanting all tubes.
10. Rinse each tube 2 times with 4mls of PBS buffer.
11. Add 2mls of crystal violet stain (0.08%) into each tube and let stand for 10 minutes.
12. Decant each tube.

13. Rinse each tube 3 times with 4mls of water.
14. Observe each tube. Any biofilm present in the control or patient tube will retain the stain and color the inside of the tube.
15. Add 2mls of 30% Acetic Acid to each tube.
16. Cap each tube and mix by inversion two times.
17. Read color produced on the Densichek Photometer.
18. Record the reading on the Worksheet prepared next to each patient ID number.
19. The Densichek can be blanked with 2mls of water or 30% Acetic Acid if necessary.
20. Take the Worksheet and apply the reading rules to arrive at the result for each patient and control as Negative, Weak 1+, Moderate 2+ and Strong 3+.
21. Average the results for each set of tubes.
22. Rules Defining Results – Photometer Readings

    Negative = or LT Negative Control
    Weak 1+ GT Negative Control to 1.4
    Moderate 2+ 1.5 – 3.0
    Strong 3+ GT 3.0

    LT is less than, GT is greater than
23. Enter, review and release results in the computer, follow result data entry procedure.

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