



Counting Rules for Membrane Filtration Methods for Coliform Bacteria

NOTE: The following examples in this document pertain only to fecal coliforms, although the rules are applicable to total coliforms as well.

1.0 General Information and Equation

Select the membrane filter with the number of colonies in the ideal counting range and report as count per 100 mL according to the general formula:

$$\text{CFU}/100 \text{ mL} = (\# \text{ of colonies counted} \div \text{sample volume filtered in mL}) \times 100$$

(CFU = colony forming units).

2.0 Colony Counts Within the Ideal Counting Range

The ideal range of colonies that are countable on a membrane filter depends on the method and/or regulatory guidelines. An ideal range of 20-60 colonies is used for fecal coliform testing, and an ideal range of 20-80 colonies is used for total coliform methods.

Example 1: Sample volumes of 15, 5, and 2 mL produced colony counts of 110, 40, and 10 respectively. Which filter(s) should be chosen to calculate results? The 5 mL sample dilution produced a colony count of 40, which falls in the 20 - 60 ideal range. Since results are reported on a 100 mL sample scale, (see section 1.0 above), the reportable result would be calculated using the $(40 \div 5) \times 100 = 800 \text{ CFU}/100 \text{ mL}$.

3.0 More Than One Count in the Ideal Range

If more than one filter has ideal colony counts, carry results to reporting units, and then average the results to arrive at final reporting value.

Example 2: Sample volumes of 75, 50, 25, 10, and 1 mL yield colony counts of 100, 59, 26, 12, and 2, respectively. The 50 mL and 25 mL sample dilutions yield colony counts in the required range. Calculate each to a final result: $(59 \div 50) \times 100 = 118$; $(26 \div 25) \times 100 = 104$. Then average the results: $(118 + 104) \div 2 = 111 \text{ CFU}/100 \text{ mL}$.

Example 3. The same rule applies if two replicates are analyzed that meet the ideal range. If replicate 50 mL dilutions result in colony counts of 24 and 36 colonies, the results would be: $(24 \div 50) \times 100 = 48$ and $(36 \div 50) \times 100 = 72$. Take the average of the two results to arrive at final reportable result: $(72 + 48) \div 2 = 60 \text{ CFU}/100 \text{ mL}$.

4.0 All Counts Below the Lower Ideal Range

When all counts are below the lower ideal range, select the most nearly acceptable count and report as an estimated count.

Example 4. Sample volumes of 75, 50, and 25 mL produce colony counts of 15, 9, and 6. Select the most nearly acceptable count and report as an estimated count. Thus, the reportable result is $(15 \div 75) \times 100 = 20 \text{ CFU}/100 \text{ mL}$.

Example 5. Sample volumes of 100, 10 and 1 mL produce colony counts of 17, 1, and 0, respectively. Since a 100 mL volume was filtered report that value as 17 CFU/100 mL. Note: It is not an estimate due to the 100 mL sample volume.

5.0 All Counts Above the Upper Ideal Range

When all MF counts are above the upper ideal range but < 200 total colonies, calculate results using the smallest volume filtered and report as an estimated value.

Example 6. Sample volumes of 10, 5, and 2 mL produce colony counts of TNTC (Too Numerous to Count), 196, and 90. Since all are above the ideal range, calculate estimated result based on smallest volume filtered: $(90 \div 2) \times 100 = 4,500$ CFU/100 mL.

Example 7. Sample volumes of 10, 1 and 0.1 mL produce colony counts of TNTC, TNTC, and TNTC. Use the upper ideal range (60 for fecal coliforms) with the smallest volume (0.1 mL) and calculate the result: $(60 \div 0.1) \times 100 = >60,000$ CFU/100 mL.

6.0 Counts Above and Below the Ideal Range

When MF counts are above and below the reporting range, select the most nearly acceptable count to the ideal range.

Example 8. Sample volumes of 90, 10 and 1 produce colony counts of 62, 12, and 1. Since the 62 colony count is the most nearly acceptable value, use it to calculate the result: $(62 \div 90) \times 100 = 69$ CFU/100 mL. This would be reported as an estimated count of 69 CFU/100 mL.

Example 9. Sample volumes of 90, 10, and 1 mL produce colony counts of 180, 18, and 2. Select the count closest to the ideal colony count range. This would be 18 and calculate the estimated result:
 $(18 \div 10) \times 100 = 180$ CFU/100 mL.

7.0 Miscellaneous Examples (Including 100 mL Sample Results)

The ideal range for colony counts on a filter, e.g., 20 - 60 for fecal coliforms under the CWA, takes precedence over any other counting rule.

Example 10. Sample volumes of 100, 10 and 1 produce colony counts of 165, 39, and 18. Since the colony count for the 10 mL dilution meets the ideal range of 20 – 60, it must be used to calculate the result: $(39 \div 10) \times 100 = 390$ CFU/100 mL. Even though a 100 mL portion was filtered, it did not meet the ideal range and cannot be used as the reportable result, because a filter meeting that range is available.

Example 11. Sample volumes of 100, 10 and 1 produce colony counts of 100, 19, and 2. Since the colony count for the 10 mL dilution is the most nearly acceptable to the range of 20 – 60, it must be used to calculate the result: $(19 \div 10) \times 100 = 190$ CFU/100 mL. This would be an estimated result. Even though a 100 mL portion was filtered, the colony count for the 10 mL sample dilution is closer to the ideal range and thus, must be used as the reportable result.

7.1 Membranes with no colonies present. These results will be reported as less than values.

Example 12. Sample volumes of 10, 5, and 1 mL produce colony counts of 0, 0, and 0. Insert 1 colony into the general equation using the largest volume and calculate result: $(1 \div 10) \times 100 = 10$. This would be reported as a less than value, i.e., < 10 CFU/100 mL.

7.2 Colony counts based on a sample volume of 100 mL filtered. This result will be an actual value, not an estimated value.

Example 13. A sample volume of 100 mL is filtered and produces 6 colonies. The result would be 6 CFU/100mL.

Questions concerning these rules can be addressed by contacting the Office of Environmental Laboratory Certification at 803-896-0970.