FIAlab 100
Determination of Inorganic Ammonia by Continuous Flow Gas Diffusion and Fluorescence Detector Analysis.

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1. Scope and Application

1. This method is designed for the determination of ammonia [CAS 7664-41-7] in wastewater, natural water and Kjeldahl digestant samples. A method range of 50 – 10,000 µg/L NH_3-N, a Method Limit of 50 µg/L NH_3-N, and a Method Detection Level 12 µg/L NH_3-N of was determined in a validation study

2. Summary of Method

1. This method is designed for the determination of ammonia in wastewater, natural water and Kjeldahl digestant samples by means of Flow Injection Analysis (FIA).
2. The sample is introduced to the analyzer where it is made alkaline with sodium hydroxide. Ammonia is separated from the sample matrix by passage through a gas diffusion cell.
3. After separation in the gas diffusion cell, ammonia is reacted with o-phthalaldehyde to form a fluorescent compound. The reaction product is detected by a fluorimeter and the response is directly proportional to the concentration of ammonia.

3. Definitions

1. Ammonia Stock Standard Solution: A concentrated solution containing method analyte prepared in the laboratory using assayed reference materials or purchased from a reputable commercial source.
2. Calibration Blank: A volume of reagent water acidified with the same acid matrix as in the calibration standards. The calibration blank is a zero standard and is used to calibrate the ammonia analyzer
3. Continuing Calibration Verification (CCV): A calibration standard which is analyzed periodically to verify the accuracy of the existing calibration for those analytes.
4. Calibration Standard: A solution prepared from the dilution of stock standard solutions. These solutions are used to calibrate the instrument response with respect to analyte concentration.
5. Detection Limit (DL), also called Method Detection Limit (MDL): The minimum concentration of an analyte that can be identified, measured, and reported with 99% confidence that the analyte concentration is greater than zero.
6. Dynamic Range (DR): The concentration range over which the instrument response to an analyte is first order linear or second order quadratic.
7. Flow Injection Analysis (FIA): An automated wet chemistry analysis method where the sample is injected into a continuously moving liquid carrier stream. The injected sample is processed by mixing it with reagent stream(s) and a signal proportional to the analyte concentration is provided by an in-line detector.
8. Initial Precision and recovery (IPR): When maintenance or modifications are performed on the instrument or before new analysts run any samples, verify their capability with the method. Prepare and analyze four laboratory fortified blanks (LFB) within a single batch, calculate the mean and standard deviation and compare to the limits listed in this method for IPR.

9. Instrument Performance Check (IPC) Solution: A solution of method analyte, used to evaluate the performance of the instrument system with respect to a defined set of method criteria.
10. Laboratory Fortified Blank (LFB) also called Ongoing Precision and Recovery (OPR): An aliquot of reagent water or other blank matrix to which known quantities of the method analytes are added. The LFB is processed and analyzed exactly like a sample, and its purpose is to determine whether the methodology is in control, and whether the laboratory is capable of making accurate and precise measurements.
11. Laboratory Fortified Sample Matrix/Duplicate (LFM/LFMD) also called Matrix Spike/Matrix Spike Duplicate (MS/MSD): An aliquot of an environmental sample to which known quantities of ammonia is added in the laboratory. The LFM is analyzed exactly like a sample, and its purpose is to determine whether the sample matrix contributes bias to the analytical results. The background concentrations of the analytes in the sample matrix must be determined in a separate aliquot and the measured values in the LFM corrected for background concentrations (Section 9.1.3).
12. Laboratory Reagent Blank (LRB): A volume of reagent water or other blank matrix that is processed exactly as a sample including exposure to all glassware, equipment, solvents and reagents, sample preservatives, surrogates and internal standards that are used in the extraction and analysis batches. The LRB is used to determine if the method analytes or other interferences are present in the laboratory environment, the reagents, or the apparatus.
13. Minimum Reporting Level (MRL): The minimum concentration that can be reported by a laboratory as a quantitated value for a method analyte in a sample following analysis. This concentration must not be any lower than the concentration of the lowest calibration standard for that instrument.
14. Ongoing Precision and Recovery (OPR): Reagent water spiked with known quantities of ammonia, performed at least once per sample batch to demonstrate proficiency with the method. (Also referred to as a Laboratory Fortified Blank). These results are compared to the recovery limits listed in this method for LFB/OPR
15. Sample Batch: A sample batch is defined as a minimum of one (1) to a maximum of twenty (20) analytical samples run during a normal analyst's daily shift.
16. Solid Sample: For the purpose of this method, a sample taken from material classified as soil, sediment or sludge for TKN analysis.
17. Water Sample: For the purpose of this method, a sample taken from one of the following sources: surface, ground, storm runoff, industrial or domestic wastewater.

4. Interferences

1. Sample with high suspended solids will need to be pre-filtered with a 0.45 μm filter prior to analyses.
2. A very strongly acidic sample matrix with pH of 0.5 or less may result in reduced response, due to incomplete conversion of ammonia into the volatile basic form.
3. pH of all samples should be checked before analyses are performed.

4. Presence of chlorine in the sample may need to be neutralized . Refer to the Declorination Note below.

NOTE: Dechlorinating reagent: Dissolve 3.5 g sodium thiosulfate ($Na_2S_2O_3$ 5 H_2O) in DI water and dilute to 1 L. Prepare fresh weekly. Use 1 mL reagent to remove 1 mg/L residual chlorine in 500-mL sample.

5. Samples with pH ≤ 0.5 should be pH adjusted to 6.0 with 6 M NaOH before analyses.
6. Deionized water used in reagents and standards must be high quality and ammonia-free to avoid interference.

5. Safety

1. This method does not address all safety issues associated with its use. The laboratory is responsible for maintaining a safe work environment.
2. Each laboratory is responsible for maintaining compliance with OSHA regulations regarding the safe handling of the chemicals specified in this method. Material Safety Data Sheets (MSDS) should be made available to all personnel using the method.
3. All waste materials should be disposed of in a responsible manner, in accordance with federal, state and other local regulations.
4. The following chemicals have the potential to be highly toxic or highly hazardous, for detailed explanations consult the MSDS:

    4.1. o-phthalaldehyde

    4.2. sodium hydroxide

6. Equipment and Supplies

Note: references to specific brands or catalog numbers are included as examples only and do not imply endorsement of the product. Such reference does not preclude the use of other vendors or suppliers.

1. Balance:
2. Sample weighing balance 0.1 g resolution for preparation of solid samples.
3. Analytical balance with a 0.0001 g resolution for preparation of standards
4. Glassware:
5. Class A volumetric flasks.
6. Volumes dependent on tasks performed. Suggested volumes: 50 mL, 100 mL, 500 mL, 1000 mL.
7. Class A glass volumetric pipettes.
8. Volumes dependent on tasks performed. Suggested volumes: 1 mL, 5 mL, 10 mL, 25 mL, 50 mL.
9. Class B glass or plastic transfer pipettes

10. Volumes dependent on tasks performed. Suggested volumes: 1-10 mL, 1-25 mL
11. Appropriate glass or plastic beakers
12. Volumes dependent on tasks performed. Suggested volumes: 125 mL, 250 mL, 500 mL, 1000 mL, 2000 mL
13. Brown glass or plastic storage containers for samples and standards. Refer to current promulgated 40 CFR Part 136 Table II for samples storage and preservation requirements.
14. Flow injection analysis apparatus or equivalent consisting of:
15. Automated flow injection analyzer, FIAnlyzer -1000 or equivalent
16. Gas diffusion cell, equipped with a Teflon® or polypropylene membrane
17. Flow-through heater
18. Fluorescence detector, FIAlab PMT-FL or equivalent, equipped with a 365 nm light source, 365 nm excitation filter and 430 nm emission filter
19. Autosampler, Cetac ASX-260/520, AIM-3200/3300 or equivalent

7. Method Range

Table 1: Method Range for 3 Inch Sample Loop			
Sample Loop	**Flow Cell**	**Reporting Limit Concentration of Ammonia as N (µg N / L)**	**Method Detection Limit Concentration of Ammonia as N (µg N / L)**
3 inch	100 µL	50 - 10,000	12
Note: Other Sample Loop Lengths (inch) and Flow Cells (µL) can be used to determine Ammonia as N concentrations with different dynamic ranges for different Reporting Limits and MDLs.			

8. Reagents and Standards

8.1 List of chemicals

1. Type 1 Ammonia Free Deionized (DI) Reagent Water (H_2O) [CAS - 7732-18-5].

Note: Sigma Aldrich Part Numbers provided for reference. Other suppliers may be used as long as purity and efficacy of chemical is maintained.

2. Ammonium Chloride (NH_4Cl) 99.99% [CAS - 12125-02-9]. Sigma-Aldrich P/N 326372 or Equivalent.

3. Diethylenetriaminepentaacetic Acid ≥ 99% [CAS - 67-43-6]. Sigma-Aldrich P/N 32319 or Equivalent.
4. Sodium Hydroxide, (NaOH) ≥ 98% [CAS - 1310-73-2]. Sigma-Aldrich P/N S5881 or Equivalent.
5. Sodium Tetraborate Decahydrate ($Na_2B_4O_7 \cdot 10H_2O$) ≥ 99.5 % [CAS - 1303-96-4]. Sigma-Aldrich S9640 or Equivalent.
6. Sodium Phosphate dibasic Heptahydrate ($Na_2HPO_4 \cdot 7H_2O$) ≥ 98% [CAS - 7782-85-6]. Sigma-Aldrich S9390 or Equivalent.
7. Sulfuric Acid (H_2SO_4) concentrated [CAS - 7664-93-9]. Sigma-Aldrich 258105 or Equivalent.
8. o-Phthalaldehyde ($C_8H_6O_2$) ≥ 97 % [CAS - 643-79-8]. Sigma-Aldrich P1378 or Equivalent.
9. Sodium Sulfite (Na_2SO_3) ≥ 98 % [CAS - 7757-83-7]. Sigma-Aldrich S4672 or Equivalent.
10. Ethanol (200 Proof) (CH_3CH_2OH) [CAS - 64-17-5]. Sigma-Aldrich 459828 or Equivalent.
11. Sodium Thiosulfate ($Na_2S_2O_3$ 5 H_2O) Sigma-Aldrich 217247 or Equivalent.

8.2 Preparation of Reagents

Note:

Care must be taken to avoid ammonia contamination in the laboratory. It is recommended that only ammonia-free cleaning products be used in the laboratory.

All solutions must be made with Type 1 ammonia-free DI reagent water.

Such water is best prepared by passage through an ion exchange column containing a strongly acidic cation exchange resin mixed with a strongly basic anion exchange resin.

Regeneration of the column should be carried out according to the manufacturer's instructions.

Carrier: Deionized water, ammonia free.

Note: May be adjusted to ~ pH 3 when the peak height has been reduced due to asymmetrical peak shape. Add 0.1 mL of Sulfuric Acid (H_2SO_4) concentrated to 1 liter Deionized water, ammonia free

Carrier for Kjeldahl digestant samples: 0.1 mL of Sulfuric Acid (H_2SO_4) concentrated to 1 liter Deionized water, ammonia free.

Reagent 1: 50 mM diethylenetriaminepentaacetic acid in 0.3 M sodium hydroxide (500mL).

1. Dissolve 6.0 g sodium hydroxide in 400 mL of deionized water.

Note: Diethylenetriaminepentaacetic acid will neutralize some of the sodium hydroxide. The remaining solution will be 0.05 M sodium hydroxide.

2. Add 9.8 g diethylenetriaminepentaacetic acid to the sodium hydroxide solution, Mix well to dissolve.
3. Add deionized water to a total volume of 500 mL.

4. Mix well and store in a plastic bottle.
5. Prepare fresh daily.

Reagent 1 for Kjeldahl digestant samples or samples run with sulfuric acid solution carrier: 50 mM diethylenetriaminepentaacetic acid in 0.9 M sodium hydroxide (500mL).

1. Dissolve 18.0 g sodium hydroxide in 400 mL of deionized water.

Note: Diethylenetriaminepentaacetic acid will neutralize some of the sodium hydroxide. The remaining solution will be 0.65 M sodium hydroxide.

2. Add 9.8 g diethylenetriaminepentaacetic acid to the sodium hydroxide solution, Mix well to dissolve.
3. Add deionized water to a total volume of 500 mL.
4. Mix well and store in a plastic bottle.
5. Prepare fresh daily.

Reagent 2: 6 mM o-phthalaldehyde solution (500 mL)

1. Dissolve 10 g sodium tetraborate decahydrate OR 24.5 g sodium phosphate dibasic heptahydrate in 500 mL water to create a buffer solution.
2. Dissolve 0.4 g o-phthalaldehyde in 2mL ethanol. Add 2 mL of this solution to the buffer solution.
3. Dissolve 120 mg sodium sulfite in 2 mL water. Add 1.5 mL of this solution to the buffer solution.
4. Mix well and store in a dark glass bottle.

Note: Use of high quality laboratory bottles is important.

8.3 Preparation of standards

Standard Stock Solution: 1000 mg N / L (1000 mL). Purchase standard or prepare as per instructions below.

1. Dissolve 3.819 g of anhydrous ammonium chloride, dried at 105°C, in 800 mL carrier water in a Class a 1000 mL volumetric flask.
2. Add carrier water to a total volume of 1000 mL.
3. Mix well and store in a plastic bottle. Shelf life of 360 days or as per manufacturer's directions.

Stock Solution #1: 10 mg N / L (1000 mL).

1. Transfer 10 mL of Standard Stock Solution with a Class A pipette to a 1000 mL Class A volumetric flask.
2. Add carrier water to a total volume of 1000 mL.
3. Mix well and store in a plastic bottle. Shelf life of 1 week.

Standard working solutions:

1. Standard's dynamic range.

Table 2: Standard's Dynamic Range			
Setup	**Sample Loop**	**Flow Cell**	**Standards Range µg N-NH4 / L**
1	3 Inch	100 µL	50 - 10,000

2. Dilute standard stock solution with carrier water to the desired number of standards. Concentrations selected for standards must meet the following criteria:

   2.1. A minimum of 5 standards and a calibration blank for a quadratic calibration slope.

3. Suggested Standards' dilutions and preparation.

   3.1. Utilizing Class A pipettes transfer the aliquot of Stock Solution #1 to a Class A volumetric flask as listed in Table 2.

   3.2. Dilute to the volumetric mark with carrier water, mix well by inversion and transfer to a plastic bottle. Prepare fresh each day of use.

Table 3: Standards' Dilutions		
mL of Stock Solution 10 mg N / L	**Volumetric Flask (volume mL)**	**Concentration of Final Standard (µg N / L)**
Use As Is	NA	10,000
500	1000	5,000
100	1000	1,000
70	1000	700
50	1000	500
10	1000	100
5	1000	50

9. Sample Collection, Preservation and Storage

1. Samples should be collected in plastic or glass bottles. All bottles must be thoroughly cleaned and rinsed with reagent water. Volume collected should be sufficient to insure a representative sample, allow for replicate analysis (if required), and minimize waste disposal.

2. Samples must be preserved with H_2SO_4 to a pH <2 and cooled to ≤ 6°C at the time of collection.
3. Samples should be analyzed as soon as possible after collection. If storage is required, preserved samples are maintained at ≤ 6°C and may be held for up to 28 days.

Refer to current promulgated 40 CFR part 136 Table II and subsequent footnotes for all updates applicable to these analytes.

10. Quality Control

1. Each laboratory using this method is required to operate a formal quality control (QC) program. The minimum requirements of this program consist of an Initial Demonstration of Capability (consisting of an MDL study, and analysis of four IPR samples), a LRB, LFB/OPR, LFM, LFMD, and CCV for each Sample Batch and the use of control charts to determine Ongoing Demonstration of Capability. The laboratory is required to maintain performance records that define the quality of the data that are generated.
2. A LRB, LFB/OPR, LFM, LFMD, and CCV must be run with each sample batch. Failure of any QC sample in sample batch will require a corrective action and may require the sample batch to be reanalyzed.

10.1 Initial Precision and Recovery (IPR)

1. Initial precision and recovery (IPR) – to establish the ability to generate acceptable precision and accuracy, the laboratory shall perform the following operations:
2. Determine the concentration of ammonia in four LFBs, run within a single analytical batch.
3. Then, using the results of the set of four analyses, compute the mean percent recovery ($\bar{X}$) and the standard deviation of the percent recovery (s). Use the following equation for calculation of the standard deviation of the percent recovery:

$$s = \sqrt{\frac{\sum x^2 - \frac{\left(\sum x^2\right)}{n}}{n-1}}$$

where:

n = Number of samples

x = % recovery in each sample

4. Calculate the Relative Standard Deviation (RSD), using the mean ($\bar{X}$) and standard deviation (s) calculated in step 3:

$$RSD = \frac{s}{\bar{X}} * 100\%$$

5. Compare the RSD and $\bar{X}$ to the following limits for initial precision and recovery:

Mean percent recovery ($\bar{X}$): 86% - 121%.

RSD of the percent recoveries: Must not exceed 10%

6. If the RSD and $\bar{X}$ meet the acceptance criteria, system performance is acceptable and analysis of samples may begin. If, however, the RSD exceeds the precision limit or $\bar{X}$ falls outside the range for recovery, system performance is unacceptable. In this event, correct the problem and repeat the test.

10.2 Method Detection Limit, Blank (MDL_B)

1. Method Detection Limit (MDL_B) – MDL_B must be established for all analytes, using Type 1 Ammonia Free Deionized (DI) Reagent Water.
2. To determine MDL values, take seven replicate aliquots of the reagent water and process through the entire analytical method.
3. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

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

$$\text{If } \bar{X} < 0, \text{then use } 0$$

where, $t_{n-1,1-\alpha=(0.99)}$ = *Student's t value for a* 99% *confidence level and a standard deviation estimate with* $n-1$ *degrees of freedom* $[t = 3.14$ *for seven replicates*$]$

S_b = *standard deviation of the replicate analyses*

4. MDLs should be determined every six months, when a new operator begins work or whenever there is a significant change in the background or instrument response.
5. MDL values must be below the lowest reporting limit for the analyses. (See Table 4-Figure 1)

MDLs must be determined as per the current promulgated 40 CFR part 136 Appendix B.

10.3 Method Detection Limit, Spike (MDL_S)

1. Method Detection Limit (MDL_S) -- MDL_S must be established for all analytes, using Type 1 Ammonia Free Deionized (DI) Reagent Water fortified at a concentration of two to three times the estimated instrument detection limit.
2. To determine MDL values, take seven replicate aliquots of the fortified reagent water and process through the entire analytical method.
3. Perform all calculations defined in the method and report the concentration values in the appropriate units. Calculate the MDL as follows:

$$MDL = t \times S$$

where, t = *Student's t value for a* 99% *confidence level*

and a standard deviation estimate with $n-1$ *degrees of freedom*

$[t = 3.14$ *for seven replicates*$]$

S = *standard deviation of the replicate analyses*

4. MDLs should be determined every six months, when a new operator begins work or whenever there is a significant change in the background or instrument response.
5. MDL values must be below the lowest reporting limit for the analyses. (See Table 4-Figure 1)

MDLs must be determined as per the current promulgated 40 CFR part 136 Appendix B.

10.4 Laboratory Reagent Blank (LRB):

1. Carrier water that is analyzed as a sample. Analyzed at a frequency of no less than once per sample batch. The blank result must be below the lowest calibration standard or the method level. See Table 11-Figure 6.

10.5 Laboratory Fortified Blank (LFB):

1. A sample of known concentration of ammonia in carrier water. Analyzed at a frequency of no less than once per sample batch. The concentration of the LFB should be between 10% and 50% of the calibration range set by the calibration standards.
2. The ongoing recovery must be between 85% and 122%.

Percent Recovery LFB calculation:

$$\left(\frac{Experimental\,Value}{Expected\,Value}\right) * 100 = \text{ Percent Recovery LFB}$$

$Experimental\,Value$ = LFB Concentration determined experimentally

$Expected\,Value$ = Known LFB concentration

10.6 Laboratory Fortified Matrix Spikes (LFM and LFMD) or Matrix Spikes (MS/MSD):

1. A duplicate set of ammonia water or Kjeldahl digestant samples spiked with a known amount of ammonia so that the overall concentration of the spiked sample is within the dynamic calibration range of the instrument. Analyzed at a frequency of no less than once per sample batch. A Relative Percent Difference will be calculated for each LFM/LFMD or MS/MSD set.
2. The LFM/LFMD or MS/MSD recovery must be between 85% and 120%.
3. The RPD must not exceed 16%.Percent Recovery LFM Calculation

$$\left(\frac{\text{Spiked Sample Result - (d x Unspiked Sample Result)}}{\text{Concentration of Spike}}\right)*100 = \text{Percent Recovery LFM}$$

Spiked Sample Result = LFM concentration determined experimentally

Unspiked Sample Result = Concentration of sample before spiking

d=Dilution Correction

Relative Percent Difference (RPD) Calculation

$$\left(\frac{(LFM - LFMD)}{\left(\frac{LFM + LFMD}{2}\right)}\right)*100 = \text{RPD}$$

LFM = Concentration determined for LFM

LFMD = Concentration determined for LFM duplicate

10.7 Continuing Calibration Verification (CCV):

1. Check standards will be prepared so as to have a concentration between the ML and upper calibration standard. They must be run at a minimum frequency of once for each batch of 20 or fewer samples including QC samples.

$$\left(\frac{A-B}{A}\right)*100 = \text{CCV Concentration Difference}$$

$A = \text{Standard Concentration}$

$B = \text{Concentration Determined for CCV Sample}$

2. If the CCV Concentration Difference exceed ±10% of the reported Standard Concentration value:

   2.1. The analysis will be stopped.

   2.2. Only sample values in the batch between acceptable CCV Concentration Difference values can be reported.

   2.3. The instrument recalibrated before continuing analysis.

10.8 Dynamic Range (DR)

1. The DR must be determined initially and with each day of use. This is performed by calibrating the instrument for a quadratic range as per the manufacturer's directions.

10.9 Relative Standard Error (RSE)

1. The RSE must be determined initially and with each day of use. This is performed by calibrating the instrument for a quadratic range as per the manufacturer's directions.

$$\%RSE = 100 \times \sqrt{\sum_{i=1}^{n} \frac{\left[\frac{x_i' - x_i}{x_i}\right]^2}{n-p}}$$

1. The true concentration of each calibration standard. This is x_i
2. The measured concentration of each calibration standard. This is x_i'
3. The number of standard levels in the curve. This is n
4. The type of curve (average, linear or quadratic)

   the type of curve determines the value of p.

   For an average curve, p=1, for linear p=2 and quadratic p=3

2. A RSE of ≤ 22% must be obtained with each calibration.

10.10 Instrument Performance Check (IPC) Solutions

1. Solutions consisting of the LRB, LFB, LFM/LFMD and CCV ran per each batch of samples. These results will be charted on control charts to determine analyses performance for the laboratory.

10.11 Sample Batch

1. A group of samples which behave similarly with respect to the sampling or the testing procedures being employed and which are processed as a unit. For QC purposes, if the number of samples in a group is greater than 20, then each group of 20 samples or less will all be handled as a separate batch. A batch cannot span between laboratory work days (24 hrs.). New batches must be started each laboratory work day.
2. Sample Batch: Typical sample analysis sequence.

   2.1. Instrument Start Up

   2.2. Calibration blank

   2.3. Calibration Standards, Quadratic

   2.4. LRB

   2.5. LFB

   2.6. Sample used for LFM/LFMD

   2.7. LFM

   2.8. LFMD

   2.9. Samples (First half of batch)

   2.10. CCV

   2.11. Samples (Second half of batch)

   2.12. CCV

11. Instrument Calibration and Standardization

1. Prepare a series of at least 5 standards (quadratic), covering the desired range, and a blank by diluting suitable volumes of standard solutions (Table 1-3). A minimum of 100 mL of standard is needed.
2. Set up analyzer as shown in FIAlab manufacturer's manual.
3. Place appropriate standards in the sampler in order of increasing concentration and perform analysis.
4. After the calibration has been established, it must be verified at a minimum frequency of once for each batch of 20 or fewer samples including QC samples.
5. If the CCV Concentration Difference exceed ±10% of the reported Standard Concentration value:

   5.1. The analysis must be stopped.

   5.2. Only sample values in the batch between acceptable CCV Concentration Difference values can be reported.
6. The instrument must be recalibrated before continuing analysis.

12. Sample Analysis Procedure

1. Set up the FIA analyzer and establish initial operating conditions. If the analyzer is computerized, select the appropriate method and set up a sample table for the run.
2. Adjust the heater set point if necessary and allow the heater temperature to stabilize.
3. Verify that the reagents are flowing smoothly through the analyzer and that the flow cell is free of air bubbles.
4. Load the autosampler with ammonia standards, QC samples and unknown samples.
5. Begin analysis.

13. Data Analysis and Calculations

1. Prepare a calibration curve by plotting instrument response against standard concentration.
2. Compute sample concentration by comparing sample response with the standard curve. Multiply answer by appropriate dilution factor.
3. Report only those values that fall between the lowest and the highest calibration standards. Samples exceeding the highest standard should be diluted and reanalyzed.
4. Report results in µg NH_3-N/L.

14. Method Performance

1. Maintain control charts for all QC parameters of the instrument and for each analysis batch. All analyses that fall outside the control limits must be reviewed by the laboratory QC staff before they are accepted.

2. Results obtained from statistical report *"Ammonia-001,Determination of Inorganic Ammonia in Wastewater and Kjeldahl Nitrogen Digestant by Flow Injection Gas Diffusion and Fluorescence Detector Analysis, Final Data and Statistical Validation Report 2/15/18"* provided in the Tables and Figures section.

15. Pollution Prevention

1. The quantity of chemicals purchased should be based on expected usage during its shelf life and disposal cost of unused material. Actual reagent preparation volumes should reflect anticipated usage and reagent stability.
2. For information about pollution prevention that may be applicable to laboratories and research institutions, consult "Less is Better: Laboratory Chemical Management for Waste Reduction".

16. Waste Management

1. Each laboratory is responsible for maintaining compliance with regulations regarding the safe handling and disposal of the chemicals specified in this method.

17. References

1. Aminot A., Kérouel R., Birot D., "A flow injection-fluorometric method for the determination of ammonium in fresh and saline waters with a view to in situ analyses", *Water Research*, 35(7), 2001, 1777-1785.
2. Less is Better: Laboratory Chemical Management for Waste Reduction. Available from the American Chemical Society, Department of Government Regulations and Science Policy, 1155 16th Street, NW, Washington, DC 20036

18.Tables and Figures

Table 4: Method Detection Limit and Method Limit				
Laboratory Name	**Individual Laboratory Pooled MDL**	**Overall Pooled MDL**	**Total Laboratories Minimum Level (not rounded)**	**Reporting Method Limit**
1	27.1028	12.2745	39	50
2	4.5413			
3	11.8246			
4	20.9661			
6	28.4678			
7	10.0953			
8	6.0706			
9	5.3284			
10	15.5804			
11	2.8266			
12	17.2399			
13	18.8200			

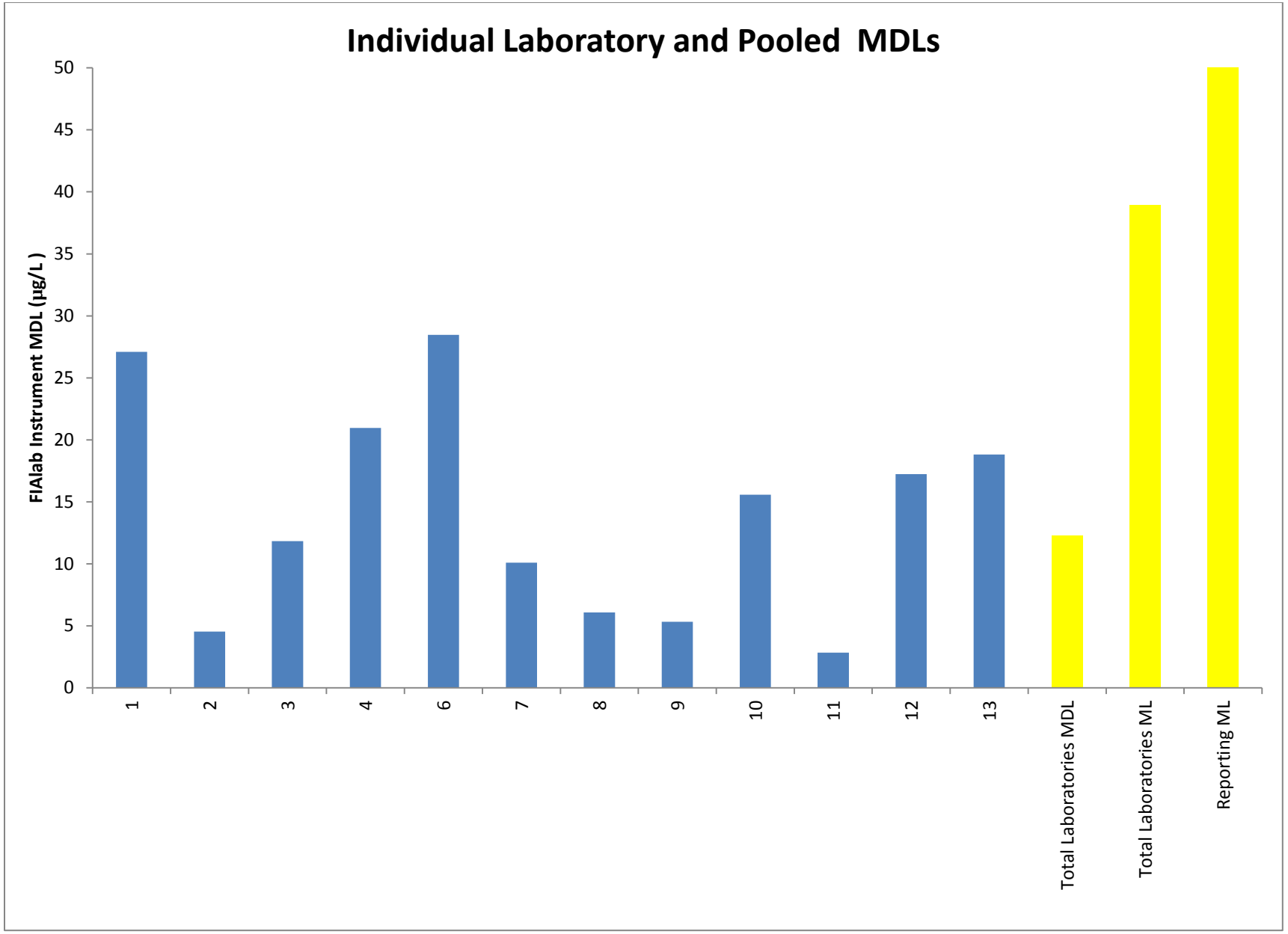

Figure 1: MDL Chart

Table 5 Matrix Spike/Matrix Spike Duplicate Individual Laboratory Results					
Laboratory	**Matrix Spike Recovery**	**Matrix Spike Duplicate Recovery**	**Individual Laboratory Mean**	**Individual Laboratory Standard Deviation**	**Individual Laboratory Relative Percent Difference (RPD)**
1	91.30%	102.44%	96.87%	7.88%	11.50%
2	98.86%	99.80%	99.33%	0.66%	0.95%
3	94.92%	99.62%	97.27%	3.33%	4.84%
4	97.80%	98.09%	97.95%	0.20%	0.29%
6	105.32%	104.09%	104.70%	0.87%	1.17%
7	93.57%	93.69%	93.63%	0.09%	0.13%
8	101.12%	116.87%	108.99%	11.13%	14.45%
9	109.18%	121.76%	115.47%	8.89%	10.89%
10	113.27%	110.02%	111.65%	2.30%	2.91%
11	104.34%	100.74%	102.54%	2.55%	3.51%
12	104.01%	100.69%	102.35%	2.35%	3.25%

Table 6 Matrix Spike/Matrix Spike Duplicate Pooled Laboratory Results						
All Laboratory Mean	**Pooled Within Laboratory Standard Deviation (S_w)**	**Between Laboratory Standard Deviation of the Mean Results (S_b)**	**RPD (Max)**	**Combined Standard Deviation (S_c)**	**All Laboratory MS/MSD Lower Limit**	**All Laboratory MS/MSD Upper Limit**
102.80%	5.18%	6.83%	15.52%	8.04%	85.10%	120.49%

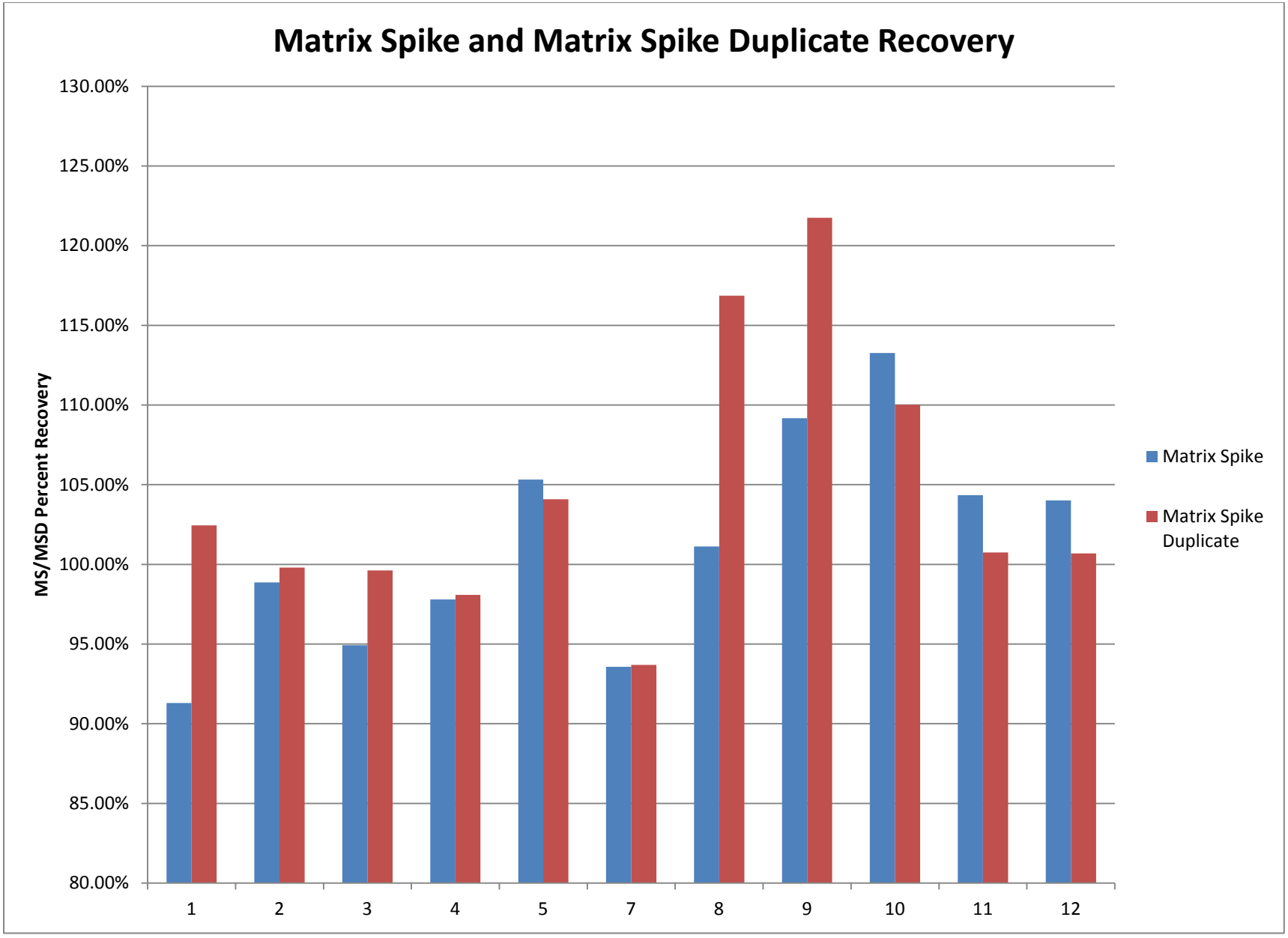

Figure 2: Matrix Recovery

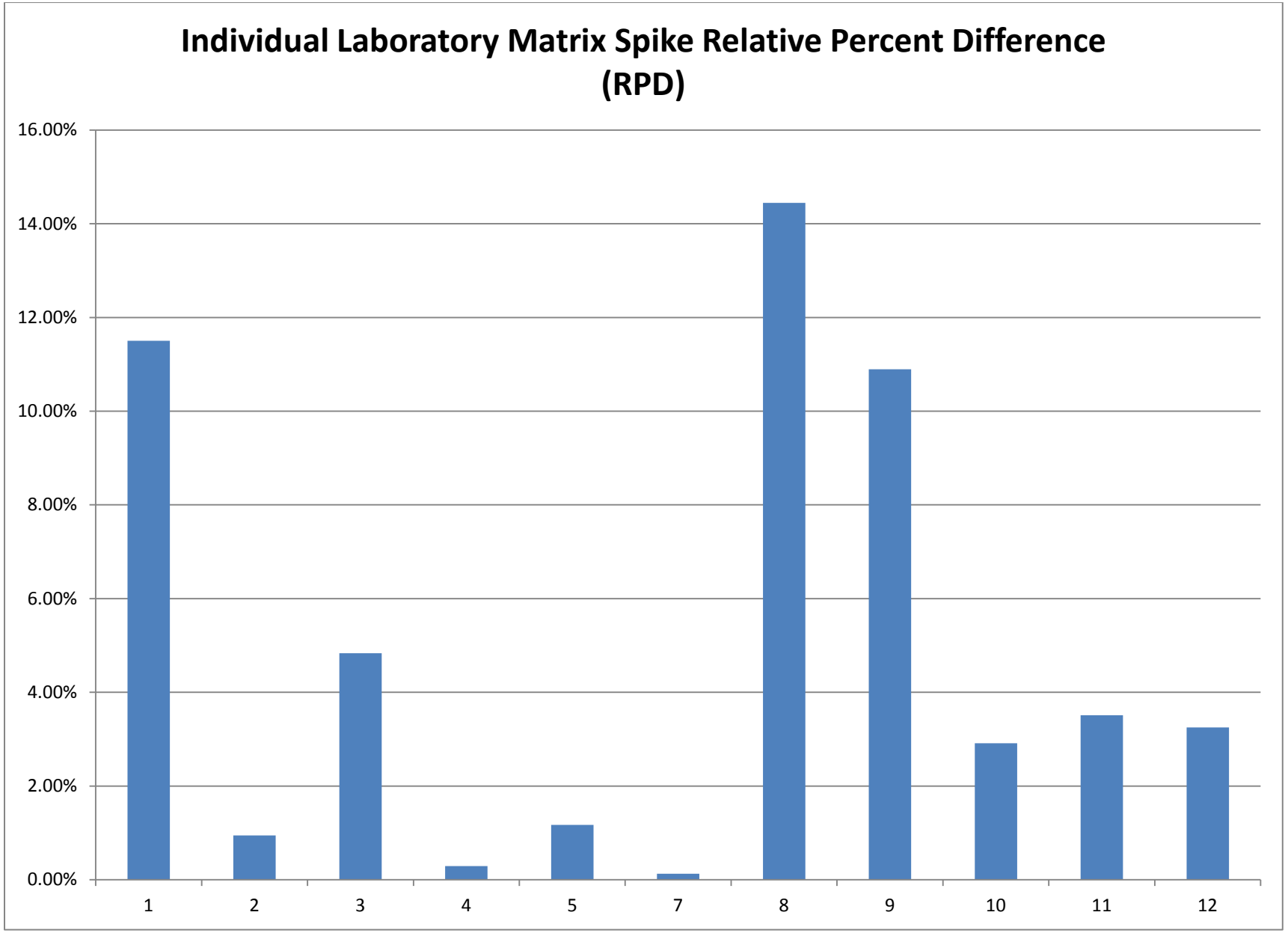

Figure 3: Matrix Spike Relative Percent Difference

Table 7: LFB Percent Recovery and % RSD					
Laboratory	**Single Lab Average % Recovery**	**Single Lab STDEV**	**Total Lab Average % Recovery**	**Total Lab STDEV**	**Total Lab % RSD**
2	112.31%	0.15036	102.48%	0.05820	5.68%
3	100.06%	0.04122			
7	92.81%	0.04806			
8	97.79%	0.03001			
9	99.23%	0.01951			
10	105.95%	0.02099			
11	108.73%	0.03458			
12	101.99%	0.01405			
13	103.42%	0.02750			

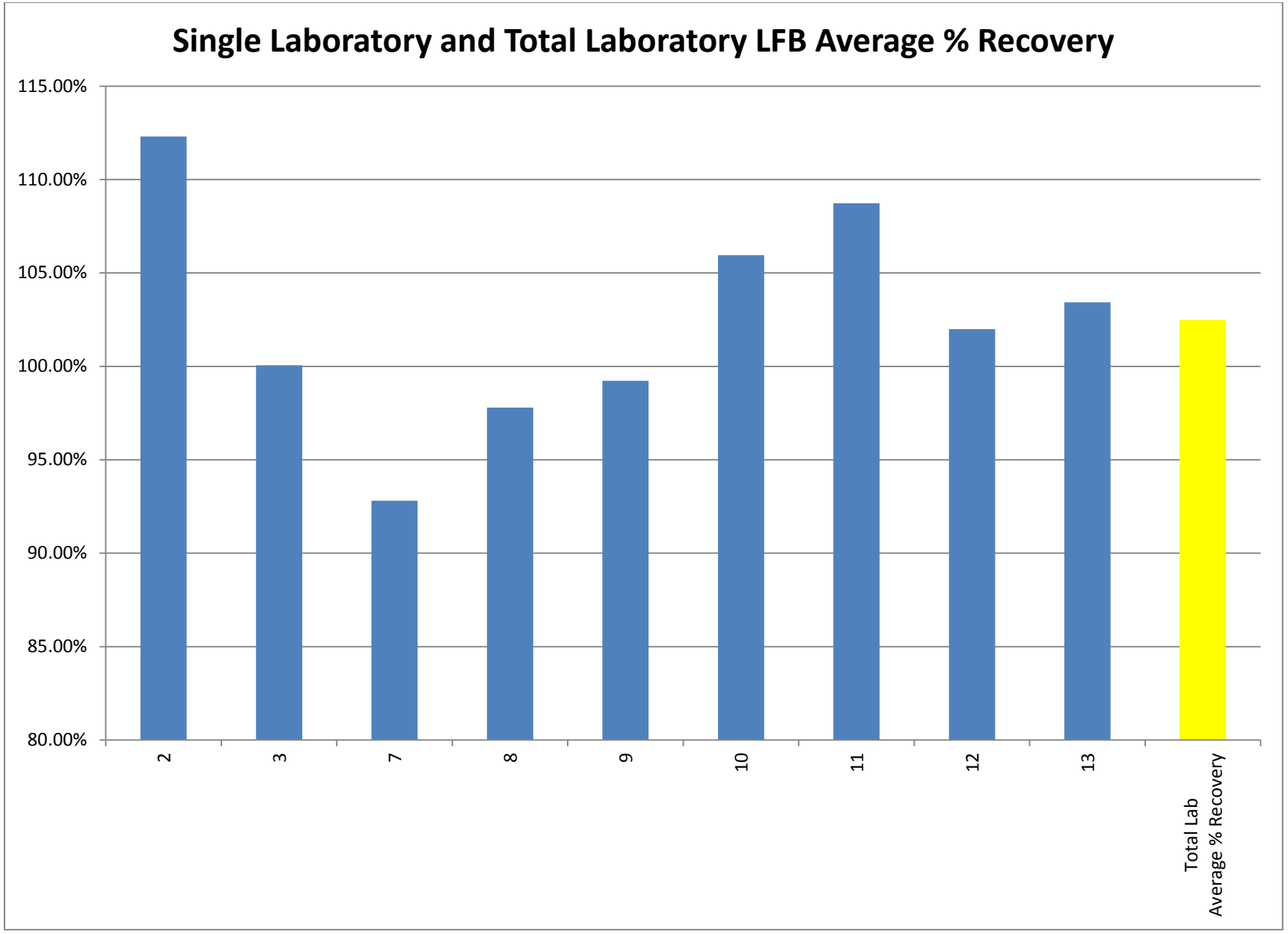

Figure 4: Average LFB % Recovery

Table 8: % RSE Calibration Check			
Laboratory	**Individual Laboratory Pooled % RSE**	**All Laboratory's % RSE (pooled)**	**% RSE Cal Max**
1	17.01	14.42 %	22 %
2	4.96		
3	14.17		
4	19.70		
5	15.68		
6	6.15		
7	15.35		
8	4.88		
9	9.63		
10	15.23		
11	9.62		
12	13.38		
13	25.71		

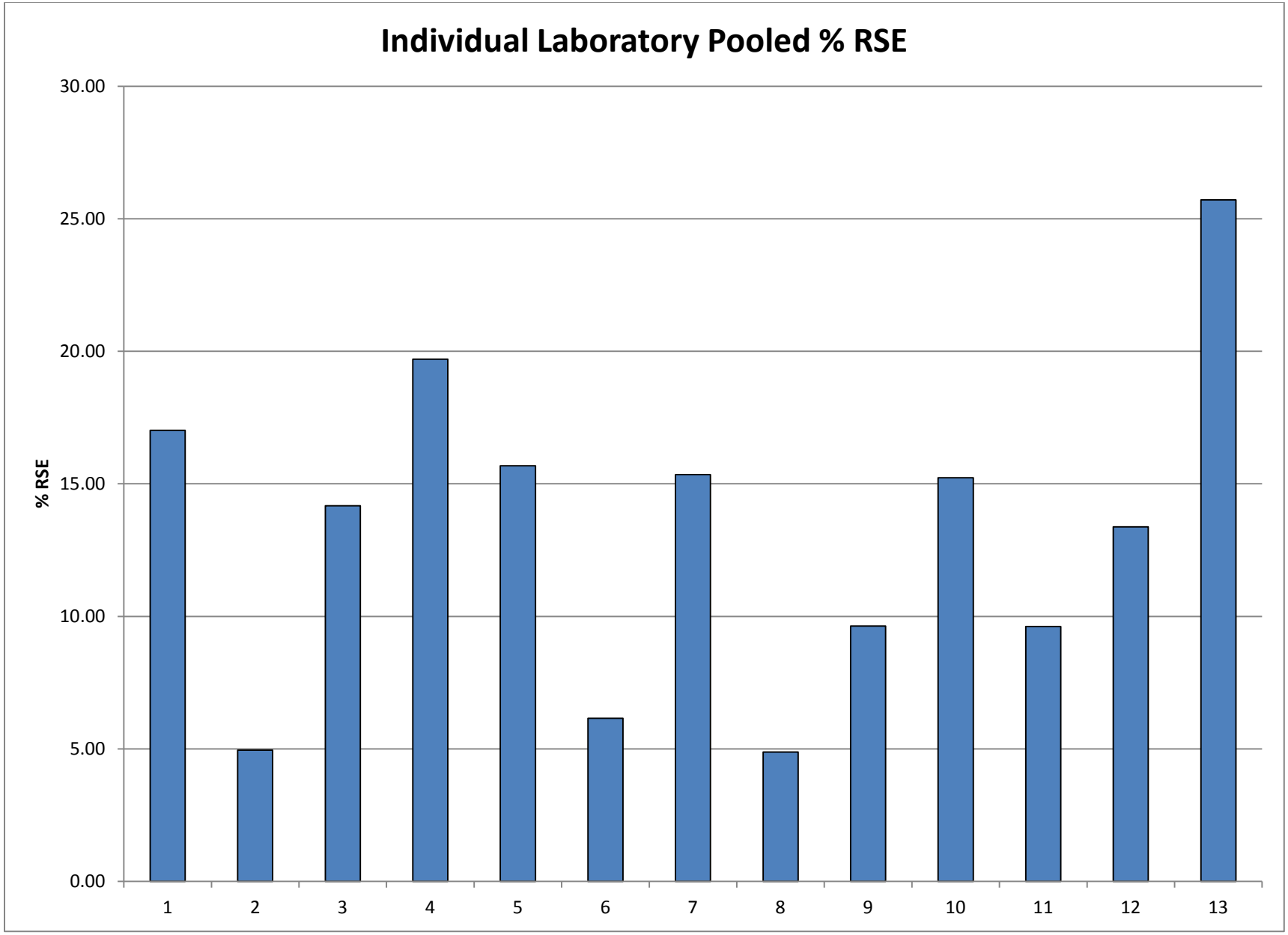

Figure 5: Individual Laboratories Pooled % RSE Values

Table 9: Low Concentration CCC				
Laboratory	**Single Lab Bias**	**Single Lab Precision**	**Total Lab Bias**	**Total Lab Precision**
1	3.82	6.25	2.13	5.82
5	1.08	3.26		
6	6.05	7.21		

Table 10: High Concentration CCC				
Laboratory	**Single Lab Bias**	**Single Lab Precision**	**Total Lab Bias**	**Total Lab Precision**
2	-33.91	39.26	22.50	123.37
3	-4.57	56.88		
7	-108.68	131.81		
8	-65.33	91.80		
9	19.55	28.88		
10	151.57	177.53		
11	235.13	273.17		
12	16.63	20.97		
13	32.90	63.81		

Table 11: Laboratories LFB Concentrations IPR-OPR Table													
Laboratory	**Individual Laboratory IDC Lower Limit**	**Individual Laboratory IDC Upper Limit**	**All Laboratory Mean**	**Pooled Within Laboratory Standard Deviation (S_w)**	**RSD**	**RSDIPR,max**	**S_b**	**S_{ic}**	**IPR High (Pooled)**	**IPR Low (Pooled)**	**OPR High (Pooled)**	**OPR Low (Pooled)**	**S_{oc}**
1	93.29%	103.07%	103.71%	0.05919	5.71%	9.53%	0.07026	0.07617	121.23%	86.19%	122.53%	84.88%	0.08964
2	53.61%	187.81%											
3	83.57%	119.86%											
5	68.46%	156.26%											
6	35.61%	164.08%											
7	82.99%	108.10%											
8	83.52%	114.28%											
9	87.40%	109.48%											
10	93.39%	117.11%											
11	94.99%	118.18%											

12	95.72%	109.65%	[redacted]
13	96.42%	112.12%	

Table 12 Laboratory Reagent Blank								
Laboratory	**Measurement 1 (ug/L)**	**Measurement 2 (ug/L)**	**Measurement 3 (ug/L)**	**Measurement 1 Below ML**	**Measurement 2 Below ML**	**Measurement 3 Below ML**	**ML (ug/L)**	**IDL (ug/L)**
1	-11.19	-9.8	-10.58	**Pass**	**Pass**	**Pass**	50	33
2	8.74	8.22	7.99	**Pass**	**Pass**	**Pass**		
3	21.42	21.04	23.6	**Pass**	**Pass**	**Pass**		
4	-4.58	6.42	5.81	**Pass**	**Pass**	**Pass**		
5	11.78	11.51	11.88	**Pass**	**Pass**	**Pass**		
6	17.42	21.1	22.65	**Pass**	**Pass**	**Pass**		
7	0.06	-0.71	1.37	**Pass**	**Pass**	**Pass**		
8	12.04	11.18	17.47	**Pass**	**Pass**	**Pass**		
9	15.39	19.34	14.49	**Pass**	**Pass**	**Pass**		
10	3.18	2.31	0.12	**Pass**	**Pass**	**Pass**		
11	6.59	7.08	5.87	**Pass**	**Pass**	**Pass**		
12	-1.4	-4.11	-6.11	**Pass**	**Pass**	**Pass**		

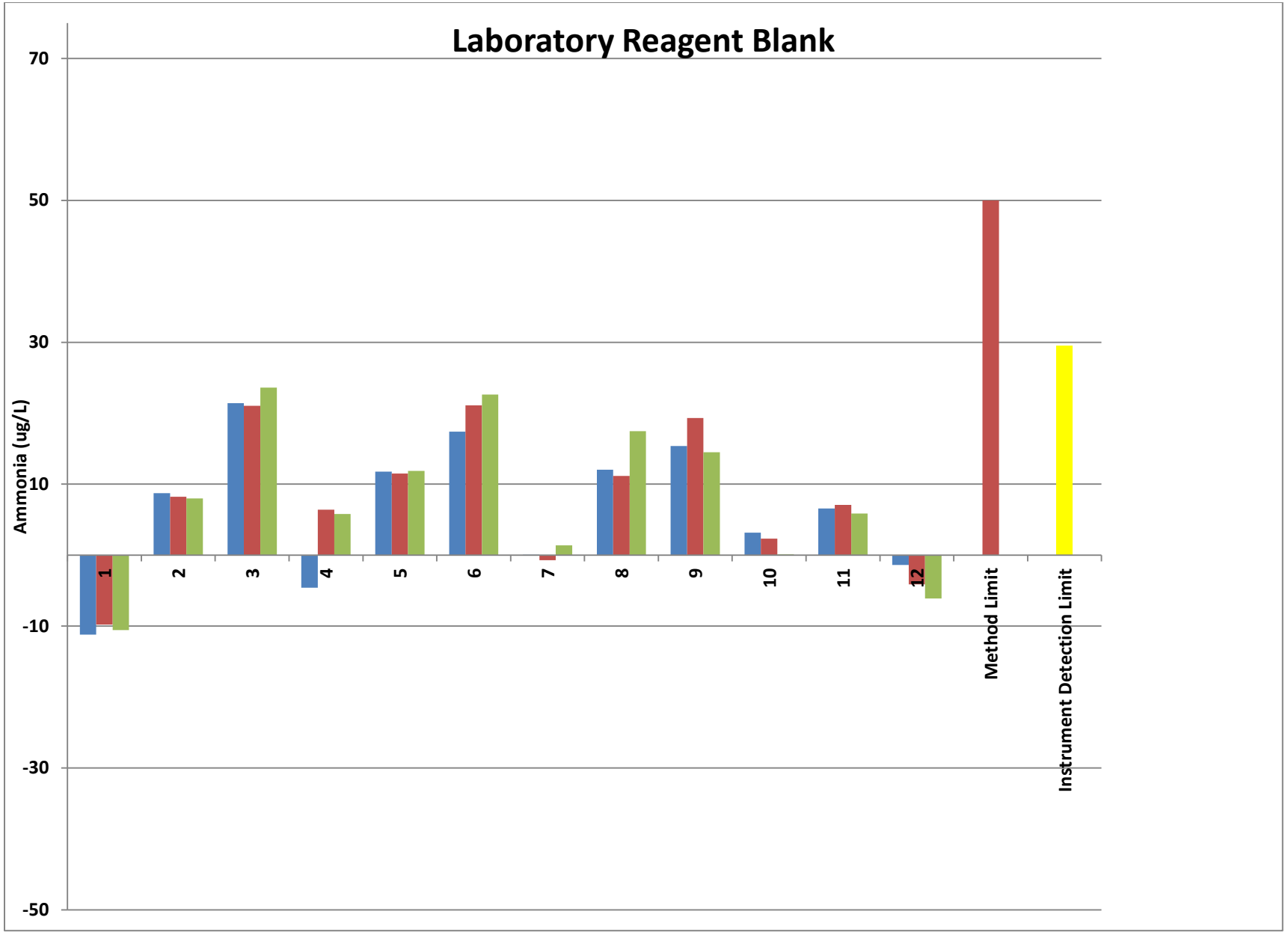

Figure 6: LRB-IDL-ML