How’s that *new* LOD Coming?

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WWOA Board of Directors
DNR LabCert
The LOD Procedure has changed

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**ACTION:** Final rule.

**DATES:** This regulation took effect on September 27, 2017.
EPA has revised the procedure for determination of the MDL primarily to address laboratory blank contamination and to better account for intra-laboratory variability. The suggestion for these revisions came first from The NELAC Institute. EPA proposed to adopt these revisions.

The majority of commenters supported the revised MDL procedure. All lab associations commented in favor. Comments not in favor of the MDL revision were received from individual laboratories, individuals, one utility, and two state government departments.

As a result of the comments, EPA has made minor clarifications to the MDL procedure.
Why Change It?

1. Bottom line is that it is only based on precision (replicates):
   \[ \text{LOD} = \left( \frac{\text{Std. Deviation}}{\# \text{ reps}} \right) \times \left( \frac{t\text{-value}}{\# \text{ reps}} \right) \]

2. It doesn’t matter what recovery you get on the replicates.
What’s wrong with precision?

• Precision is based on the instrument condition, analyst technique, reagent quality, and other issue such as background contamination.

• And with the LOD formula, the better the precision, the LOWER the LOD.

• So... if ONE analyst analyzes 7 replicates all in the same day, and even within the same hour, unless there is background contamination, variability is minimized and the LOD will be ↓↓↓↓.

• How does that compare with “real life”: analyst variability, instrument condition, reagents, etc.?
If you analyze a 0.2 ppm standard and measure 0.021 (on average).

Then if you measure a standard at the LOD (0.02 ppm), wouldn’t you expect to measure 0.002?
The Good, The Bad, & the Ugly

• **Good:** If you only do BOD and/or TSS in your lab...you don’t have to worry about this!

• **Bad:** Didn’t fix the precision issue.

• **Ugly:** Allows LOD to be raised to the level of background contamination. And that might have permit issues.
What has NOT changed

- It’s still based on precision (standard deviation).
- You still need to analyze spiked blanks to determine the LOD.
- You still have to do something annually (the something has changed).
- It remains in your best interest to perform a “reasonableness” check.
WHAT’S NEW?

• Clearly specifies that MDL is inappropriate for (WET), Micro, BOD/cBOD), color, pH, specific conductance, and titration methods.

• Provides options/alternatives to determine the initial spiking level to determine the MDL.

• Requires assessment of routine blanks in addition to replicate spiked blanks.

• Requires MDL “spikes” be separated and analyzed over time. Over at least 3 separate calendar days.

• Discourages outlier rejection of replicate spikes. Exclusion ONLY if can document a valid reason.
• All instruments in use must be incorporated.

• If you use more than one OR add an instrument: Must prepare/analyzer (on different calendar dates) at least 2 spikes and 2 blanks per instrument.

• One prep sample may be analyzed on multiple instruments so long as still have 7 spikes from at least 3 separate batches.

• No more “validation” of the MDL.
  i.e. Spike level > MDL > 10% spike level
Definition - What are we determining?

Original (1984) and Initially Proposed (Feb 2015)

MDL = the minimum concentration of a substance that can be measured and reported with 99% confidence that the analyte concentration is greater than zero and is determined from analysis of a sample in a given matrix containing the analyte.

August 2017

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.

NR149 (existing and proposed revisions)

LOD = the lowest concentration or amount of analyte that can be identified, measured, and reported with confidence that the concentration is not a false positive value.

Analyte is greater than 0

Soapbox time

Method blanks consider both contamination and normal background “noise”. Why address contamination? Everything becomes “ND”!

Analyte is distinguishable from method blanks

Analyte is not a false positive
Wait...WHY do we have to do this?

We follow orders, son. We follow orders or people die, it's that simple.

Are we clear?
Housekeeping: A few notes

- WI uses "LOD". 40 CFR uses "MDL". We consider MDL equivalent to LOD.
- Regulation discusses "spiked samples"; you known better as LOD replicates, standards, or spiked blanks (LCS).
- Method blank here is designated as "MB"
- Creates terms: MDL\_B (LOD\_B) for LOD based on [method] blanks
- MDL\_S (LOD\_S) for LOD based on LOD replicates (actually spiked blanks).
- SD designates the standard deviation
- QTR means calendar quarter (3 months)
b. Process a minimum of 7 spiked blanks PLUS minimum of 7 method blanks
   – at least 3 batches on 3 separate calendar days
   – should not use outlier rejection (except for gross & documented failures)

i. if multiple instruments used, must be spread out over

ii. Minimum per instrument: 2 spikes, 2 method blanks on different days

iii. CAN analyze single prep on multiple instruments

d. Calculations
   i. Calculate SD of spiked samples and blanks
   ii. LOD$_S$ = $t \times$ SD$_S$
1. Determine Initial [LOD]

d. Calculating the LOD_B
   i. \( LOD_B = \)

C. \( LOD_B = \text{Mean}_{MB} + (t \times \text{SD}_B) \)

NOTE: if \( \text{Mean}_{MB} < 0 \), use “0”

OPTION: IF \( \geq 100 \text{ MB} \) THEN \( LOD_B = 99^{\text{th}} \text{ %ile MB} \)

\( 99^{\text{th}} \text{ percentile} = 0.99 \times \text{the number of blanks} \)

For 100-150 blanks, \( MB_{0.99} = 2^{\text{nd}} \text{ highest blank} \)

e. \( LOD = \text{Greater of } (LOD_S, LOD_B) \)
Minimum 7 LOD replicates. At least 3 batches. 3 separate calendar dates

Use at least 8

Existing LOD (MDL) (LODₜ)

Minimun 7 blanks. At least 3 batches. 3 separate calendar dates

Use at least 50 blanks

*new* “Blank” LOD (LODₐ)

Whichever is greater...

...is the LOD
One year (up to 13 mos) later...

Initial 8 replicates PLUS 8 replicates from the past year.

Existing LOD* (MDL) (LOD$_s$)

Collect all of your blank data over past 2 years.

*new* “Blank” LOD (LOD$_b$)

This is our recommended initial LOD

Whichever is greater...

...is the LOD*

* Be sure to use the t-value for n=16

* Option to continue using existing LOD if certain criteria met
Year 3…and future years…

24 replicates (8 from each of the most current 3 years).

Existing LOD* (MDL) (LOD$_S$)

* Be sure to use the t-value for n=24

Collect all of your blank data over at least the past year (up to 3 years).

*new* “Blank” LOD (LOD$_B$)

Whichever is greater…

...is the LOD
...is the $\text{LOD}_B$.

The $\text{LOD}_S$ is simply the age-old calculation for LOD.

With the new protocol, in addition to the familiar LOD calculation (now $\text{LOD}_S$), you calculate the $\text{LOD}_B$.

The new LOD is set at the greater of $\text{LOD}_B$ & $\text{LOD}_S$.

Let’s go back and review how the $\text{LOD}_B$ is calculated for common wastewater tests (NH3, TP, Chloride).
Breaking down the $\text{LOD}_B$ for the WWTP =

- $\text{Mean}_{MB} + (t \times \text{SD}_{MB})$
- if $\text{Mean}_{MB} < 0$, $\text{Mean}_{MB} = 0$

So, this takes the AVERAGE method blank, and adds to it the standard deviation times the $t$-value for the number of blanks and allows this as a “maximum” predicted background contamination level.

Thus, if your blanks are high and variable in concentration, this will probably dictate your “new” LOD.

**NOTE:** If > 100 MB results can use 99th percentile MB
• If you have 50 blanks, and the average is 0.020 ppm. And the Standard deviation is 0.0008 ppm.

• The t-value for 50 is about 2.4.

• So the LOD\(_B\) would be 0.020 + (0.0008 x 2.4) = 0.020 + (0.00192) = 0.02192 = 0.022

• This kind of consistent, low level blank data would probably have no impact and the new LOD would remain the LOD\(_S\).
Total Phos Example #2

- If you have 50 blanks, and the average is 0.03 ppm. And the Standard deviation is 0.01 ppm...
- The t-value for 50 is about 2.4.
- So the $\text{LOD}_B$ would be $0.03 + (0.01 \times 2.4) = 0.03 + (0.024) = 0.054 = 0.054$
- But if your blanks are elevated and even slightly variable, blank data would probably dictate the new LOD.
2. ONGOING DATA COLLECTION

a. If any samples analyzed in a given QTR prep/analyze a minimum of 2 spiked samples, in separate batches, on each instrument.

d. If method is changed in any way *that can reasonably be expected to affect sensitivity* (e.g., LOD) must re-determine initial LOD and re-start ongoing data collection.

**This is an important piece.**

The key to the new protocol is that everything must be maintained the same:

- Replicate spike concentration,
- Instrument operating condition,
- Reagent quality
3.e. If a new instrument is added, analyze $\geq 2$ spiked blanks and $\geq 2$ MB

- If both MB $< \text{existing LOD}$ then the LOD$_B$ is validated.

- Combine the new spiked blanks to existing data and re-calculate LOD$_S$.

- If new LOD$_S$ $\geq 0.5X$ AND $< 2X$ existing LOD, LOD$_S$ is validated.

- Repeat initial LOD if either LOD$_B$ or LOD$_S$ not validated.
You purchase a Hach DR3900 to replace a Genesys 10.

**Existing LOD$_B$ = 0.015 ppm.**

You analyze 2 blanks: 0.013 and 0.014 ppm
Both are <0.015, so LOD$_B$ is verified.

**Existing LOD$_S$ = 0.020 ppm.**

You analyze 2 spikes:
Combined LOD = 0.039
LOD$_S$ is verified.
3. ONGOING ANNUAL VERIFICATION

a. At least every 13 months, re-calculate $\text{LOD}_s$ and $\text{LOD}_B$ from data collected.

b. Include data within the last 24 months, but only data at the same spike level. Only documented gross failures may be excluded.

   **BUT...** must have at least 7 spikes over 3 calendar days

c. Include the initial LOD spikes if the data were generated within past 24 months.

d. Only use data with acceptable calibration and QC

   *If method is changed in any way that can reasonably be expected to affect sensitivity use only data collected after the change.*
3. ONGOING ANNUAL VERIFICATION

e. Ideally use all MB data from last 24 months. **OPTION** *(use whichever provides more data)*

- Use only the last 6 months of data,
- Use the 50 most recent method blanks

**OR**

**IF** Verified LOD is within 0.5x-2x existing LOD AND < 3% MB are numerical results > existing LOD, **THEN** you may **CHOOSE** to keep existing LOD OR use new, verified LOD

**ELSE** *if > 3% MB are > LOD* MUST use new, verified LOD
Got all

That?

Let’s go thru some examples
<table>
<thead>
<tr>
<th>MDL Study Sample Number</th>
<th>Result</th>
<th>% Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Replicate 1</td>
<td>0.028</td>
<td>93%</td>
</tr>
<tr>
<td>Replicate 2</td>
<td>0.027</td>
<td>90%</td>
</tr>
<tr>
<td>Replicate 3</td>
<td>0.032</td>
<td>107%</td>
</tr>
<tr>
<td>Replicate 4</td>
<td>0.031</td>
<td>103%</td>
</tr>
<tr>
<td>Replicate 5</td>
<td>0.027</td>
<td>90%</td>
</tr>
<tr>
<td>Replicate 6</td>
<td>0.029</td>
<td>97%</td>
</tr>
<tr>
<td>Replicate 7</td>
<td>0.027</td>
<td>90%</td>
</tr>
<tr>
<td>Replicate 8</td>
<td>0.031</td>
<td>103%</td>
</tr>
<tr>
<td>Replicate 9</td>
<td></td>
<td>0%</td>
</tr>
<tr>
<td>Replicate 10</td>
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<td>0%</td>
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</table>

Average: 0.029, 96%
Standard Deviation: 0.002

Student's t-value to use: 2.998
Calculated LOD: 0.006 mg/L
75 TP blanks (most recent 6 months) from a WWTP

<p>| | | | | |</p>
<table>
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<th></th>
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<tr>
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<td>0.0010</td>
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Mean: 0.00044
SD: 0.00152
Range: -0.003-0.004
### Pine Stump WWTP Initial LOD

**Manual hotplate**

<table>
<thead>
<tr>
<th>blanks</th>
<th>spikes</th>
<th>LOD spike level 0.03 ppm</th>
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</thead>
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<tr>
<td>75 blanks over most recent 6 months</td>
<td>0.028</td>
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<tr>
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<td></td>
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<tr>
<td><strong>mean</strong></td>
<td>0.00044</td>
<td>0.029</td>
</tr>
<tr>
<td><strong>SD</strong></td>
<td>0.00152</td>
<td>0.002</td>
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</table>

**MDL**

\[
\text{MDL}_S = 2.998 \times 0.029 = 0.006
\]

\[
\text{MDL}_B = 0.00044 + (2.378 \times 0.00152) = 0.004
\]

If had > 100 blanks, could use the 99th percentile blank.

**MDL**

\[
\text{MDL} = \text{MDL}_S = 0.006
\]

**MDL** > **MDL**

**MDL** = **MDL**

**MDL** = 0.006
Ongoing spike data collection records for a wastewater lab may look something like this.
### Pine Stump WWTP

**Ongoing Blanks**

150 TP blanks (1 calendar year) from a WWTP

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<td>-0.001</td>
<td>-0.001</td>
<td>-0.002</td>
<td></td>
</tr>
</tbody>
</table>

**Mean:** 0.0004  
**SD:** 0.0021  
**Range:** -0.004-0.007  
**99th %ile:** 0.007
## Pine Stump WWTP Annual Verification

### Current LOD = 0.006

- **New LOD\(_S\) = 0.0091**
  
  \[
  0.0035 \times 2.602 = 0.0091
  \]

- **New LOD\(_B\) = 0.0053**
  
  \[
  0.0004 + (2.3516 \times 0.0021)
  \]

- **New LOD = 0.009**
  
  0.009 > 0.005

- **Acceptable range = 0.003 to 0.012**
  
  **LOD is verified**

Can keep LOD at 0.006 OR switch to 0.009

---

### Table:

<table>
<thead>
<tr>
<th>MDL Sample ID</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>QTR 1 A</td>
<td>0.034</td>
</tr>
<tr>
<td>QTR 1 B</td>
<td>0.031</td>
</tr>
<tr>
<td>QTR 2 A</td>
<td>0.026</td>
</tr>
<tr>
<td>QTR 2 B</td>
<td>0.028</td>
</tr>
<tr>
<td>QTR 3 A</td>
<td>0.037</td>
</tr>
<tr>
<td>QTR 3 B</td>
<td>0.030</td>
</tr>
<tr>
<td>QTR 4 A</td>
<td>0.036</td>
</tr>
<tr>
<td>QTR 4 B</td>
<td>0.025</td>
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<tr>
<td>Initial LOD 1</td>
<td>0.028</td>
</tr>
<tr>
<td>Initial LOD 2</td>
<td>0.027</td>
</tr>
<tr>
<td>Initial LOD 3</td>
<td>0.032</td>
</tr>
<tr>
<td>Initial LOD 4</td>
<td>0.031</td>
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<tr>
<td>Initial LOD 5</td>
<td>0.027</td>
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<td>Initial LOD 7</td>
<td>0.027</td>
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<tr>
<td>Initial LOD 8</td>
<td>0.031</td>
</tr>
</tbody>
</table>

| Mean:         | 0.0299 |
| SD:           | 0.0035 |
...but remember that you will now have to catalog the following:

- Collect and retain all blank data.... Including dates, blank value and LOD at the time (plus whether exceeded LOD)
- Remember to add Quarterly LOD spikes to your “TO DO” list
- If your blanks run high and variable, your LOD$_B$ may be your LOD and it may not be suitable for permit compliance
- Do a little more analysis annually to determine whether to keep or change the existing LOD
Capturing blank data

- Could be as simple as a handwritten list.

- Could be an Excel (or Google docs...or...) spreadsheet.

- Can your instrument handle it? Hach WIMS?
How many blanks do you need?

• A lab doing phosphorus 3 times/week is going to generate 156 blanks.
• Even if an analysis is only performed once weekly, you would generate 52 blanks per year.
• Consequently, you should have at least 50 and many will have more than 100 blanks.
• The procedure allows you to use only the most recent 50 blanks or 6 months of blank data...whichever yields more data points.

If you run Total Phosphorus at least 3 times/week, in 6 months = at least 72 blanks.
So unless you run < 3x/week, you will need 6 months data
Thanks...Questions?

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