Testing of Consumable Materials for Fecal Coliform MF Method Policy

(NC WW/GW LCB 08/06/2021)

Before a new lot of consumable materials are used for the Fecal Coliform MF method, those materials must be tested and compared to those currently in use to ensure they are reliable. Consumable materials included in this requirement are: membrane filters and/or pads (often packaged together) and media. **It is recommended that only one consumable be tested at a time.**

At a minimum, make single analyses on five positive samples that will yield 20-60 colonies for both the current lot and the new lot.

There are two options for determining acceptance of results:

Option 1:

> Follow the acceptance criteria described in Standard Methods 9020 B 5. *f* 2) a) and b).

Option 2:

> Compare the average colony count of each five-sample set and evaluate against your routine sample duplicate acceptance criterion.

<u>The following is provided as guidance in performing the required testing followed by evaluation according to the duplicate acceptance criterion option.</u>

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. 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 (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	43
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	37
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 comparison of results must adhere to 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 criterion 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 criterion 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.