

Synopsis of E. coli Counting Rules

One Acceptable Count

Assume that filtration of volumes of 50, 30, and 20 ml produced colony counts of 200, 110, and 40, respectively.

Count the colonies on all filters and select the membrane filter(s) with 20 – 80 coliform colonies.

After selecting the membrane filter within the range of 20 – 80 colonies, in this case the MF with a 40 colony count, apply the general calculation formula as follows:

$$\frac{20}{40} \times 100 = 200 \text{ Colonies per } 100 \text{ ml}$$

More Than One Acceptable Count

If there are acceptable counts within the range of 20 – 80 colonies for more than one dilution, independently carry counts to final reporting units, then average them for final reported value.

For example, assume that volumes of 50, 30, and 20 ml produced coliform colony counts of 75, 30, and 8, respectively. In this example, two volumes, 50 and 30, produce colonies in the acceptable counting range.

Independently carry each MF count to a count per 100 ml:

$$\frac{75}{50} \times 100 = 150/100 \text{ ml}$$

$$\frac{30}{30} \times 100 = 100/100 \text{ ml}$$

Then calculate the arithmetic mean of these counts to obtain the final reported value:

$$(150 + 100) \div 2 = 125$$

Report as: 120/100 ml (See 'Significant Figures' example #2)

If Counts From All Membranes are Zero, Calculate Using Count from Largest Filtration Volume Only

For example, sample volumes of 25, 15, and 10 ml produced colony counts of 0, 0, and 0, respectively, and no actual calculation is possible, even as an estimated report. Calculate the number of colonies per 100 ml that would have been reported if there had been one colony on the filter representing the largest filtration volume, thus:

$$\frac{1}{25} \times 100 = 4$$

Report as: < (Less Than) 4 colonies per 100 ml.

If All MF Counts are Below the Lower Limit, Select the Most Nearly Acceptable Count

For example, assume a count in which sample volumes of 40, 35 and 25 ml produced colony counts of 14, 11, and 7, respectively.

Here no colony count falls within recommended limits. Calculate on the basis of the most nearly acceptable plate count, 14, and report with a qualifying remark:

$$\frac{14}{40} \times 100 = 35$$

Report as: Estimated Count, 35 per 100 ml.

If All Membrane Counts are Above the Upper Limit, Calculate Count with Smallest Volume Filtered

For example, assume that the volumes 40, 35, and 25 ml produced colony counts of TNTC, 150, and 100 colonies. Since all colony counts are above the recommended limit, use the colony count from the smallest sample volume filtered and estimate the count as:

$$\frac{100}{25} \times 100 = 400$$

Report as: Estimated Count 400 per 100 ml.

If Colonies are Too Numerous To Count, Use Upper Limit Count with Smallest Filtration Volume

Assume that the volumes 50, 30, and 20 ml, all produced too many colonies to show separated colonies, and that the laboratory bench record showed TNTC (Too Numerous To Count).

Use 80 colonies (Upper Limit Count for E.coli) as the basis of calculation with the smallest filtration volume, thus:

$$\frac{80}{20} \times 100 = 400$$

Report as: > (Greater Than) 400 per 100 ml.

Reporting Results – Significant Figures

In plate count and MF methods, the number of significant digits which can be reported is dictated by the method itself as follows: within the acceptable counting range of the method itself, i.e., 20-60, 20-80, 20-100 or 30-300 the actual number of colonies observed is the best estimate of the true density. The number of significant figures is equal to the number of colonies.

TABLE II-C-2

Number of Significant
Figures (S.F.) Reported

Actual Colony Count	Pour Plate/ Spread Plate Method	Membrane Filtration Method
1-9	1 S.F.	1 S.F.
10-99	2 S.F.	2 S.F.
100-300	3 S.F.	-----

Since plate counts must be limited to the number of significant figures obtainable by the method, the non-zero number which is not significant should be treated by the standard scientific convention. See the following examples:

- 1- If the insignificant digit is less than 5, replace it with a zero, e.g., 124 becomes 120, 3530 becomes 3500.
- 2- If the insignificant digit is five, round the preceding significant digit to the nearest even number, e.g., with two S.F., 125 becomes 120, 135 becomes 140, 3450 becomes 3400, and 3550 becomes 3600.
- 3- If the insignificant digit is greater than 5, drop the digit and increase the preceding significant number by one, e.g., 127 becomes 130, 406 becomes 410, 3480 becomes 3500.

For a complete description of the E. coli reporting and counting rules, see pp. 69-70 and 75- 79, respectively, of EPA's publication Microbiological Methods for Monitoring the Environment – December 1978.