Ammonia as Nitrogen (Example using MDL_s and MDL_b)

Review EPA's "*Definition and Procedure for the Determination of the Method Detection Limit, Revision 2*" before proceeding. Click Here

Click "Blue-gray References" to launch to EPA Guidance. From Guidance press "Alt" + "Left Arrow" or "Right-Click" and chose "Previous View" to return the examples.

Estimate Initial MDL Determination - *Procedure (1)*

Use 1 of 6 ways to estimate the initial MDL_s concentration.

Initial MDL Determination - *Procedure (2)*

Prepare MDL spikes based on the estimate above.
(Typically 0.1 to 0.03 mg/L range)

Use reagent grade/lab water to make up standard dilutions.

To prepare a 0.03 mg/L concentration in 1000 mL volumetric flask:

Using a purchased or prepared 100 ppm (mg/L) standard,
pipet and dilute 0.3mL of the 100 mg/l standard to 1000mL with reagent water.

100 mg/L / 1000mL X 0.3mL = 0.03 mg/L

Analyze at least seven MDL spikes as you would samples. (8 spikes are used in the example below.)

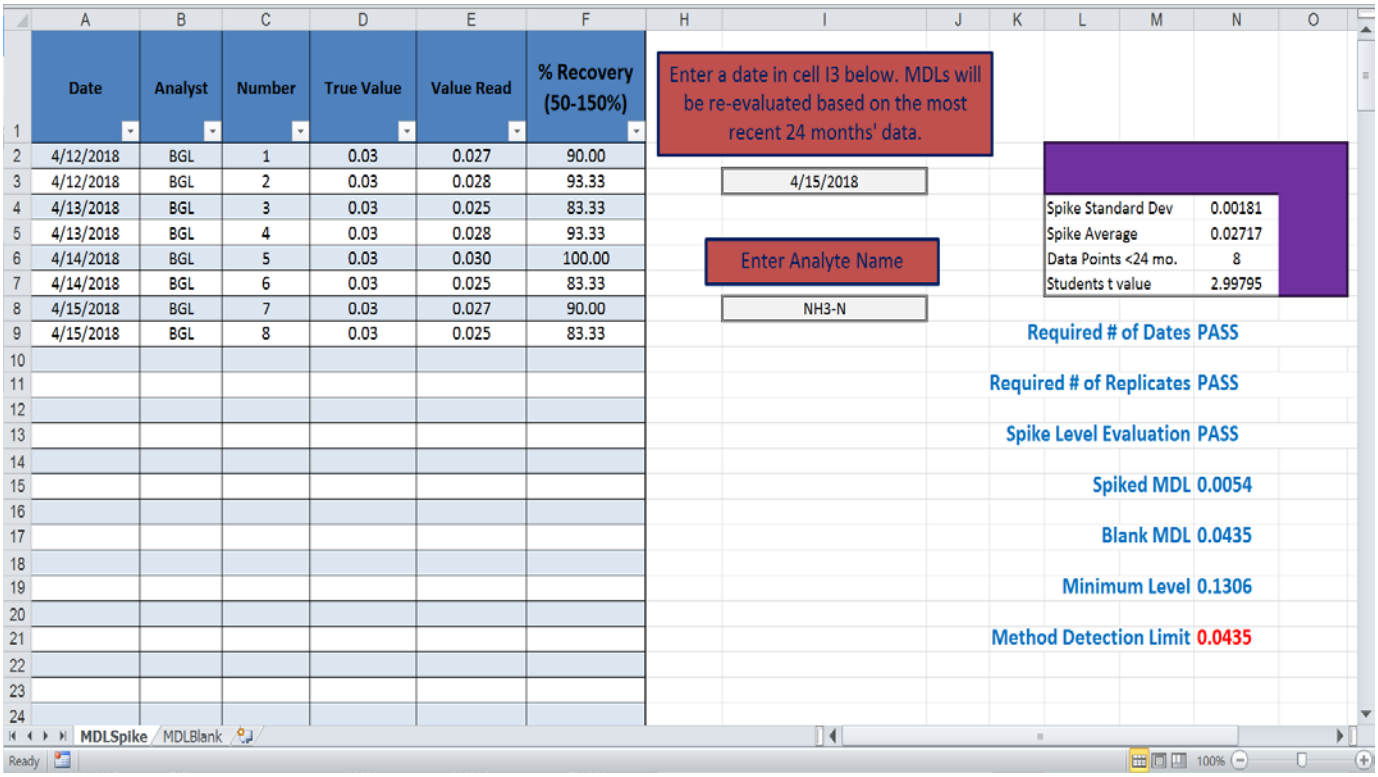
- Prepare and analyze a total of at least seven spikes at the same concentration on at least 3 different calendar dates.

Date	Analyst	Number	Spike mg/L
04/12/18	BGL	1	0.027
04/12/18	BGL	2	0.028
04/13/18	BGL	3	0.025
04/13/18	BGL	4	0.028
04/14/18	BGL	5	0.030
04/14/18	BGL	6	0.025
04/15/18	BGL	7	0.027
04/15/18	BGL	8	0.025

Record and evaluate MDL spiking level. *Procedure (2) (c)*

- Proceed only if all spikes produce a number greater than "0".

Calculate the Initial MDL_s. *Procedure (2) (d) (ii)*
(Calculations may be performed manually or using a suitable statistical spreadsheet.)



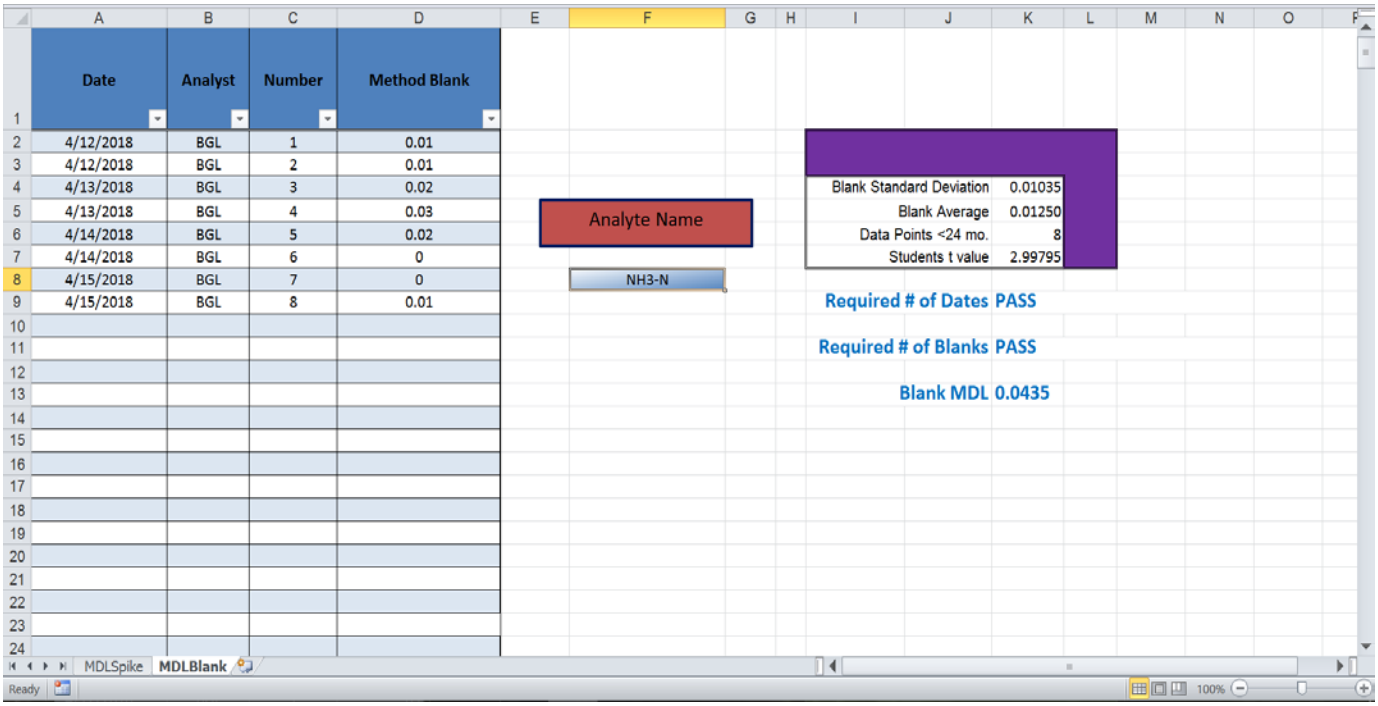
Prepare method blanks using reagent grade/lab water. *Procedure (2) (b)*

Analyze at least seven method blanks as you would samples. (8 blanks are used in the example below.)

- Prepare and analyze a total of at least 7 method blanks on at least 3 different calendar dates.

Date	Analyst	Number	Method Blank, mg/L
04/12/18	BGL	1	0.01
04/12/18	BGL	2	0.01
04/13/18	BGL	3	0.02
04/13/18	BGL	4	0.03
04/14/18	BGL	5	0.02
04/14/18	BGL	6	0.0
04/15/18	BGL	7	0.0
04/15/18	BGL	8	0.01

Calculate the Initial MDL_b. *Procedure (2) (d) (iii)*
(Calculations may be performed manually or using a suitable statistical spreadsheet.)



Initial MDL Calculation *Procedure (2) (e)*

The Initial MDL is the greater of the MDL_s and MDL_b values as calculated above.

Ongoing Data Collection - *Procedure (3)*

Analyze at least 2 spikes (the same concentration as the initial MDL spikes) in separate batches per quarter. Analyze method blanks as required by normal laboratory batch QC. (A minimum of 2 method blanks in separate batches are also required.)Record all values in a log book or keep a running log in a spreadsheet.

Ongoing Annual Verification - *Procedure (4)*

At least once every 13 months re-calculate using all data generated within the past 24 months. *Procedure (4) (a) and (4) (f)*
(If necessary change the reported MDL to the new value.)

Ideally, use all method blank results form the last 24 months for the MDLb calculation. Optionally use the last 6 months of method blanks or the 50 most recent, whichever criteria yield the greater number of method blanks. Procedure (4) (e)

Total Suspended Solids (Example using MDL_b only)

Initial MDL Determination - *Procedure (2)*

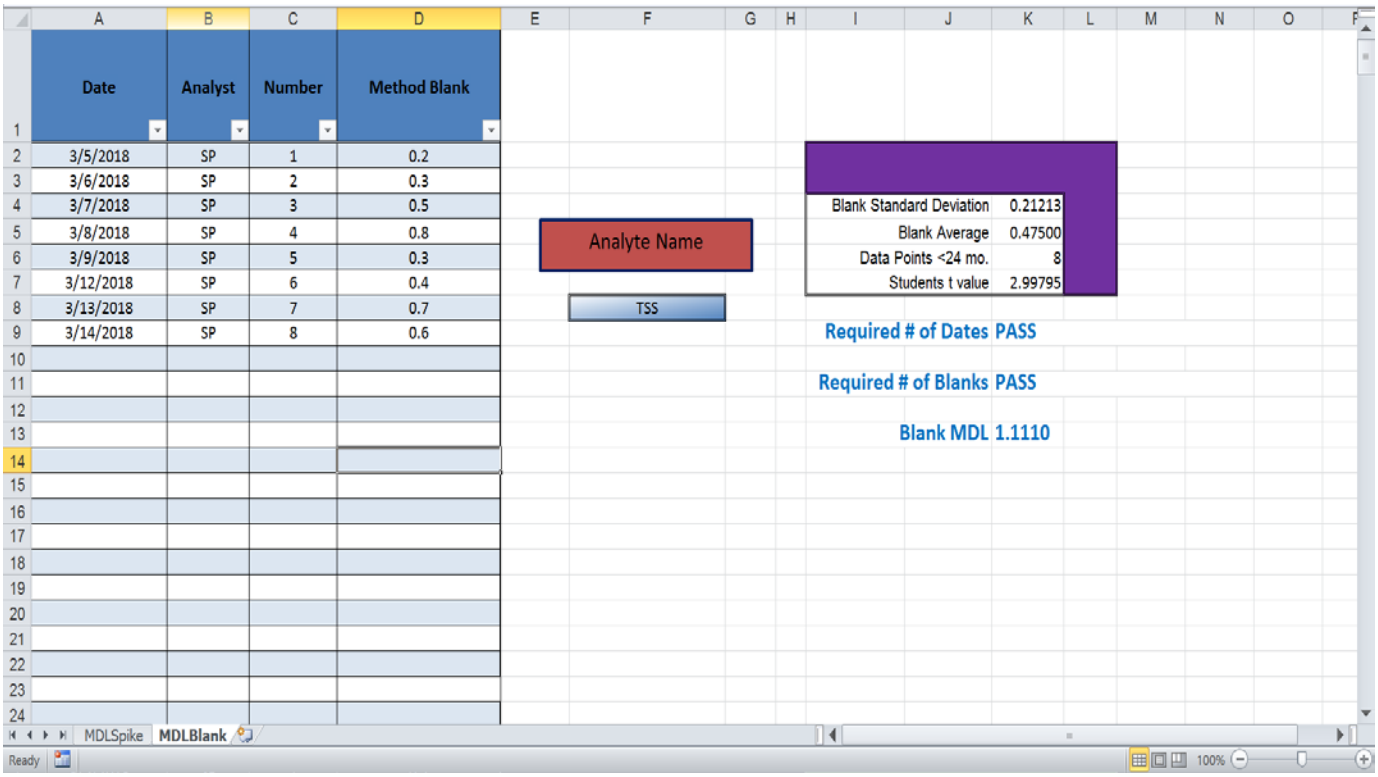
Prepare method blanks using reagent grade/lab water. *Procedure (2) (b)*

Analyze at least seven method blanks as you would samples. (8 blanks are used in the example below.)

- Prepare and analyze a total of at least 7 method blanks on at least 3 different calendar dates.

Date	Analyst	Number	Method Blank, mg/L
03/05/18	SP	1	0.2
03/06/18	SP	2	0.3
03/07/18	SP	3	0.5
03/08/18	SP	4	0.8
03/09/18	SP	5	0.3
03/12/18	SP	6	0.4
03/13/18	SP	7	0.7
03/14/18	SP	8	0.6

Calculate the Initial MDL_b. *Procedure (2) (d) (iii)*
(Calculations may be performed manually or using a suitable statistical spreadsheet.)



Initial MDL Calculation - *Procedure (2) (d) (iii)*

The Initial MDL is equal to the MDL_b value as calculated above.

Ongoing Data Collection - *Procedure (3)*

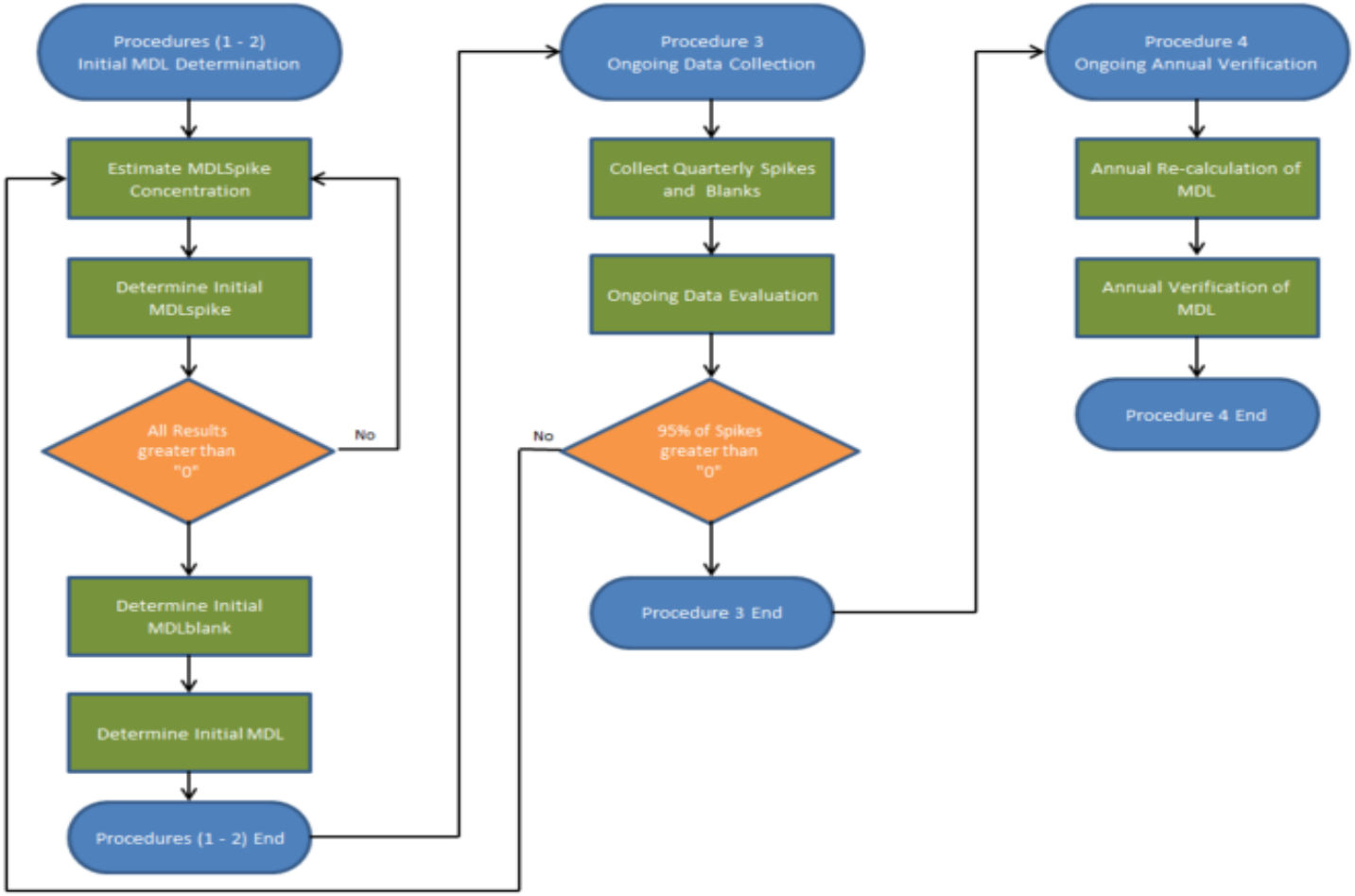
Analyze method blanks as required by normal laboratory batch QC. (A minimum of 2 method blanks in separate batches.) Record all values in a log book or keep a running log in a spreadsheet.

Ongoing Annual Verification - *Procedure (4)*

At least once every 13 months re-calculate using all data generated within the past 24 months. *Procedure (4) (a) and (4) (f)*
(If necessary change the reported MDL to the new value.)

Ideally, use all method blank results form the last 24 months for the MDLb calculation. Optionally use the last 6 months of method blanks or the 50 most recent, whichever criteria yield the greater number of method blanks. Procedure (4) (e)

Method Detection Limit Workflow Diagram



United States Environmental Protection Agency

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Definition and Procedure for the Determination of the Method Detection Limit, Revision 2

This document contains the text of Revision 2 of the method detection limit procedure from 40 CFR 136 Appendix B; but formatted as a more user friendly stand-alone document.

Please address questions or comments to:

CWA Methods Team
Engineering and Analytical Support Branch/EAD (4303T)
Office of Science and Technology
U.S. Environmental Protection Agency
1200 Pennsylvania Avenue
Washington, DC 20460

https://www.epa.gov/cwa-methods

DEFINITION AND PROCEDURE FOR THE DETERMINATION OF THE METHOD DETECTION LIMIT
REVISION 2

Definition

The method detection limit (MDL) is defined as the minimum measured concentration of a substance that can be reported with 99% confidence that the measured concentration is distinguishable from method blank results.

Scope and Application

The MDL procedure is designed to be a straightforward technique for estimation of the detection limit for a broad variety of physical and chemical methods. The procedure requires a complete, specific, and well-defined analytical method. It is essential that all sample processing steps used by the laboratory be included in the determination of the method detection limit.

The MDL procedure is *not* applicable to methods that do not produce results with a continuous distribution, such as, but not limited to, methods for whole effluent toxicity, presence/absence methods, and microbiological methods that involve counting colonies. 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. Except as described in the addendum, for the purposes of this procedure, "spiked samples" are prepared from a clean reference matrix, such as reagent water, spiked with a known and consistent quantity of the analyte. 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.

Procedure

To return: "Alt" + "Left Arrow" or "Right-Click" and "Previous View"

(1) Estimate the initial MDL using one or more of the following:

(a) The mean determined concentration plus three times the standard deviation of a set of method blanks.

(b) The concentration value that corresponds to an instrument signal-to-noise ratio in the range of 3 to 5.

(c) The concentration equivalent to three times the standard deviation of replicate instrumental measurements of spiked blanks.

(d) That region of the calibration where there is a significant change in sensitivity, i.e., a break in the slope of the calibration.

(e) Instrumental limitations.

(f) Previously determined MDL.

It is recognized that the experience of the analyst is important to this process. However, the analyst should include some or all of the above considerations in the initial estimate of the MDL.

(2) Determine the initial MDL

Note: The Initial MDL is used when the laboratory does not have adequate data to perform the Ongoing Annual Verification specified in Section (4), typically when a new method is implemented or if a method was rarely used in the last 24 months.

(a) Select a spiking level, typically 2 – 10 times the estimated MDL in Section 1. Spiking levels in excess of 10 times the estimated detection limit may be required for analytes with very poor recovery (e.g., for an analyte with 10% recovery, spiked at 100 micrograms/L, with mean recovery of 10 micrograms/L; the calculated MDL may be around 3 micrograms/L. Therefore, in this example, the spiking level would be 33 times the MDL, but spiking lower may result in no recovery at all).

(b) Process a minimum of seven spiked samples and seven method blank samples through all steps of the method. The samples used for the MDL must be prepared in at least three batches on three separate calendar dates and analyzed on three separate calendar dates. (Preparation and analysis may be on the same day.) Existing data may be used, if compliant with the requirements for at least three batches, and generated within the last twenty four months. The most recent available data for method blanks and spiked samples must be used. Statistical outlier removal procedures should not be used to remove data for the initial MDL determination, since the total number of observations is small and the purpose of the MDL procedure is to capture routine method variability. However, documented instances of gross failures (e.g., instrument malfunctions, mislabeled samples, cracked vials) may be excluded from the calculations, provided that at least seven spiked samples and seven method blanks are available. (The rationale for removal of specific outliers must be documented and maintained on file with the results of the MDL determination.)

(i) If there are multiple instruments that will be assigned the same MDL, then the sample analyses must be distributed across all of the instruments.

(ii) A minimum of two spiked samples and two method blank samples prepared and analyzed on different calendar dates is required for each instrument. Each analytical batch may contain one spiked sample and one method blank sample run together. A spiked sample and a method blank sample may be analyzed in the same batch, but are not required to be.

(iii) The same prepared extract may be analyzed on multiple instruments so long as the minimum requirement of seven preparations in at least three separate batches is maintained.

(c) Evaluate the spiking level: If any result for any individual analyte from the spiked samples does not meet the method qualitative identification criteria or does not provide a numerical result greater than zero, then repeat the spiked samples at a higher concentration. (Qualitative identification criteria are a set of rules or guidelines for establishing the identification or presence of an analyte using a measurement system. Qualitative identification does not ensure that quantitative results for the analyte can be obtained.)

(d) Make all computations as specified in the analytical method and express the final results in the method-specified reporting units.

(i) Calculate the sample standard deviation (S) of the replicate spiked sample measurements and the sample standard deviation of the replicate method blank measurements from all instruments to which the MDL will be applied.

(ii) Compute the MDL_s (the MDL based on spiked samples) as follows:

$$MDL_S = t_{(n-1,\ 1-\propto=0.99)} S_s$$

where:

MDL_s = the method detection limit based on spiked samples

$t_{(n-1,\ 1-\alpha\ =\ 0.99)}$ = the Student's *t*-value appropriate for a single-tailed 99th percentile *t* statistic and a standard deviation estimate with n-1 degrees of freedom. See Addendum Table 1.

S_s = sample standard deviation of the replicate spiked sample analyses.

(iii) 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 x 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:

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

where:

MDL_b = the MDL based on method blanks

$\overline{X}$ = 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. See Addendum Table 1.

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, as described in Section (2)(d)(iii)(B).

(e) Select the greater of MDL_s or MDL_b as the initial MDL.

(3) Ongoing Data Collection

(a) During any quarter in which samples are being analyzed, prepare and analyze a minimum of two spiked samples on each instrument, in separate batches, using the same spiking concentration used in Section 2. If any analytes are repeatedly not detected in the quarterly spiked sample analyses, or do not meet the qualitative identification criteria of the method (see Section 2(c) of this procedure), then this is an indication that the spiking level is not high enough and should be adjusted upward. Note that it is not necessary to analyze additional method blanks together with the spiked samples, the method blank population should include all of the routine method blanks analyzed with each batch during the course of sample analysis.

(b) Ensure that at least seven spiked samples and seven method blanks are completed for the annual verification. If only one instrument is in use, a minimum of seven spikes are still required, but they may be drawn from the last two years of data collection.

(c) At least once per year, re-evaluate the spiking level.

(i) If more than 5% of the spiked samples do not return positive numerical results that meet all method qualitative identification criteria, then the spiking level must be increased and the initial MDL re-determined following the procedure in Section 2.

(d) If the method is altered in a way that can be reasonably expected to change its sensitivity, then re-determine the initial MDL according to Section 2, and the restart the ongoing data collection.

(e) If a new instrument is added to a group of instruments whose data are being pooled to create a single MDL, analyze a minimum of two spiked replicates and two method blank replicates on the new instrument. If both method blank results are below the existing MDL, then the existing MDL_b is validated. Combine the new spiked sample results to the existing spiked sample results and recalculate the MDL_s as in Section 4. If the recalculated MDL_s does not vary by more than the factor specified in Section 4(f) of this procedure, then the existing MDL_s is validated. If either of these two conditions is not met, then calculate a new MDL following the instructions in Section 2.

(4) Ongoing Annual Verification

(a) At least once every thirteen months, re-calculate MDL_s and MDL_b from the collected spiked samples and method blank results using the equations in Section 2.

(b) Include data generated within the last twenty four months, but only data with the same spiking level. Only documented instances of gross failures (e.g., instrument malfunctions, mislabeled samples, cracked vials) may be excluded from the calculations. (The rationale for removal of specific outliers must be documented and maintained on file with the results of the MDL determination.) If the laboratory believes the sensitivity of the method has changed significantly, then the most recent data available may be used, maintaining compliance with the requirement for at least seven replicates in three separate batches on three separate days (see Section 2b).

(c) Include the initial MDL spiked samples, if the data were generated within twenty four months.

(d) Only use data associated with acceptable calibrations and batch QC. Include all routine data, with the exception of batches that are rejected and the associated samples reanalyzed. If the method has been altered in a way that can be reasonably expected to change its sensitivity, then use only data collected after the change.

(e) Ideally, use all method blank results from the last 24 months for the MDL_b calculation. The laboratory has the option to use only the last six months of method blank data or the fifty most recent method blanks, whichever criteria yields the greater number of method blanks.

(f) The verified MDL is the greater of the MDL_s or MDL_b. 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. (The range of 0.5 to 2.0 approximates the 95th percentile confidence interval for the initial MDL determination with six degrees of freedom.)

ADDENDUM: DETERMINATION OF THE MDL FOR A SPECIFIC MATRIX

The MDL may be determined in a specific sample matrix as well as in reagent water.

(1) Analyze the sample matrix to determine the native (background) concentration of the analyte(s) of interest.

(2) If the response for the native concentration is at a signal-to-noise ratio of approximately 5-20, determine the matrix-specific MDL according to Section 2 but without spiking additional analyte.

(3) Calculate MDL_b using the method blanks, not the sample matrix.

(4) If the signal-to-noise ratio is less than 5, then the analyte(s) should be spiked into the sample matrix to obtain a concentration that will give results with a signal-to-noise ratio of approximately 10-20.

(5) If the analytes(s) of interest have signal-to-noise ratio(s) greater than approximately 20, then the resulting MDL is likely to be biased high.

Table 1: Single-Tailed 99th Percentile *t* Statistic

Number of replicates	Degrees of freedom (n-1)	$t_{(n-1, 0.99)}$
7	6	3.143
8	7	2.998
9	8	2.896
10	9	2.821
11	10	2.764
16	15	2.602
21	20	2.528
26	25	2.485
31	30	2.457
32	31	2.453
48	47	2.408
50	49	2.405
61	60	2.390
64	63	2.387
80	79	2.374
96	95	2.366
100	99	2.365

Documentation

The analytical method used must be specifically identified by number or title and the MDL for each analyte expressed in the appropriate method reporting units. Data and calculations used to establish the MDL must be able to be reconstructed upon request. The sample matrix used to determine the MDL must also be identified with MDL value. Document the mean spiked and recovered analyte levels with the MDL. The rationale for removal of outlier results, if any, must be documented and maintained on file with the results of the MDL determination.

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