Increasing Efficiency of Fecal Coliform Testing through EPA-Approved Alternate Method Colilert*-18

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Introduction

The 21 SM 9221 E multiple-tube fermentation method for fecal coliform analysis requires a large time and reagent investment for the performing laboratory. In late 2010, the EPA approved an alternative procedure for the determination of fecal coliforms designated as Colilert*-18. However, as of late 2016, only two VELAP-certified laboratories in the Commonwealth of Virginia have been certified in this method.

Method Descriptions

21 SM 9221 E requires the inoculation of sample with a series of 3 dilutions with 5 A-1 media broth-filled tubes per dilution (15 total tubes). These tubes are placed in an incubator for 3 hours at 35.0±0.5°C for 3 hours, then transferred to another incubator at 44.5±0.2°C for 15 hours. The tubes are then visually analyzed for change in clarity to determine the end result in MPN/100mL. Inoculating the 15 tubes per sample is time-consuming to the laboratory personnel. Following the two-step incubation process is also cumbersome, compared to the one-step process. In addition, a method blank per batch is required for this method, as well as a duplicate sample to be run per 10% of total samples analyzed.[1]

IDEXX Colilert*-18 method requires the Colilert*-18 reagent snap pack to be introduced to a 100mL sample, allow time to dissolve the reagent, then the sample is sealed in a Quantitray. The sample is then incubated at 44.5±°C for 18 hours.[2] The Colilert*-18 method provides a 1-step incubation process that is simpler for lab personnel. The setup procedure also requires less time and effort for lab personnel to accomplish. IDEXX comparator provides less human error in determining positive/negative results for this test.

Cost Comparison

To compare costs of the two tests, we must consider the cost of materials, personnel hours, and additional quality control materials and hours.

21 SM 9221 E

Per test, the media tube (5 tubes of A-1 Double Strength Media broth and 10 tubes of A-1 Single Strength media broth) cost for 21 SM 9221 E is $38.55 and the costs of materials (pipet tips, sample bottles) is **$**1.27, bringing the total to **$15.52**.[3,4]

21 SM 9221 E requires greater quality control per batch of samples analyzed. VELAP accreditation requires each lot of purchased prepared medium to have a verified appropriate response by testing with known positive and negative control cultures. This method requires, per batch, a method blank as well as a duplicate sample per 10% total samples required.[1] The cost of quality control per test is $**19.06**. This estimate takes into account the cost of performing quality control on the amount of media tubes required to perform 200 tests (1000 double strength and 2000 single strength).

The time required to perform the setup of one sample for analysis varies from one analyst to another, but has been estimated to be **17 minutes** for 21 SM 9221 E. That includes the transfer time from the first incubator to the second and the additional quality controls required by 21 SM 9221 E. Estimated labor cost, based on $19/hr, is $**5.38** for 21 SM 9221 E.[4]

Colilert*-18

The Colilert*-18 costs of materials for a single test 1 Quantitray ($1.73 ea), 1 sample bottle ($0.60 ea) and 1 reagent snap pack ($5.41 ea) are **$7.54.**[3]

VELAP accreditation requires each lot of purchased prepared medium to have a verified appropriate response by testing with known positive and negative control cultures. Colilert*-18, however, does not require either of the aforementioned quality controls that 21 SM 9221 E requires (duplicate sample and method blank), although it does *recommend* a duplicate sample.[2] The cost of quality control for one test, using this method, is **$0.33**. This estimate takes into account the cost of performing quality control on the amount of Colilert*-18 snap packs to perform 200 tests. The cost would be reduced, per test, with larger orders.

The time required to perform the setup of one sample for analysis has been estimated to be **3 minutes** for Colilert*-18. Again, this time varies between analysts. The estimated labor cost, based on $19/hr, is **$0.95**.

Cost Comparison Chart

Conclusion	21 SM 9221 E	Colilert*-18	Difference
Time spent per test (est. avg.)	17 min	3 min	14 min
Labor costs per test (based on est. avg. at $19/hr)	$5.38	$0.95	$4.43
Cost of materials per test	$15.52	$7.54	$7.98
Cost of Quality Control per test	$19.06	$0.33	$18.73
Total	$39.96	$8.82	$31.14

EPA Accreditation Steps

1. Initial demonstration of laboratory capability
2. Proficiency Testing
3. QC check: Positive and Negative
4. Testing actual wastewater samples: Side-by side testing of Colilert*-18 and approved 40 CFR Part 136 method on seven representative effluents, with QC check of one duplicate per day.
5. Standard Operating Procedure

Citations and Notes

[1]Method 9221 E, Standard methods for the examination of water and waste water, 22nd edn. American Public Health Association, Washington, DC, 2012.

[2]Colilert*-18 Procedure. ©2017 IDEXX Laboratories, Inc. https://www.idexx.com/resource-library/water/Colilert*-18-procedure-en.pdf

[3]These prices assume the laboratory has available equipment such as micropipettes, Quantitray sealer, various incubators, etc. These costs reflect only the disposable materials.

[4]This estimate is based on a batch containing a single sample. The addition of more samples would change the amount of time and cost per test, based on the number of samples, because of quality control requirements.