**[lab name]**

**Initial and Ongoing Demonstrations of Capability for Microbiology Using an Existing Sample**

 **[#]**

**In Compliance with V1M5 1.6.2 and 1.6.3**

**VERSION #1.0 Effective date: January 1, 2024**

**APPROVED BY**

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**New SOP**

**Revision History**

|  |  |
| --- | --- |
| Version number and effective date | Revisions made |
| V 1.0 January 1, 2024 | Conforms to TNI 2016 standards. |
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# Introduction and Scope

The Standard in module 5 requires that analysts undergo an Initial Demonstration of Capability before being allowed to work without direct supervision, and all authorized analysts provide Ongoing Demonstrations of Capability on an annual basis.

The Standard stipulates analysis of a single sample in quadruplicate (V1M5 1.6.2.2.(b)). The results are to have the mean and standard deviation computed, then compared to what is allowed by the method.

However, unlike chemistry, microbiological enumeration methods do not provide any information regarding precision or accuracy. The difficulty is in the ability to prepare solutions of known bacterial density. Any procedure the laboratory could use either poorly estimates the density, or it has no ability to know in advance of testing by the analyst. Many laboratories resort to purchasing QC solutions from vendors as they have established criterion, but it is expensive, and the purchase must be of 4 identical solutions to meet the Standard.

For ongoing demonstrations, the laboratory can choose to use PT analyses, but if the laboratory has more than one authorized analyst, one can do the PT and all others must perform a different procedure.

Many laboratories have samples that consistently provide positive enumerations, which would seem to be used for both Demonstrations, but the precision is not known, and the actual density may depend on when the sample was collected.

This procedure provides a mechanism to create a precision criterion for an existing sample to use for all Demonstrations. The focus on precision as enumeration methods are at best fair estimates of actual density and accuracy, being not known in advance, is always based on the collected analyses of multiple analysts/labs. A procedure for determining accuracy is provided that is based on the outcome of the determination of a precision criterion.

# Procedure Overview

Prior to the use of the selected sample for any Demonstrations, the laboratory must determine the precision criterion by the process described in section 4. Once established, the criterion can be used for all Demonstrations for at least a year from the date the criterion is established. When a Demonstration is scheduled, the analyst will analyze at least 4 replicate aliquots. The Demonstration may include a known sterile sample or, if desired, a known Negative Control consisting of non-target organisms. If these additional samples are included, then the identity of all samples is to be blind to the analyst.

The results provided by the analyst are processed by the procedure in Section 5 and compared to the criterion. To assist with this, an Excel workbook is used with all formulas and displayed outcomes.

If the analyst is within the established criterion, then the Demonstration is successful.

# Establishing a Precision Criterion

This procedure is based on the determination of R as found in *Standard Methods*… section 9020B 9.(e). It is suitable for all enumeration methods including Multiple Tube, Enzyme Substrate, Membrane Filter, and HPC. Two options are provided, and the laboratory may select either one. The selection must be recorded in the record of the Demonstration for each analyst.

## Option 1 – Establish Criterion for a Single Demonstration

This procedure allows for the quick determination of the criterion but is not suitable for Demonstrations over time as it does not capture the variability of the sample over time. If that is desired, then follow Option 2.

Collection sufficient volume of sample in a single sterile container to cover both the determination of the precision criterion and testing the analyst. For methods using 100mL per sample, this will mean collecting at least 4 liters. If you collect in individual sample containers, the outcome is affected by the variability of the bacterial density, and you will see larger criterion than if a single container is used.

To compute the criterion, begin by analyzing at least 15 pairs (30 total samples) using the method and an authorized analyst. Do this in advance of the test analyst performing the method but so that the time difference is less than the specified holding time for the sample.

Have the analyst prepare 4 aliquots of the same sample by the analytical method.

When the incubation period is over for the paired samples, determine the quantitative value for each sample, then place each value in the Excel workbook in the Compute R spreadsheet. The workbook uses the following equations.

Equation Computation of log difference

$$Log difference\_{Pair x}=Log\_{MPN1}-Log\_{MPN2}$$

Where Log = Log to the base 10

Equation Computation of R

$$R=\frac{\sum\_{n}^{1}Log difference\_{n}}{n}$$

Where n = the number of sample pairs

The acceptance criterion is computed as 3.27 times R.

## Option 2 – Establish Criterion for Multiple Determinations with a 12-month Period

In advance of the testing period, collect sufficient samples for at least 5 pairs of analysis (10 total samples or 1 liter of sample). Analyze the samples using the selected method and record the results in the Excel workbook as described in Option 1. The computation will happen as described above.

At least one month to 3 months after the first set of pairs, collect an additional 5 pairs of samples and conduct the method again. Add the results to the workbook, which will change the computed value.

Repeat the above at least once more and add those values to the workbook. Additional pairs of samples may be analyzed and added to the workbook.

All the above must include at least 15 pairs and be completed within 6 months.

# Performing Evaluations in Demonstrations of Capability

## Evaluation Against the Precision Criterion

The four individual analyses reported by the analyst are placed in the workbook worksheet DoC Evaluation. The worksheet computes the difference between each analysis in the following fashion.

* First result/Second result. Third result/Fourth result.
* First result/Third result. Second result/Fourth result.
* First result/Fourth result. Second result/Third result.

The individual pairs and the combined pairs are compared to the computation of the criterion. All values meeting the criterion are marked as PASS with a green color coding. Failed results are marked as FAIL with a red color coding.

As required by the Standard, the mean and standard deviation are computed and displayed. Since there are no criterion for these, they are simply noted.

## Evaluation for Accuracy

The induvial results from the analyst are compared against the mean of the set used to compute R and + the standard deviation of the set times the appropriate Student’s t value. Results within those limits are marked as PASS with green color coding and those that do not are marked as FAIL with red color coding.

## Evaluation of Other Types of Samples

If sterile or Negative Control samples are analyzed, then success is when no positive results are reported. Any positive result is considered a failure of the Demonstration.

## Final Evaluation

The Demonstration is considered successfully completed if there are no precision or accuracy tests marked FAIL.

# References

* *Management and Technical Requirements for Laboratories Performing Environmental Analyses*, The NELAC Institute (TNI), Rev 2.1, September 1, 2016
* *Standard Methods for the Examination of Water and Wastewater*, 22nd edition, section 9020B.9.(e)

# Definitions and Acronyms

Words specific to this document or used outside of their dictionary definition are defined here. Acronyms can be defined in the text above on their first appearance.

## Definitions

## Acronyms

# Appendices