Now! Test Negative Control Protocol

1) Don proper PPE and follow aseptic technique as a measure of good practice.

2) Draw up 0.5 mL of supplied pre-packaged sterile water (emptied in the provided ziplock bag or a sterile container, e.g. urine cup) using the supplied graduated pipette.

3) Add the water to the provided cuvette with the growth medium.

4) Tightly close the cuvette lid and invert gently a couple times for mixing.

5) Place vials in the block incubator and allow 12 or more hours of incubation. The incubator should be set to 37°C.

6) After incubation, the cuvette needs to be allowed to cool down. One of two methods can be employed for cooling down the sample:

**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.

7) Before adding Reagent A, switch the power source of the fluorometer at the upper right corner to “ON”.

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

9) ‘Gently’ invert the cuvette 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.

10) 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.

11) Press the “Measure” button on the fluorometer.

12) Press “Blank” (timer will start counting seconds).

13) Press “Measure” and wait 10 minutes to get the reading.
14) 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.

15) To begin testing a new sample, press “Return” twice to return to the main menu of the fluorometer.

INTERPRETATION OF RESULTS

- A numerical value should be less than “200.”
- A value greater than “200” may indicate:
  1. The cuvette was not allowed to cool long enough (Step 6).
  2. The sample was contaminated during testing with Gram-negative bacteria.
  3. Bubbles were present when the cuvette was placed in the fluorometer and caused auto-fluorescence.
  4. There is an issue with either the consumables of the NOW! Test or the fluorometer.
- If the result is greater than 200, run a second negative control.
- If a result greater than 200 is again reported, contact Healthmark.