ALTA

AGRICULTURAL LABORATORY TESTING ASSOCIATION

Overview:

- **Illinois Soil Testing Association (ISTA) was founded in 1981 address Illinois growers' needs for quality soil test information. ISTA rebranded as the Agriculture Laboratory Testing Association (ALTA).**
- **ALTA's mission is to promote the interests of the Ag testing industry and advance high-quality soil & plant-tissue analysis data for farm profitability, and sustainability in the US.**
- **ALTA is committed to ensuring the quality of data to agricultural communities by encouraging the development, use, and acceptance of proven agricultural testing methods.**

Determining the Method Detection Limit

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ALTA Webinar
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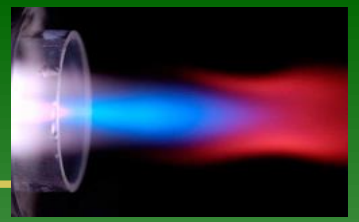
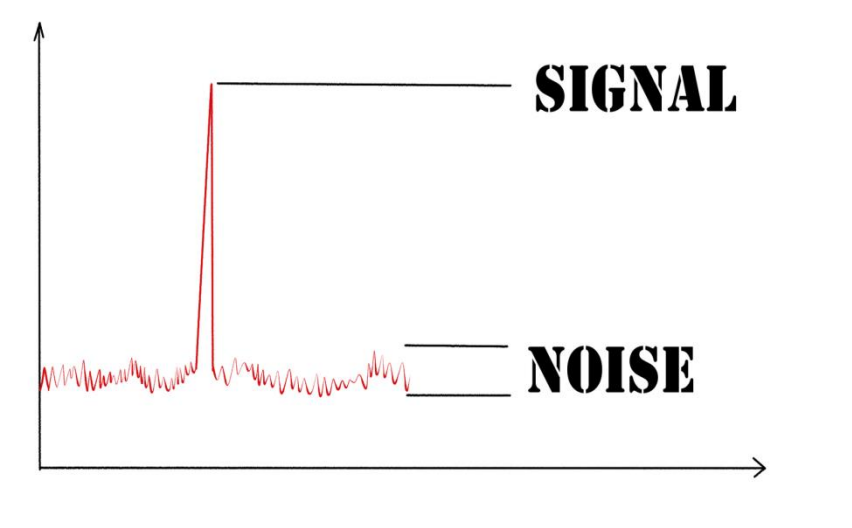
Overview



Chemical analysis is based on the quantification of an analyte or substance utilizing a defined method.

Analytical methods typically employ an instrument for quantification. At very low analyte concentrations it is increasingly difficult to separate the detector signal from the noise.



The Method Detection Limit establishes the protocol for defining the minimum measured signal for the method.

Factors influencing the MDL

Sample matrix

Preparation steps (extraction / dilution)

Instrument technology (sensor / detector)

Instrument (age, maintenance)

Analyst skill

Environmental conditions

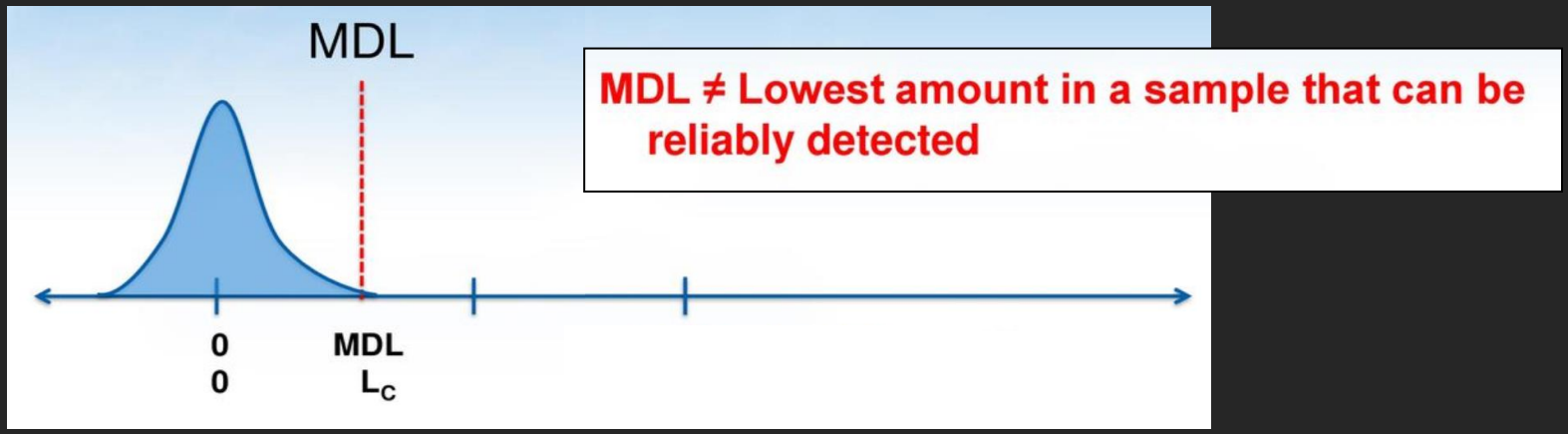
Importance of MDL

Although quantification of the MDL is of less importance for soil testing methods (e.g. Mehlich 3, SOM and etc.) it of high importance to manure analysis, environmental testing and the assessment of contaminants.

It is critical in identifying the presence of analyte and quantifying the concentration with defined statistical certainty.

The Method Detection Limit

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 the method blank. MDL ? DL[1]



[1] Detection Limit (DL) is the smallest analyte concentration that can be demonstrated to be different from zero or a blank concentration with 99% confidence (Probability of the false positive rate (Type I error) is 1%).

Additional definitions

Instrument Detection Limit (IDL): is the analyte concentration that is required to produce an instrument signal greater than three times the standard deviation of the noise level. *Not to be confused with MDL.*

Limit of Detection (LOD) is defined as the lowest concentration for reliable reporting of a non-detect of a specific analyte in a specific matrix with a specific method at 99% confidence (Probability false negative rate, Type II error is 1%). *LOD ≥ 2 x MDL*

Limit of Quantification (LOQ): is the lowest analyte concentration that can be quantitatively detected with some predefined level of accuracy and precision. Similar term, Practical Quantitation Limit (PQL) is the quantitation limit with >99.9% confidence in the result.

MDL, DL, LOD, and LOQ

Range where detection is quantified as an estimate

MDL **LOD** **LOQ**

Quantification range

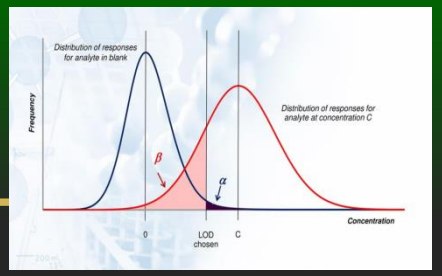
Not *Detectable*

0 **2 x MDL** **3 x MDL** **High Cal**

Low false positive
High false negative
Estimated values

Low false positive
Low false negative
Estimated values

Detection limit and errors



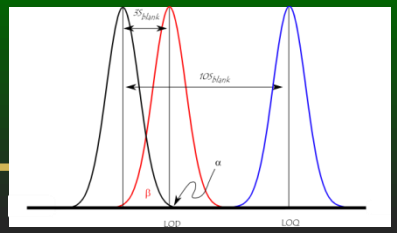
At low analyte concentrations, two errors are common:

- False positives, reporting detection when no analyte is present
- False negatives, reporting nothing detected when analyte is actually present

		Actual State	
		Absent	*Present*
Measured Result	*Absent*	**True Negative** **probability = 1 - α**	**False Negative** **probability = β**
	Present	**False Positive** **probability = α**	**True Positive** **probability = 1 - β**

Darcy Wilkins, Dionex , 2008

MDL US-EPA - 40 CFR 136 Appendix B



1984-2017

MDL defined as the minimum concentration of a substance measured and reported with 99% confidence that the analyte concentration is greater than zero as determined in a sample in a given matrix with the analyte.

7 low level spikes, 2 – 10 times the expected MDL, MDL = $T_{(n-1,1-\alpha=0.99)}$ *(S)*

2017-Present

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 the method blank results.

US-EPA MDL changes

- Use of blanks in assessing MDL over 3 days
- The MDL now requires that the samples used to calculate the MDL are representative of laboratory performance throughout the year, rather than on a single date.
- A laboratory has the option to pool data from multiple instruments to calculate one MDL that represents multiple instruments. Recalculate every 13 months.

US-EPA MDL 40 CFR 136 Appendix B

Procedure

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

(a) The mean determined concentration plus three times the stdev of five method blanks.

(b) The concentration equivalent to three times the stdev of replicate instrumental measurements of spiked blanks.

(c) Instrumental limitations. It is recognized analyst experience is important, but should include one or all of the above in the MDL estimate.

(edited)

(2) Determine the initial MDL

Note: The Initial MDL is used when the laboratory has insufficient data, or when a new method is implemented or rarely used.

(a) Select a spiking level, typically 2 – 10 times the estimated MDL in Section 1.

(b) Process a minimum of 7 spiked samples and 7 method blank samples through all steps of the method. Samples used must be prepared in at least 3 batches on 3 separate calendar dates and analyzed on 3 separate dates. (Preparation and analysis may be on the same day.)

(c) Evaluate spiking level: If any result for any individual analyte from the spiked samples does not meet method quality, repeat the spiked samples using a higher concentration[1].

[1] Existing lab analysis 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, as 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.)

(edited)

(2) continued

(d) Complete 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_s) of both the replicate spiked sample measurements (MDL_s) and the sample replicate method blank measurements (MDL_b).

(ii) Compute the MDL_s as follows: $MDLs = (n-1, 1-\propto=0.99)S_s$
t (n-1, 1-α = 0.99) = the Student's *t*-value for a single-tailed 99th percentile *t* statistic and S_s estimate with n-1 degrees of freedom.
S_s = sample standard deviation of the replicate spike.

(iii) Compute the MDL_b (MDL method blanks)

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

(edited)

(2) *iii* continued

If method blanks give numerical results, then calculate the MDL_b as:

$MDL_b = X_b + (n-1,1-\propto=0.99)\ S_b$

X_b= mean of the method blank results

t (n-1, 1-α = 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 statistics Table.

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

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

(edited)

MDL Overview

The MDL US-EPA protocol, is an iterative process based on an estimate of the MDL followed by an analysis of a replicated low level spike and blank solutions and a statistical evaluation of the results.

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It provides high statistical confidence in quantifying the limit of method detection and quantification.

MDL Estimate

Spike and Blank Evaluation

Calculation of MDL

MDL example, N combustion liquid

Total nitrogen MDL by combustion analyzer was determined using a spiked Tris buffer solution in accordance with Sections 1(b) (MDL_e) and 2(b) (MDL_i) above.

(i) Total N replicate results of five laboratory spiked blanks:

Results (n=5)	N %
Mean	0.0332
Stdev (*s*)	0.0012

MDL_e= 3 x stdev = 0.0037 %

Nitrogen example continued

(ii) MDL_i : Seven spiked blanks were prepared based on 3 x MDL_e concentration of 0.0037 % N. Seven spiked blanks and seven unspiked blanks were analyzed. Data was tabulated and MDL_i determined as follows:

Results	Spiked blank		blank	
(n=7)	X_{sb}	S_{sb}	X_b	S_b
N %	**0.0150**	**0.0015**	**0.0010**	**0.0050**

Student's *t*-value n=7, single-tailed 99th, t = 3.143.

$MDL_s = 3.143 \times 0.0015 = 0.0047$

$MDL_b = 0.021 + (3.143 \times 0.0050) = 0.0017$

$MDL_s > MDL_b$

Total Nitrogen MDL_i = 0.0047 % $LOQ = 3 \times MDL_i = 0.014$ %

MDL example, Phosphorus water ICP-OES

Phosphorus MDL by ICP-OES was determined using a spiked solution in accordance with Sections 1(b) (MDL_e)

(i) P replicate results of five laboratory spiked blanks:

Results (n=5)	P mg/l
Mean	0.0334
Stdev (*s*)	0.2811

MDL_e= 3 x stdev = 0.843

Phosphorus example continued

(ii) MDL_i : Nine spiked blanks were prepared based on 3 x MDL_e with a mean concentration of 0.843 mg/L. Nine spiked blanks and nine unspiked blanks were analyzed. Data was tabulated and MDL_i determined as follows:

Results	Spiked blank		blank	
(n=9)	X_{sb}	S_{sb}	X_b	S_b
P mg/L	**0.777**	**0.231**	**0.021**	**0.280**

Student's *t*-value n=9, single-tailed 99th, t = 2.896.

$MDL_s = 2.896 \times 0.231 = 0.669$

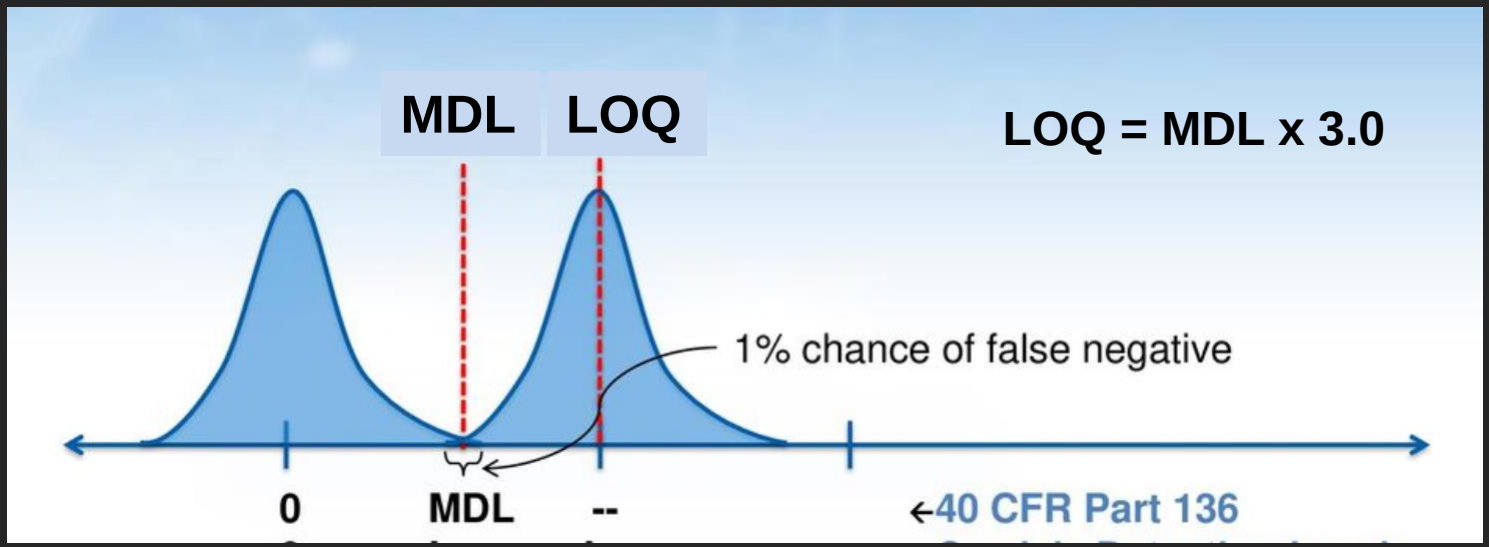
$MDL_b = 0.021 + (2.896 \times 0.280) = 0.832$

$MDL_s < MDL_b$

Phosphorus MDL_i = 0.832 mg/l LOQ = 3 x MDL_i = 2.49 mg/l

MDL vs LOQ

The Limit of Quantification (LOQ) represents that analyte concentration with 99% confidence (Probability false negative, Type II error is 1%).



Summary comments

The MDL quantifies the base level of method performance the laboratory.

It is subject to changes in laboratory instrumentation, reagents and changes to the SOP, and should be re-evaluated yearly or any time operational changes are made to the method.

MDL resources

ANALYTICAL DETECTION LIMIT GUIDANCE, & Laboratory Guide for Determining Method Detection Limits. Wisconsin Department of Natural Resources Laboratory Certification Program, April 1996 dnr.wi.gov/regulations/labcert/documents/guidance/-lodguide.pdf

Detected or not? Defining Laboratory Analytical Limits. 2018. by Michael Brisson and Dereck Popp. synergist.aiha.org/201711-detected-or-not

Definition and Procedure for the Determination of the Method Detection Limit, Revision 2, December 2016, US-EPA, Office of Water, EPA 821-R-16-006. www.epa.gov/sites/default/files/2016-12/documents/mdl-procedure_rev2_12-13-2016.pdf

Setting Meaningful Detection and Quantitation Limits. 2007. J. Schibler, D. Moore Dionex Corporation https://slideplayer.com/slide/12708496/

How to calculate LOD and LOQ, www.youtube.com/watch?v=suvnbRH2y-0

ALTA Webinar - August

Soil scooping is integral to the assessment of soil fertility in the Midwest.

A webinar on soil scooping is scheduled August 24, 2021, 10:00 am CDT.

Thank you for your time and attention

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