

NC DENR/DWQ LABORATORY CERTIFICATION

Testing of Consumable Materials for Fecal Coliform MF Method

Standard Methods requires that before a new lot of consumable materials are used for the Fecal Coliform MF method, those materials be tested to ensure they are reliable. North Carolina policy requires the testing of the following consumable materials before they can be used for sample analyses: membrane filters and/or pads (often packaged together) and media. **Test only one consumable at a time.**

REQUIREMENT: It is required that when a new lot of culture medium, pads, or membrane filters is to be used, a comparison of the current lot in use (reference lot) against the new lot (test lot), be made. As a minimum, make single analyses on five positive samples. Ref: Standard Methods, 18th Edition - Method 9020 B.3d. (page 9-7).

The following is provided as guidance in performing the required testing. Let's say you got a new batch of membrane filters in. We will call the currently used filters lot #1 and the new filters lot #2.

1. Select a culture positive sample.

What you want is something that will yield 20-60 colonies when a reasonable sample volume is filtered. This may be a stream sample or a sample taken somewhere within the waste treatment plant. If the concentration is high enough that greater than 60 colonies are obtained when 1 mL is filtered then the solution is too strong and must be diluted. Any time a sample is diluted be sure it is done with the BUFFERED dilution water used for rinsing the funnels.

2. Test the culture positive to determine the appropriate volume to use.

When collecting the culture positive sample do not think about it as a sample. You do not have to be concerned with a sterile sample bottle or 6 hour hold time. Collect enough sample so that you have plenty to work with, probably more than your normal fecal bottle holds. Set a series of dilutions using the currently used materials, in this example filter lot #1. Do not use the materials you want to prove are OK at this point. All you are trying to do is to determine the volume of sample that will yield 20-60 colonies. Put the rest of the sample in the refrigerator. For example:

Volume used	Colonies obtained on lot #1 filters
50 ml	TNTC
25 ml	138
10 ml	50
5 ml	22
1 ml	4

Based on this preliminary testing it appears that a 10 ml volume would probably be appropriate to use and will yield the desired 20-60 colonies. Remember when you do the actual consumable test, the culture positive sample will be 24 hours old and the results you obtain may be lower than the initial results yielded, but not so significantly lower as to change your dilution choice. It is better to have your initial results on the high side of the 20-60 range for this reason. In this example the 5 ml volume would probably be too low and would likely yield less than 20 colonies the next day.

3. Perform the consumable test

Once you determine the appropriate volume, in this case 10 mls, take the remaining culture positive sample from the refrigerator, bring to room temperature and set five 10 ml plates with the currently used filters, lot #1, and five 10 ml plates with the new filters, lot #2.

4. Determine acceptability of new material

For example:

Lot #1-current filters	Colonies obtained on lot #1 filters
10 ml	48
10ml	45
10 ml	50
10 ml	44
10 ml	<u>43</u>
Average:	46

Lot #2-new filters	Colonies obtained on lot #2 filters
10 ml	40
10ml	45
10 ml	38
10 ml	46
10 ml	<u>37</u>
Average:	41

When determining the acceptability of the new material, compare the average of the five replicates for lot #1 to the average of the five replicates for lot #2; that is 46 vs. 41 colonies. The recommended acceptance criteria would be your current acceptance criteria used for your Fecal Coliform duplicates. If the test and reference materials check within what you have determined is acceptable for duplicates of samples, the test material would be considered acceptable to use. This may be a calculated acceptance criteria based on 3 times the standard deviation of the mean or a set value like 20% RPD. No matter how you determine your duplicate acceptance criteria make sure you **use colony counts not final calculated values** in doing this. Other factors to consider when determining if a new material is suitable include:

Are the colonies obtained typical, that is normal looking blue colonies?

Are the colonies evenly distributed across the membrane surface?

Are there an unusual number of non typical colonies present?

Is there a pattern to the colony recoveries? For example are all the plates for the test materials significantly lower in counts than the reference lot?

It is recommended that new consumables be tested as soon as possible after receipt to avoid problems if the materials are not acceptable. Once you determine that the new material is acceptable to use; you may begin to do so. Document the date the new lot # is put into use.