Laboratory Procedure Manual

Analyte: **Fluoride, ionic**

Matrix: **Water**

Method: **Fluoride Ion-specific Electrode**

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Public Release Data Set Information

This document details the Lab Protocol for testing the item shown in the following table:

Data File Name	Variable name	Description
FLDEW_H	LBDWFL	Fluoride in water (mg/L), average 2 values

1 SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

a. ***Analyte.***

Fluoride, ionic

Clinical Relevance.

Fluoride, the ionic form of the element fluorine, is ubiquitous in nature. The most common source of human exposure to fluoride is drinking water in which the ion may be present naturally or added in controlled amounts for the prevention of dental caries. There are no known adverse health effects associated with low fluoride levels in water such as those found in public fluoridated water supplies which typically range from 0.7 to 1.2 mg/L. Concentrations above 2 mg/L may cause some degree of dental fluorosis but only if chronically ingested by children with developing teeth. Ingestion of water with fluoride concentrations above 10 mg/L for 10 or more years may cause some degree of skeletal fluorosis.

b. ***Assay Principle.***

Fluoride concentrations in water samples and appropriate aqueous standards are measured electrometrically using the ion-specific electrode. The limit of detection (LOD) of the electrode is 0.019 mg/L, a concentration far below the concentration typically found in water. The linear portion of the standard curve extends from 0.1 mg/L to 19,000 mg/L at the electrode.

2 SAFETY PRECAUTIONS

a. ***Reagent Toxicity / Carcinogenicity.*** Some of the reagents, namely sodium hydroxide and acetic acid, used in this procedure are toxic. Safety precautions must be taken to avoid dermal and inhalation exposure to these reagents. Spills of these reagents must be treated using the acid or base cleanup kits. The reagents used in the analysis of water for fluoride are not carcinogenic.

b. ***Radioactive Hazards.*** This procedure does not use radioactive materials. There are no radioactive hazards associated with it.

c. ***Biological Hazards.*** This assay involves handling of drinking water samples. The risk of exposure to biological hazards is minimal. Nevertheless the analyst is expected to wear gloves and a lab coat during the analytical procedure.

d. ***Chemical Hazards.*** MSDSs for sodium hydroxide and acetic acid are readily accessible on the internet. For example visit the following sites: http://www.msdssearch.net/MSDSSearch.asp, or http://msds.ehs.cornell.edu/msdssrch.asp. Hardcopies are maintained on file.

e. ***Mechanical hazards.*** There are no unusual mechanical hazards associated with this method. Analysts should know and follow the manufacturer’s recommendations concerning the safe handling of instruments and other equipment.

f. ***Protective equipment.*** Standard safety precautions should be followed when performing this

procedure including the use of a lab coat, appropriate gloves and the use of chemical fume hoods as needed.

g. ***Training.*** Training in the use of the multistep analytical method is required. All analysts must demonstrate proficiency in the analysis before analyzing water samples.

h. ***Disposal of Wastes.*** All waste disposals must be in compliance with policies of the Biological and Chemical Safety Committees of Georgia Regents University. Discard waste reagents into an appropriate container marked for waste handling. Place all disposable items, including vials containing water samples and pipette tips that come in contact with the water samples, in an appropriate container.

3 COMPUTERIZATION; DATA SYSTEM MANAGEMENT

a. ***Software and knowledge requirements.*** The initial, handwritten fluoride concentrations of the standards and their associated millivolt values are entered into an Excel spreadsheet. Using the linear regression program, the values for the standards are used to determine the slope and intercept of the standard curve from which the fluoride concentrations of the water samples are determined. Knowledge of and experience with the Excel software and its linear regression program and statistical summary program are required for performing these functions.

b. ***Sample information.*** All samples are analyzed in runs that include analytical standards, QC bench standards, and QC unknowns (if any). Each water sample is identified by a unique code that is generated by NHANES/CDC personnel. Each run is recorded as a file that contains sample ID, analyst's name, volumes, date of analysis and other information. A program generating the database containing this information has been developed by NHANES/CDC/Westat personnel and this fluoride research laboratory.

c. ***Data maintenance.*** Following each analytical run, the standards and samples are processed as described above on an Excel spreadsheet, which includes sample file number, sample ID and date assayed. The original handwritten mV and other analytical data are maintained in secure files in the fluoride laboratory for at least two years as are copies of the corresponding Excel spreadsheets and the laboratory reports of the water concentrations sent to the NHANES/CDC/Westat team.

d. ***Information security.*** Information security is provided at multiple levels. Information and analytical findings recorded on paper are maintained in the laboratory which is locked when laboratory personnel are not present. The data accessed via computers require individual login passwords that default to locked conditions during extended periods of nonuse. In addition, the fluoride laboratory is located in the Hamilton Wind of the Research and Education Building, entry into which requires use of a "smart card." Confidentiality of the results is protected by use of coded ID numbers only. No personal identifiers are ever used.

4 COLLECTION, STORAGE, AND HANDLING PROCEDURES; CRITERIA FOR SPECIMEN REJECTION

a. ***Special requirements.*** There are no special requirements for this assay.

b. ***Sample collection.*** The specimen for these analyses is drinking water. The water samples are collected in the field in a standardized manner by NHANES personnel. The collections are made directly from the tap after allowing the water to run for 5-10 seconds. Samples should be refrigerated as soon as possible after collection, and frozen for longer term storage if necessary. Care should be taken not to completely fill the tube (maximum tube volume is 10.0 mL) to allow for expansion of the water while in the freezer.

c. ***Sample handling.*** After collection, water samples should be frozen at approximately –20°C and shipped with dry ice by overnight air. A packing list showing the code numbers must be included with the samples and the GRU fluoride research laboratory should be notified before shipment. Unless special arrangements are made, shipment schedules should avoid having samples arrive at the laboratory on weekends or holidays since sample handling at those times may not be possible. After receipt, samples are stored frozen at approximately –20°C until they are prepared for fluoride analysis.

d. ***Sample quantity.*** A minimum of 1.0 mL of water is needed for duplicate analyses. Vials containing less than 1.0 mL of water will be analyzed only once.

e. ***Unacceptable specimens.*** Criteria for defining a water sample as unacceptable for analysis include: (1) use of improper collection materials or techniques leading to elevated fluoride contamination; (2) sample volumes less than the required minimum; or (3) improper shipment or storage of samples leading to thawing for more than one day, leaking or similar problems. All samples are logged in at receipt and problems with shipment or storage are identified at that point. If a sample must be rejected as unacceptable, a description of the problem must be entered into the database and associated with that sample.

f. ***Long-term stability.*** Long-term stability of ionic fluoride in refrigerated or frozen water appears to be on the order of several months. The purpose of maintaining water samples at low temperatures before analysis is to prevent growth of microorganisms.

5 PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTION OF INADEQUATELY PREPARED SLIDES

Not applicable for this procedure.

6 PREPARATION OF REAGENTS, CALIBRATORS (STANDARDS), CONTROLS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

a. ***Reagents and Standards. Identification and handling.***

All reagents and standards must be prepared with ACS certified chemicals and deionized water (dH_2O) and stored at room temperature in tightly sealed plastic bottles.

(1) TISAB I (Total Ionic Strength Acetate Buffer I)

This buffer solution is added to all standards and water samples prior to analysis with the ion-specific electrode for the purpose of adjusting the pH and ionic strength of all solutions to uniform values. A stock solution of TISAB I can be prepared in the laboratory as described in the next paragraph or it can purchased from Orion Research.

(2) Preparation of TISAB I

Place a one-liter plastic TriPour beaker containing approximately 700 mL of dH_2O on a magnetic stirrer.

Add a medium sized magnet spin bar and activate the spinning process. Slowly add 57 mL of glacial acetic acid, 58 grams of sodium chloride, and 0.30 grams of sodium citrate to the water. The solution will be warm and strongly acidic. Titrate the solution to pH 5.2 by slowly adding 10N NaOH. Finally, add more dH_2O to a final volume of 1.00 liter. Transfer the solution to a properly labeled one-liter plastic bottle for future use.

(3) TISAB II

TISAB II differs from TISAB I only in that it contains CDTA, a chelator of aluminum and several other divalent cations that may form complexes with fluoride. It is purchased from Orion Research. It is recommended that TISAB II be used for samples suspected or known to contain significant amounts of aluminum which can complex fluoride ions and renders them undetectable by the fluoride electrode. TISAB II, however, can be routinely used instead of TISAB I for the analysis of all water samples.

(4) Preparation of Stock Standards

Use only the volumetric flasks dedicated to the preparation of fluoride analytical standards. Prepare a stock standard containing a fluoride concentration of 100.0 mg/L by adding 221.1 mg of sodium fluoride to a final volume of 1.000 L. beginning with the 100.0 mg/L standard and using the serial dilution method, made a series of stock standards with fluoride concentrations of 50.00, 10.00, 5.000, 2.500, 1.000, 0.500 and 0.250 mg/L. These standards are stored in one-liter plastic bottles at room temperature. The fluoride concentrations appear to be stable for at least 10 years.

(5) Preparation of Analytical Standards for Direct Analysis of Water Samples

Three sets of three analytical standards are prepared for each analytical run. These standards are used to generate the standard (calibration) curve from which the concentrations of fluoride in the water samples will be determined. They fluoride stock standards to be used are 0.50, 1.00 and 2.50 mg/L. The standards are prepared for analysis by adding 250 µL of TISAB (I or II) to 500 µL of each stock standard solution. Similarly, the water samples are prepared for analysis by adding TISAB (250 µL) to each water sample (500 µL). One set of the three analytical standards is read at the beginning of the run, one set is read at or near the mid-point of the run, and one set is read at the end of the run.

b. ***Controls.***

1. ***Quality Control Bench Standards.*** One set of three bench fluoride standards, 0.50, 1.00 and 2.50 mg/L are prepared using TISAB as described in the preceding paragraph. They are read at the beginning of each analytical run. The purpose of the bench standards, whose fluoride contents are known to the analyst, is to confirm that the analytical procedure is under control as judged by: (1) historical records of millivolt reading for equivalent concentrations of fluoride and (2) the slope, intercept and linear regression r^2 values of the relationship of the mV readings to the concentrations of fluoride.

2. ***Quality Control Blind Standards.*** The concentrations of fluoride in blind quality control

standards are not known to the analyst. They are prepared and inserted into an analytical run by the laboratory director at his discretion. When blind quality control standards are used there will be a minimum of one and a maximum of three such standards. The concentrations of fluoride in the blind standards will be within the range of the analytical standards, i.e. from 0.25 to 2.50 mg/L.

c. ***Major Instrumentation and Other Equipment.***

Potentiometer. Orion Star, model A211 or similar pH/mV meter.

Ion-Specific Electrode. Orion, 9409 fluoride electrode.

Reference Electrode. Accumet minature calomel reference electrode.

Rotary Shaker. New Brunswick rotary shaker.

Desktop Computer and Printer with appropriate software.

7 CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

a. ***Cleaning and calibration***

When not in use the tips (sensing ends) of the fluoride and reference electrodes are stored in 0.01 mM NaF and in saturated KCl solutions, respectively. The fluoride and reference electrodes are rinsed with dH2O and dried with Kimwipes at the beginning of each analytical run and after reading each standard and each sample.

b. ***Calibration Curve***

A calibration curve for this assay is based on the analysis of the set of standards described in section 6.a.5 above. A set of three fluoride standards ranging in value from 0.50 to 2.50 mg/L is analyzed at the beginning, middle and end of each run.

c. ***Verification***

Initial. The initial accuracy of this method was established by analyzing a series of standards prepared as described above. The resulting calibration curves (mV vs ln of the concentration of fluoride) were linear with r^2 values >0.98.

Daily. Prior to assaying each run of water samples, the mV results from the fluoride bench standards and first set of analytical standard are reviewed for acceptable accuracy and precision as judged by the slope, intercept and r^2 of the standard curve and by comparison with historical mV readings from previous runs.

8 PROCEDURE OPERATING INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

8.1 Fluoride Analysis: Determine the Millivolt Potential

1. All standards and water samples are prepared by adding 250 µL of TISAB to 500 µL of each standard and each sample. The solutions are placed together in a 5

mL disposable Microbeaker and mixed by gentle swirling.

2. "Warm up" the fluoride and reference electrodes. This is done by placing the electrodes in the standard solutions starting with the lowest and then the progressively higher standards. Rinse with dH_2O and dry the electrodes between exposures to the solutions. Repeat this process 3-5 times until the millivolt readings for each standard are reproducible and then record the millivolt readings. Then read the bench standards. The TISAB-buffered samples can now be read.
3. Place the electrodes in contact with the 750 μL buffered solution. Gently rotate the Microbeaker every 10-15 seconds until a stable millivolt reading is obtained. Record the mV reading. Stability is usually obtained within 2-5 minutes.
4. Remove the electrodes from the solution, rinse them with dH_2O and dry with KimWipes.
5. Drop a small piece of pH paper into the solution that was just analyzed and record the pH which should be between 5 and 6. Lower pH values cause the formation of hydrofluoric acid (HF) which is not detected by the electrode. High pH values cause an overestimation of the fluoride concentration because the electrode responds equally well to hydroxyl ions.

8.2 Calculation of the Fluoride Concentrations

1. Open the StatView or Excel spreadsheet for "Direct Analysis" and enter the name of the study, the kind of samples being analyzed, the date, the analyst's initials, etc.
2. Next column: Enter the identification names or code numbers of the samples.
3. Next column: Enter the concentrations of the fluoride standards.
4. Next column: Enter the millivolt readings for the standards and samples.
5. Convert the amounts of fluoride in the standards to their natural logarithms (ln).
6. Perform a linear regression analysis with millivolts of the standards and samples on the X axis (independent variable) and the ln of the standards on the Y axis (dependent variable). Select "predicted values."
7. The output will be the natural logarithms of the amounts of fluoride in the samples.
8. Calculate the concentrations of fluoride in the samples by taking the antilogarithms.

9 REPORTABLE RANGE OF RESULTS

9.1 Linearity Limits

The mV responses of the fluoride electrode to ionic fluoride concentrations are linear, on semi-logarithmic plots, from 3 micromoles per liter to 1,000 millimoles per liter.

9.2 Limit of Detection (LOD)

The lower LOD is approximately 0.10 mg/L. Fluoride concentrations less than 0.10 mg/L are recorded as "less than lower LOD." The upper LOD is 19,000 mg/L. Thus, the

upper LOD will not be encountered when analyzing drinking water using the method described in this Procedure Manual.

9.3 Precision

Precision has been estimated by repetitive analysis of water samples and fluoride standards (see section 6.a.5 above) for each analytical run. Precision has been found to be within ±10% for each standard.

9.4 Accuracy

The accuracy of fluoride analysis of water using the fluoride electrode in this laboratory was established monthly for more than 10 years by participating in the CDC's Fluoride Proficiency Program. During this period several hundred water samples were analyzed. Except for 22 results from this laboratory all results were within the target range (target concentration ±5%). The results that were outside the target range were just barely outside that range. Accuracy is also shown by consistency of mV readings for given standards at the beginning, middle and end of each run.

9.5 Analytical Specificity

This method uses the fluoride-specific electrode. The only other ion to which the electrode responds is the hydroxyl ion. Interference by hydroxyl ions is eliminated by adjusting the pH of the analyzed solution to approximately 5.0. At this pH the hydroxyl ion concentration, 10^{-9} moles/L, is far below the electrode's lower LOD (ca. 5×10^{-6} moles/L).

9.6 Carryover

The fluoride electrode is rinsed and cleaned with deionized water and dried (see above) between the analysis of every standard and sample. There has been no evidence of carryover as judged by duplicate analyses of water samples and by repeated analyses of analytical standards at the beginning, middle and end of each run.

9.7 Freeze-Thaw and Storage Stability

Water samples are received from Westat on dry ice in the frozen state. They are then placed in the freezer to await thawing at room temperature followed immediately by preparation for analysis. There are no cases in which a sample is thawed, frozen again and then thawed again or repeatedly put through freeze-thaw cycles.

10 QUALITY ASSURANCE AND CONTROL

10.1 Quality Assurance

10.1.1 Safety

Georgia Regents University maintains ongoing Biosafety, Radiation Safety and Chemical Safety programs to assure safe working conditions throughout the University campus. The office telephone numbers for these programs are 706-721-2663, 706-721-9826 and 706-721-

9643, respectively. They can be called to answer questions or provide assistance as needed. Classes pertaining to each of these programs are offered on a periodic or as-needed basis.

10.1.2 Specimen Handling

Introduction

The goal of specimen handling is to optimize the accurate and reliable measurement of fluoride in water and plasma samples to be analyzed in the 2012-2017 “Analysis of Fluoride in Water and Plasma” project conducted by NHANES-CDC.

10.1.3 Identification of Specimens

The labeled and coded water and plasma samples are shipped periodically from Westat on dry ice to Dr. Whit ford’s laboratory for determination of their fluoride concentrations. Personal identifiers (e.g., names of survey participants) are not included on test specimens. NHANES uses a random internal specimen ID number to identify and track individual samples. The Integrated Survey and Information System (ISIS) system at Westat and the National Center for Health Statistics (NCHS) maintain information that links the specimen ID number to the survey participant’s name.

10.1.4 Questions on Analytical Method for Fluoride in Water

Dr. Whitford and Ms. Danielle Riley address questions concerning the analytical methods. Detailed information concerning the methods can be found in the appropriate sections above.

10.1.5 IRB Review

Introduction

Under a congressional mandate (Section 306 of the Public Health Service Act 42 U.S.C.242k) since 1960, NCHS has collected data on the health of the people of the United States through interviews and extensive physical examinations. Seven surveys using health examination procedures have been completed since 1960. As in previous NHANES programs, the survey’s primary purpose is to produce descriptive statistics that can be used to measure and monitor the health and nutritional status of the civilian, noninstitutional U.S. population. Data collection for this survey involves about 7,000 survey participants per year, of whom about 5,000 per year are expected to be examined.

Human Subjects Review

The NCHS Ethics Review Board (ERB), as defined by the CDC institutional review board (IRB) criteria, has approved collection and laboratory analysis of all human specimens that are part of the current NHANES survey. The IRB of Georgia Regents University has determined that the analysis of water and plasma for fluoride in Dr. Whitford’s laboratory is not “human research.”

10.2 Quality Control

The fluoride quantitative analytical procedures involve several operations, or steps, each of which is subject to some inaccuracy or imprecision or to the possibility of a mistake. The immediate aim of quality control is to ensure that the analytical values produced are sufficiently reliable for their intended purpose.

A good quality control program monitors the following five parameters:

1. **Clerical Error:** This includes properly documented acknowledgment of transmittal and receipt of specimens (for example, “logging in”), proper labeling of all specimens, correct assignment of laboratory values to the proper subject ID number, and maintenance of proper records for all specimens for future reference.

2. **Techniques:** This includes continued assurance that all personnel performing an assay understand the principles underlying a particular assay and are cognizant of the proper technique for that assay; that all personnel use the same technique for a particular assay; that there is ready access to a current technique manual; and that periodic review is undertaken to ensure use of the most current and reliable techniques.

3. **Reagents and Materials:** This includes confirmation of commercial standards and controls before they reach the bench; proper labeling of reagents, particularly those prepared in the laboratory; ensuring all reagents in use are not outdated; having an adequate supply of current reliable reagents; proper calibration of equipment, such as pipettes; and proper washing of glassware.

4. **Bench Performance:** This includes the use of controls and standards for each assay performed, a technique based on sound statistical principles which allows the technologist performing the assay to detect error outside of previously determined limits before reporting data; documentation of daily bench performance for detection of less obvious errors (particularly those which tend to accumulate over time, so-called “drift”); and established procedures to be followed wherever error is found to exceed previously determined limits.

5. **Instrumentation:** This includes periodic preventive maintenance of all instruments in use in the laboratory and documentation that each instrument is maintaining a previously determined level of each performance at each check.

10.2.1 Bench Controls for Direct Analysis of Water

For each run of water fluoride analysis three bench standards will be analyzed. The fluoride concentrations of these standards will be 0.50, 1.00 and 2.50 mg/L. They will be analyzed at the beginning of each run (i.e. before the analytical standards that are used to establish the standard curve and before analysis of the water samples) to ensure that the analytical system is under good control.

10.2.2 Quality Control Records

Quality control results will be evaluated by Dr. Whitford and also sent to Westat and CDC for evaluation for the degree of analytical adequacy and control. Records of all quality control results are maintained for at least 2 years. A QC logbook is maintained that documents out-of-control conditions and remedial actions taken to correct out-of-control conditions.

10.2.3 Proficiency Testing

There is no proficiency testing programs known to this laboratory for the determination of fluoride concentrations in drinking water using the ion-specific fluoride electrode.

11. REMEDIAL ACTION IF CALIBRATION OR QC SYSTEMS FAIL TO MEET ACCEPTABLE CRITERIA

11.1 Calibration.

System calibration and general readiness is assessed before each analytical run from a review of the mV readings of the bench standards and the first set of analytical standards. This is done by (1) determining the slope of the mV vs. fluoride concentration curve of the bench standards and mV changes for 2-fold and 5-fold changes in fluoride concentrations of the bench standards and (2) by comparison of the mV readings of the bench standards to historical mV readings of the same standards. The mV change for a 2-fold change in concentration should be 17 ±1 mV; the change for a 5-fold in concentration should be 41 ±1 mV. When corrective actions are indicated, they are performed and the system is re-evaluated with additional bench standards until acceptable results are obtained before water samples are analyzed.

11.2 Quality Control.

If the results from analysis of three QC bench samples are outside the acceptable limits, ±3 standard deviations of the historical means, and a reason is identified for the apparent problem, the problem is indicated and the run is scheduled for repeat sample preparation and analysis for samples that have sufficient volume remaining. If the problem is not identified, sample preparation and analysis is suspended until the problem or problems are discovered and corrected. Any questionable sample identified by QC or individual sample evaluation that cannot be confirmed by repeat analysis is not included in the reportable database of results.

12 LIMITATIONS OF METHOD: INTERFERING SUBSTANCES AND CONDITIONS

The only non-fluoride ion to which the fluoride electrode responds is the hydroxyl ion. The addition of TISAB I or II buffers the standard and water samples to pH 5. This reduces the concentration of hydroxyl ions orders of magnitude below the electrode's LOD for fluoride and hydroxyl ions. A pH of 5 also eliminates interference by hydrogen ions which combine reversibly with fluoride ions to form hydrofluoric acid (pK_a = 3.4), a compound not sensed by the electrode.

13 REFERENCE RANGES (NORMAL VALUES)

There are no “normal” fluoride concentrations in drinking water. Public water supplies to which fluoride is added for the prevention and control of dental caries have concentrations close to 0.7 mg/L as recommended by the CDC in 2011. Well water samples and bottled water products have concentrations that can range from 0.1 to 4 mg/L or more.

14 CRITICAL CALL RESULTS (“PANIC VALUES”)

Not applicable for this procedure.

15 SPECIMEN STORAGE AND HANDLING DURING TESTING

Water samples are received frozen and stored frozen at approximately -20°C until analysis. After samples are aliquoted for analysis the remainder of water, if any, is returned to the freezer at -20°C for later repeat analysis if required.

16 ALTERNATE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM FAILS

No alternative method is available. The test system, however, is robust and durable. During the past 40+ years of experience the only problem that delayed analytical progress was breakage of the miniature calomel reference electrode. For that reason, a backup reference electrode, fluoride electrode and potentiometer are always available.

17 TEST RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Not applicable at this time.

18 TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

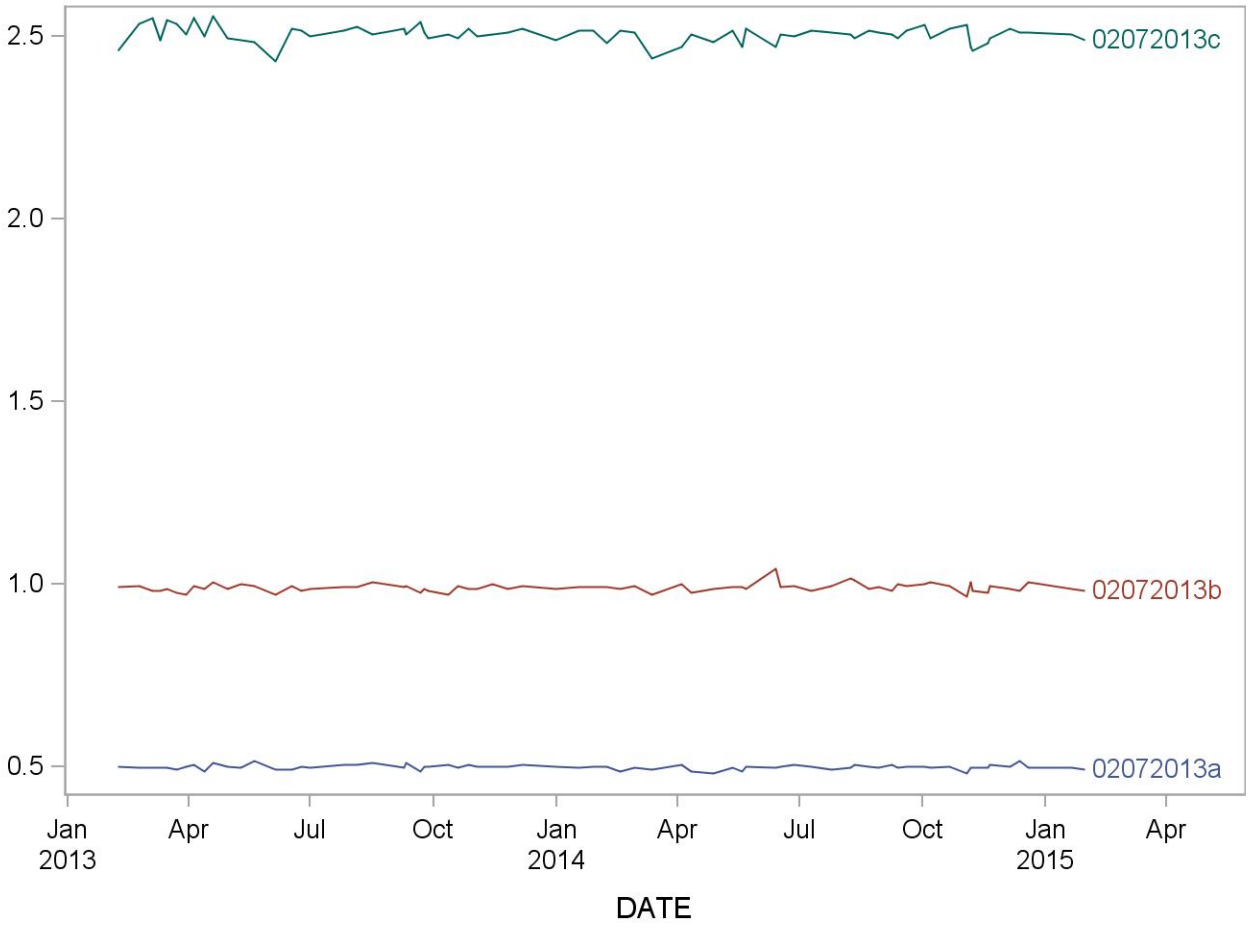
Following analysis residual volumes of water, if sufficient for further analyses, are held in storage at approximately -20°C in the fluoride laboratory. Water samples are not transferred of referred. Water samples are accounted for as described in sections 3 and 4 above.

19. SUMMARY STATISTICS and QC GRAPHS

See following pages

2013-2014 Summary Statistics and QC Chart for Fluoride (water) mg/L

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
02072013c	168	07FEB13	30JAN15	2.503	0.025	1.0
02072013a	168	07FEB13	30JAN15	0.497	0.007	1.5
02072013b	168	07FEB13	30JAN15	0.989	0.012	1.2



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