CHESAPEAKE BAY PROGRAM DATA ANALYSIS ISSUES TRACKING SYSTEM

ISSUE TRACKING NUMBER: 051

CATEGORY CODE: Analytical Methods (AM)

ISSUE TITLE: Comparison of field preservation methods for nitrate-nitrite and dissolved organic carbon analyzed at the Department of Health and Mental Hygiene

DATE OF INTRODUCTION OF THIS TO THE SYSTEM: September 2011

STATEMENT OF ISSUE:
The procedures used by the Maryland Department of Natural Resources(DNR)for preserving dissolved nitrate-nitrite(NO23)and dissolved organic carbon(DOC) samples under the non-tidal monitoring programs do not conform to the EPA Region 3 policy for ambient surface water monitoring, which is to follow Clean Water Act requirements. According to Table 2(Required Containers, Preservation Techniques, and Holding Times) in Title 40 Code of Federal Regulations Part 136, "Guidelines Establishing Test Procedures for the Analysis of Pollutants under the Clean Water Act", NO23 samples are required to be preserved by cooling to $\leq 6^{o}C$ and acidifying with sulfuric acid (H_2SO_4) to pH<2. DOC samples are also required to be preserved by cooling to $\leq 6^{o}C$, but must be acidified with hydrochloric acid (HCl) (Code of Federal Regulations, 2007).

Rather than using acid preservation as specified by Title 40, DNR preserves NO23 and DOC samples either by icing them at $\leq 4^{\circ}C$ for short holding times or by freezing at $-20^{\circ}C$ for longer holding times. The regulation prohibits freezing of samples "unless data demonstrating that sample freezing does not adversely impact sample integrity is maintained on file and accepted by the regulatory authority", i.e., EPA Region 3. In 2010, DNR conducted a study to evaluate the impact of their preservation techniques on sample integrity.

DNR's Annapolis Field Office staff collects water samples and filters them in the field at 55 non-tidal fresh water stations as part of the CORE/Trend monitoring program (Appendix A) and at 13 non-tidal fresh water stations as part of the non-tidal network (Appendix B) (Although there are 55 historical CORE/Trend non-tidal stations one of these stations is no longer included in the analyses that are performed under the CORE/Trend program, so there were 54 candidate sites included in this study.) DNR initiated field-filtering in July 2005 for both the CORE/Trend and non-tidal network programs. Funding for the CORE/Trend program and the non-tidal network is provided by the U.S. Environmental Protection Agency under Sections 106 and 117 of the Clean Water Act and the results and recommendations described herein only apply to field-filtered samples collected under those two grants starting in July 2005.

DNR has monitored non-tidal fresh water streams in Maryland since the early 1970s in response to the Clean Water Act. The original DNR lab that analyzed samples for NO23, DOC, and other nutrients implemented preservation methods based on refrigeration, since samples were analyzed within 24 hours. Subsequently, the Maryland Department of Health and Mental Hygiene (DHMH) laboratory, which assumed responsibility for laboratory work continued this practice as the monitoring program expanded. As a result, field office staff preserved samples by keeping them on ice as opposed to preserving them with acid. In addition, several parameters analyzed under the monitoring programs have a maximum holding time of 48 hours and are delivered to the lab within 24 hours, so preservation by acidification did not seem warranted.

Field office staff adopted freezing as an option for preserving samples collected under the non-tidal network program following a study by the Chesapeake Biological Laboratory (CBL)in Solomons, MD that demonstrated there were no differences in the results between freezing samples and preserving them with acid (CBL received approval from EPA Region III to preserve samples by freezing; however their study only applied to estuarine samples and may not be applicable to fresh water systems.) Freezing is occasionally used for the non-tidal network stations, because sample collection is not always pre-planned, which occasionally makes it impossible to deliver samples to the lab within 24 hours of collection (e.g., storm event samples may be collected during Fridays and weekends when it would not be possible to deliver samples to the lab within 24 hours). Because of the historical precedent regarding refrigeration and the assumption on the part of DNR that freezing would provide similar results to acidification of fresh water samples, field office staff never adopted the requirement described in 40 CFR Part 136 regarding acid preservation of NO23 and DOC.

PROPOSED SOLUTION:
Two lines of evidence were pursued to test for differences in the concentrations of NO23 and DOC. The first part of the study involved analyzing data collected from a subset of the 55 CORE/Trend stations that would provide a cross section of low to high NO23 and DOC concentrations. The second part of the study involved comparing the NO23 and DOC concentrations of de-ionized (DI) water that was used by field staff to clean sampling equipment and water used by DHMH as laboratory blanks. Field samples were preserved by three methods: 1) maintaining samples at $\leq 4^{\circ}C$ for up to 24 hours, 2) freezing, and 3) acidification. All statistical analyses were performed and all graphs were prepared using SAS® software. NO23 samples were analyzed by DHMH using EPA Method 353.2 and DOC samples were analyzed using Standard Method 5310 B.

Samples Analyzed
CORE/Trend Samples
Ambient water quality samples were collected at 15 fresh water non-tidal stations in the CORE/Trend network over a period of three months from May through July 2010 (Data from a minimum of 15 stations were required to obtain sufficient power to statistically discern differences among the preservation methods.) The 15 stations were selected on the basis of providing a range of concentrations to ensure that differences among preservation methods were not a function of NO23 or DOC concentration. Using 15 stations also provided a range of environmental conditions. The range of NO23 and DOC in CORE/Trend data (2005-2009) was used as the basis for selecting the 15 stations used in this study. To ensure that a suitable range of concentrations was obtained, the CORE/Trend data for 54 stations were downloaded from DNR's internal data base for July 2005 through July 2009. The data were averaged by station for each month and variable and ranked from lowest to highest concentration. Stations were selected for each month in the low, middle, and high concentration range.

Five two person crews collected and field-processed samples at the CORE/Trend stations on 25 May 2010, 29 June 2010, and 27 July 2010. Samples were collected over a three month time period to allow DHMH sufficient time to process the samples in addition to their regular workload. Samples from each station were placed in a polyethylene churn splitter to ensure they were well mixed. Field crews then filtered and sub-sampled water out of the churn splitter for each of the three preservation methods (iced, frozen, and acidified). The sub-samples for each preservation method and station (replicate 1 and replicate 2) were placed in polyethylene bottles. Sub-sampling allowed replicate variability (field plus laboratory) to be examined. Summary statistics for NO23 and DOC

field data are provided by preservation method in Table 1 and Table 2, respectively. Field data for NO23 and DOC are presented in Appendix C.

Field De-ionized Water and Laboratory Blanks

De-ionized (DI) water is used by the field office to rinse filtration equipment before use, after use, and between samples. DI water is also used to rinse filter pads during filtration and as a final rinse after acid washing equipment. DI water is generated from tap water using a Thermo Scientific DIamond TII RO/DI system. Feed water is first run through a General Electric Smart Water external carbon pre-filter before entering the reverse osmosis system.

One iced, one frozen, and one acidified de-ionized water sample were generated each sampling day by each field crew for a total of six samples per month or 18 for the project. Replicates of the DI treatments were not made. Analysis of DI water treatments provided a control for possible contamination of the field samples. Field DI water results are presented in Table 3. DHMH analyzed blanks on each day samples were collected. The results for laboratory blanks associated with frozen, iced, and acidified sampling runs are presented in Tables 4, 5, and 6, respectively.

Sample Treatment

Iced Samples

Iced samples were put on ice immediately after they were filtered and sub-set and were delivered to DHMH the day after the samples were collected. DHMH analyzed the iced samples within 24 hours of the collection time.

Acid-preserved Samples

A 95% concentrated 36 normal (gram equivalent weight of a solute per liter of solution) solution of sulfuric acid was added to the NO23 samples for acid preservation after they were sub-set. A 37.4% solution of 12.1 normal hydrochloric acid was used for the DOC samples. Of the 35 H_2SO_4-preserved NO23 samples analyzed by the lab (30 field samples and 5 de-ionized water blanks) 32 had four drops of acid added and three had three drops of acid added. Of the 36 HCl-preserved DOC samples analyzed by the lab (30 field and 6 de-ionized water blanks) two had four drops of acid added, 14 had three drops of acid added, and 20 had two drops of acid added. The amount of acid added was determined in the field by testing the pH of each 100 milliliter sample bottle with a strip of litmus paper to ensure that each sample pH was 2 or less. As a result the maximum amount of acid solution added to a sample was 0.20 milliliters, assuming one drop is approximately 0.05 milliliters. The acid-preserved samples were then placed in a cooler with ice and taken back to the field office.

Acid-preserved samples that were collected in May were brought to DHMH two days after they were collected. Samples collected in June and July were taken to DHMH the day after they were collected. Rather than neutralizing acidified samples prior to analysis, the standards, reagents, and carrier were acidified to match the pH of the samples i.e., DHMH matched the matrix of the samples. The samples were analyzed by DHMH one week after they were received.

Frozen Samples
The samples to be frozen were placed in a cooler along with the iced samples and brought back to the field office where they were stored in a freezer which is kept at approximately -20°C. Frozen samples collected in May were delivered to DHMH along with the acidified samples two days after they were collected. Samples collected in June and July were taken to DHMH the day after they were collected. Frozen samples were analyzed by DYMH one week after they were received.

STATISTICAL ANALYSIS METHODS AND RESULTS:
CORE/Trend Samples
The NO23 and DOC data were analyzed statistically using SAS® PROC GLM, a General Linear Model. The model included two main effects (month and preservation method), a nested effect (station nested within month), and a crossed effect (preservation and station) nested within month. The main effects (month and preservation method) control for the effect of each on concentration independent of the other variables in the model. The nested effect removes the station-to-station variance to get a more precise assessment of the difference among the preservation methods. The nested effect was used because not all stations were sampled in all three months. The crossed effect nested within month tested to determine if the effect of preservation method was the same across stations and months, i.e., tests if there are location and season effects.

Differences among the preservation methods were also explored using Tukey's Studentized Range Test of Honest Significant Differences (HSD) (SAS, 1989). The Tukey HSD test compares the means of values after controlling for the Type 1 experiment-wise error rate, which occurs when multiple comparisons are made (A type 1 error occurs when one incorrectly rejects the null hypothesis of no difference when it is true – a false positive. Performing multiple comparisons on the same data increases the likelihood that one of those comparisons will be significant when it is in fact not significant.)

•NO23 Results
The results for the NO23 data are presented graphically in Figure 1. Figure 1 shows that the values for all three preservation methods are nearly identical; however, this could be a result of y-axis scaling. It is clear that spatial and temporal variability are very large compared to differences among the methods. There does not appear to be any bias associated with any of the preservation methods.

P-values for all four model terms were significant. The main effects (month and preservation method) and nested effect (station within month) were significant at $p<0.0001$. The crossed effect (preservation method and station within month) was significant at $p=0.0013$. The significance of month and terms involving station and month indicates that there are seasonal and spatial differences in NO23 concentrations. The significance of the preservation method term indicates that there are differences in NO23 concentration based on the preservation method that is used.

While the p-value for preservation method indicates there are significant differences among the methods, it does not indicate which method produced those results. Tukey's HSD test grouped the mean of iced and frozen samples together and acid-preserved separately. The difference of 0.005 mg/L between the mean concentrations of iced and frozen NO23 exceeded the method detection limit of 0.003 mg/L; however, the difference is not statistically significant at $p=0.05$. The difference of 0.019 mg/L between iced (the current preservation method) and

acid-preserved is well above the MDL and accounts for the overall significant result in the general linear model.

A comparison of the mean squares for station nested within month (17.541) and preservation method (0.003) indicates that preservation method has far less variability compared to the seasonal and spatial differences and that a change in preservation methods would be lost in the differences for station and season. For NO23, the difference in concentrations between iced and frozen preservation methods is not statistically significant and is biologically unimportant.

The results of the statistical analysis, which detected a significant difference among the preservation methods, appear to conflict with the graphical results in Figure 1, which shows that the preservation methods produce nearly identical results. As an independent confirmation of the statistical results, a second statistical test (the Friedman test) was used to test for significant differences among the preservation methods. The Friedman test is similar to an Analysis of Variance, except that it is a non-parametric method that is based on ranks. The Friedman test also detected a significant difference among the preservation methods ($p=0.0063$), which confirms the original result.

The difference between iced and acid-preserved (0.019 mg/L) and between frozen and acid-preserved (0.014 mg/L) may be statistically significant because the mean square error is comparatively small (0.0002), which resulted from good repeatability in the laboratory results for the duplicate samples, i.e., the differences between acid-preserved and the other methods appear large because the error (difference between replicate samples) is small.

•DOC Results

The results for DOC are presented graphically in Figure 2. Figure 2 indicates that for 11 of the stations, the symbols for the three preservation methods overlap. The high degree of overlapping symbols could be the result of scaling on the y-axis, which has a range of 10 mg/L. Of the 30 records for DOC (15 stations with replication) acid-preserved samples had the highest concentration 17 times, iced samples had the highest concentration 11 times, and a frozen sample was highest one time (iced and acid-preserved were tied once).

All four terms in the general linear model were statistically significant. Month, preservation method, and station nested within month were significant at $p<0.0001$ and the crossed effect was significant at $p=0.0003$. The statistically significant results for month and effects that include station indicate that there are seasonal and spatial differences in DOC concentrations. The significant result for preservation method indicates that there are differences in DOC concentration as a result of the preservation method that is used.

Tukey's HSD test grouped the mean DOC concentrations of acid-preserved and iced samples together and frozen samples in a separate Tukey group. The difference of 0.06 mg/L between acid-preserved and iced samples is less than the MDL of 0.14 mg/L and would be difficult to detect in the laboratory. The difference between acid-preserved and frozen samples of 0.15 mg/L only slightly exceeds the MDL and would also be difficult to detect in the laboratory. The difference between frozen samples and the other two preservation methods is statistically significant at the $p=0.05$ level. It is the author's opinion that the small differences that were detected in this study for DOC are biologically unimportant. In addition, the difference between preservation with acid (EPA method) and iced (primary current DNR method) are less than the MDL and are not statistically significant.

The difference between the mean squares for station nested within month (35.105) and preservation method (0.171) indicates that seasonal and spatial variability greatly exceed differences associated with preservation method.

Field and Laboratory Blank Samples

Field DI water samples and laboratory blanks were also evaluated statistically using SAS® PROC GLM. The model for the field DI water included only the main effects of "MonthTeam", and preservation method, there were no interaction or nested effects. The data for month and team were combined into one variable because not all teams were represented in all three months. MonthTeam controlled for the effect of different teams collecting samples in different months and for possible differences in how each team may have handled the samples. Preservation method tested for differences in concentration resulting from preservation method. The means were also compared using the Tukey HSD test.

The model for the laboratory blanks included only month and preservation method. Month controlled for possible differences that may have resulted from analyzing the samples during different months. Preservation method examined possible differences in the blanks that were used in each run associated with each preservation method. Although the laboratory blanks were not preserved, acid was added to the blank water for the acidified samples to match the pH of the samples. The means were compared using the Tukey HSD test.

•NO23 Results

Neither of the terms in the model for the field data was significant. A difference among months was detected in the laboratory blanks (p=0.035). The Tukey HSD test grouped May and June together and June and July in a second Tukey group. These results indicate that concentrations of NO23 in laboratory blank water used in May differed from water used in July (the concentrations for all three months were slightly negative).

The results for the field de-ionized water blank samples and the laboratory blank samples are presented in Figures 3, 4, and 5 for iced, acidified, and frozen NO23, respectively. NO23 concentrations in all blanks associated with iced samples were below the method detection limit (MDL) (horizontal line) during all three months. One acidified field sample was slightly higher than the MDL in June. All blanks associated with the frozen samples were below the MDL.

•DOC Results

None of the terms in the model for the field blanks or the laboratory blanks was significant. The results for the field de-ionized water blank samples and the laboratory blank samples for iced, acid-preserved, and frozen DOC are presented in Figures 6, 7, and 8, respectively. The concentrations of DOC detected in most of the field and laboratory blanks exceeded the MDL of 0.14 mg/L, but was less than the reporting limit of 0.5 mg/L. The highest concentration (0.65 mg/L) was detected in a laboratory blank associated with the May run of iced samples. Unlike NO23, the concentrations of DOC for all field and laboratory blanks were positive. It is unclear what may have caused the contamination of the DOC blanks. De-ionized water used in the field, as well as the field sub-samples were kept in polyethylene bottles, which may have contributed trace amounts of DOC. The relatively higher concentrations of DOC for the acid-preserved field de-ionized blanks suggests that the extra handling associated with acid preservation may have introduced some contamination. Although the mean concentration of the acid-preserved field blank DOC samples was higher than

iced and frozen samples, the differences were not significant possibly as a result of the small sample size.

SENSE OF THE RESOURCES NEEDED TO RESPOND:
Members of the Analytical Methods and Quality Assurance Workgroup (AMQWA) and data analysts are requested to review this document and provide comments by 30 June 2011.

PRIORITY RANKING:
Three (medium). A decision regarding the need to change the historic methods for preserving samples (icing and delivering to the laboratory for analysis within 24 hours) or freezing is needed for NO23 and DOC so that a waiver from the methods outlined in 40 CFR, Part 136, Table 2 can be requested from the U.S. Environmental Protection Agency, Region 3.

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ACTIONS TO DATE:
Presented the findings of this study to the Analytical Methods and Quality Assurance Workgroup at its 28 September 2010 meeting. Prepared a draft report on 3 August 2011. Analyses performed in PreservationStudy.SAS program.

OVERALL RESOLUTIONS SUMMARY OF ACTIONS:

RECOMMENDED ACTIONS:
Based on the analyses described in this report, it is the author's recommendation that field office staff continue to preserve filtered NO23 and DOC samples collected under Sections 106 and 117 of the Clean Water Act by keeping them on ice if they can be delivered to and analyzed by the laboratory within 24 hours or by freezing at -20°C if they cannot be delivered within 24 hours. This would provide consistency with the historical data for NO23 and DOC. The difference in NO23 concentrations between acid-preserved and either iced or frozen samples is statistically significant, but insignificant relative to the seasonal and spatial variability. Acid-preserved and iced DOC samples were in the same Tukey HSD group, so preservation by either method would not result in a significant difference.

ACTION NUMBER: 01
1. Designated Respondent: Analytical Methods and QA Workgroup (AMQAW)
2. Action: Review report and determine if changing in procedure is warranted.
3. Resources Needed: None
4. Due Date: None
5. Action Item Resolution Summary:

   This report was discussed at the Sept. 26, 2011 AMQAW meeting where members agreed that the statistically-significant differences found among acidified, chilled and frozen NO23 samples were small enough to continuing to chill and/or freeze the samples as in the past. They noted that the mean differences between acid and iced DOC samples were less than the method detection limit and differences between acid and frozen only slightly

exceeded the method detection limit. Further comparisons of the magnitudes of differences between treatments to the routine differences measured by laboratory duplicate data was suggested but not attempted.

In June 2011 and March 2012, Mary Ellen Ley requested written approval from EPA Region III to use freezing as an alternative preservation technique for NO23 and DOC samples. Joe Slayton of Region III responded in an e-mail saying:

"My understanding is that the use of NPDES methods has routinely been encouraged for ambient monitoring. Given the CBP's split sample program and strong lab quality system requirements coupled and the realities that ambient monitoring often demands greater method sensitivity and other special challenges (salt for instance) I do not think that that formal approval, beyond that required internal to the CB's monitoring program, should be necessary. I am copying WPD folks on this note as this definitely needs their input."

As of September 2013, no further direction from Region III has occurred on this subject and the AMQAW recommendation stands.

Table 1. Summary statistics for NO23 (mg/L).

Preservation method	N	Mean	Std. Deviation	Range
Acid	30	1.553	1.565	4.661
Frozen	30	1.567	1.561	4.605
Iced	30	1.572	1.568	4.645

Table 2. Summary statistics for DOC (mg/L).

Preservation method	N	Mean	Std. Deviation	Range
Acid	30	3.536	2.269	8.410
Frozen	30	3.386	2.175	8.200
Iced	30	3.479	2.200	8.000

Table 3. Field blank data for frozen, iced, and acidified samples.

Date	DOC (mg/L)	NO23 (mg/L)	Preservation method
5/25/2010	0.28	-0.001	Frozen
5/25/2010	0.16	-0.002	Frozen
5/25/2010	0.46	-0.006	Iced
5/25/2010	0.36	-0.002	Iced
5/25/2010	0.41	-0.005	Acid
5/25/2010	0.29	NA[1]	Acid
6/29/2010	0.23	0.001	Frozen
6/29/2010	0.27	-0.002	Frozen
6/29/2010	0.17	0.003	Iced
6/29/2010	0.15	0.002	Iced
6/29/2010	0.27	0.004	Acid
6/29/2010	0.44	-0.003	Acid
7/27/2010	0.19	-0.005	Frozen
7/27/2010	0.19	0.001	Frozen
7/27/2010	0.14	0.001	Iced
7/27/2010	0.27	-0.005	Iced
7/27/2010	0.19	-0.008	Acid
7/27/2010	0.22	0.001	Acid

[1]Data are not available.

Table 4. Laboratory blank data for frozen samples.

Date	DOC (mg/L)	NO23 (mg/L)
5/25/2010	0.27	0.001
5/25/2010	0.30	0.000
5/25/2010	0.22	0.000
5/25/2010	0.18	0.000
5/25/2010	0.33	NA[1]
5/25/2010	0.30	NA
5/25/2010	0.22	NA
6/29/2010	0.39	-0.004
6/29/2010	0.11	-0.001
6/29/2010	0.21	-0.003
6/29/2010	0.31	0.000
6/29/2010	0.20	NA
6/29/2010	0.27	NA
6/29/2010	0.18	NA
6/29/2010	0.14	NA
7/27/2010	0.35	-0.003
7/27/2010	0.30	-0.001
7/27/2010	0.20	-0.002
7/27/2010	0.10	0.000
7/27/2010	0.20	NA
7/27/2010	0.20	NA
7/27/2010	0.20	NA

[1]Data are not available.

Table 5. Laboratory blank data for iced samples.

Date	DOC (mg/L)	NO23 (mg/L)
5/25/2010	0.65	0.001
5/25/2010	0.21	-0.005
5/25/2010	0.12	0.000
5/25/2010	0.24	-0.005
5/25/2010	0.27	NA[1]
5/25/2010	0.25	NA
5/25/2010	0.33	NA
5/25/2010	0.25	NA
5/25/2010	0.25	NA
6/29/2010	0.24	0.002
6/29/2010	0.37	0.002
6/29/2010	0.15	-0.003
6/29/2010	0.10	-0.004
6/29/2010	0.14	NA
6/29/2010	0.20	NA
6/29/2010	0.20	NA
6/29/2010	0.27	NA
6/29/2010	0.32	NA
7/27/2010	0.48	-0.005
7/27/2010	0.37	-0.002
7/27/2010	0.17	-0.004
7/27/2010	0.31	-0.004
7/29/2010	0.25	NA
7/29/2010	0.12	NA
7/29/2010	0.41	NA

[1]Data are not available.

Table 6. Laboratory blank data for acidified samples.

Date	DOC (mg/L)	NO23 (mg/L)
5/25/2010	0.27	-0.004
5/25/2010	0.30	0.001
5/25/2010	0.22	0.001
5/25/2010	0.17	0.001
5/25/2010	0.18	NA[1]
5/25/2010	0.33	NA
5/25/2010	0.30	NA
5/25/2010	0.22	NA
5/25/2010	0.28	NA
6/29/2010	0.39	-0.004
6/29/2010	0.42	-0.001
6/29/2010	0.26	-0.0003
6/29/2010	0.11	0.000
6/29/2010	0.21	NA
6/29/2010	0.31	NA
6/29/2010	0.20	NA
6/29/2010	0.18	NA
6/29/2010	0.14	NA
7/27/2010	0.35	-0.005
7/27/2010	0.20	-0.002
7/27/2010	0.10	-0.003
7/27/2010	0.20	-0.006
7/29/2010	0.20	NA
7/29/2010	0.20	NA

[1]Data are not available.

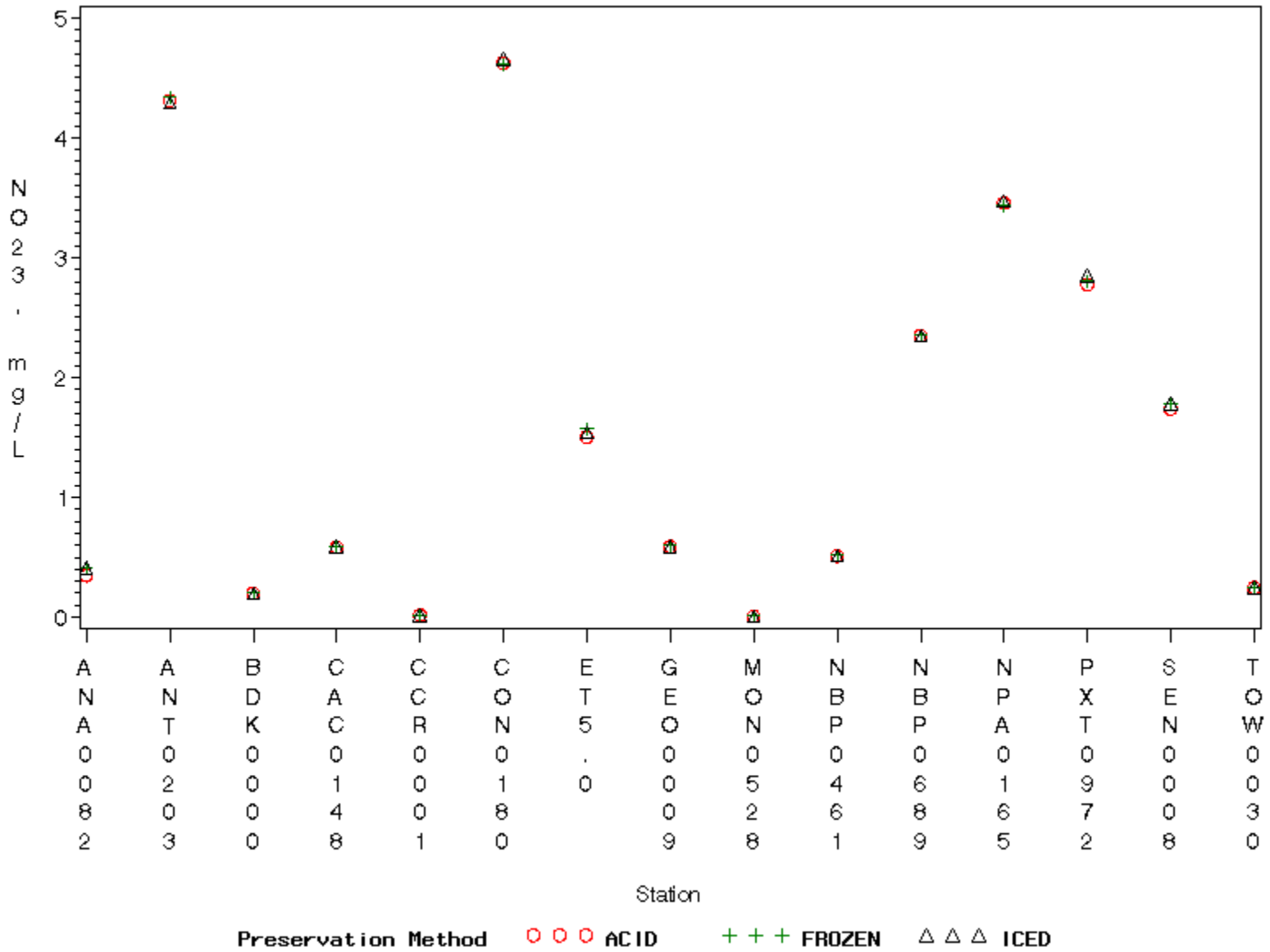

Figure 1. Comparison of preservation methods for NO23 for the 15 CORE/Trend stations.

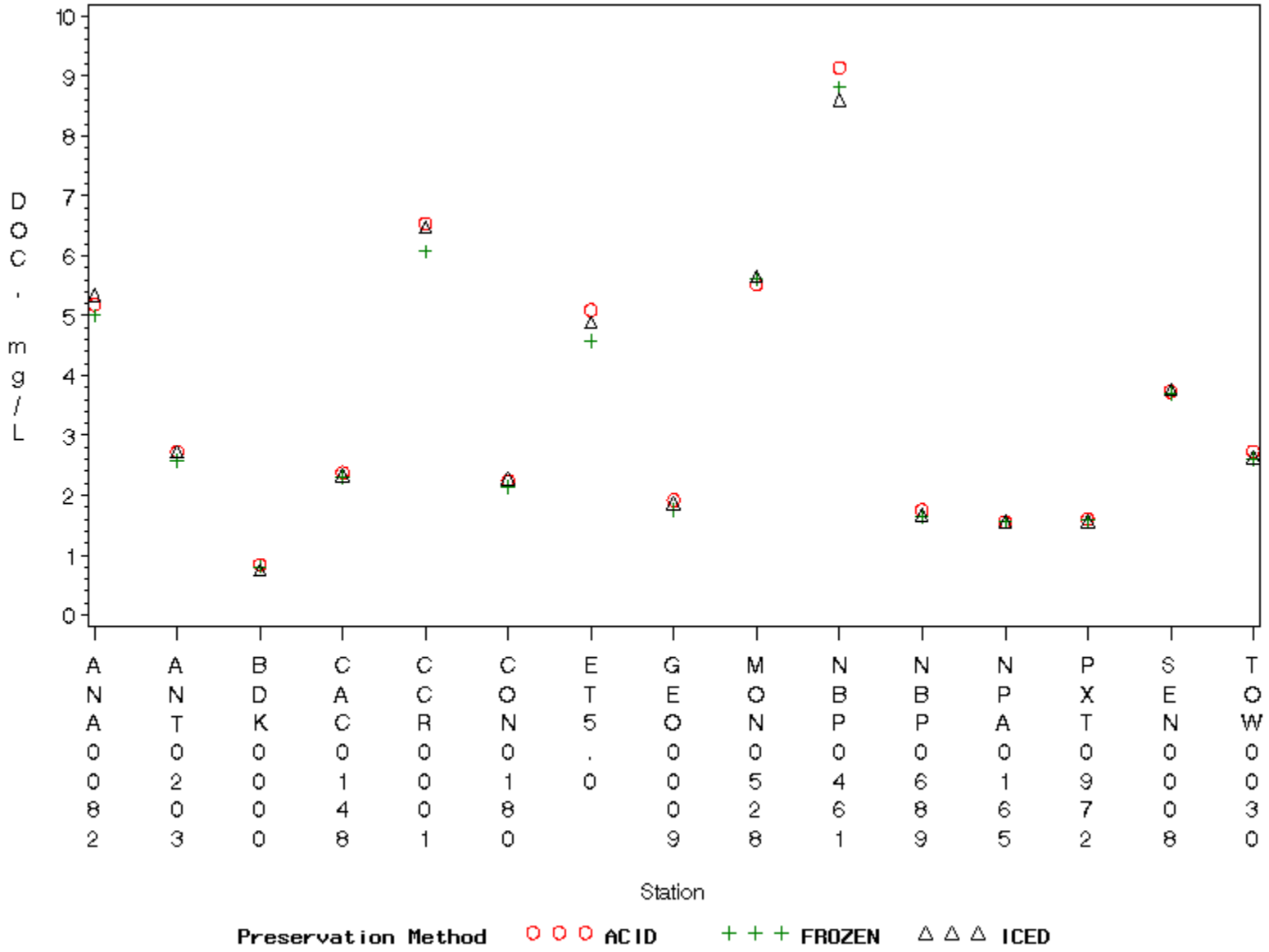

Figure 2. Comparison of preservation methods for DOC for the 15 CORE/Trend Sites.

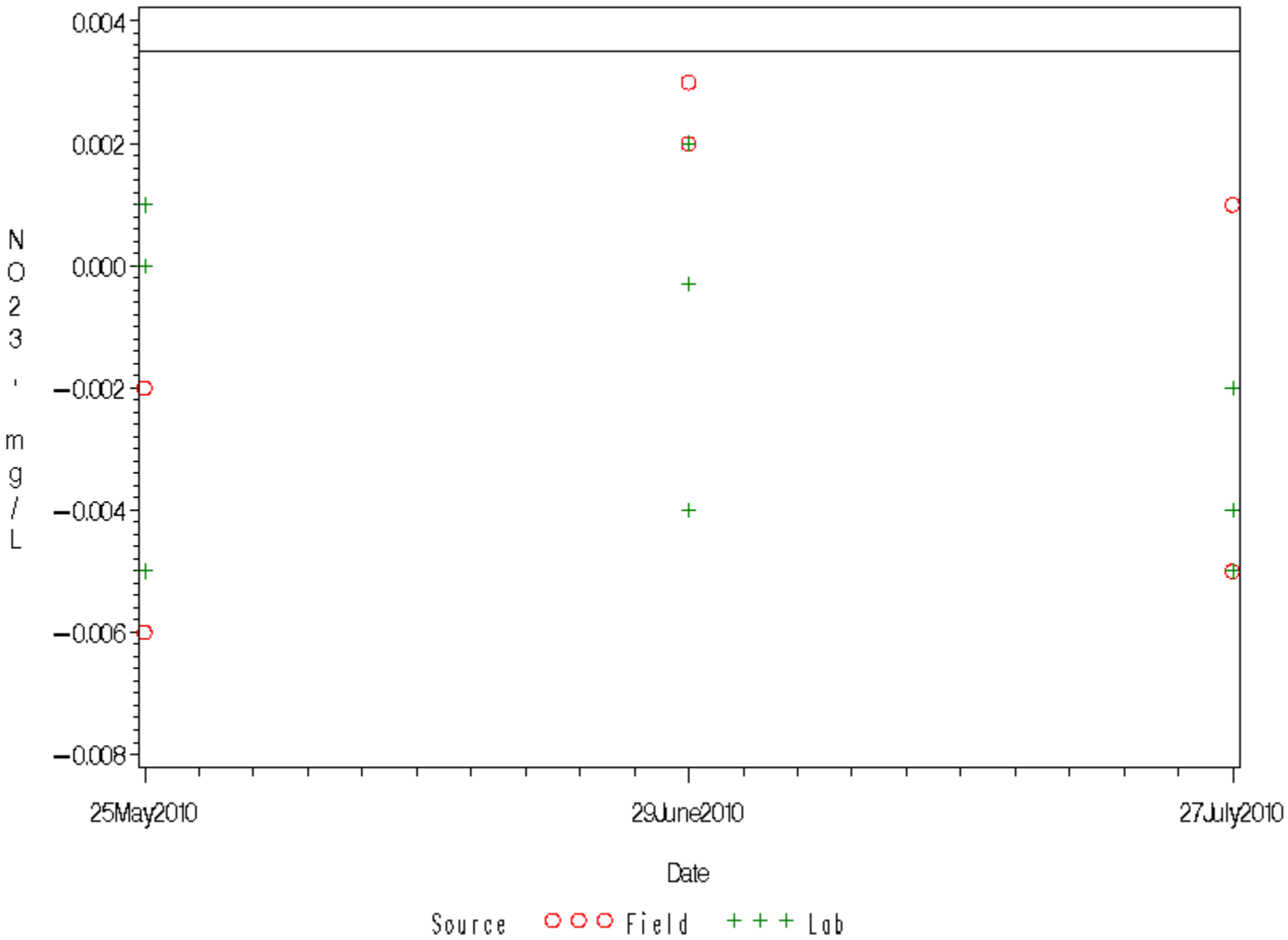

Figure 3. Comparison of field and laboratory blanks for iced nitrite-nitrate. Horizontal line shows the method detection limit.

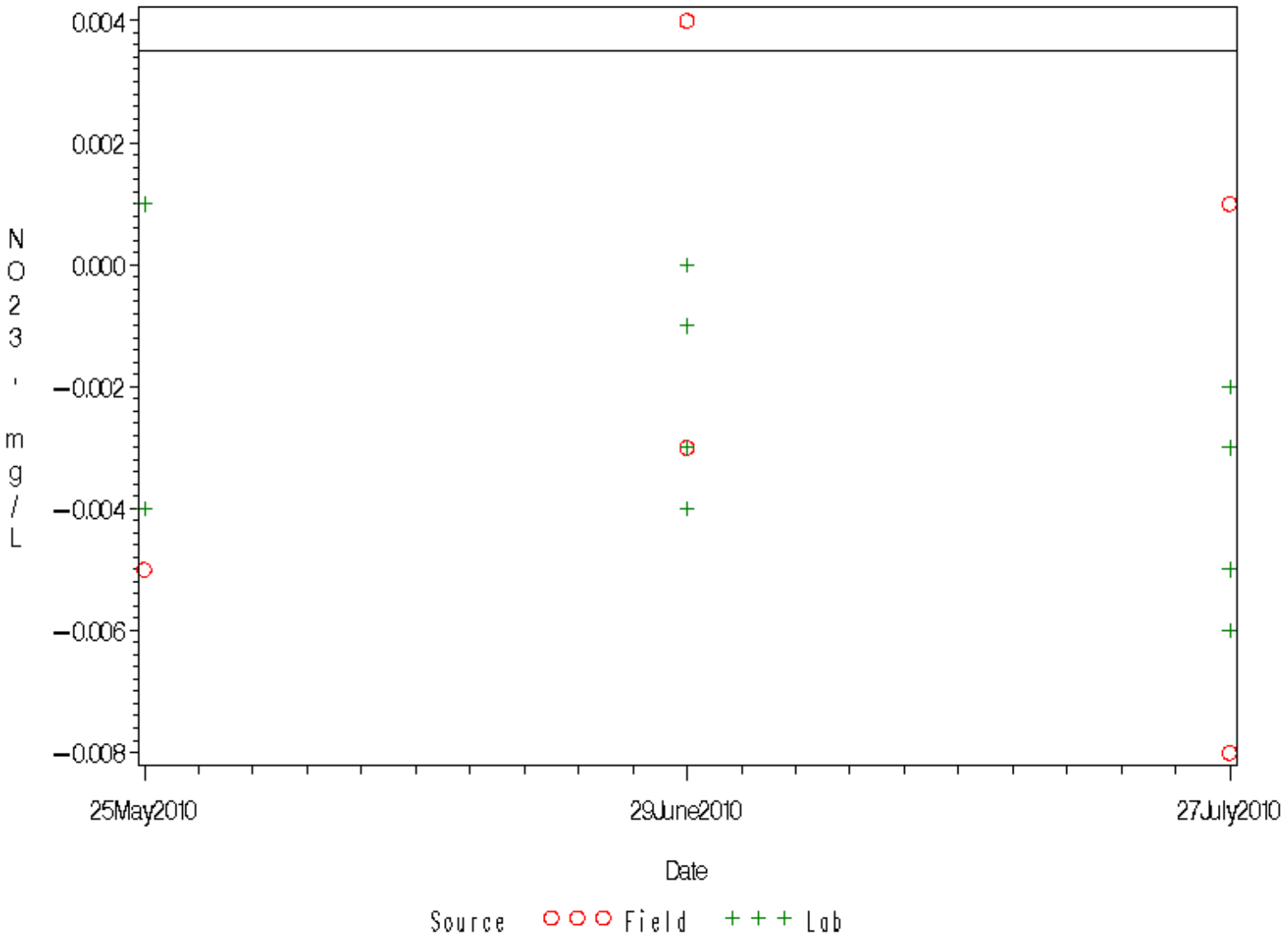

Figure 4. Comparison of field and laboratory blanks for acidified nitrate-nitrite. Horizontal line shows the method detection limit.

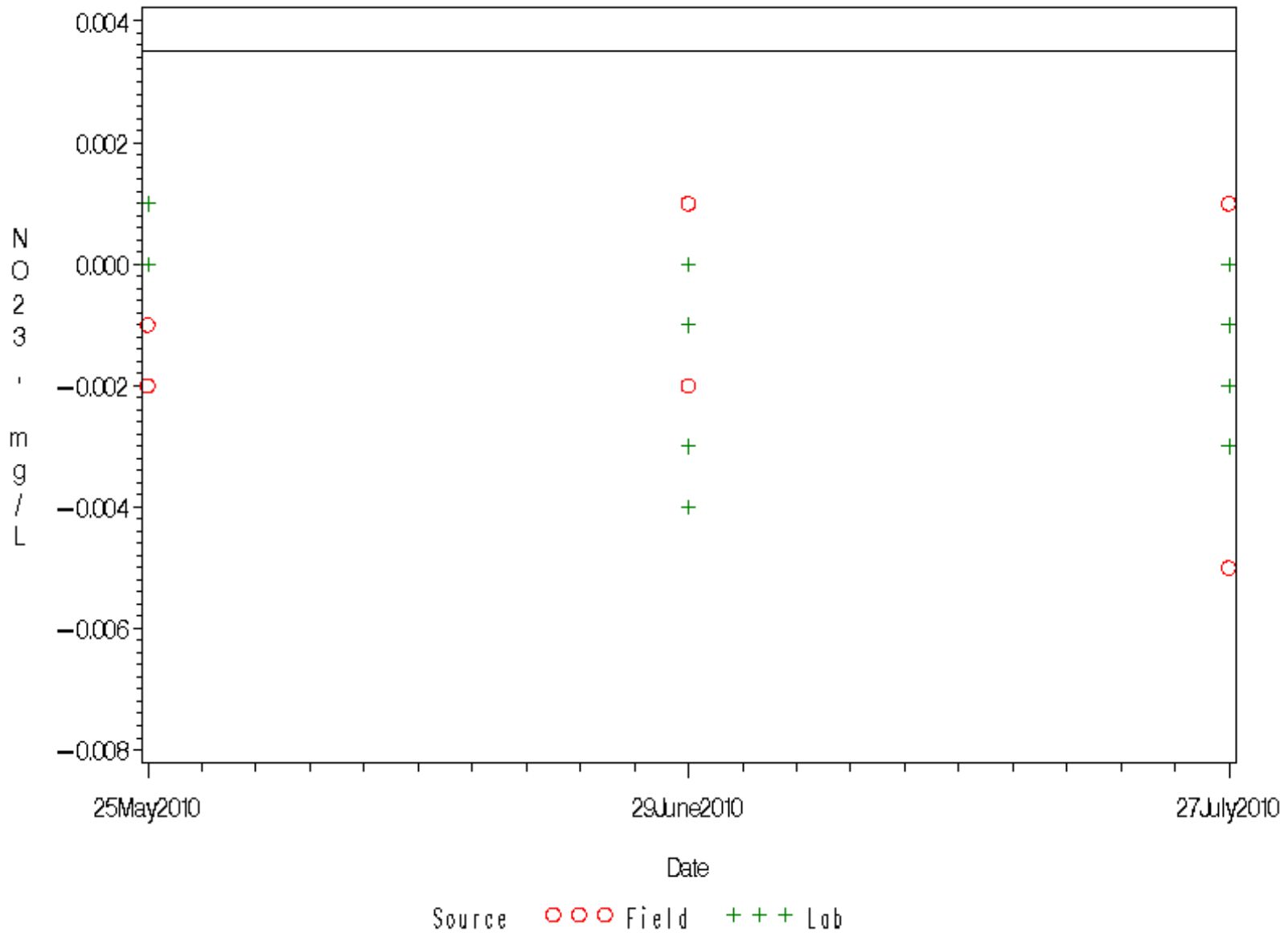

Figure 5. Comparison of field and laboratory blanks for frozen nitrate-nitrite. Horizontal line shows the method detection limit.

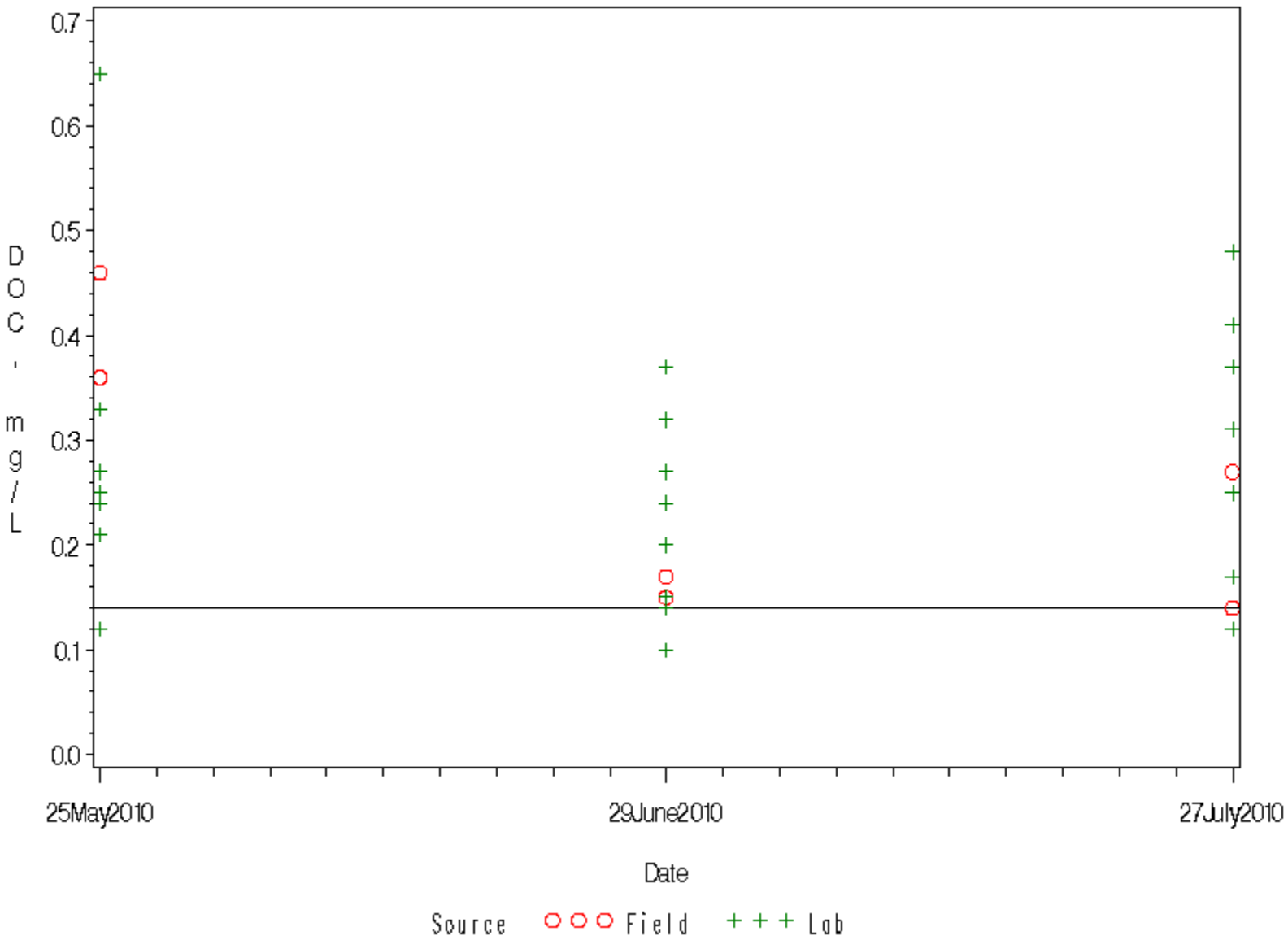

Figure 6. Comparison of field and laboratory blanks for iced dissolved organic carbon. Horizontal reference line shows the method detection limit.

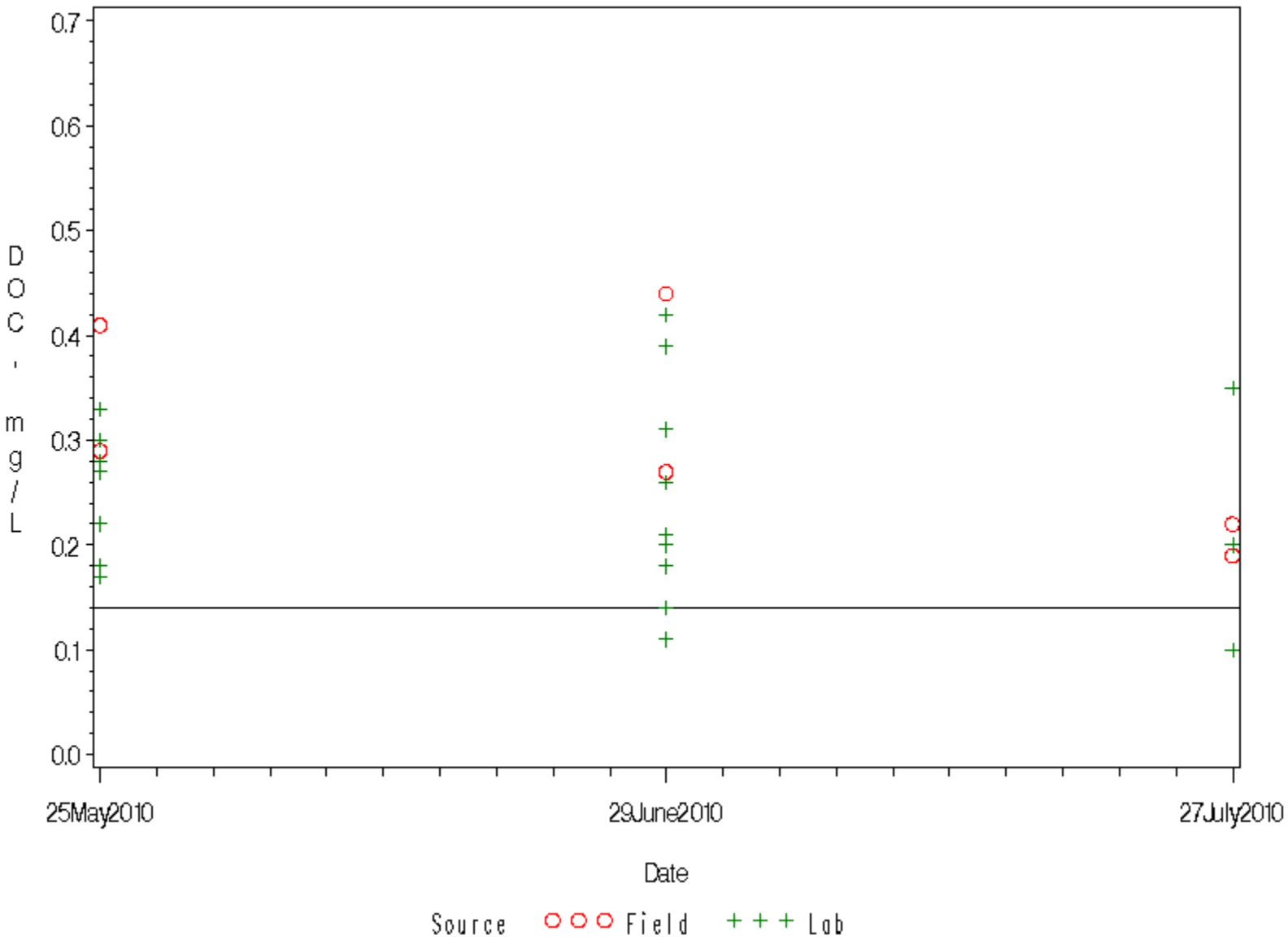

Figure 7. Comparison of field and laboratory blanks for acidified dissolved organic carbon. Horizontal line shows the method detection limit.

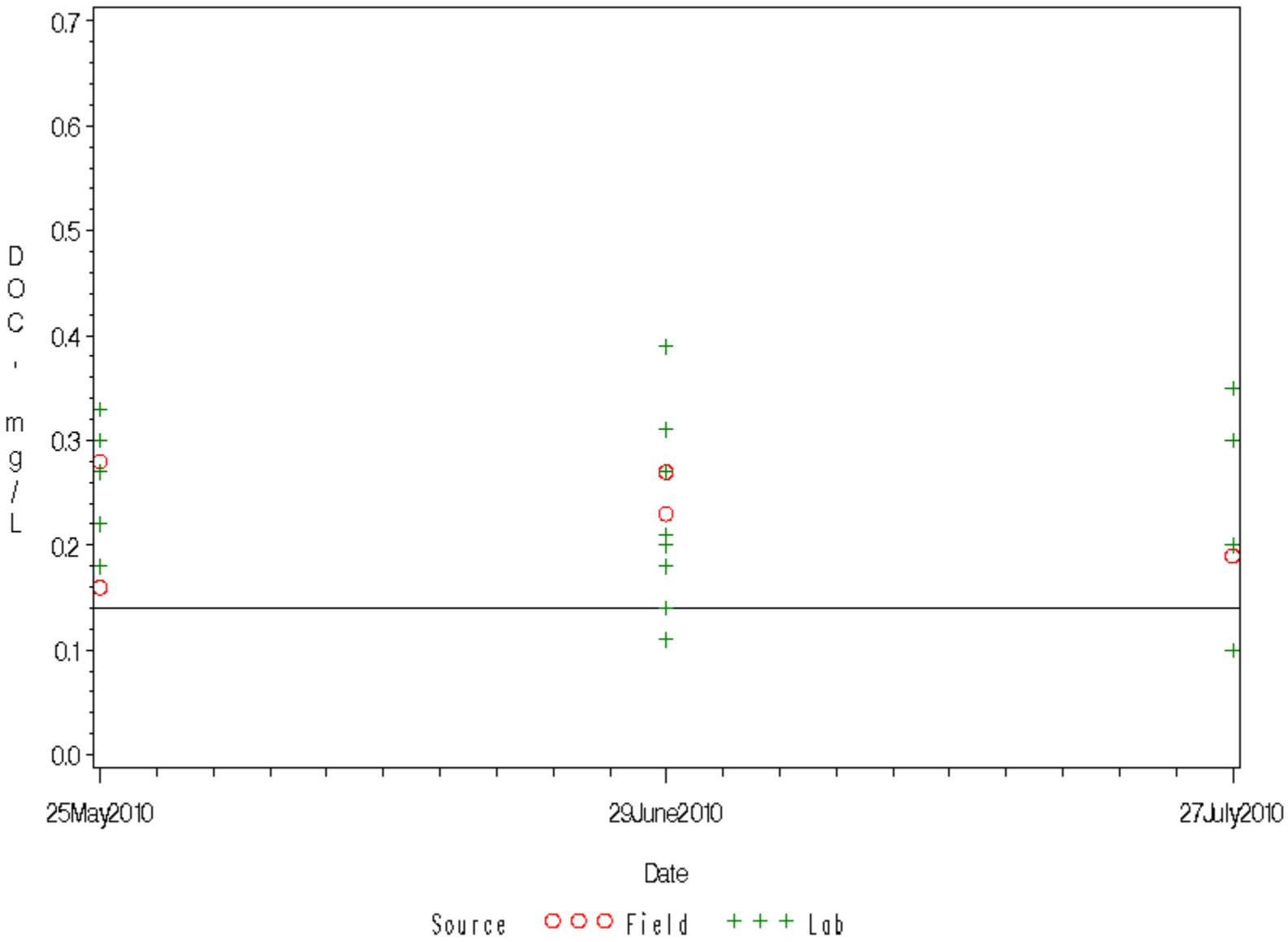

Figure 8. Comparison of field and laboratory blanks for frozen dissolved organic carbon. Horizontal line shows the method detection limit.

References

Guidelines establishing test procedures for the analysis of pollutants, Title 40 Code of Federal Regulations, Part 136, 12 March 2007, pp. 11200-11239.

SAS Institute, Inc., *SAS/Stat*® User's Guide, Version 6, Fourth Edition,Volume 2, Cary, NC: SAS Institute Inc., 1989, 846 pp.

Appendix A

1) ANA0082
2) ANT0044
3) ANT0203
4) ANT0366
5) BDK0000
6) BEL0053
7) BPC0035
8) CAC0031
9) CAC0148
10) CAS0479
11) CB1.0
12) CCR0001
13) CJB0005
14) CON0005
15) CON0180
16) DER0015
17) GEO0009
18) GUN0125
19) GUN0258
20) GUN0478
21) GWN0115
22) JON0184
23) LYO0004
24) MON0020
25) MON0155
26) MON0269
27) MON0528
28) NBP0023
29) NBP0103
30) NBP0326
31) NBP0461
32) NBP0534
33) NBP0689
34) NPA0165
35) PAT0176
36) PAT0285
37) PIS0033 (no longer analyzed as part of the CORE/Trend program)
38) POT1184
39) POT1471
40) POT1472
41) POT1595
42) POT1596
43) POT11830
44) POT2386
45) POT2766
46) PXT0809
47) PXT0972
48) RCM0111
49) SAV0000
50) SEN0008
51) TF1.0
52) TOW0030
53) WIL0013
54) YOU0925
55) YOU1139

Appendix B

1) ANT0047
2) BEL0053
3) CAC0148
4) DER0015
5) GEO0009
6) GUN0258
7) GWN0115
8) MON0546
9) NPA0165
10) PXT0972
11) TF1.2
12) TUK0181
13) WIL0013

Appendix C

StationName	SampleDate	RepNumber	PresMethod	NO23	NO23_G	DOC	DOC_G
GEO0009	5/25/2010	1	FROZEN	0.607		1.72	
GEO0009	5/25/2010	2	FROZEN	0.594		1.79	
ET5.0	5/25/2010	1	FROZEN	1.565		4.52	
ET5.0	5/25/2010	2	FROZEN	1.585		4.65	
ANT0203	5/25/2010	1	FROZEN	4.327		2.55	
ANT0203	5/25/2010	2	FROZEN	4.351		2.61	
CCR0001	5/25/2010	1	FROZEN	0.02		6.05	
CCR0001	5/25/2010	2	FROZEN	0.019		6.09	
TOW0030	5/25/2010	1	FROZEN	0.256		2.61	
TOW0030	5/25/2010	2	FROZEN	0.249		2.62	
GEO0009	5/25/2010	1	ICED	0.595		1.85	
GEO0009	5/25/2010	2	ICED	0.596		1.89	
ET5.0	5/25/2010	1	ICED	1.552		4.85	
ET5.0	5/25/2010	2	ICED	1.54		4.93	
ANT0203	5/25/2010	1	ICED	4.268		2.55	
ANT0203	5/25/2010	2	ICED	4.322		2.93	
CCR0001	5/25/2010	1	ICED	0.019		6.55	
CCR0001	5/25/2010	2	ICED	0.018		6.42	
TOW0030	5/25/2010	1	ICED	0.252		2.64	
TOW0030	5/25/2010	2	ICED	0.251		2.63	
GEO0009	5/25/2010	1	ACID	0.592		1.83	
GEO0009	5/25/2010	2	ACID	0.59		2.02	
ET5.0	5/25/2010	1	ACID	1.505		5.06	
ET5.0	5/25/2010	2	ACID	1.508		5.14	
ANT0203	5/25/2010	1	ACID	4.279		2.65	
ANT0203	5/25/2010	2	ACID	4.345		2.82	
CCR0001	5/25/2010	1	ACID	0.023		6.5	
CCR0001	5/25/2010	2	ACID	0.019		6.59	
TOW0030	5/25/2010	1	ACID	0.251		2.9	
TOW0030	5/25/2010	2	ACID	0.248		2.58	
BDK0000	6/29/2010	1	ICED	0.204		0.71	
BDK0000	6/29/2010	2	ICED	0.205		0.82	
NBP0461	6/29/2010	1	ICED	0.517		8.5	
NBP0461	6/29/2010	2	ICED	0.521		8.71	
CON0180	6/29/2010	1	ICED	4.658		2.2	
CON0180	6/29/2010	2	ICED	4.656		2.35	
PXT0972	6/29/2010	1	ICED	2.84		1.6	
PXT0972	6/29/2010	2	ICED	2.858		1.55	
CAC0148	6/29/2010	1	ICED	0.598		2.29	
CAC0148	6/29/2010	2	ICED	0.593		2.37	
BDK0000	6/29/2010	1	FROZEN	0.207		0.75	
BDK0000	6/29/2010	2	FROZEN	0.205		0.87	
NBP0461	6/29/2010	1	FROZEN	0.522		8.95	
NBP0461	6/29/2010	2	FROZEN	0.512		8.67	
CON0180	6/29/2010	1	FROZEN	4.618		2.12	
CON0180	6/29/2010	2	FROZEN	4.605		2.18	

StationName	SampleDate	RepNumber	PresMethod	NO23	NO23_G	DOC	DOC_G
PXT0972	6/29/2010	1	FROZEN	2.815		1.63	
PXT0972	6/29/2010	2	FROZEN	2.794		1.55	
CAC0148	6/29/2010	1	FROZEN	0.595		2.28	
CAC0148	6/29/2010	2	FROZEN	0.59		2.35	
BDK0000	6/29/2010	1	ACID	0.201		0.82	
BDK0000	6/29/2010	2	ACID	0.202		0.87	
PXT0972	6/29/2010	1	ACID	2.784		1.64	
PXT0972	6/29/2010	2	ACID	2.769		1.57	
CAC0148	6/29/2010	1	ACID	0.587		2.34	
CAC0148	6/29/2010	2	ACID	0.588		2.43	
CON0180	6/29/2010	1	ACID	4.669		2.2	
CON0180	6/29/2010	2	ACID	4.585		2.29	
NBP0461	6/29/2010	1	ACID	0.51		9.06	
NBP0461	6/29/2010	2	ACID	0.517		9.23	
MON0528	7/27/2010	1	ICED	0.013		5.6	
MON0528	7/27/2010	2	ICED	0.013		5.71	
NBP0689	7/27/2010	1	ICED	2.347		1.71	
NBP0689	7/27/2010	2	ICED	2.364		1.64	
ANA0082	7/27/2010	1	ICED	0.412		5.24	
ANA0082	7/27/2010	2	ICED	0.415		5.45	
NPA0165	7/27/2010	1	ICED	3.481		1.54	
NPA0165	7/27/2010	2	ICED	3.472		1.61	
SEN0008	7/27/2010	1	ICED	1.77		3.74	
SEN0008	7/27/2010	2	ICED	1.799		3.8	
MON0528	7/27/2010	1	FROZEN	0.013	L	5.62	
MON0528	7/27/2010	2	FROZEN	0.016	L	5.6	
NBP0689	7/27/2010	1	FROZEN	2.356		1.7	
NBP0689	7/27/2010	2	FROZEN	2.344		1.6	
ANA0082	7/27/2010	1	FROZEN	0.408		4.89	
ANA0082	7/27/2010	2	FROZEN	0.406		5.12	
NPA0165	7/27/2010	1	FROZEN	3.435		1.58	
NPA0165	7/27/2010	2	FROZEN	3.435		1.52	
SEN0008	7/27/2010	1	FROZEN	1.789		3.73	
SEN0008	7/27/2010	2	FROZEN	1.772		3.67	
MON0528	7/27/2010	1	ACID	0.009	L	5.52	
MON0528	7/27/2010	2	ACID	0.008	L	5.53	
NBP0689	7/27/2010	1	ACID	2.367		1.76	
NBP0689	7/27/2010	2	ACID	2.331		1.76	
ANA0082	7/27/2010	1	ACID	0.35		5.15	
ANA0082	7/27/2010	2	ACID	0.35		5.22	
NPA0165	7/27/2010	1	ACID	3.459		1.58	
NPA0165	7/27/2010	2	ACID	3.454		1.54	
SEN0008	7/27/2010	1	ACID	1.754		3.73	
SEN0008	7/27/2010	2	ACID	1.726		3.74	