



TNI V1M4 2016 Standard Update Guidance on Detection and Quantitation

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This document was prepared to provide guidance on the detection and quantitation section (1.5.2) of Module 4 of the 2016 TNI Standard Volume 1, i.e., V1M4. This document is not intended to be an official interpretation of the Standard, nor is it to be used in place of the Standard. This document is only intended to help users of the Standard understand the changes and implement them in their laboratory. If there are questions regarding the use and implementation of the Standard, contact the appropriate accreditation body. Standard Interpretation Requests may be made through the TNI website.

This guidance document covers determination and verification of the LOQ (Limit of Quantitation) and Limit of Detection, hereafter called DL (Detection Limit). Note: Volume 1, Module 2 defines Limit of Detection as “The minimum result, which can be reliably discriminated from a blank with a predetermined confidence level. Also used is Detection Limit.” This is comparable, but less specific than EPA’s definition of the Method Detection Limit (MDL), “the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results.” TNI uses DL to ensure there is no confusion with the Limit of Detection published in the Department of Defense Quality Systems Manual for Environmental Laboratories (DOD QSM).

This guidance is written as a set of procedural recommendations that will allow the requirements of the Standard to be met in a relatively productive and efficient manner. One key assumption is that the laboratory will be following EPA’s revised procedure for determining an MDL according to the procedure in Appendix B of 40 CFR Part 136. It is not a requirement of the Standard to follow the EPA MDL

procedure. However, as pointed out in a NOTE to section 1.5.2.1.1, following the EPA MDL procedure is an acceptable way to meet the TNI requirements regarding detection limits and is likely the easiest way to be in compliance with the TNI requirements. While the EPA procedure is only required for wastewater analyses conducted under Part 136, the procedure can be applied to other matrices such as air, drinking water, or soils. As stated in Section 1.5.2.1.1 f) of the TNI standard, the TNI procedure applies to all quality system matrices, as defined in Module 2. Some laboratories believe that certain methods (e.g., Methods 300.1 and 351.2) have different requirements, but a careful reading of these methods indicate the methods use the word “should,” so the EPA and TNI requirements would override what is in the methods. Also note that section 1.5.1 (a) requires an initial DL and LOQ determination as part of the initial method validation.

Note: Language quoted from the standard is shown in grey text boxes.

1.0 Overview of Section 1.5.2

Section 1.5.2 includes subsections 1.5.2.1, Detection Limit and 1.5.2.2, Limit of Quantitation, making it appear that these are two separate requirements. However, the two requirements are meant to be used together, with one set of activities that achieve the requirements of both subsections. Note that there are two distinct processes: the determination of the DL and initial verification of the LOQ, and the periodic verification and annual recalculation of the DL and LOQ. The table below summarizes the steps in the procedure.

	Step	Description	Section	TNI Standard Section	Comments
Initial determination of the DL and verification of the LOQ	1.1	Select a quantitation limit (LOQ)	2.2	1.5.2.2	LOQ must be at or above the low calibration standard
	1.2	Analyze at least 7 blanks and 7 spikes	2.3	1.5.2.2.1(a)	Ensure at least 3 batches over 3 days and at least 2 spikes per instruments. Tabulate all blank data.
	1.3	Evaluate the results	2.4	1.5.2.2.1(c)	Spikes must meet qualitative ID criteria Results must be above 0 and meet recovery limits
	1.4	Calculate DL _s and DL _b . Determine DL	2.5	1.5.2.1.1(c)	According to EPA: DL _s = s*t; DL _b = X + (s*t) DL = greater of DL _s or DL _b
	1.5	Verify the LOQ	2.6	1.5.2.2.1(c)	LOQ ≥ spike level and > DL
Ongoing recalculation of the DL and verification of the LOQ	2.1	Analyze at least one spike per instrument every quarter in which samples are analyzed.	3.0	1.5.2.1.2 and 1.5.2.2.2	Spike at same level as initial DL study Note the EPA procedure requires 2 spikes per quarter
	2.2	Evaluate Data	3.1	1.5.2.1.1(d) and 1.5.2.1.2	Results must be above DL and meet recovery limits
	2.3	Tabulate blank data	3.2	1.5.2.1.2	Use all data from the last 13 months
	2.3	Recalculate DL	3.3	1.5.2.4	The EPA procedure requires the newly calculated DL be used if it is < 0.5 or > 2 x the initial DL; otherwise, the lab may or may not change the DL
	2.4	Verify the LOQ		1.5.2.4	LOQ must be > DL and meet recovery criteria

Terms in the Calculation of DL

DLs = Detection limit from spikes
DLb = Detection limit from blanks
s = standard deviation
t = Student t value
X = Mean blank concentration

2.0 Initial Determination of the DL and Verification of the LOQ

1.5.2.1 If a mandated test method or applicable regulation includes protocols for determining detection limits, they shall be followed. The laboratory shall document the procedure used for determining the DL. If the method or regulation does not contain specific directions for determination of the detection limit, the following requirements shall apply.

1.5.2.2 If a mandated test method or applicable regulation includes protocols for determining quantitation limits, they shall be followed. The procedure used for determining the LOQ shall be documented by the laboratory. The laboratory shall select an LOQ for each analyte, consistent with the needs of its clients, and greater than the DL.

Although the two sections above could be seen as two separate activities, in fact they are intertwined. The LOQ is established first, since the LOQ must be at or above the spiking level. If a DL has already been determined, then the LOQ must be set at a concentration that is greater than the DL and at or above the lowest calibration standard. An LOQ is required for each *quality system matrix of interest, technology, method, and analyte*. For example, as specified in 1.5.2.2. b) if a laboratory performs the analytical method 8270 and uses preparation methods 3510 (separatory funnel) and 3520 (CLLE) for aqueous samples, and preparation method 3540 (Soxhlet) for soils, then 3 separate LOQ verifications will be required. The LOQ for preparation method 3510 may well be determined to be the same as that for 3520, but separate initial determinations are required. However, there is no firm relationship of the MDL to the LOQ other than a statement in 1.5.2.2.1 c) that the LOQ must be greater than the DL.

2.1 Exceptions

1.5.2.1 DL determinations are not required for methods/analytes for which a detection limit is not applicable such as pH, color, odor, temperature, or dissolved oxygen. DL determinations based on low level spikes are not required for analytes for which no spiking solutions are available.

1.5.2.2 An LOQ is required for each quality system matrix of interest, technology, method, and analyte, except for any component or property for which spiking solutions are not available or a quantitation limit is not appropriate, such as pH, color, odor, temperature, dissolved oxygen, or turbidity.

Module 4 of the TNI Standard is for chemical testing and thus does not apply to asbestos, microbiology, radiochemistry or toxicity testing. The EPA MDL procedure states:

The MDL procedure also is *not* applicable to measurements such as, but not limited to, biochemical oxygen demand, color, pH, specific conductance, many titration methods, and any method where low-level spiked samples cannot be prepared. MDL determinations using

spiked samples may not be appropriate for all gravimetric methods (e.g., residue or total suspended solids), but an MDL based on method blanks can be determined in such instances.

The laboratory should note that certified reference materials are available for several analytes that are determined gravimetrically (e.g., TSS) and titrimetrically (e.g., Residual Chlorine). Thus, DLs and LOQs may need to be established for these analytes since non-detects may be expected in some samples. Please contact your Accreditation Body for information concerning requirements for these analytes.

2.2 Step 1: Selection of the LOQ

1.5.2.2 The laboratory shall select an LOQ for each analyte, consistent with the needs of its clients, and greater than the DL.

Since the DL has not yet been determined, the laboratory may select any LOQ consistent with the needs of its clients. The LOQ does not have to be as low as can possibly be analyzed by the method and instrument. For example, in a sufficiently clean environment, an ICPMS could have an LOQ in the low part per trillion range for iron. This would be of no value for environmental analysis, and most labs will select an LOQ for iron in the part per million range. However, as discussed later in this section, the LOQ must also be at or greater than the lowest calibration standard.

2.3 Step 2 - Initial Verification of the LOQ

1.5.2.2 a) Each selected LOQ shall be verified through analysis of initial verification samples. An initial verification sample consists of a spiked matrix blank at or below the selected LOQ.

In some instances, assuming that the performance of the method is adequate, it is recommended to spike at a concentration half that of the LOQ. The reason for this is that the LOQ verification samples may also be used to calculate the DL. Spiking at a concentration below the LOQ makes it more likely that a DL will be 2-3 times below the LOQ. If the laboratory is seeking the lowest possible LOQ or the LOQ is less than 2-3X the DL, spiking at half the LOQ concentration is not recommended.

1.5.2.2 b) All sample processing and analysis steps performed for routine sample analysis shall be included in the LOQ verification testing.

Essentially, the LOQ verification spikes must be treated in the same way and go through the same steps that are performed for sample processing and analysis.

1.5.2.2 c) The LOQ must be at or above the lowest corresponding calibration standard concentration with the exception of methods using a single point calibration.

As noted above, the LOQ must be at or above the lowest calibration standard. (If the LOQ verification is performed using spikes at half the LOQ, then the spiking level may be below the lowest calibration standard, but in that case it is recommended to include an additional calibration standard at least as low as the spiking level).

1.5.2.2 d) The laboratory shall establish acceptance criteria for accuracy for the LOQ verification spikes

These accuracy criteria may come from a method or a Quality Assurance Plan. If these documents do not include acceptance criteria then the laboratory determines its own criteria. The acceptance criteria should be reasonable; in other words, choosing acceptance criteria of 0-200% for everything may meet the letter of the Standard, but not the intent. Most methods will have performance criteria for Laboratory Control Samples (LCS), but since these samples are generally at a higher level, the acceptance limits may not be appropriate for LOQ spikes. A reasonable first approximation for the LOQ verification could be 10-20% wider than the LCS. For example, if the LCS recovery criterion is 70-130%, then 60 -140% or 50-150% is reasonable for the LOQ verification acceptance limits.

The laboratory may analyze the LOQ verification spikes first, and then develop the recovery acceptance criteria based on comparative methods or laboratory statistical process control (e.g. control charting) of the results obtained. LOQ verification data must be provided to clients upon request. If the acceptance limits are too wide, a client may decide that the laboratory performance is inadequate to meet their needs. Note there is no quantitative criterion for recovery at the calculated DL, nor is there one in the EPA procedure.

1.5.2.2.1 a) A minimum of seven (7) low level spikes at or below the LOQ concentration shall be processed through all steps of the method. Both preparation and analysis of these low-level spikes shall include at least three (3) batches on three (3) separate days.

The seven (minimum) low level spikes are processed through the entire method, and the preparation and analysis must both be spread over at least three separate days, although the preparation and analysis of an individual spiked blank may be performed on the same day.

- i. If there are multiple instruments that will be assigned the same LOQ, then these low-level spikes shall be distributed across all of the instruments.
- ii. A minimum of two (2) low level spikes prepared and analyzed on different days shall be tested on each instrument.

As an example, assume a laboratory has four instruments. The following set of analyses would meet the requirements:

Monday	Prepare extracts 1 and 2	Analyze extract 1 on instruments a and b
Tuesday	Prepare extracts 3 and 4	Analyze extract 2 on instruments a and b Analyze extract 3 on instruments c and d
Wednesday	Prepare extracts 5, 6 and 7	Analyze extract 4 on instruments c and d Analyze extracts 5, 6 and 7 on any instrument

1.5.2.2.1 b) Existing data may be used if compliant with the requirements for at least three (3) batches, generated within the last two (2) years and representative of current operations.

This is a very important point – samples that laboratories are currently analyzing in order to meet existing requirements such as the current TNI LOQ and DL verifications, Department of Defense LOD requirements, Drinking Water requirements, or SW-846 requirements, may well meet the requirements of the new LOQ standard. This is especially the case since there is a period of time available before the TNI Standard is implemented. If the low-level spikes analyzed for these or other programs are i) spiked with an analyte concentration at or below the desired LOQ, ii) give results above the DL that meet the qualitative identification criteria in the method, iii) are within the laboratory established recovery criteria, and iv) are analyzed across at least 3 separate batches and days, then they will be suitable as LOQ verification spikes. It is highly recommended to plan ahead and design your current low-level spike analyses such that they meet the requirements for the LOQ verification.

2.4 Step 3 - Evaluation of the Results of the LOQ Verification Samples

1.5.2.1.1 Initial determination of the DL

The laboratory DL procedure, unless following a mandated test method or procedure, at a minimum, shall incorporate language addressing the following requirements:

d) results from low level spikes used in the DL determination shall meet qualitative identification criteria in the method, and shall be above zero;

1.5.2.2.1 c) The LOQ is verified if the following criteria are met

i) All results are quantitative (above zero and meet the qualitative identification criteria of the method; e.g., recognizable spectra, signal to noise requirements, and presence of qualifier ions).

The qualitative identification criteria required differ from method to method, but should be those used to determine if an analyte is present. For example, a GC/MS method might require that the quantitation and two qualifier ions maximize within a 2-scan range and that the mass spectrum obtained be fully recognizable, while an ICP method may have very little in the way of qualitative identification criteria.

ii) The mean recovery of each analyte is within the laboratory established accuracy acceptance criteria

The results are evaluated against the laboratory established recovery criteria.

iii) The LOQ is greater than the established DL and at or above the spiking concentration.

If the DL has not been determined yet, this part iii does not apply immediately. If there is an established DL, then the comparison is made and the LOQ adjusted if necessary. The LOQ must be greater than the DL. Note that this adjustment DOES NOT require reanalyzing spiked samples at a higher concentration.

1.5.2.1.1 Initial determination of the DL

The laboratory DL procedure, unless following a mandated test method or procedure, at a minimum, shall incorporate language addressing the following requirements:

- a) e) the DL procedure shall include criteria for and evaluation of false positive rates in routine method blanks;

The TNI standard does not describe the criteria for evaluating method blanks, but the EPA procedure states:

Compute the MDL_b (the MDL based on method blanks) as follows:

- (A) If none of the method blanks give numerical results for an individual analyte, the MDL_b does not apply. A numerical result includes both positive and negative results, including results below the current MDL, but not results of "ND" (not detected) commonly observed when a peak is not present in chromatographic analysis.
- (B) If some (but not all) of the method blanks for an individual analyte give numerical results, set the MDL_b equal to the highest method blank result. If more than 100 method blanks are available, set MDL_b to the level that is no less than the 99th percentile of the method blank results. For "n" method blanks where $n \geq 100$, sort the method blanks in rank order. The $(n * 0.99)$ ranked method blank result (round to the nearest whole number) is the MDL_b . For example, to find MDL_b from a set of 164 method blanks where the highest ranked method blank results are ... 1.5, 1.7, 1.9, 5.0, and 10, then $164 \times 0.99 = 162.36$ which rounds to the 162nd method blank result. Therefore, MDL_b is 1.9 for $n = 164$ (10 is the 164th result, 5.0 is the 163rd result, and 1.9 is the 162nd result). Alternatively, you may use spreadsheet algorithms to calculate the 99th percentile to interpolate between the ranks more precisely.
- (C) If all of the method blanks for an individual analyte give numerical results, then calculate the MDL_b as:

$$MDL_b = \bar{X} + t_{(n-1, 1-\alpha=0.99)} S_b$$

Where:

MDL_b = the MDL based on method blanks

\bar{X} = mean of the method blank results (use zero in place of the mean if the mean is negative)

$t_{(n-1, 1-\alpha=0.99)}$ = the Student's t-value appropriate for the single-tailed 99th percentile t

statistic and a standard deviation estimate with n-1 degrees of freedom.

S_b = sample standard deviation of the replicate method blank sample analyses.

Note: If 100 or more method blanks are available, as an option, MDL_b may be set to the concentration that is greater than or equal to the 99th percentile of the method blank results.

2.5 Step 5 - Determination of the DL

1.5.2.1.1 c) the DL determination shall include data from low level spikes and routine method blanks prepared and analyzed over multiple days; at least one low level spike and routine method blank must be analyzed on each applicable instrument; a minimum of seven (7) replicates is required for both low level spikes and routine method blanks;

Determination of the DL requires results for a set of method blanks (DL_b) as well as the spiked samples (DL_s) using the spikes from the LOQ determination. For an existing method, just use the routine method blanks; there is no need to run additional method blanks. If validating a new method (or a new analyte in an existing method) the same requirement for at least three batches analyzed over three separate days applies, and a minimum of seven method blanks is required.

The TNI procedure does not provide equations for calculating the DL. The EPA MDL procedure has these equations.

$$DL_s = ts;$$

where t is Student's t value and s is the standard deviation of the results for the spiked samples.

Note: With this procedure, many laboratories are likely to have more than 7 spike or blank results. Appendix 1 contains an expanded Student t Table to help with this calculation.

Then, calculate the DL_b based on at least seven method blank results.

If all the method blanks give numerical results calculate the DL_b as follows:

$$DL_b = X + ts;$$

where X is the mean of the blank results, t is Student's t value, and s is the standard deviation of the blank results. Numerical results include both positive and negative values. The EPA MDL procedure requires the laboratory to use 0 as the mean if the MDL_b is calculated as a negative number. If all of the blank results are "ND" then the DL_b is zero and the DL will be based on the spike results.

If some of the results are “ND” and some are numerical results, as stated in the EPA MDL procedure, two options are available:

- 1) Set the DL_b equal to the highest method blank result.
- 2) If more than 100 method blanks are available, it is recommended to set DL_b to the level that is no less than the 99th percentile of the blank results. When using this approach to set the DL_b , all results including the “ND” results, are included. The 99th percentile is the more robust statistic and ensures a 99% confidence interval, consistent with the EPA definition of the MDL. The 99th percentile equation in Excel is “=PERCENTILE(A1:Axxx,99)”, where xxx is the number of blanks.

Finally, as stated in the EPA procedure, compare DL_s and DL_b – the higher of the two becomes the DL.

2.6 Step 5 - Verification of the LOQ Based on the Determined DL

1.5.2.2.1 c) The LOQ is verified if the following criteria are met

iii) The LOQ is greater than the established DL and at or above the spiking concentration.

If the LOQ is less than or equal to the DL, the LOQ shall be raised to greater than the DL.

The main determinant of the LOQ is the spiking concentration; the LOQ must be at or above the spiking concentration used for the DL replicates. There is a secondary requirement, that the LOQ must be greater than the DL.

3.0 Annual Recalculation of the DL and Ongoing Verification of the LOQ

1.5.2.1.2 Ongoing verification of the DL
A minimum of one (1) verification spike and one (1) blank shall be analyzed on each instrument during each quarter in which samples are being analyzed....

1.5.2.2.2 Ongoing Verification of the LOQ
The laboratory shall prepare and analyze a minimum of one (1) verification sample spiked at the same concentration as the initial LOQ verification on each instrument during each quarter in which samples are being analyzed

Assuming that the same low-level spikes or samples spiked at the same concentration were used for the determination of the DL and the initial verification of the LOQ, then the ongoing verifications may be carried out using one set of low-level spikes.

Note that if different spike concentrations were used for the initial DL determination and initial LOQ verification, then different spike concentrations would be required for the ongoing verifications of the DL and LOQ as well.

The TNI standard requires one spike sample be analyzed per instrument per quarter. However, the EPA procedure requires at least two spikes in separate batches per quarter on any instrument that is used to analyze samples. Thus for those laboratories who analyze various sample types using one method, then

two spikes would be required. It is important to note that the spiking concentration of the ongoing verification samples must be the same as for the initial verification of the LOQ. If for some reason it is necessary to use a different concentration, then a new initial study is required. Note: A single extract may be analyzed on one or more instruments.

The TNI Standard does not require quarterly DL verification if data is not being reported below the LOQ, but keep in mind that the EPA MDL procedure does require quarterly verification in any quarter in which samples are analyzed. The section above does not discuss blanks, but references section 1.5.2.1.1 (e) which states the DL procedure shall include “criteria for evaluating false positive rates in routine blanks.” The EPA procedure contains more details including options for only using the most recent 50 blanks or the last six months whichever is greater. These options are not allowed under TNI which states in section 1.5.2.4 that “all data representative of the current operations shall be used, if generated in the last 2 years.”

3.1 Acceptance Criteria for the Quarterly Verification Spikes

1.5.2.1.1 d) results from low level spikes used in the DL determination shall meet qualitative identification criteria in the method, and shall be above zero;

1.5.2.1.2 In the event that verification fails, the laboratory shall perform a new DL study within thirty (30) calendar days.

For a spike analysis to be acceptable as a DL verification sample, the result must be above zero, and any qualitative identification criteria in the method must be met. (Note: The laboratory may need to modify the way they record sample data since the results may be below the laboratory’s LOQ) If DL verification samples are to be used for LOQ verification they must also meet the criteria listed in 1.5.2.2.2 a). If these criteria are not met, then the laboratory must perform one of the corrective actions as listed in 1.5.2.2.2 b) (See section 3.3 below) and document a technically valid reason for the corrective action. The technically valid reason shall be appropriate for the corrective action selected. Examples of a technically valid reason are: incorrect preparation, instrument failure, calibration error, instrument performance indications show a change in sensitivity, etc. If the spiking level must be raised and a new initial study performed within 30 days, the existing DL and LOQ are used for reporting during this 30 day (or less) period.

The requirement in section 1.5.2.1.2 is only applicable for the analyte/s that failed.

3.2 Corrective Action

1.5.2.2.2 b) If a continuing LOQ verification test does not meet this requirement, the laboratory shall take corrective action and document a technically valid reason for the corrective action.

Corrective action shall be one of the following:

- (i) correcting method or instrument performance and repeating the verification test;
 - (ii) evaluating the laboratory established control limits to ensure they reflect current performance;
- or
- (iii) raising the spiking level (and the quantitation limit if the spiking level is above it) and repeating the initial verification study within thirty (30) calendar days of the initial failure.

Any samples analyzed in a batch associated with a failing LOQ verification shall be reanalyzed or reported with qualifiers.

If a LOQ verification does not meet this requirement, it is considered a nonconformance and shall be evaluated per V1M2 section 4.9 and documented appropriately. If a repeat of the initial verification of the LOQ is required see section 1.5.2.2.1 for requirements. This will also meet the requirements of the initial determination of the detection limit found in section 1.5.2.1.1.

3.3 Annual Assessment of the Quarterly Spike and Blank Results

1.5.2.4 Documentation

At least once per year, the laboratory shall tabulate all results of the ongoing verification sample testing. All data representative of the current operations shall be used, if generated within the last two (2) years. A minimum of seven (7) samples is required.

- a) The laboratory shall record the analytical and preparation methods used, dates of preparation and testing, the batch identifiers, the testing instrument, quality system matrix, technology, analyte, concentration in the spiked sample with units, and the test result (if any) for each LOQ and/or DL verification test.
- b) For each analyte, the laboratory shall record the percent recovery, the number of results (n), the mean and standard deviation of the percent recovery, and the spiking concentration of the spiked samples with units. These data shall be provided to clients upon request.

Ongoing verification data must be collected following the analysis of an initial study. All data used to establish the initial study must be used in the ongoing documentation if it is within the last 24 months.

The results from the quarterly spikes are collected and tabulated. This documentation is intended to be adequate to unequivocally identify the samples used in the quarterly verifications including appropriate preservation if utilized. Once collected, the number of samples, and the mean and standard deviation of the results are calculated summarized for laboratory customers and/or assessors to review as needed.

3.4 Updating the LOQ

If the DL has been changed, then the LOQ may also need to be changed, based on the requirement that the LOQ shall be above the DL.

1.5.2.2.2 a the quantitated result shall be greater than the DL and meet the laboratory established accuracy criteria as established by Section 1.5.2.2 d)

The EPA MDL procedure states:

If the verified MDL is within 0.5 to 2.0 times the existing MDL, and fewer than 3% of the method blank results (for the individual analyte) have numerical results above the existing MDL, then the existing MDL may optionally be left unchanged. Otherwise, adjust the MDL to the new verification MDL.

While this is not a TNI requirement, it seems prudent to include this action when appropriate. Usually the LOQ will remain unchanged. However, if the DL has increased it may also be necessary to raise the LOQ, since the LOQ must be greater than the DL.

Appendix 1: Student's t Table for 99% Confidence

Degrees of Freedom = Number of Spikes or Blanks - 1	Student's t
6	3.143
7	2.998
8	2.896
9	2.821
10	2.764
11	2.718
12	2.681
13	2.650
14	2.624
15	2.602
16	2.583
17	2.567
18	2.552
19	2.539
20	2.528
21	2.518
22	2.508
23	2.500
24	2.492
25	2.485
26	2.479
27	2.473
28	2.467
29	2.462
30	2.457
40	2.423
60	2.390
80	2.374
100	2.364
1000	2.330