Disclaimer: The information in this guidance document does not supplant the provisions of the USEPA approved methods, Standard Methods for the Examination of Water and Wastewater, or 40 CFR, Part 141 or Part 136. This document is a tool to help laboratories understand and comply with the calculation requirements used during membrane filtration procedures. If there is any disagreement between the contents of this document and any of the above regulations or methods, the methods shall prevail. The examples given in this document are for illustrative purposes only, meant to aide individuals in visualizing applications of the regulatory requirements. These examples do not represent all regulatory requirements.

Question: For membrane filtration methods how do I determine the density?

Calculate as described in the following sections unless the method specifies otherwise. Volume used in calculation refers to volume of original sample. Compute the count using membrane filters (plates) within the following acceptable ranges:

Total Coliforms	20 to 80
Fecal Coliforms	20 to 60
E. coli	20 to 80 (20 to 60 for method SM 9222D/9222G)
Fecal streptococci	20 to 60
Enterococci	20 to 60

Use equation:

$$\text{Colony Forming Units (“CFU”)/100-mL} = \frac{\text{CFU counted x 100}}{\text{mL sample filtered}}$$

CASE 1: ONE OR MORE PLATES CONTAIN COUNTS WITHIN ACCEPTABLE RANGE

If one or more filters fall within the acceptable range use all filters within the range to calculate the total count and report as number per 100-mL.

EXAMPLE 1: One plate within acceptable range

Filter 1 volume = 100 mL	CFU = TNTC
Filter 2 volume = 10 mL	CFU = 40
Filter 3 volume = 1.0 mL	CFU = 9
Filter 4 volume = 0.1 mL	CFU = 0

$$\frac{(40 \times 100)}{10} = \textbf{400 coliforms / 100 mL}$$

EXAMPLE 2: Duplicate plates within acceptable range

If there are acceptable counts on replicate plates, carry counts independently to final reporting units, then calculate the arithmetic mean of these counts to obtain final reported value.

Filter 1 volume = 1 mL	CFU = 26
Filter 2 volume = 1 mL	CFU = 36

$$\frac{(26 \times 100)}{1} = 2600 \text{ coliforms / 100 mL, and}$$

$$\frac{(36 \times 100)}{1} = 3600 \text{ coliforms / 100 mL, then}$$

$$\frac{(2600 + 3600)}{2} = \textbf{3100 coliforms / 100 mL}$$

EXAMPLE 3: More than one plate within acceptable range

If more than one dilution, independently carry counts to final reporting units, then average for final reported value:

        Filter 1 volume = 0.1 mL   CFU = 75
        Filter 2 volume = 0.03 mL   CFU = 30

$$\frac{(75 \times 100)}{0.1} = 75,000 \text{ coliforms / 100 mL, and}$$

$$\frac{(30 \times 100)}{0.03} = 100,000 \text{ coliforms / 100 mL, then}$$

$$\frac{(75,000 + 100,000)}{2} = \textbf{87,500 coliforms / 100 mL}$$

CASE 2: NO COUNTS ON ALL PLATES

If all filters result in no coliforms, then select the largest volume and calculate as less than the total number if the largest volume had 1 coliform:

EXAMPLE 4: All plates result in no counts

        Filter 1 volume = 100 mL   CFU = Lab accident, no data available
        Filter 2 volume = 25 mL   CFU = 0
        Filter 3 volume = 10 mL   CFU = 0

$$\frac{1 \times 100}{25} = \textbf{< 4 coliforms / 100 mL}$$

CASE 3: TOO NUMEROUS TO COUNT ON ALL PLATES

If all filters result in Too Numerous to Count (TNTC), then select the smallest volume and calculate as greater than the total number if the smallest volume had the upper limit of the acceptable range.

EXAMPLE 5: All plates Too Numerous to Count

        Filter 1 volume = 1.0 mL   CFU = TNTC
        Filter 2 volume = 0.3 mL   CFU = TNTC
        Filter 3 volume = 0.01mL   CFU = TNTC

$$\frac{(60 \times 100)}{0.01} = \textbf{> 600,000 coliforms / 100 mL}$$

CASE 4: NO PLATES WITHIN THE ACCEPTABLE RANGE

If there are no filters with a count in the acceptable range, sum the coliforms on all filters, divide by the total volume filtered and report as number per 100 mL.

EXAMPLE 6: All results below acceptable range

    Filter 1 volume = 50 mL   CFU = 15
    Filter 2 volume = 25 m   CFU = 6
    Filter 3 volume = 10 mL   CFU = 0

$$\frac{[(15 + 6 + 0) \times 100]}{(50 + 25 + 10)} = \textbf{25 coliforms / 100 mL estimated}$$

EXAMPLE 7: All results above acceptable range, without plates containing >200 colonies

    Filter 1 volume = 50 mL   CFU = 105
    Filter 2 volume = 25 mL   CFU = 92
    Filter 3 volume = 10 mL   CFU = 85

$$\frac{[(105 + 92 + 85) \times 100]}{(50 + 25 + 10)} = \textbf{332 coliforms / 100 mL estimated}$$

EXAMPLE 8: Results above and below acceptable range

    Filter 1 volume = 100 mL   CFU = 82
    Filter 2 volume = 10 mL   CFU = 18
    Filter 3 volume = 1.0 mL   CFU = 0

$$\frac{[(82 + 18 + 0) \times 100]}{(100 + 10 + 1)} = \textbf{90 coliforms / 100 mL estimated}$$

EXAMPLE 9: Results above countable range, with plates containing >200 colonies

    Filter 1 volume = 50 mL   CFU = TNTC
    Filter 2 volume = 25 mL   CFU = TNTC
    Filter 3 volume = 10 mL   CFU = 83

$$\frac{(83 \times 100)}{10} = \textbf{830 coliforms / 100 mL estimated}$$

Report as estimated based on non-ideal colony counts.

Some methods may handle some of the above scenarios differently than described in this guidance document. For example, for case 4 some methods have the analyst calculate the estimated value based on the most acceptable result. Whatever the guidelines chosen, the laboratory must use the same guidelines for all samples and may not "pick and choose" systems based on giving the laboratory results within a facility's permit limit or trigger level.

The qualifiers "<", ">" and "estimated" should be used in your analytical data and reports if the data is reported to another facility. The results are to be included in average calculations, such as monthly

averages for DMR data, without the qualifier. In the case of the "greater than" sign the average result should be flagged with a statement that indicates the number of "greater than" occurrences. Also, if the less than value is equal to or greater than the permit limit the laboratory should flag the data and indicate the number of occurrences.

References:

Microbial Laboratory Guidance Manual For The Final Long Term 2 Enhanced Surface Water Treatment Rule (EPA 815-R06-006), February 2006.

Microbial Methods for Monitoring the Environment Water and Wastes (EPA-600/8-78-017), December 1978.

Standard Methods for the Examination of Water and Wastewater, 20th Edition, 1998