The NOW! Test Validation Testing

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The NOW! Test is a test to check flexible endoscopes for gram negative bacteria, after reprocessing. It is a fluorometric diagnostic system that can be used to provide a fast diagnosis (~12 hours) of low levels of gram negative bacteria, claim for this test being <10 Colony Forming Units (CFU). Testing can be done in the endoscopy clinic or within the facility, thus not requiring sending the sample to a laboratory for testing.

The NOW! Test detects gram negative bacteria like *E. coli, Pseudomonas aeruginosa, Klebsiella, Salmonella, Helicobacter*, *Serratia*, *Legionella*, associated with patient infection after endoscopic procedures. It also detects the multidrug resistant CRE strains of these organisms. Gram negative bacteria act as indicators for bacterial contamination in endoscopes and reduce the risk of false positives associated with the gram positive bacteria occurring normally as skin flora like *Staphylococcus epidermidis, Streptococcus salivarius*.

BACKGROUND

Due to the complex physical design of flexible endoscopes, any slight deviation from the recommended reprocessing protocol can lead to the survival of microorganisms in the suction channels (*Alfa, M.J., et al. American Journal of Infection Control, 2006).* If microorganisms survive the steps of endoscope reprocessing, it could potentially lead to subsequent patient infection. Nosocomial outbreaks linked to endoscopes contaminated with gram negative bacteria have been frequently reported (*Muscarella L.F., Infect Control Hosp Epidemiol. May 2002)*.

Gram negative bacteria replicate more easily in the presence of moisture, and have been implicated in endoscope associated infections more frequently than gram positive bacteria (*Alfa M.J. et al. J Hosp Infect 1991).* Contamination and colonization of automated endoscope reprocessors (AERs) may also result in exposure of the endoscopes to pathogens.

There is a need to have a rapid test that detects microbial growth inside the lumens of reprocessed endoscopes. This led to the conception of the NOW! Test. The kit comprises a sensitive test specific to gram negative bacteria.

WORKING PRINCIPLE OF THE NOW! Test

The NOW! Test works by detecting an enzyme mechanism typical to the gram negative bacteria. The test utilizes a fluorogenic substrate which, when hydrolyzed by a specific enzyme present in gram negative bacteria, produces fluorescence that is then read by the fluorometer.

The reading correlates to the amount of the bacterial enzyme present, which in turn relates directly to the number of gram negative bacterial cells present.

INDEPENDENT THIRD PARTY LABORATORY TESTING

The test was validated by two independent testing laboratories. The purpose of this study was to evaluate the sensitivity of the NOW! Test in the detection of gram negative bacteria. Laboratory 1 tested the kit with the CRE strains of three different gram negative bacteria (*Escherichia coli ATCC BAA-2469, Pseudomonas aeruginosa ATCC BAA 2110,* and *Klebsiella pneumoniae ATCC BAA-1705)* and Laboratory 2 tested the kit with three non-CRE strains of bacteria (*Escherichia coli ATCC #8739, Pseudomonas aeruginosa ATCC #9027,* and *Klebsiella pneumoniae ATCC #4352)*. Side by side traditional culturing was done by these labs to verify the sensitivity.

Both the laboratories performed the same protocol for testing. The protocol was as follows:

Bacteria to be tested:

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

Each organism was diluted and analyzed with both the fluorometer and by culturing on nutrient agar.

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 CFU level by plating a full mL of the dilution level, and incubating for 24-48 hours at 37°C. Count the colonies formed.

Testing Protocol

- Prepare a stock solution of sterile growth medium at a concentration of 150 mg/mL.
- Dispense 49.5 µL of the growth medium into as many cuvettes as needed, aseptically.

- Add 500 µL of a water sample (or bacterial standard) to a growth medium containing cuvette.
- Mix well, place into the block incubator set at 37^{o}C, and incubate 8-12 hours.
- Remove the cuvette from the incubator and let the cuvettes cool down.
- Turn on the fluorometer
- Add 2 drops of Reagent A to the cuvette and gently invert it a few times to help mix the reagent with the sample. Make sure there are no air bubbles in the cuvettes, as these will interfere with the reading of the fluorescence and may produce inaccurate test results.
- Place the cuvette in the fluorometer so the hinge is facing the right, and the pointy side of the cuvette is lined up with the black line in the reader.
- Press "Measure" and then "360"
- Press "Blank", and wait for it to finish measuring.
- Press "Measure". The fluorometer will take an initial reading after a few seconds, and then the final reading after 10 minutes. Record the final reading. The initial reading doesn't need to be recorded.

<u>Direction for conducting the negative control</u>

- Prepare cuvettes with sterile growth medium as mentioned above.
- Add 500 µL of sterile water to the cuvette.
- Close the lid, mix gently and place into the block incubator set at 37^{o}C, and incubate overnight.
- Remove the cuvette from the incubator and let it cool down.
- Turn on the fluorometer, and place the cuvette in the fluorometer so the hinge is facing the right, and the pointy side of the cuvette is lined up with the black line in the reader
- Press "Measure" and then "360"
- Press "Blank", and wait for it to finish measuring.
- Press "Measure". The fluorometer will take an initial reading after a few seconds, and then the final reading after 10 minutes.

RESULTS

For both the labs, the negative controls readings were below 300, most of them being under 200. The bacterial sample dilutions of 10 CFU or higher had readings above 300.

Following are the Summary Tables of each organism tested from the laboratories:

RESULTS FROM LABORATORY 1:
The first column indicates the targeted number of CFU in the dilution series. The second column indicates the actual number of CFU in the side by side traditional culturing of the same dilution. The third column indicates the final reading on the fluorometer.

Data Table for *E.coli* BAA 2469

Theoretical CFU	Actual Average CFU	Fluorometer Reading
10	9	470
10	10	377
5	8	252
5	6	290
1	2	252
1	2	270
5	6	246
5	9	247
~2.5	4	213
~2.5	2	189
<1	1	222
<1	1	249
Negative Control '0'	0	167
Negative Control '0'	0	137

Data Table for *Pseudomonas aeruginosa* BAA 2110

Theoretical CFU	Actual Average CFU	Fluorometer Reading
10	5	470
10	3	465
5	2	296
5	2	244
1	1	471

1	1	231
5	6	295
5	4	222
~2.5	3	227
~2.5	4	192
<1	1	203
<1	1	202
Negative Control ‘0’	0	193
Negative Control ‘0’	0	126

Data Table for *Klebsiella pneumoniae* BAA 1705

Theoretical CFU	Actual Average CFU	Fluorometer Reading
10	17	609
10	16	1644
5	13	320
5	11	248
1	2	164
1	3	861
5	10	1062
5	10	1387
~2.5	5	1124
~2.5	4	336
<1	1	188
<1	1	232
Negative Control ‘0’	0	110
Negative Control ‘0’	0	124

RESULTS FROM LABORATORY 2:
The first column indicates the actual dilution in the serial dilutions. The second column indicates the number of CFU in the side by side traditional culturing of the same dilution. The third column indicates the final reading on the fluorometer.

Data Table for *E. coli* ATCC #8739

Dilutions	Average Counts (CFU)	Fluorometer Readings
10:10	~29	2,490
9:10	~22	1,991
8:10	~19	2,211
7:10	~15	2,220
6:10	~14	2346
5:10	~10	1,584
4:10	~8	1,987
3:10	~8	1,813
2:10	~5	1,682
1:10	~1	1,511
Neg. Control	0	122

Data Table for *Pseudomonas aeruginosa* ATCC #2097

Dilutions	Average Counts (CFU)	Fluorometer Readings
10:10	~9	299
9:10	~7	267
8:10	~7	181
7:10	~6	218
6:10	~4	247
5:10	~5	81
4:10	~3	91
3:10	~1	105

2:10	~2	249
1:10	~1	49
Neg. Control	0	8

Data Table for *Klebsiella pneumoniae* ATCC # 4352

Dilutions	Average Counts (CFU)	Fluorometer Readings
10:10	~26	2,138
9:10	~24	2,359
8:10	~20	1,817
7:10	~22	1,917
6:10	~18	1,826
5:10	~14	1,636
4:10	~10	1,271
3:10	~10	807
2:10	~6	559
1:10	~3	332
Neg. Control	0	22

ADDITIONAL NEGATIVE CONTROLS

Negative Control	Fluorometer Reading
1	118
2	141
3	38
4	127
5	50
6	190

INTERPRETATION OF RESULTS

Based on the above independent laboratories' results and Healthmark's internal testing, a numerical value greater than 300 strongly indicates the presence of more than 10 CFU of gram negative bacteria. If the readings are over 300, further steps including reprocessing and investigation of reprocessing procedures (perhaps involving Risk Management, Infection Control, etc.) should be undertaken. One of these steps may be culturing of the endoscope for bacterial contamination and species identification.

A numerical value between 200 and 300 likely indicates the presence of Gram negative bacteria (but could be due to insufficient cooling of the cuvette or air bubbles in the cuvette). Reprocess the endoscope and retest, ensuring that sufficient time for cooling has occurred, according to the IFU, and that the mixing of Reagent A in the cuvettes is done gently to avoid air microbubbles in the solution.

Other contaminants (such as loose debris) in the recaptured water can cause auto fluorescence. This also necessitates a reprocessing of the scope as such debris should not be present in a clean endoscope.

DISCUSSION

The importance of endoscope sampling cannot be undermined when keeping patient safety in mind. The need for endoscope sampling is especially pronounced if the facility has recently adopted a new cleaning or disinfection protocol, if there is a recent nosocomial outbreak that needs to be investigated or if a perfect quality assurance program is not in place in the facility. The innovative NOW! Test caters perfectly to these concerns and comes at a time when the awareness of these issues is on a steep rise.

REFERENCES

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