Laboratory Procedure Manual

Analyte: **Fluoride**

Matrix: **Water**

Method: Fluoride in Water using ISE

Method No: 3048.1-01

Revised: **09/26/2019**

As performed by:

Inorganic and Radiation Analytical Toxicology Branch (IRATB)
Division of Laboratory Sciences
National Center for Environmental Health

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Important Information for Users

CDC periodically refines these laboratory methods. It is the responsibility of the user to contact the person listed on the title page of each write-up before using the analytical method to find out whether any changes have been made and what revisions, if any, have been incorporated.

Public Release Data Set FLDEW_K Information

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

Data File Name	Variable name	Description
FLDW_K_R	LBXWFL	Fluoride – water (mg/L)

1. Summary of Test Principle and Clinical Relevance

Fluoride concentrations in water samples and aqueous calibrators are measured potentiometrically using an ion selective electrode (ISE). The electrode is calibrated using fluoride solutions with known concentrations. Calibrators and samples are diluted with a total ionic strength adjustment buffer (TISAB) and are measured under the same conditions. The TISAB contains an acetic acid/acetate buffer that adjusts the pH of the solution to approximately pH 5. At this pH the formation of HF is negligible and the concentration of OH-, the only other anion that the electrode responds to, is insignificant. It also contains NaCl to establish a high and constant ionic strength and a complexing agent that inhibits cations that could interfere by forming complexes with fluoride.

Fluoride is a natural element found at different concentrations in drinking water as well as in soil. Water and water-based beverages are the primary source of dietary fluoride. Approximately 80% of dietary fluoride comes from tap and bottle water (1-2).
Fluoride is voluntarily added to some drinking water systems as a public health measure for reducing the incidence of cavities among the treated population. The decision to fluoridate a water supply is made by the state or local municipality and is not mandated by EPA or any other federal entity. The Centers for Disease Control and Prevention (CDC) provides recommendations about the optimal levels of fluoride in drinking water in order to prevent tooth decay. For community water systems that add fluoride to their water, the Public Health System (PHS) recommends a fluoride concentration of 0.7 mg/L (parts per million [ppm]) to maintain tooth decay prevention benefits and reduce the risk of dental fluorosis (3). 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 (3, 4). EPA's current fluoride enforceable standard (maximum contaminant level) is 4.0 mg/L. The standard is set to this concentration to prevent severe skeletal fluorosis. The EPA's secondary maximum contaminant level (SMCL) for fluoride is 2.0 mg/L (4, 5). Concentrations above 2 mg/L may cause some degree of dental fluorosis but only if chronically ingested by children with developing teeth. Children are at risk for fluorosis in the permanent teeth from birth through 8 years of age (3, 6).

2. Safety Precautions

Wear protective gloves, laboratory coats, and safety glasses during all steps of this method. Discard residual aliquotted sample(s) containing buffer into an appropriate waste container. Make sure that the residual waste is within the appropriate pH range prior to disposing of the waste down the sink. Refer to method DLS 3500 *SOP for Handling Corrosive Liquid Laboratory Waste* for more information. Place waste (pipet tips, vials, gloves, etc.) that come into contact with reagents and water samples in biohazardous autoclave bags even though they do not come into contact with biological samples. This is a precautionary measure used to eliminate uncertainty about the safety of waste that is being disposed of. Keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces when work is finished.

Reagents used in this analytical method include those listed in Section 7. Safety Data Sheet documents (SDS) for these chemicals are readily accessible as hard copies in the lab. If needed, SDS for other chemicals can be viewed at http://www.ilpi.com/msds/index.html or in https://chats.cdc.gov/Pages/Home.

a. **Waste Disposal:** Operators of this method must take the Hazardous Chemical Waste Management for CDC Workers course upon initial hire and yearly refreshers thereafter.

1) Waste to be Placed into Biohazard Autoclave Bags & Pans:

1. Place all disposable items, including vials containing water samples and pipette tips that come in contact with the water samples, in an appropriate container. This eliminates questions on behalf of facilities personnel as to whether or not the waste that they are disposing of is safe.

2) Waste discarded down drain:
Waste collected in dedicated waste containers during sample preparation and sample analysis can be disposed down the drain after verifying that the pH of the accumulated waste is between 5 and 11. This is the required range for disposal down the drain. If the pH is outside that range, the waste must be treated as described in the standard operating procedure DLS 3500 prior to discarding it down the drain. The chemical composition of the waste is approximately 50% TISAB and 50% tap water or deionized water.

3. Computerization: Data System Management

Data is collected using LabX software. The analytical run can be controlled either directly from the keypad of the meter or by using the software.

Non-computerized data collection:

Note: This method of data collection is to be used only when the software is inaccessible. Individual results are printed through a small USB printer, and attached to the pages of the laboratory notebook assigned to this method. The pages are scanned and attached to the STARLIMS run and placed in the run folder located here:

\\cdc.gov\project\CCEHIP_NCEH_DLS_IRATB_COMMON\Nutritional\Instruments\Mettler Toledo\ISE

The results, date and time of analysis, and sample IDs are transcribed into an Excel worksheet to allow importation into STARLIMS. The analyst enters the data. This data is reviewed by another analyst prior to being reviewed by the lab chief or team leader. The final review of the data is by the QA QC department; thus creating a four-fold level of review.

Data collection by batch using LabX, computerized:

The analytical run results are stored in LabX in the dedicated instrument computer. The run/batch results are stored by date in the results section of LabX. The laboratory set up a customized export template in LabX to allow data import into STARLIMS. The run results file are saved here:

\\cdc.gov\project\CCEHIP_NCEH_DLS_IRATB_COMMON\Nutritional\Instruments\Mettler Toledo\ISE

4. Specimen Collection, Storage, and Handling Procedures; Criteria for Specimen Rejection

a. Specimen handling conditions for NHANES samples are outlined in the NCHS (National Center for Health Statistics) NHANES water sample collection protocol. The protocol discusses collection and transport of samples and any special equipment required.

b. Samples collected in the field are refrigerated then shipped to the laboratory on cold packs overnight. Store samples refrigerated at approximately 2-8° C.
c. 9 mL of water is the preferred minimum aliquot size to allow for repeat analyses.
d. Ensure samples, calibrators and QC are at ambient temperature (approximately 20°C to 25°C) before measurement.
e. Every attempt is made to ensure that samples derived from outside direct CDC control are collected, stored, and transported in a manner that maintains the integrity of the sample as it was collected.
f. 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

5. Types of Quality Control Materials

a. Bench Quality Control Materials

Two bench quality control (QC) materials are made from tap water. See the "Quality Control Materials" section of this method for details on preparation. The two water QC pools used for this method are designated as:

QC level	QC Designation ID
low pool	LW-yy###
high pool	HW-yy###

where yy is the last two digits of the production year and ### is the assigned pool identification number.

QC material that is to be used for bench quality control purposes will need to be "characterized" as described in the section "Establish QC Limits for Each QC Pool". .

b. Reference Materials:

National Institute of Standards and Technology (NIST) SRM (Standard Reference Material) 3183 (Fluoride Anion (F^-) Standard Solution) is available for this method. Follow the instructions for use within the certificate of analysis.

Certified reference material (CRM) for fluoride in water (product QC3162) was obtained from Sigma-Aldrich. This CRM is traceable to NIST SRM 3183.

6. Preparation of Reagents, Calibration Standards, Controls, and All Other Materials

a. Reagent Preparation

TISAB II

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 render them undetectable by the fluoride electrode. All standards and samples with this method are prepared with TISAB II in a 1:1 ratio.

b. Preparation of Calibration Standards

For this analytical method, there are five calibrators. The Fluoride concentration of the calibrators are as follows: S1= 0.1 mg/L, S2 = 1.0 mg/L, S3 = 2.0 mg/L, S4 = 5.0 mg/L, S5 = 10.0 mg/L. Calibrators are prepared using deionized water. S0 is deionized water.

Note: Use dedicated volumetric flasks for the preparation of fluoride analytical standards to avoid contamination.

Preparation of 10.0 mg / Fluoride stock standard:

1. Add 500 µL of 1000 mg/L fluoride standard to a 50 mL volumetric flask.
2. Fill to 50 mL with deionized water and mix thoroughly.
3. Store at ambient temperature and label appropriately. Expiration date is 1 year from the date of preparation.

Preparation of 5.0 mg / Fluoride stock standard:

1. Add 250 µL of 1000 mg/L fluoride standard to a 50 mL volumetric flask.
2. Fill to 50 mL with deionized water and mix thoroughly.
3. Store at ambient temperature and label appropriately. Expiration date is 1 year from the date of preparation.

Preparation of 2.0 mg / Fluoride stock standard:

1. Add 100µL of 1000 mg/L fluoride standard to a 50 mL volumetric flask.
2. Fill to 50 mL with deionized water and mix thoroughly.
3. Store at ambient temperature and label appropriately. Expiration date is 1 year from the date of preparation.

Preparation of 1.0 mg / Fluoride stock standard:

1. Add 50 µL of 1000 mg/L fluoride standard to a 50 mL volumetric flask.
2. Fill to 50 mL with deionized water and mix thoroughly.
3. Store at ambient temperature and label appropriately. Expiration date is 1 year from the date of preparation.

Preparation of 0.1 mg/L Fluoride stock standard:

1. Add 1000 µL of 5.0 mg/L Fluoride standard to a 50 mL volumetric flask.
2. Fill to 50 mL with deionized water and mix thoroughly.
3. Store at ambient temperature and label appropriately. Expiration date is 7 days from date of preparation.

c. Quality Control Materials

Pools preparation:

Using a graduated cylinder transfer 26 liters of tap water into a pre-cleaned 30-L carboy. Repeat this process to transfer 26 L of tap water to another pre-cleaned 30-L carboy. Label the carboys for the low and high QC pools. Take an aliquot from each carboy to assay for fluoride concentration. Analyze the aliquot for each pool. Record these results for future recovery calculations. Use these results to determine the concentration of

fluoride that must be added to each pool to reach the target fluoride concentration for each pool. While stirring the pools on large stir plates, spike each pool with calculated volumes of fluoride standard (the spiking standard used must be traceable to NIST). Stir pools for at least 2 hours after spiking to ensure homogeneity. Remove an aliquot from each pool to test prior to dispensing the pool into individual vials.

Dispensing and storage:

While maintaining constant stirring of each pool, aliquot approximately 9.0 mL of water into pre-labeled 10 mL polypropylene cryovials that are labeled and sequentially numbered. A sufficient quantity of vials are pre-labeled to provide QC materials for 1000 or more analytical runs.

Stability studies assessing long-term (1 year) storage conditions of laboratory prepared quality control materials for this analytical method are pending. In the interim, short-term storage (2 weeks or less) at approximately 2-8°C and long-term storage (greater than 2 weeks) at approximately ≤-70°C is recommended.

d. Establish QC Limits for Each Pool

When a QC pool material is prepared, an analytical run to assess the homogeneity of the pools is performed after the quality control pools are aliquotted into individual vials. Vials are randomly chosen and randomly analyzed. The first and last vials dispensed are always included in the homogeneity study. Unlike the characterization of the QC, the homogeneity study should be completed in a single analytical run. Once analysis is complete, the data is statistically evaluated to determine homogeneity. If the pool does not vary from beginning to end or problem vials can be identified and eliminated, the characterization of the QC is the next step. If problems exist, the source(s) of the problem has to be identified and the pool has to be re-made and re-aliquotted.

Characterization of each QC pool establishes statistical control limits for each pool. At least 20 analytical runs are needed for a QC characterization. The preference for QC characterizations is that the runs are distributed among all of the instruments that will be used to analyze the method. When available, previously characterized QC samples or pools with target values assigned by outside laboratories are also analyzed in QC characterization runs to evaluate each run. Once analysis of all characterization runs is complete, SAS is used to calculate the characterization statistics that will be used for the statistical evaluation of all future analytical runs.

7. Instrumentation, Equipment, and other Materials

a. Instrumentation, Software, and Equipment

The following materials are obtained from the manufacturer (Mettler-Toledo, Columbus, OH) or other reseller unless otherwise specified:

1) SevenExcellence pH/Ion meter S500-F-kit (with PerfectION comb F-), Item # 30046251, or equivalent
2) Software LabX pH Express, Item # 30247987, or equivalent
3) License LabX pH 1 instrument, Item # 30247989, or equivalent]
4) License Lab X Import/Export, Item # 11153105, or equivalent
5) uMix magnetic stirrer, Item # 30040000, or equivalent
6) Metter-Toledo USB-P25 printer, Item # 11124301, or equivalent
7) Epson Ribbon Cartridge, Item # 68017663, (Epson item # ERC-09), or equivalent

8) USB printer paper, set of 5 rolls, Item # 72456, or equivalent
9) USB printer paper roll self-adhesive, set of 3 rolls, Item # 11600388, or equivalent

b. Other Materials

The following materials are obtained from the manufacturer (Mettler-Toledo, Columbus, OH) or other reseller unless otherwise specified:

1) Deionized (DI) water, high purity (≥ 18 M cm resistivity), ELGA PURELAB Classic ID, or equivalent
2) Ion Electrolyte A Reference filing solution, pk of 5, Hanna instruments Item # 51344750, or equivalent
3) Fluoride ISE 1000 ppm Standard, Hanna instruments Item # HI4010-03, or equivalent
4) Fluoride ISE 1 ppm Standard with TISAB II, Hanna instruments item # HI4010-11
5) Fluoride ISE 2 ppm Standard with TISAB II, Hanna instruments item # HI4010-12
6) Fluoride ISE 10 ppm Standard with TISAB II, Hanna instruments item # HI4010-10
7) TISAB II for Fluoride ISEs, Hanna instruments item # HI4010-00
8) 30 mL Nalgene™ Lab Quality Wide Mouth HDPE Bottle with 28mm Cap, item# 312104-0001., or equivalent
9) 15 mL polypropylene tube with conical bottom tubes to pour TISAB II, working calibrators, and DI water in the sample preparation, Greiner Bio-One., item# 188271., or equivalent
10) 50 mL volumetric polypropylene flask for standards stocks preparation United States Plastic Corp., or equivalent
11) 200 mL volumetric polypropylene flask for standards stocks preparation United States Plastic Corp., or equivalent
12) Nalgene externally-threaded cryovials (5 and/or 10 mL), Fisher Scientific, or equivalent
13) 1000 mL narrow mouth high-density polyethylene bottle, Item # 69062, United States Plastics Corporation, or equivalent.
14) 10 or 20 L carboy for waste collection and disposal, United States Plastic Corp., or equivalent
15) BH Tips 100-5000 µL Sartorius Part# 780304, or equivalent
16) BH Tips 1-10 ml Sartorius Part# 780308, or equivalent

8. SevenExcellence Operating Instructions

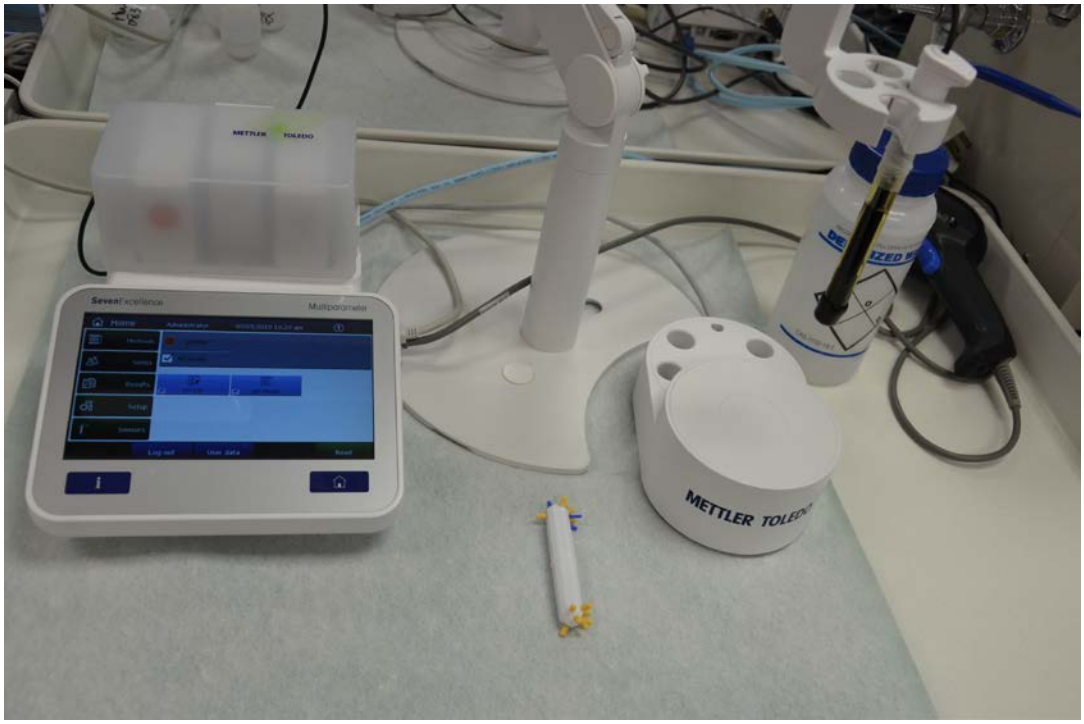
NOTE: Initial method set-up is done by following the instructions of the instrument user manual. Refer to the users' manual for specific instructions regarding instrument set up.

a. Preliminary Steps

1) Turn the Ion meter on if it is powered off.
2) Identify and gather the necessary specimen tubes containing the water samples for the batch ("run") to be analyzed.
3) Likewise, for each batch run, remove an adequate number of low and high bench QC vials "LW-yyxxx" and "HW-yyxxx" from the refrigerator. For an explanation of the nomenclature, see the *Bench Quality Control Materials* section. 4 mL of each is needed for each measurement. Allow all samples and QC materials to reach room temperature for a minimum of 20 minutes after thawing prior to analysis.

4) Prepare water samples and QC materials on the day of analysis. One run is defined as the analysis of a contiguous set of samples bracketed by bench QC materials at the beginning and end of the set. The total analysis time for a run cannot exceed 24 hours.

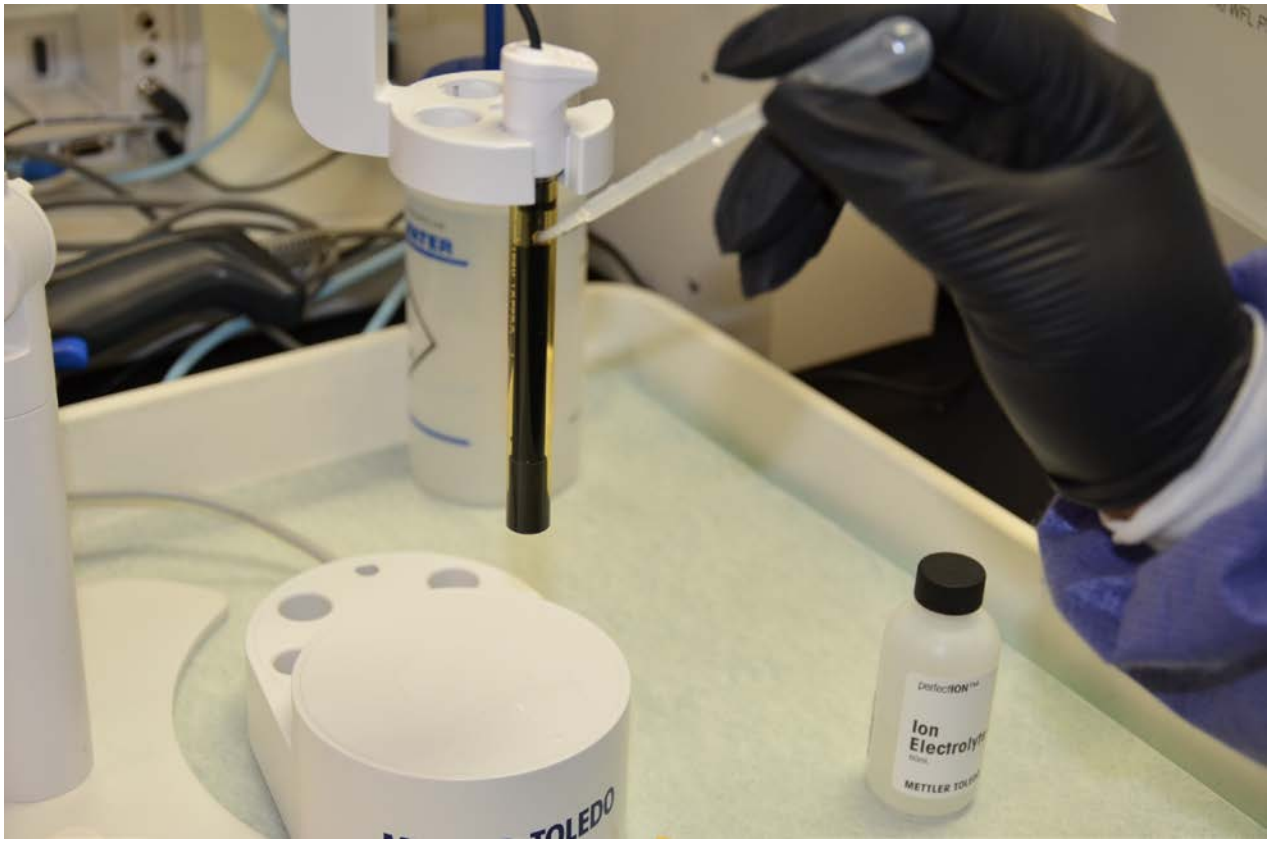
Figure 8-1 SevenExcellence set up



b. Pre- Analytical Steps

1) Remove the protective cap from the electrode and set it aside.
2) Add filling solution (Ion electrode A) each day before using the electrode as shown in Figure 8-2.
3) Check the waste container. If it is full, prepare the full container for disposal.
4) Arrange the samples in run order starting with deionized water, beginning QC, samples, and ending QC.
5) Let samples and QC sit at room temperature for at least 20 minutes after removing them from the refrigerator. If they are removed from the freezer, let them sit at room temperature for at least 20 minutes after thawing is complete.

Figure 8-2 Electrode set up



c. Direct Calibration without LabX method

1) Prepare the calibrators by adding 4 mL of the calibrator and 4 mL of TISAB II to individual 30 mL HDPE bottles. Start the direct calibration with the less concentrated standard (0.1 mg/L) to the more concentrated standard (10.0 mg/L). All samples and calibrators should be at the same temperature.
2) On the home screen (Figure 8-3) of the SevenExcellence Ion meter touch “pH/Ion”
3) The module settings will appear on the screen
4) Review the calibration settings (Figures 8-5-A and 8-5-B) before starting the direct calibration.
    a. calibration mode = linear
    b. number of standards = 5
    c. the concentrations are 0.1 mg/L, 1.0 mg/L, 2.0 mg/L, 5.0 mg/L, 10 mg/L.
5) Touch “Back” to return to the module settings screen (Figure 8-4).
6) Touch “Calibrate” on the module settings screen (bottom right corner) to start the direct calibration.

Figure 8-3 SevenExcellence LCD Home Screen

Figure 8-4 Module Settings Screen

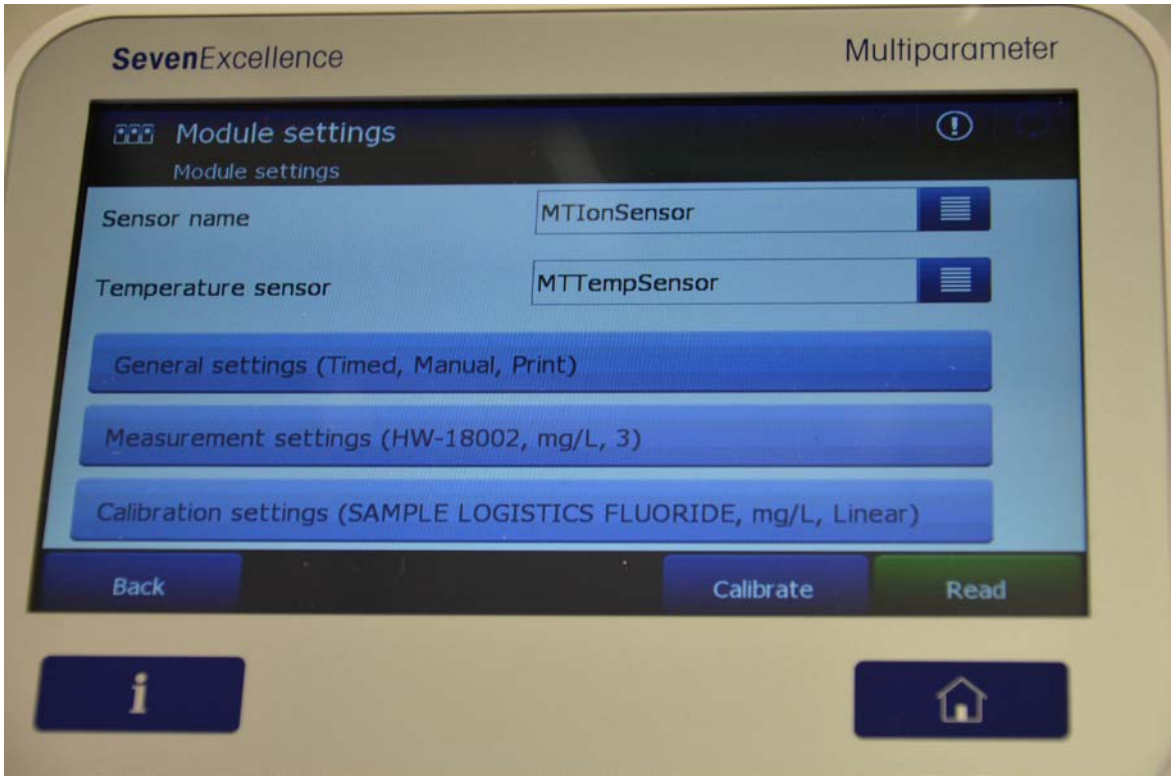


Figure 8-5-A Calibration settings A

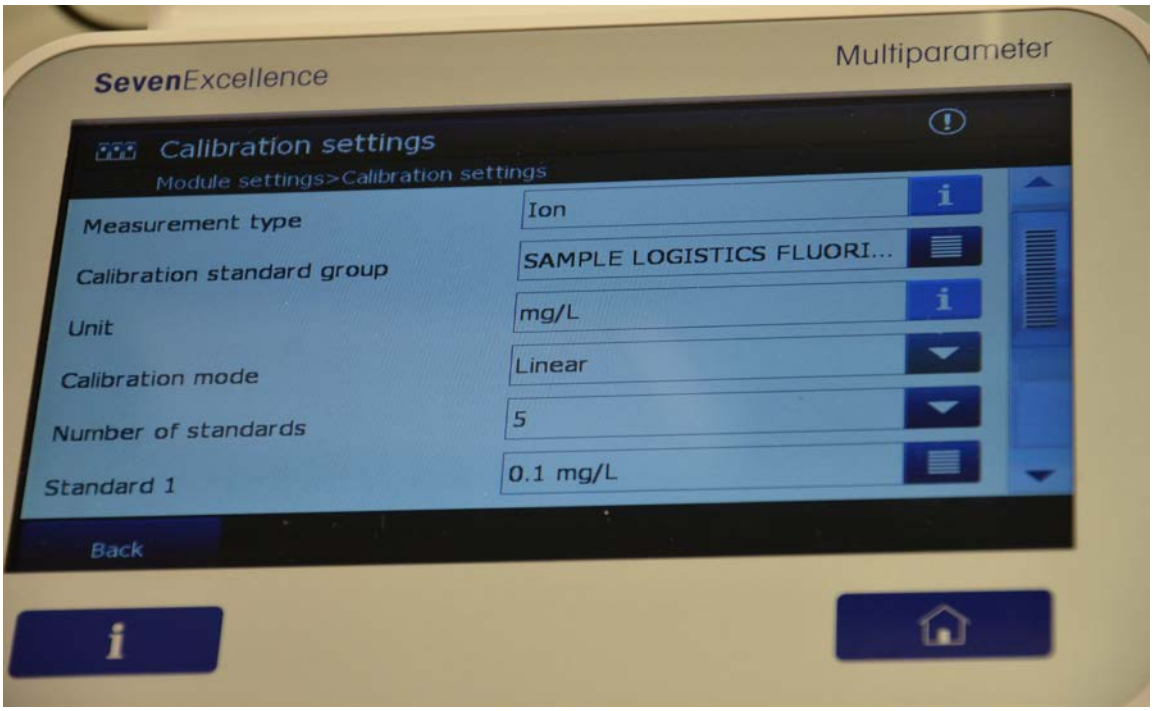
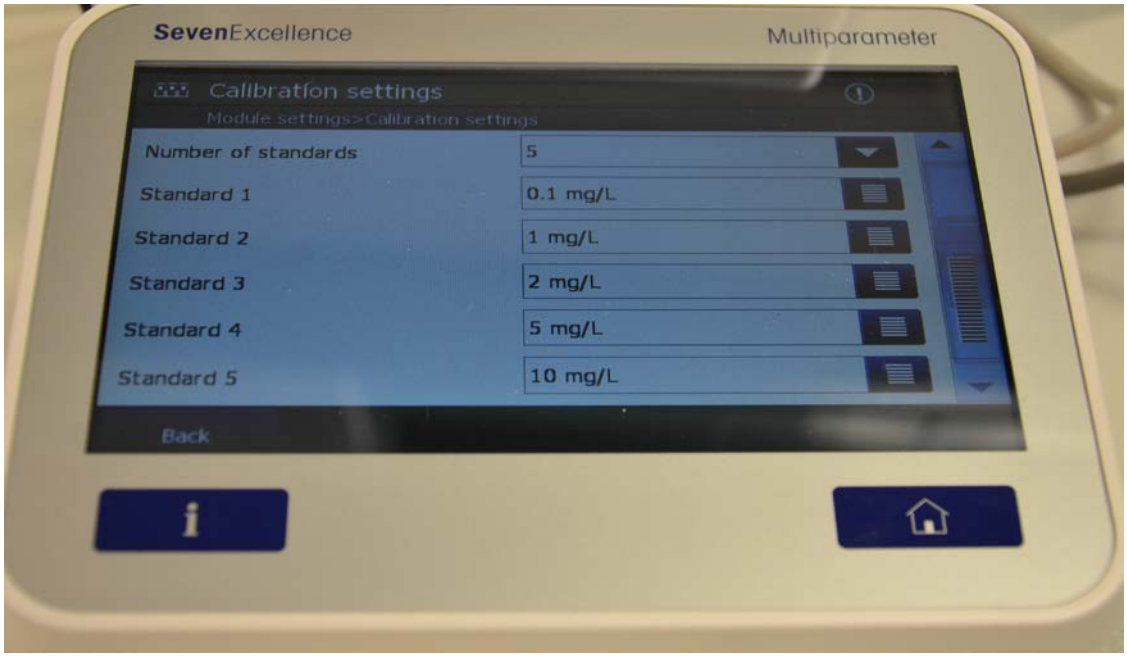
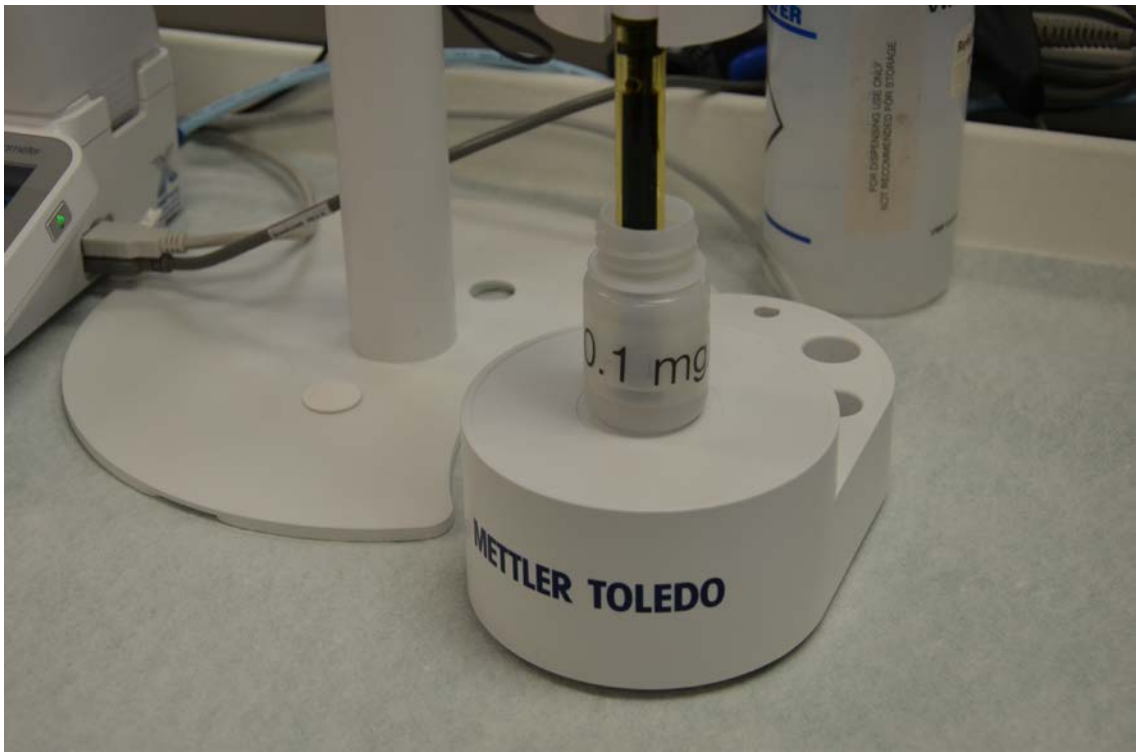


Figure 8-5–B Calibration Settings B



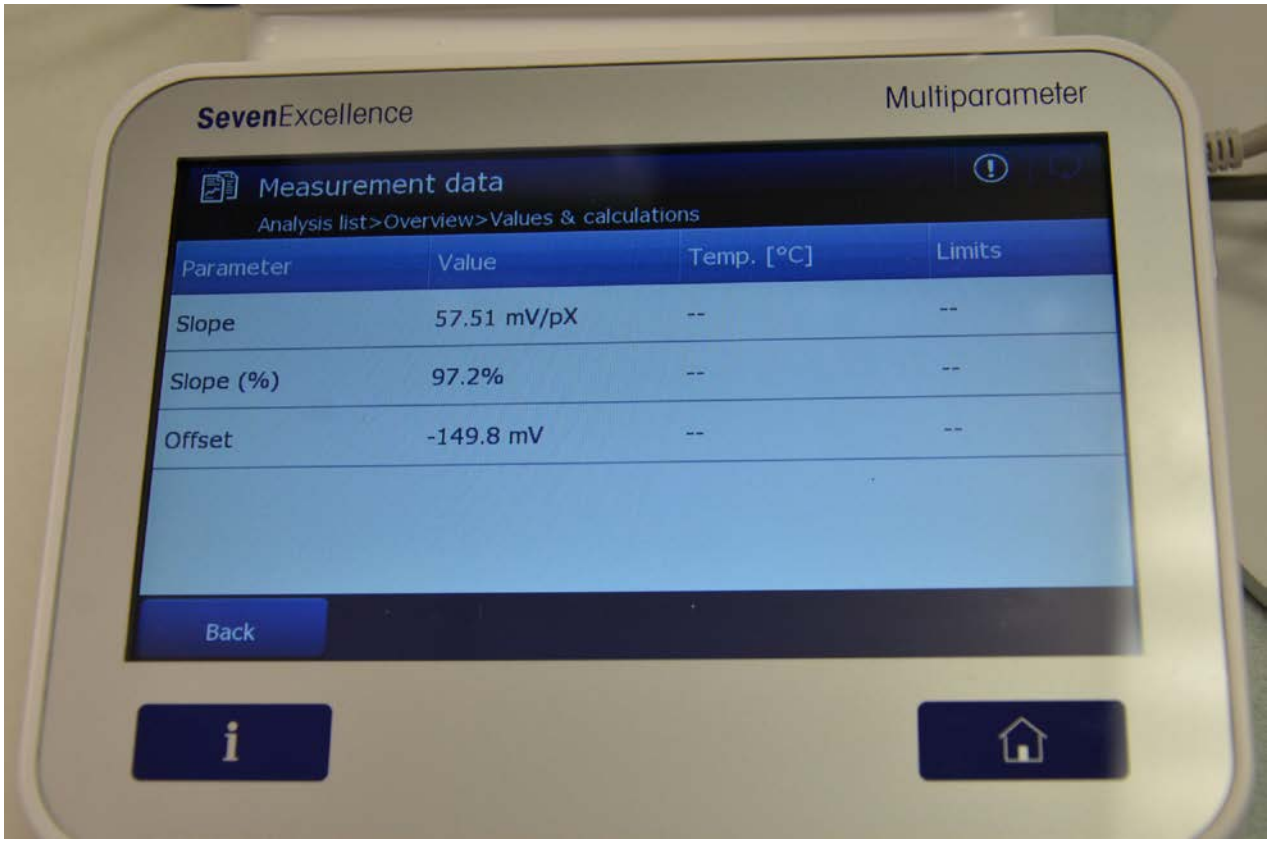
7) An “Immerse sensor in buffer /standard 0.1 mg/L.” prompt will appear on the screen.
8) Thoroughly mix the prepared calibrator sample before placing it on the uMix magnetic stirrer.
9) Place the 30 mL HDPE bottle with the least concentrated calibration (0.1 mg/L calibrator) solution on the uMix magnetic stirrer. Immerse the sensor in the solution and touch “OK” on the LCD screen.

Figure 8-7 Standard placement on uMix magnetic stirrer



10) Wait until the analysis of the standard is completed (approximately 300 seconds – 600 seconds).
11) Review the millivolts reading on the screen. The millivolts will be stable approximately after 200 seconds [approximately 3 minutes] from the start time of the analysis.
12) When the standard analysis is complete, remove the sensor from the solution and rinse it with deionized water. Blot the sensor dry using kimwipes.
13) An "*immerse sensor in buffer / standard 1.0 mg/L*" prompt will appeared on the screen.
14) Repeat steps 8-12 until all five calibrators are tested.
15) Review the slope information (Figure 8-8) before proceeding with sample analysis. A slope of 95% or greater is required to continue with sample analysis.
16) If the slope does not meet the requirements, calibrators from a different lot will be prepared and the calibration test repeated until a passing test is achieved."
17) Touch "Back" on the LCD o return to the module settings screen.

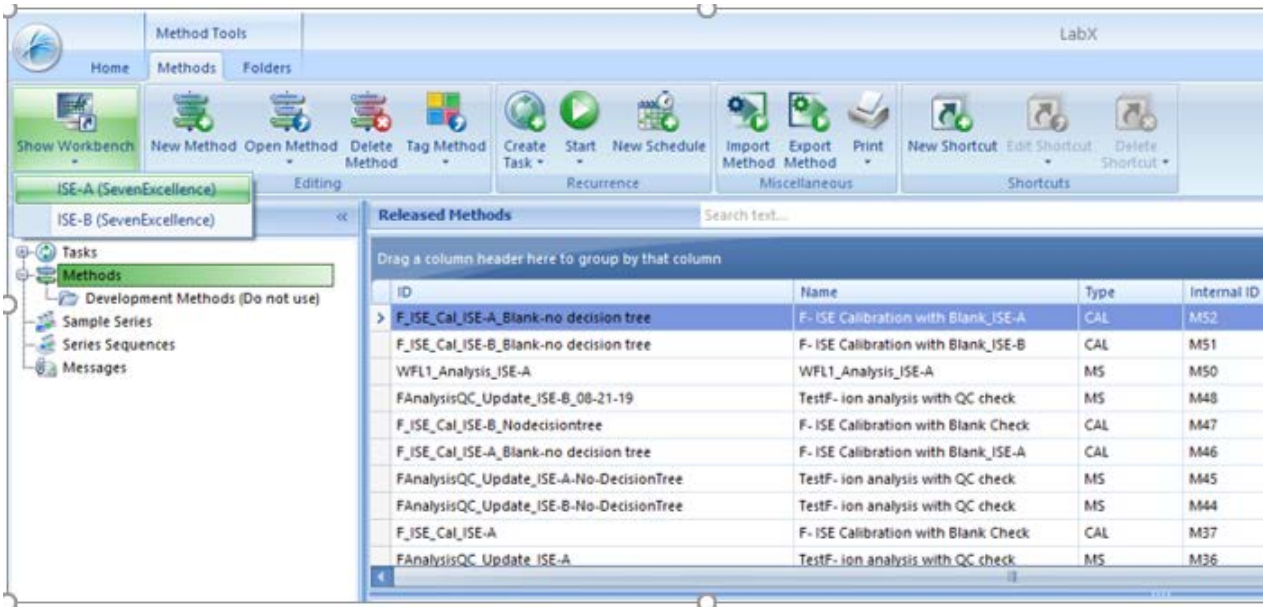
Figure 8-8 Measurement Data



d. Direct Calibration using LabX method

1) Prepare the calibrators by adding 4 mL of the calibrator and 4 mL of TISAB II to 30 mL HDPE bottles.
2) Thoroughly mix each prepared calibrator sample before placing it on the uMix magnetic stirrer.
3) Start the direct calibration with the least concentrated standard (0.1 mg/L) and progress to the more concentrated standard (10.0 mg/L). All samples and calibrators should be at the same temperature.
4) In LabX click on “Show workbench”. Select the ISE meter name that will be calibrated for the analytical run. (See figure 8-9)

Figure 8-9 LabX view of methods section



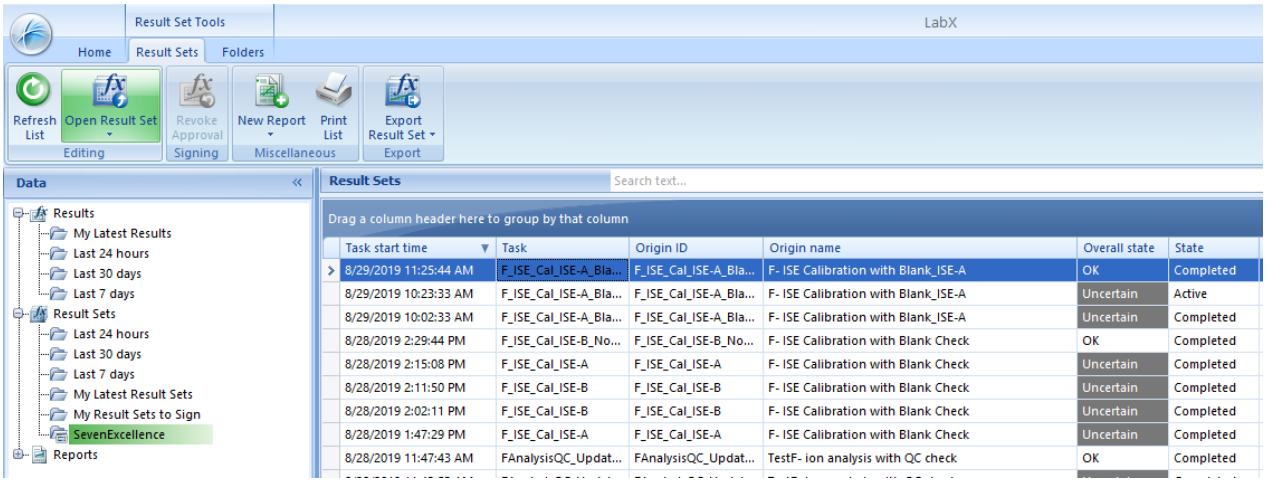
5) The workbench window will appear on the screen of the instrument computer. Select the calibration method that corresponds to the meter to start the calibration. (See figure 8-10)

Figure 8-10: ISE-A Workbench view Calibration method

6) Follow the instructions that will appear on screen of the instrument computer and the LCD screen of the meter
7) Thoroughly mix the prepared sample before placing it on the uMix magnetic stirrer.
8) Place the 30 mL HDPE bottle containing the blank sample (3048-DIH2O) solution on the uMix magnetic stirrer.
9) When the meter completes the analysis of the blank (approximately 300 seconds – 600 seconds).
10) Remove the sensor from the solution and rinse it with deionized water. Blot the sensor dry using kimwipes.
11) An "Immerse sensor in buffer /standard 0.1 mg/L." prompt will appear on the screen.

12) Place the 30 mL bottle containing 0.1 mg/L calibrator solution (4 mL of the calibrator and 4 mL of TISAB II) on the uMix magnetic stirrer. Immerse sensor in the solution and touch "OK" on the LCD screen or in LabX.
13) When the standard analysis is completed (approximately 300 seconds – 600 seconds), remove the sensor from the solution and rinse it with deionized water. Blot the sensor dry with kimwipes.
14) An “immerse sensor in buffer / standard 1.0 mg/L” prompt will appeared on the screen.
15) Repeat steps until all five calibrators have been analyzed. Review the slope information (Figure 8-8) before proceeding with sample analysis. A slope of 95% or greater is required to continue with sample analysis.
16) If the slope does not meet the requirements, calibrators from a different lot will be prepared and the calibration test repeated until a passing test is achieved.
17) In LabX, click on “Results sets” then click on “SevenExcellence”. When the list of tasks appear on the screen, click on the calibration task completed to create the Calibration report. (See Figure 8-11).

Figure 8-11: SevenExcellence’s Results set view from LabX



18) Open the results, select the “Raw data” tab, and click on “Print Data Tab” to open the Calibration Raw results report.
19) The results report window will appear on the screen. (See Figure 8-13).
20) Click on “Export as PDF” to save the calibration report in the C drive of the instrument computer.
21) Calibration report needs to be saved with the analytical run data in this location:

\\cdc.gov\project\CCEHIP_NCEH_DLS_IRATB_COMMON\Nutritional\Instruments\Mettler Toledo\ISE

Figure 8-12: Results Editor view

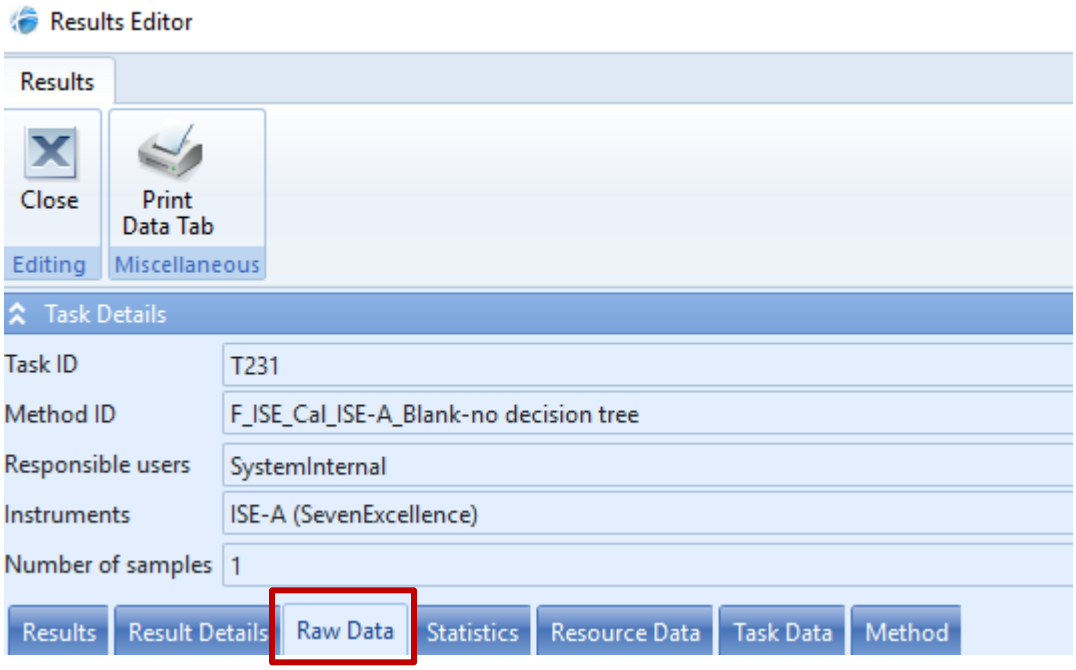
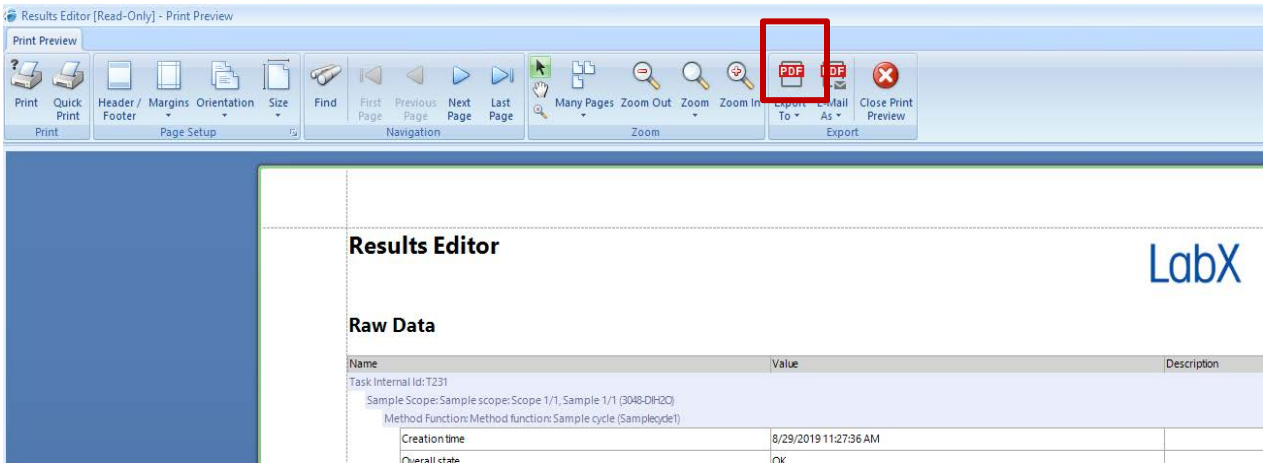


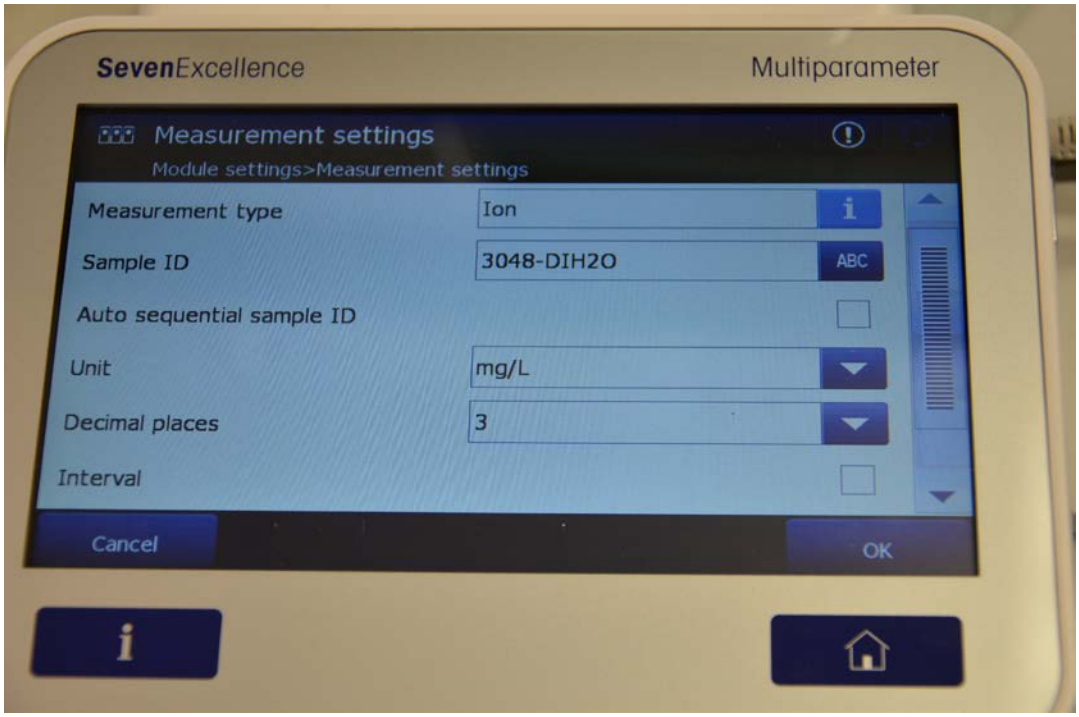
Figure 8-13: Raw data Calibration report view



Analyzing Samples in SevenExcellence Direct measurement

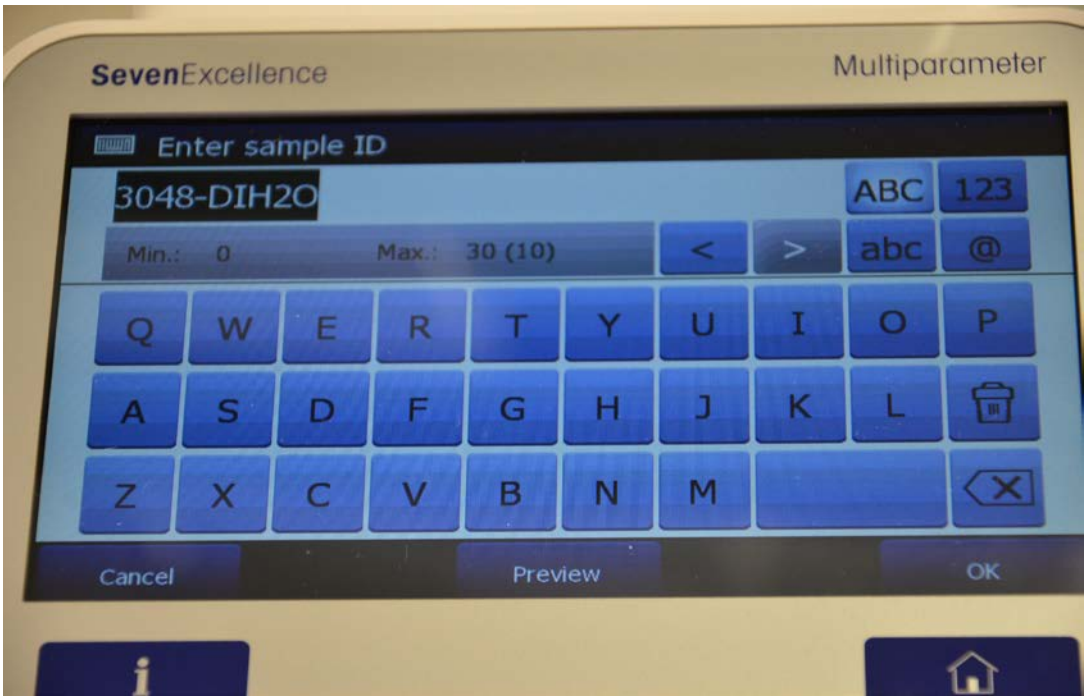
1) Empty the waste container if needed.
2) On the module settings screen touch “Measurement settings”.
3) Touch the sample ID field as shown in Figure 8-14-A to change the name.
4) Scan the barcode of the next sample to be analyzed.

Figure 8-14-A Sample ID Entry



5) Touch “OK” (Figure 8-15-B) after the name has been entered using the barcode reader from the meter.

Figure 8-14-B Sample ID Entry



6) Touch “Save” (Figure 8-16).

Figure 8-15 Module Settings

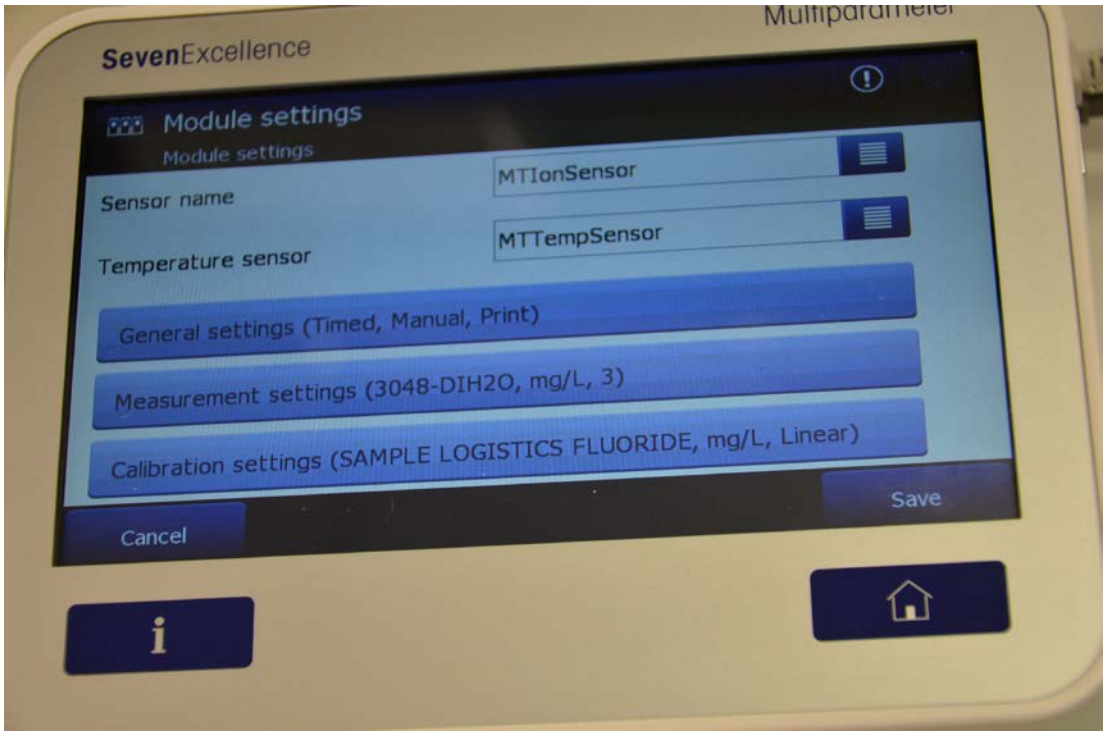
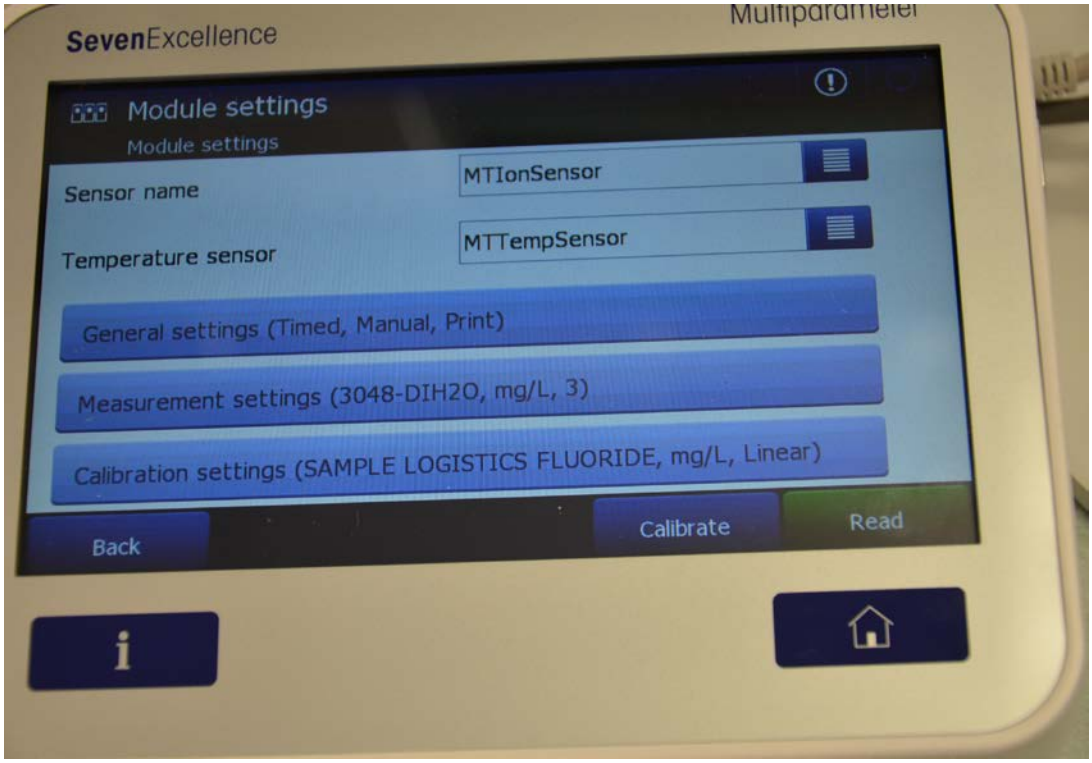


Figure 8-16 Module settings screen “Read” button bottom right corner



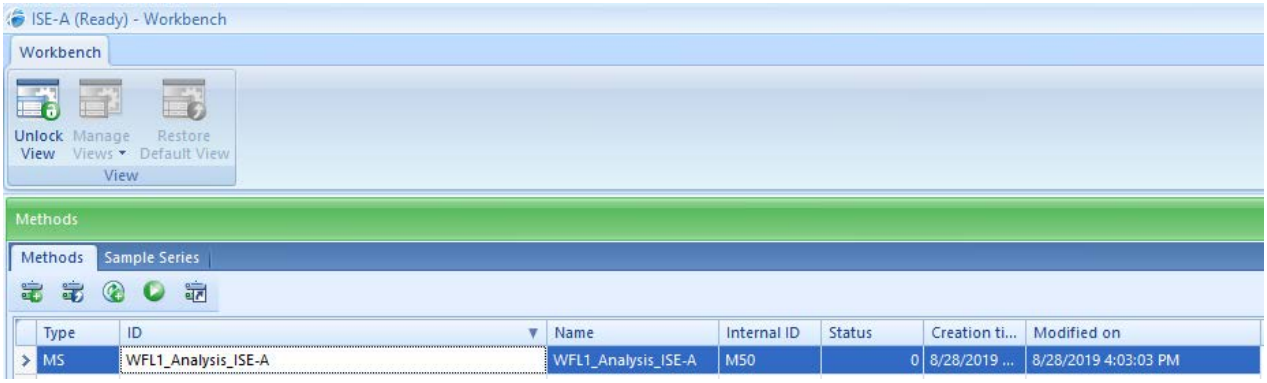
7) Mix the contents of the 30 mL HDPE bottle thoroughly.

8) Place the 30 mL HDPE bottle with the sample (4 mL of the sample and 4 mL of TISAB II) on the uMix magnetic stirrer.
9) Immerse sensor in the sample and touch “OK” on the LCD screen. The instrument will begin taking a measurement.
10) When the measurement is completed the fluoride concentration of the sample will appear on the screen.
11) Remove the sensor from the solution and rinse it with deionized water. Blot dry the sensor with kimwipes.
12) Touch OK to return to the Module settings screen and change the sample ID to the ID of the next sample to be tested.
13) Repeat steps 2-10 for all subsequent samples in the run.

e. Analyzing Samples using LabX measurement method

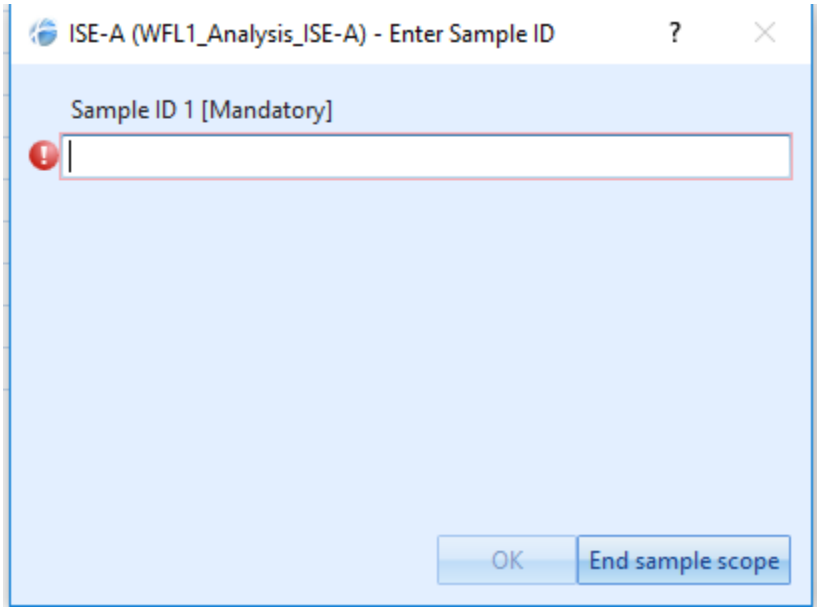
1) Empty the waste container if needed.
2) In LabX click on “Show workbench”. Select the ISE meter name that will be used for that will be calibrated for the analytical run. (See figure 8-9)
3) The workbench window will appear on the screen of the instrument computer. Select the Measurement method that corresponds to the meter to start the sample analysis. (See figure 8-17)

Figure 8-17: ISE-A Workbench view Fluoride measurement method



4) Follow the instructions that appear on screen of the instrument computer and the LCD screen of the meter
5) Mix the blank sample (3048-DIH2O) solution thoroughly in the 30 mL HDPE bottle.
6) Place the 30 mL HDPE bottle containing the blank sample (3048-DIH2O) solution on the uMix magnetic stirrer. When the meter completes the analysis of the blank (approximately 300 seconds – 600 seconds) remove the sensor from the solution and rinse it with deionized water. Blot the sensor dry using kimwipes.
7) An "Enter Sample ID." prompt will appear on the screen.

Figure 8-18: Sample ID prompt screen



8) Scan the sample ID of the next sample to be analyzed using the barcode reader of the meter.
9) Mix the prepared sample before placing it on the uMix magnetic stirrer.
10) Place the 30 mL bottle with the sample to be analyzed on the uMix magnetic stirrer. Immerse sensor in the solution and touch "OK" on the LCD screen of the meter or click “OK” on the instrument computer.
11) When the analysis of the sample is completed (approximately 300 seconds – 600 seconds), remove the sensor from the solution and rinse it with deionized water. Blot dry the sensor using kimwipes.
12) An "Enter Sample ID" prompt will appear on the screen.
13) Repeat steps 8-10 until all samples are analyzed.

j. System Maintenance

1) The system maintenance consists of performing a daily calibration test to ensure the unit is operating properly. If the test fails, calibrators from a different lot will be prepared and the calibration test repeated until a passing test is achieved.

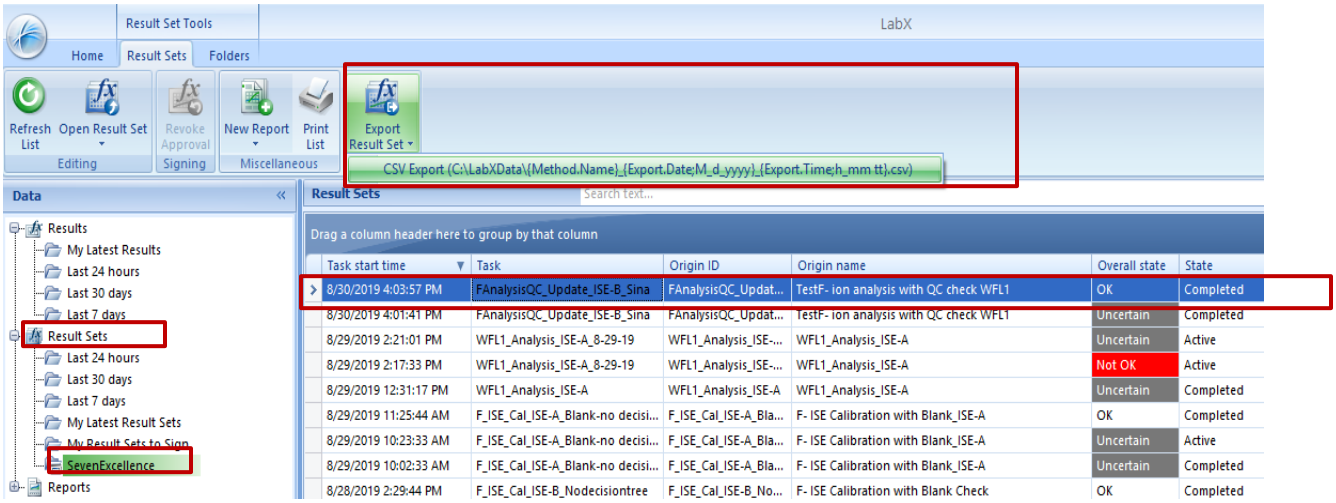
2) Preventative maintenance for the instrument should be performed once per year by a Mettler-Toledo technician or other qualified personnel.

9. Data Processing

Data processing using LabX:

1) On LabX click on “Results sets” then click on “SevenExcellence” when the list of tasks appeared on the screen, click on the task completed to export the results from the analytical run. (See Figure 8-19).
2) Save the export file with run results in the C drive on the instrument computer using the following nomenclature: "WFLYYMMDD” where "YY" is the last two digits of the year, "MM" is the month, and "DD" is the day.
3) Copy the run results file from the instrument C drive to the shared drive run folder for that date \\cdc.gov\project\CCEHIP_NCEH_DLS_IRATB_COMMON\Nutritional\Instruments\Mettler Toledo\ISE (share drive location)
4) Open the STARLIMS application and select “Pending Runs Assigned to My Labs”.
5) From the Test drop down menu select “3048 (Fluoride in water by ISE)”.
6) Click the “All Results (S)” tab. Click “[1] Upload instrument file”. Use the browser to navigate to the Excel file that is to be imported. Select your Excel Workbook file, review the data, and click “Upload”. Only recognized sample IDs will be uploaded into STARLIMS. Click "Continue".
7) Select “[2] Mark Null Results”. This will mark any QC sample that does not have a result.
8) Select “[3] Evaluate Sample QC.” This will assign each sample a Pass, Fail, or Warning such as “Re-run” or “Out of Range”. STARLIMS automatically sets all sample QC to “Pass”. If a sample does not pass, manually uncheck the “Sample QC Passed” for that sample.
9) Select “[4] Evaluate Run QC”. Choose “Proceed to next step” on the QC Evaluation box.
10) Select “Start SASQC Wizard” to begin evaluating the QC criteria. Select “Save SAS Input File” as “STARLIMS_SAS_data”. This file will be automatically overwritten each time you save the input file. Click “Save” and then “Yes” to indicate that it is okay to replace the file. Select “OK” when export is complete. The next step is to “Send to SAS Server”. Check that your run number has passed and be sure to save a copy of this Report as a PDF. Select “Finished”. A box will appear asking if you would like to proceed; select “Yes.”
11) Click “[5] Set Run QC Statuses”. Select “Proceed” if the QC passes; otherwise change the status to fail before proceeding.
12) Click “[6] Attach SAS QC File”. Select the PDF version of the SAS output previously saved.
13) Click “Set Final Wizard.” Click “Proceed.” Select the radio button “Include Result Status = “Pass”. Click “Proceed.” Verify that the correct number of results have been set final. Click “OK.” If the number of results is incorrect (due to incorrect samples IDs used or other issues), the run will have to be deleted and reimported.
14) Before Finishing Results, verify that all sample and run QC passes. The data PDF, the run Excel file, and the PDF QC file should be attached to the run.
15) Fill in “User Field 3” with the study ID and “User Field 4” with the group number.
16) Once you choose “Finish Results”, you will not be able to make revisions. When you are ready to report the results, click “Finish results”. Select the names of the individuals that you want to send your run to (the branch QA/QC officers, lab chiefs, etc.) and include your run comments in the comments section.
17) Notify the appropriate personnel via email that the run is ready to be reviewed.

Figure 8-19: LabX export report from analytical run



Data processing without LabX:

After data has been scanned into a .pdf file, then the data must be entered into and saved as an Excel workbook file. In order to compile the Excel file go to the following location:

\\cdc.gov\project\CCEHIP_NCEH_DLS_IRATB_COMMON\Nutritional\Instruments\Mettler Toledo\ISE (the file for data entry will be in this location)

1) Scan all pages of the laboratory notebook with data for the analysis date.
2) Save that file as a PDF in the run folder for that date on the shared drive using the following nomenclature: “WFLYYMMDD Scanned Results” where “YY” is the last two digits of the year, “MM” is the month, and “DD” is the day. This will also be attached as a document to the STARLIMS run.
3) Open the Excel file for data entry. Type in the data and name the .xslx file using the “WFLYYMMDD” nomenclature. Store the file in the shared drive run folder for that date.
4) Once data entry is complete, have a second analyst verify that data entry is correct. Send an email to the team lead and lab chief that data is ready for review prior to uploading to STARLIMS.
5) After the team lead or lab chief approves the data in the Excel file through email confirmation, upload the results to STARLIMS.
6) Open the STARLIMS application and select “Pending Runs Assigned to My Labs”.
7) From the Test drop down menu select “3048 (Fluoride in water by ISE)”.
8) Click the “All Results (S)” tab. Click “[1] Upload instrument file”. Use the browser to navigate to the Excel file that is to be imported. Select your Excel Workbook file, review the data, and click “Upload”. Only recognized sample IDs will be uploaded into STARLIMS. Click "Continue".
9) Select “[2] Mark Null Results”. This will mark any QC sample that does not have a result.
10) Select “[3] Evaluate Sample QC.” This will assign each sample a Pass, Fail, or Warning such as “Re-run” or “Out of Range”. STARLIMS automatically sets all sample QC to “Pass”. If a sample does not pass, manually uncheck the “Sample QC Passed” for that sample.
11) Select “[4] Evaluate Run QC”. Choose “Proceed to next step” on the QC Evaluation box.

12) Select “Start SASQC Wizard” to begin evaluating the QC criteria. Select “Save SAS Input File” as “STARLIMS_SAS_data”. This file will be automatically overwritten each time you save the input file. Click “Save” and then “Yes” to indicate that it is okay to replace the file. Select “OK” when export is complete. The next step is to “Send to SAS Server”. Check that your run number has passed and be sure to save a copy of this Report as a PDF. Select “Finished”. A box will appear asking if you would like to proceed; select “Yes.”
13) Click “[5] Set Run QC Statuses”. Select “Proceed” if the QC passes; otherwise change the status to fail before proceeding.
14) Click “[6] Attach SAS QC File”. Select the PDF version of the SAS output previously saved.
15) Click “Set Final Wizard.” Click “Proceed.” Select the radio button “Include Result Status = “Pass”. Click “Proceed.” Verify that the correct number of results have been set final. Click “OK.” If the number of results is incorrect (due to incorrect samples IDs used or other issues), the run will have to be deleted and reimported.
16) Before Finishing Results, verify that all sample and run QC passes. The data PDF, the run Excel file, and the PDF QC file should be attached to the run.
17) Fill in “User Field 3” with the study ID and “User Field 4” with the group number.
18) Once you choose “Finish Results”, you will not be able to make revisions. When you are ready to report the results, click “Finish results”. Select the names of the individuals that you want to send your run to (the branch QA/QC officers, lab chiefs, etc.) and include your run comments in the comments section.
19) Notify the appropriate personnel via email that the run is ready to be reviewed.

10. Proficiency Testing

The lab must enroll in an appropriate proficiency testing program which satisfies proficiency testing requirements as defined by the Division of Laboratory Sciences.

11. Reportable Range of Results

The reportable range for this analytical method is 0.024-10.0 mg/L.

12. Quality Control (QC) Procedures

The analyst inserts bench QC samples two times in each analytical run (a set of consecutive assays performed without interruption) so that judgments may be made on the day of analysis. Taking these samples through the complete analytical process assesses all levels of the analyte concentrations. The data from these materials are then used to estimate methodological imprecision and to assess the magnitude of any time-associated trends. The bench QC pools used in this method comprise two levels of concentrations spanning the “low-normal” and “high-normal” ranges for fluoride in water. Both of these pools are analyzed after the calibration standards are analyzed but before any patient samples are analyzed. These bench QCs must be analyzed again at the end of the run. If a second run of samples are analyzed using the same calibration curve as the first run, the QC results obtained from the second run’s own bench QC samples need to be analyzed and treated independent of the first run.

a. Precision and Accuracy

QC Results Evaluation. After completing an analytical run, consult the QC limits to determine whether the run is “in control”.. *The QC rules apply to the average of the beginning and ending analyses of each of the bench QC pools.* The QC rules are as follows:

1. If both the low-and the high-QC results are within the 2s limits, accept the run.

2. If one of the QC means is outside the 2s limits, apply the rules below and reject the run if any condition is met.
    - **Extreme Outlier –** Run mean is beyond the characterization mean +/- $4S_m$.
    - **2S Rule –** Both run means are outside the same $2S_m$ limit.
    - **3S Rule –** Run mean is outside a $3S_m$ limit**.**
    - **10 X-bar Rule –** Current and previous 9 run means are on same side of the characterization mean.
3. If one of the 4 QC individual results is outside a 2Si limit apply the rules below and reject the run if any condition is met
    - **Extreme Outlier –** One individual result is beyond the characterization mean +/- $4S_i$.
    - **R 4S Rule –** Within-run ranges for all pools in the same run exceed 4Sw (i.e., 95% range limit).
    - Note: Since runs have multiple results per pool for 2 pools, the R 4S rule is applied within runs only.

If the run is declared “out of control,” the analysis results for all patient samples analyzed during that run are invalid for reporting for the affected analytes.

S_i = Standard deviation of individual results

S_m = Standard deviation of the run means

S_w = Within-run standard deviation

13. Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria

Check to make sure that the meter and the fluoride probe are functioning properly. Perform an instrument calibration using working calibrators from a different lot. If the calibration passes, test the blank sample (3048-DIH2O) solution, a low QC and high QC sample using the LabX Fluoride Measurement method. If the QC results are within the expected values continue with the sample analysis.

If the steps outlined above do not result in correction of the "out of control" values for QC materials, consult the supervisor for other appropriate corrective actions. Do not report analytical results for analytical runs that are not within in statistical control limits.

14. Reference Ranges (Normal Values)

The Public Health Service (PHS) recommends that public water supplies contain between 0.7 to 1.2 milligrams of fluoride per liter of drinking water.

15. Action Level Results

Concentrations for fluoride that are observed to be greater than the “first upper boundary” or the “second upper boundary (defined in DLS STARLIMS as the “1UB” and “2UB’ respectively) must be reanalyzed for confirmation. The 1UB for water fluoride is 2.0 mg/L, and the 2UB is 4.0 mg/L. The result has to be marked with the appropriate comment code in STARLIMS.

16. Specimen Storage and Handling During Testing

Samples are allowed to reach room temperature during preparation. The unused portion of the sample is returned to the refrigerator.

17. Storage of Samples if Test System Fails

If the analytical system fails, we recommend that the samples be stored at approximately 2-8°C until the analytical system is restored to functionality.

18. Test Result Reporting System; Protocol for Reporting Critical Calls (If Applicable)

Not applicable

19. Transfer or Referral of Samples; Procedures for Specimen Accountability and Tracking

The exact procedure used to track samples varies with each study and is specified in the study protocol or the interagency agreement for the study. Copies of these documents are in DLS STARLIMS. In general, when samples are received, the specimen ID number is entered into the laboratory information management system (LIMS) and the samples stored in a refrigerator at approximately 2-8°C. The specimen ID is read off the vial by a barcode reader attached to the computer used to prepare the electronic specimen table for the analytical system. When the analyses are completed, the analytical results are linked to the LIMS by ID number. The analyst is responsible for keeping a notebook containing the ID numbers of samples prepared incorrectly, those with labeling problems, and those with abnormal results, together with information about these discrepancies.

20. Method Performance Documentation

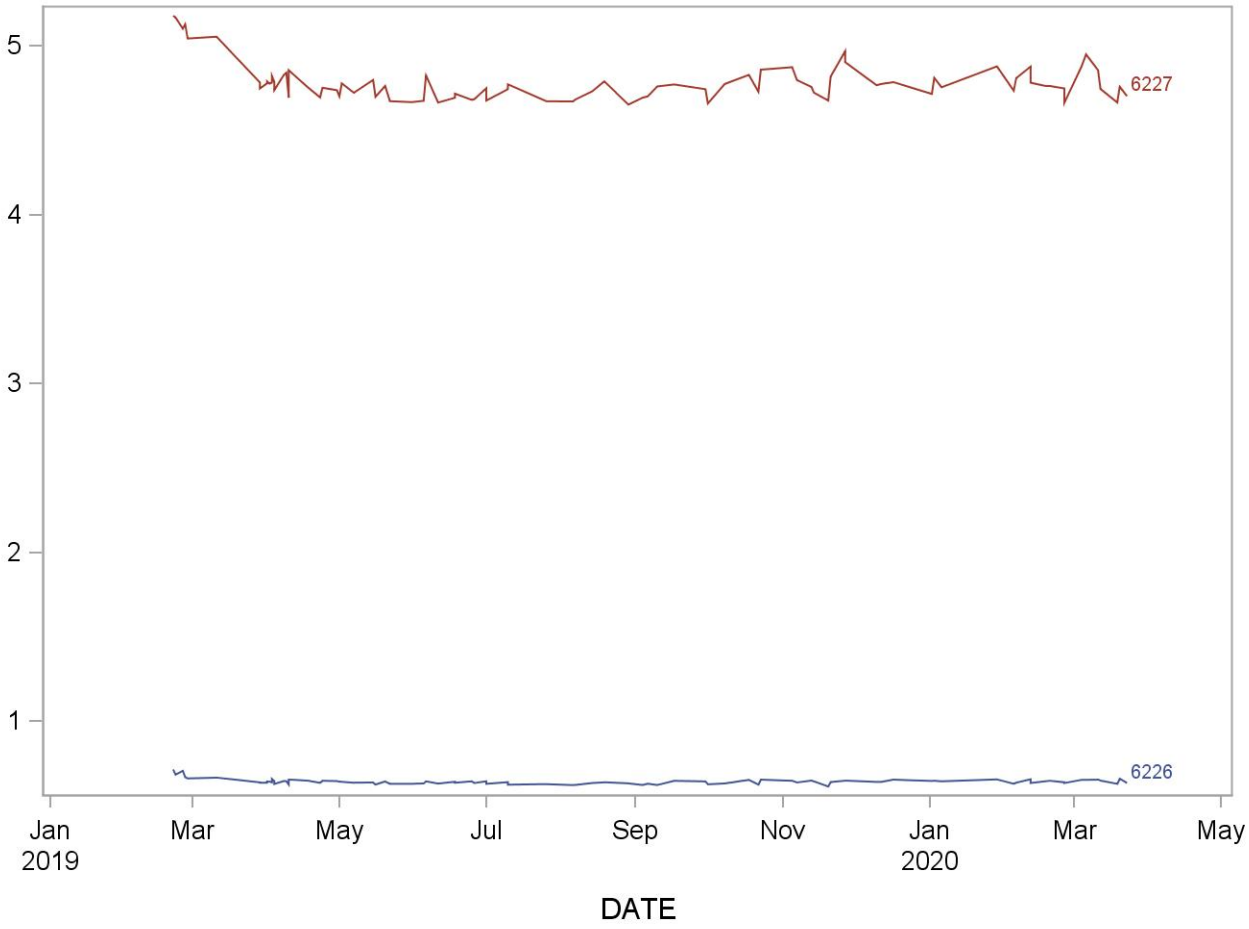
Method performance documentation for this method including accuracy, precision, sensitivity, specificity and stability is provided in Appendix A of this method documentation. **The signatures of the Branch Chief and Director of the Division of Laboratory Sciences on the first page of this procedure denote that the method performance is fit for the intended use of the method**.

21. Summary Statistics and QC Graphs

See following page.

Summary Statistics and QC Chart
LBXWFL (Fluoride (water) mg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
6226	89	21FEB19	23MAR20	0.6418	0.0156	2.4
6227	89	21FEB19	23MAR20	4.7824	0.1123	2.3



22. References

1. Palmer C, Wolfe SH; American Dietetic Association. Position of the American Dietetic Association: the impact of fluoride on health.J Am Diet Assoc. 2005 Oct;105(10):1620-8.
2. U.S. EPA (U.S. Environmental Protection Agency). 2010 “Fluoride relative source contribution analysis.“ EPA 820-R-10-0. Washington, DC U.S. EPA, Health and Ecological Criteria Divsiion, Office of Water.
3. U.S. Public Health Service Recommendation for Fluoride Concentration in Drinking Water for the Prevention of Dental Caries.” Public Health Reports, 2015 Jul-Aug;130(4):318-31.
4. Agency for Toxic Substances and Disease Registry (ATSDR). 2003, Toxicology Profile for Fluorides, Hydrogen Fluoride, and Fluorine. Atlanta, GA: U.S. Department of Health and Human Services, Public Health Service.
5. U.S. EPA (U.S. Environmental Protection Agency) 2018 Edition of the Drinking Water Standards and Health Advisories
6. Environmental Protection Agency (US) Fluoride: dose-response analysis for non-cancer effects. Washington: EPA, Office of Water, Health and Ecological Criteria Division; 2010. EPA 820-R-10-019
7. National Data Laboratory, Beltsvilee Human Nutrition Reserch,Agriculture. USDA National Nutrient Database of Selected Beverages and Foods Available at: www.nal.usda.gov/fnic/foodcomp/Data/Fluoride/fluoride.pdf
8. Jeff Prystupa (2011) Fluorine—A current literature review. An NRC and ATSDR based review of safety standards for exposure to fluorine and fluorides, Toxicology Mechanisms and Methods, 21:2, 103-170, DOI: 10.3109/15376516.2010.542931

23. Appendix

a. Appendix A: Method Performance Documentation

Accuracy compared to Reference Material

Mean concentration should be within ±15% of the nominal value except at 3*LOD, where it should be within ± 20%

Method name:	Fluoride in Water
Method #:	3048
Matrix:	Water
Units:	mg/L
Reference material:	**Fluoride in Water-QC QC3162 (traceable to NIST SRM 3183 lot 05721)**
Analyte:	**Fluoride**

Reference material	Replicate	Nominal value	Measured concentration					Mean	SD	CV (%)	Difference from nominal value (%)
			Day 1	Day 2	Day 3	Day 4	Day 5				
Level 1	1	0.420	0.409	0.406	0.413	0.423	0.405	0.41	0.01	1.65	-1.7
	2		0.412	0.411	0.415	0.426	0.410				
Level 2	1	0.210	0.206	0.207	0.209	0.215	0.202	0.2	0.00	2.21	-0.7
	2		0.209	0.208	0.212	0.215	0.202				
Level 3	1	0.140	0.141	0.137	0.140	0.144	0.135	0.1	0.00	2.38	0.3
	2		0.144	0.139	0.143	0.144	0.137				

Precision

Total relative standard deviation should be ≤ 15% (CV ≤ 15%)

Method name:	Fluoride in Water
Method #:	3048
Matrix:	Water
Units:	mg/L
Analyte:	**Fluoride**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	0.687	0.701	0.69	4.9E-05	4.9E-05	0.963272
2	0.664	0.648	0.66	6.4E-05	6.4E-05	0.860672
3	0.681	0.648	0.66	0.00027225	0.00027225	0.8831205
4	0.719	0.752	0.74	0.00027225	0.00027225	1.0819205
5	0.697	0.683	0.69	4.9E-05	4.9E-05	0.9522
6	**0.696**	**0.704**	0.70	0.000016	0.000016	0.98
7	0.709	0.673	0.69	0.000324	0.000324	0.954962
8	0.678	0.681	0.68	2.25E-06	2.25E-06	0.9234405
9	0.680	0.683	0.68	2.25E-06	2.25E-06	0.9288845
10	0.689	0.702	0.70	4.225E-05	4.225E-05	0.9674405

Grand sum 13.775 **Grand mean** 0.68875

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.0021865	0.00021865	0.014786818	2.15
Between Run	0.00838125	0.00093125	0.018875911	2.74
Total	0.01056775		0.023978115	**3.48**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2
1	5.174	5.390	5.28	0.011664	0.011664	55.799048
2	5.235	5.047	5.14	0.008836	0.008836	52.859762
3	5.150	5.047	5.10	0.00265225	0.00265225	51.9894045
4	5.201	5.087	5.14	0.003249	0.003249	52.921472
5	5.078	5.078	5.08	0	0	51.572168
6	5.108	5.170	5.14	0.000961	0.000961	52.818642
7	5.136	5.075	5.11	0.00093025	0.00093025	52.1322605
8	5.183	5.204	5.19	0.00011025	0.00011025	53.9448845
9	5.122	5.143	5.13	0.00011025	0.00011025	52.6851125
10	5.150	5.089	5.12	0.00093025	0.00093025	52.4185605

Grand sum 102.867 **Grand mean** 5.14335

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.0588865	0.00588865	0.07673754	1.49
Between Run	0.06033005	0.006703339	0.020182776	0.39
Total	0.11921655		0.079347303	**1.54**

Stability

The initial measurement can be from the same day for all stability experiments.

Freeze and thaw stability = Assess for 3 freeze-thaw cycles at ≤ -70°c; conditions mimic intended sample handling conditions

Describe condition: QC pools LW-18001 and HW-18002 were used for the measurements. Three freeze-thaw cycles were used for this experiment.

Bench-top stability = Assess short-term stability for length of time needed to handle study samples (typically at room temperature)

Describe condition: QC pools LW-18001 and HW-18002 were used for the measurements. Samples were left in the vials on the benchtop overnight.

Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler

Describe condition: QC pools LW-18001 and HW-18002 were used for the measurements. Samples were prepared with TISAB II and left on the bench ov

Long-term stability = Assess long-term stability that equals or exceeds time between date of first sample collection and date of last sample analysis

Describe condition: QC pools LW-18001 and HW-18002 will be used for measurements. This will be completed in November of 2019.

All stability sample results should be within ±15% of nominal concentration

Method name:	Fluoride in Water
Method #:	3048
Matrix:	Water
Units:	mg/L
Analyte:	**Fluoride**

Quality material 1								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	0.656	0.675	0.656	0.666	0.656	0.654	0.656	
Replicate 2	0.643	0.672	0.643	0.622	0.643	0.670	0.643	
Replicate 3	0.653	0.672	0.653	0.666	0.653	0.668	0.653	
Mean	0.650666667	0.673	0.650666667	0.7	0.650666667	0.664	0.650666667	#DIV/0!
% difference from initial measurement	--	**3.4**	--	**0.1**	--	**2.0**	--	**#DIV/0!**

Quality material 2								
	Initial measurement	**Three freeze-thaw cycles**	**Initial measurement**	**Bench-top stability**	**Initial measurement**	**Processed sample stability**	**Initial measurement**	**Long-term stability**
Replicate 1	5.059	5.027	5.059	5.051	5.059	5.150	5.059	
Replicate 2	5.039	5.007	5.039	5.030	5.039	5.108	5.039	
Replicate 3	5.059	4.986	5.059	5.010	5.059	5.108	5.059	
Mean	5.052333333	5.0	5.052333333	5.0	5.052333333	5.122	5.052333333	#DIV/0!
% difference from initial measurement	--	**-0.9**	--	**-0.4**	--	**1.4**	--	**#DIV/0!**

LOD, specificity and fit for intended use

Method name:	Fluoride in Water
Method #:	3048
Matrix:	Water
Units:	mg/L

Analytes	Limit of Detection (LOD)	Interferences successfully checked in at least 50 human samples	Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use
Fluoride	0.024	N/A	yes

b. Appendix B: Ruggedness Testing

Parameter Test #1: Effect of amount of TISAB II added to sample
Evaluating the significance of percentage difference of TISAB II present in sample on analyte recovery.

Test details:
We used eighteen vials of the high bench QC pool HW-18002 to prepare samples using different amounts of TISAB II to HW-18002:

a. 40% TISAB II to 60% HW-18002
b. 60% TISAB II to 40% HW-18002
c. 50% TISAB II to 50% HW-18002
d. 45% TISAB II to 55% HW-18002
e. 55% TISAB II to 45% HW-18002

Samples were prepared in triplicate for each TISAB II to sample amount and analyzed by the method.

Parameter Test# 1 Results. Effect of amount of TISAB II added to sample on Fluoride concentration
Analyses were performed on January 16, 2019 and January 24 2019. Results below are the average of the 3 replicates* of high bench QC (HW-18002) analyzed as samples in each run under 6 different amounts of TISAB II to HW-18002. The characterized QC mean and the acceptance levels at ±3SD are given as well. All concentrations are in mg/L.

Percentage of TISAB II to Percentage of HW-18002	Average of replicates	HW-18002 Target Mean	3SD range for HW-18002
40% TISAB II to 60% HW-1800	6.174	5.133	5.005 - 5.262
60% TISAB II to 40% HW-18002	3.916	5.133	5.005 - 5.262
50% TISAB II to 50% HW-18002	5.011	5.133	5.005 - 5.262
45% TISAB II to 55% HW-18002*	5.606	5.133	5.005 - 5.262
55% TISAB II to 45% HW-18002*	4.493	5.133	5.005 - 5.262
50% TISAB II to 50% HW-18002*	5.073	5.133	5.005 - 5.262

Experiment performed on 01/24/2019

Conclusion: The results obtained for the samples prepared with 50 % TISAB II and 50% sample (HW-18002) is the most optimal and were within the characterized QC mean of the high QC pool. The ratio of TISAB II to sample must be kept at 1:1 to achieve the best accuracy and reproducibility. The conclusion with this test was that the method is not rugged enough to withstand fluctuations in the percentage (amount) of TISAB II within the sample. Deviations in the amount of TISAB II are noticeable in terms of results obtained.

Ruggedness testing note:

There are no other alterable parameters with this ISE method.