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Now! Test Positive Control Protocol

Examples of bacteria that can be used for positive control:

1. *Klebsiella pneumoniae*
2. *Pseudomonas aeruginosa*
3. *Escherichia coli*

Preparing Bacterial Standards

I. Culture a Gram-negative bacteria sample so it is 16-18 hours old (not more than that) when it is ready to use. Use 5% Sheep's blood agar as culture medium.

II. Suspend a loop of bacteria into sterile water to match a CFU standard. (For example, using a spectrophotometer A_{600} = 0.12 is equivalent to a 0.5 McFarland Standard, or 1.5 x10^8 CFU for *E.coli*.)

III. Perform a 10-fold serial dilution keeping the final volume at 1 mL. (For example, taking 100 µL of a 10^4 CFU sample into 900 µL of sterile water gives you a 1000 CFU sample). To test for specific low CFU counts, like 1 CFU for example, you would need to make a specific dilution at 1 CFU/500µL. To keep the final volume of the dilution at 1 mL, this would mean making a 2 CFU/1mL dilution. To follow this example, you could take 200 µL of a 10 CFU/mL sample and add 800 µL of sterile water to it to get 2 CFU/mL.

IV. Verify the CFU level by plating a full mL of the dilution and incubating for 24-48 hours at 37^{o}C. Count the colonies formed.

Positive Control Testing

1) Don proper PPE as a measure of good practice.
2) Pipette 0.5 mL of the above final dilution to the provided cuvette with the growth medium. Close the lid. Invert gently a couple times for mixing.
3) Place vials in the block incubator and allow 12 or more hours of incubation. The incubator should be set to 37°C.
4) After incubation, the cuvette needs to be allowed to cool down. One of two methods can be employed:

5) Cooling Sample

a. Room temperature cooling: remove the cuvette and place in the supplied holder and allow cooling for a minimum of **1 hour**, but not greater than 3 hours. Continue to the next step.

b. Refrigerator cooling: remove the cuvette and place in the supplied holder. Place in a refrigerator (approximate temperature of 4°C) for **15 minutes**. Remove from the refrigerator at 15 minutes and continue to the next step.

6) Before adding Reagent A, switch the power source of the fluorometer at the upper right corner to "ON".

7) Add 2 drops of Reagent A to the cuvette.

8) 'Gently' invert it about four times to help mix the Reagent A with the sample. While inverting hold the cuvette at its top half. Make sure there are no air bubbles. Immediately proceed to the next steps for testing.

9) Place the cuvette in the fluorometer, line up the pointy side of the cuvette with the black line in the reader, and place the black cap firmly on the fluorometer.

10) Press the "Measure" button on the fluorometer.

11) Press "Blank" (timer will start counting seconds).

12) Press "Measure" and wait 10 minutes to get the reading.

13) At 10 minutes, the fluorometer will automatically take a reading. (A value will be displayed in the box below the timer). The value displayed before 10 minutes is disregarded. Please Note: The timer on the fluorometer will continue to run, but the reading displayed is taken exactly at the 10-minute mark.

14) To begin testing a new sample, press "Return" twice to return to the main menu of the fluorometer.

INTERPRETATION OF RESULTS

- A numerical value between 200 – 300 likely indicates the presence of Gram-negative bacteria (but could also be due to insufficient cooling of the cuvette).
- A numerical value greater than 300 strongly indicates the presence of gram-negative bacteria.
- If a value of less than 300 is reported, conduct another positive control test.
- If the second test is also below 300, contact Healthmark.