Laboratory Procedure Manual

Analyte: **Fluoride**

Matrix: **Urine**

Method: Fluoride in Urine using Ion Selective Electrode (ISE)

Method No: 3049.1

Revised: 9/28/2021

As performed by:

Inorganic and Radiation Analytical Toxicology Branch (IRATB)

Division of Laboratory Sciences

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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 Information

This document details the Lab Protocol for testing the items listed in the following table:

File Name	Variable Name	SAS Label
UR1FL_H_R UR2FL_H_R	UR1FL	Fluoride, Urine 1st collection (mg/L)
	UR2FL	Fluoride, Urine 2nd collection (mg/L)

1. Clinical relevance and test principle

A. Clinical relevance

Fluoride is a natural element found at varying concentrations in drinking water and soil. Water and water-based beverages are the primary source of dietary fluoride. Approximately 80% of dietary fluoride comes from tap and bottled 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 needed to prevent tooth decay. For community water systems that add fluoride to their water, the U.S. Public Health Service (USPHS) 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 other possible sources of fluoride exposure in addition to dietary exposure. Certain individuals run a higher risk for fluoride exposure such as those in industries such as glass manufacturing, ceramics, metallurgy, oil, and fertilizer production. It is also commonly found in rangevia inhalation or direct contact poses not just the threat of skeletal fluorosis but is also responsible for acute and chronic symptoms such as renal damage, gastrointestinal discomfort, and respiratory complications[4]. The EPA's current fluoride enforceable standard for maximum contamination is set at 4.0 mg/L to prevent severe skeletal fluorosis. In the absence of occupational exposure, the amount of fluoride in urine is expected to be minimal and vary based on geographic region and diet.

B. Test principle

Fluoride is measured in urine samples using an ion-selective electrode (ISE) which measures the activity of fluoride ions as a voltage response. The voltage response is proportional to the concentration of free fluoride ions in solution when the ionic strength is fixed. It is therefore essential that a total ionic strength adjustment buffer (TISAB) is used with each sample. In addition to adjusting the ionic strength with sodium chloride (NaCl), TISAB contains a complexing agent that binds with interfering ions and buffers the solution to a pH of 5 thus reducing OH^- and HF interferences.

2. Safety precautions

Important

Precautionary information that is important to protecting personnel and safeguarding equipment will be presented inside a box, like this one, throughout the procedure where appropriate.

Observe universal precautions; wear protective gloves, laboratory coats, and safety glasses during all steps of this analytical method. Discard residual sample aliquots in autoclave bins after analysis is complete. Place disposable plastic, glass, and paper (pipet tips, autosampler

vials, gloves, etc.) that come into contact with urine in biohazardous autoclave bags. Keep these bags in appropriate containers until sealed and autoclaved. Wipe down all work surfaces with a broad-spectrum disinfectant, or equivalent disinfecting agent when work is finished.

Reagents used in this study include those listed in Section 7. Safety Data Sheet documents (SDS) for these chemicals are readily accessible as hard copies in the laboratory, online through the manufacturer, or electronically through the CDC's Chemical Hazard Tracking System (CHaTS).

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.

i. Waste to be placed into biohazard autoclave bags and pans:

1) All residual biological samples

2) All disposable consumables that come into contact with urine (vials, caps, gloves, Kimwipes, etc.)

ii. Waste to be disposed of through online waste ticketing system (OWTS):

1) All urine samples and fluids that have come into contact with the fluoride probe must be properly disposed of. This waste is collected into a carboy. The chemical composition of the accumulated waste is approximately 50% TISAB and 50% urine. Since the accumulated waste is non-corrosive and falls within the limits defined by Dekalb County, GA (pH>6 and pH<11.5) neutralization is not needed; however, disinfection is required prior to disposal. The waste is disinfected by adding 4 mL of Lysol® I.C. (or other appropriate quaternary disinfectant) to the waste container for each liter of waste accumulated (1:250 dilution). The waste needs to be mixed and allowed to sit for a contact time of 10 minutes to allow disinfection to occur. Once disinfected, the waste is disposed through the CDC's chemical disposal system. It cannot be poured down the drain because it contains phosphates.

3. Computerization; data system management

A. Analytical run control

The analytical run can be controlled either directly from the touch-screen interface of the ISE meter or by using the LabX software on the connected computer system. This document contains instructions for both types of control: "**ISE control**" or "**LabX control**", respectively.

B. Data collection and storage

If a computer is not connected, an analytical run can be completed with the use of a connected Mettler Toledo USB-P25 printer. The printer is configured to print results automatically, but the analyst must record all calibration and sample results in a laboratory notebook. The printed data and lab notebook records must be scanned into .pdf documents and analytical results must be transcribed into an Excel template. See Sections 8.B.i and 8.B.ii for details on meter calibration and sample analysis, respectively, and Section 8.D.ii for details on data processing without use of the LabX software.

If a computer with LabX software is connected to the ISE meter, the data from analytical runs are collected and stored on the connected computer in the LabX software. Analytical run results are stored by date in the results section of LabX. The results for a run, e.g., a data set, are copied into an Excel worksheet and saved in the lab data folder on the instrument laptop. The run data is then copied into a run template so it can be uploaded into STARLIMS. See Section 8.C and Appendix D for details on data processing with the Lab X software.

All electronic run result files are saved on the Multi-user Share Tool (MUST) in the following location:

\\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. Sample collection, storage, and handling

i. Sample handling conditions are outlined in the Division of Laboratory Sciences (DLS) Policies and Procedures Manual. The protocol discusses collection and transport of samples and any special equipment required. Studies were conducted in the lab to confirm that samples thawed and refrozen three times or less are not compromised. If a sample needs to be split for analysis by different analytical methods, the appropriate amount of urine is transferred into sterile Nalgene cryovials or polypropylene centrifuge tubes labeled with unique sample identifiers.

ii. Collect urine without using preservatives.

iii. The appropriate amount of urine is dispensed into the appropriate container with the patient's unique identifier.

iv. Samples collected in the field are frozen then shipped to the laboratory on dry ice overnight. Urine samples for this method should be stored at approximately 2-8° C (4 weeks or less) or ≤ 20^{o}C for greater than 4 weeks.

v. Ensure patient samples, calibrators, and QC are at ambient temperature (approximately 20°C to 25°C) before measurement. Every attempt is made to ensure that specimens 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.

B. Criteria for sample rejection

The criteria for deeming a sample unacceptable are low volume sample volumes, suspected contamination due to improper collection procedures or collection devices, and/or contamination during sample preparation/analysis. Sample contact with dust or dirt may compromise test results. The optimum volume needed for sample analysis is ≥ 4 mL. The volume of urine used in one sample analysis is typically 3 mL. In all cases, request a second urine sample, if possible.

5. Preparation of reagents, calibrators, quality control

A. Chemicals

i. TISAB II for Fluoride ISEs: Hanna instruments item # HI4010-00

ii. Ion Electrolyte A reference filing solution: pH of 5, Hanna instruments Item # 51344750, or equivalent

B. Fluoride standards

i. Fluoride ISE 1000 ppm Standard: Hanna instruments Item # HI4010-03, or equivalent

ii. Fluoride ISE 1 ppm Standard with TISAB II: Hanna instruments item # HI4010-11

iii. Fluoride ISE 2 ppm Standard with TISAB II: Hanna instruments item # HI4010-12

iv. Fluoride ISE 10 ppm Standard with TISAB II: Hanna instruments item # HI4010-10

C. Bench quality control materials

Bench quality control (QC) materials are made from human urine collected from anonymous donors collected with the CDC IRB 3994. Section 5.C.i “Preparation of quality control materials” includes details on QC preparation. Table 1 lists the examples of urine QC pool levels and names used for this method where yy is the last two digits of the production year and ### is the assigned pool identification number.

After preparation QC material must be tested for homogeneity (see Section 5.C.ii) and characterized (see Section 5.C.iii).

Table 1 QC names for DLS 3049

QC level	QC sample name
low pool	LU-yy###
high pool	HU-yy###

i. Preparation of quality control materials

Collect human urine from anonymous donors in clean, trace metals-free urine cups. Refrigerate urine donations at approximately 2-8°C as soon as possible for periods of two weeks or less. For longer periods, freeze the urine donations until needed. Assay each donation for fluoride concentration. Assign urine donations to a “low” pool or to a “high” pool according to the concentration ranges that are needed.

Spike the pools, if necessary, using a NIST traceable fluoride standard. Obtain lab chief approval for pool concentrations prior to spiking. While maintaining constant stirring of each pool, aliquot approximately 9 mL of urine into pre-labeled 10 mL polypropylene cryovials that are labeled and sequentially numbered. Prepare a quantity of vials that can be used for analytical runs over an extended period of time (at least 2 years but up to five years or longer if proven stable). Stability studies assessing long-term storage conditions of laboratory prepared quality control materials for this analytical method have been completed and confirm samples are

stable for 1 year at -70°C. Short-term storage at approximately 2-8°C and long-term storage at approximately -70°C is recommended.

ii. QC pool homogeneity

When a pooled QC material is prepared, an analytical run to assess the homogeneity of the pools is performed after the quality control pools have been aliquoted into individual vials. Vials are randomly chosen and randomly analyzed. The first and last vials dispensed are always included in the homogeneity study. Consult with a statistician about the appropriate design of your homogeneity study prior to dispensing the pool to ensure that sufficient statistical power is obtained.

Unlike the characterization of the QC, the homogeneity study should be completed in a single analytical run. Once sample analysis is complete, the data is statistically evaluated to determine homogeneity. If the concentration of fluoride in each pool does not vary significantly from beginning to end or if 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 remedied before pool characterization can begin.

iii. QC pool characterization

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 characterization runs are distributed among all of the instruments that will be used to analyze the method. Ideally, all analysts that will run patient samples using the analytical method should complete some of the characterization runs. 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.

D. Reference materials:

i. National Institute of Standards and Technology (NIST) SRM (Standard Reference Material) 2668 (Toxic Elements in Frozen Human Urine) is available for this method. Follow the instructions for use provided in the certificate of analysis supplied by NIST.

ii. Other reference materials can be found with urine fluoride concentrations; examples are shown below:

1) ClinChek® Urine Control Level I: item # 8848 Iris Technologies, www.iristech.net

2) ClinChek® Urine Control Level II: item # 8847 Iris Technologies, www.iristech.net

E. Reagent preparation

i. TISAB II is purchased ready to use. Add TISAB II to all samples to maintain a constant ionic strength and pH to prevent interferences between samples. All standards and samples with this method are prepared with TISAB II in a 1:1 ratio of patient sample to TISAB II.

F. Preparation of intermediate calibration standards

This analytical method uses five calibration standards. The fluoride concentrations of the calibrators shown in Table 2. Calibration standards S2, S3, and S4 are purchased commercially and are pre-mixed with TISAB II solution. Calibration standards S1 and S5 are prepared in volumetric flasks described below.

Table 2 Fluoride calibration standards and concentrations

Calibration standard	Fluoride concentration (mg/L)
S1	0.25
S2	1.0
S3	2.0
S4	10
S5	20

Note: Use dedicated volumetric flasks for the preparation of fluoride analytical standards to avoid contamination. Deionized (DI) water used for the preparation of calibration materials has a resistivity ≥ 18 MΩ·cm.

i. Preparation of 20.0 mg/L Fluoride intermediate standard (S5)

1) Partially fill a 50 mL volumetric flask with DI water.

2) Add 1 mL of the 1000 mg/L fluoride standard to the flask. Use DI water to fill the flask to the mark. Mix thoroughly.

3) Store at ambient temperature and label appropriately. Expiration date is 1 year from the date of preparation.

ii. Preparation of 12.5 mg/L Fluoride intermediate stock standard

1) Partially fill a 50 mL volumetric flask with DI water.

2) Add 625 µL of the 1000 mg/L fluoride standard to the flask. Use DI water to fill the flask to the mark. Mix thoroughly.

3) Store at ambient temperature and label appropriately. Expiration date is 1 year from the date of preparation.

iii. Preparation of 0.25 mg/L Fluoride intermediate standard (S1)

1) Partially fill a 50 mL volumetric flask with DI water.

2) Add 1 mL of the 12.5 mg/L fluoride intermediate stock standard to the flask. Use DI water to fill the flask to the mark. Mix thoroughly.

3) Store at ambient temperature and label appropriately. Expiration date is 7 days from the date of preparation.

G. Working calibration standards

i. Working calibration standards S2, S3 and S4

1) Pour each into a clean 30 mL HDPE bottle

2) Add a clean stir bar to each and thoroughly mix prior to using.

ii. Working calibration standards S1 and S5

1) Aliquot S1 and S5 into separate 30 mL HDPE bottles; typical volume used is 3 mL

2) Add an equal volume of TIASB II into each bottle; typical volume used is 3 mL

3) Add a clean stir bar to each and thoroughly mix prior to using

H. Blank check, sample, and QC preparation for analysis

i. Blank check preparation – This blank is analyzed after the initial QC to ensure no residual material is left on the probe.

1) Aliquot DI H2O into a 30 mL HDPE bottle; typical volume used is 3 mL

2) Add an equal volume of TIASB II into each bottle; typical volume used is 3 mL

3) Add a clean stir bar to each and thoroughly mix prior to using

ii. Sample and QC preparation

1) Aliquot each urine specimen (sample or QC) into separate 30 mL HDPE bottles; typical volume used is 3 mL

2) Add an equal volume of TIASB II into each bottle; typical volume used is 3 mL

3) Add a clean stir bar to each and thoroughly mix prior to using

6. Equipment and instrumentation

A. Instrumentation, software and equipment

i. SevenExcellence pH/Ion meter s500-F-kit (with PerfectION comb F-): item# 30046251, or equivalent

ii. uMix magnetic stirrer: Item # 30040000, or equivalent

iii. Computer system with the following installed

1) Software LabX pH Express: Item# 30247987, or equivalent

2) License LabX pH 1 instrument: Item # 30247989, or equivalent

3) License Lab X Import/Export: Item # 11153105, or equivalent

4) License Lab X Import/Export: Item # 11153105, or equivalent

iv. Mettler Toledo USB-P25 Printer, or equivalent

B. Lab supplies

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

i. 30 mL Nalgene™ Lab Quality Wide Mouth HDPE Bottle with 28mm Cap: item# 312104-0001., or equivalent

ii. 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

iii. 50 mL volumetric polypropylene flask: for standards stocks preparation United States Plastic Corp., or equivalent

iv. 200 mL volumetric polypropylene flask: for standards stocks preparation United States Plastic Corp., or equivalent

v. Nalgene externally-threaded cryovials (5 and/or 10 mL): Fisher Scientific, or equivalent

vi. 1000 mL narrow mouth high-density polyethylene bottle: Item # 69062, United States Plastics Corporation, or equivalent

vii. 10 or 20 L carboy: for waste collection and disposal, United States Plastic Corp., or equivalent

viii. Sartorius Picus NXT® 5-120 µL: Part # LH-745041, or equivalent

ix. Sartorius Picus NXT® 50-1,000 µL: Part # LH-745081, or equivalent

x. Sartorius Picus NXT® 100-5,000 µL: Part # LH-745101, or equivalent

xi. 100-5000 µL pipette tips: Sartorius Part# 780304, or equivalent

xii. 1-10 mL pipette tips: Sartorius Part# 780308, or equivalent

xiii. Quaternary disinfectant such as Lysol® I.C.: MFR Part# LY983 or equivalent

xiv. Scienceware F371210020 Spinbar Stirring Bar: PTFE, Flea Micro, Yellow, 6.35x3mm or equivalent. Each run requires at least 1 stir bar per sample, calibrator, water blank, and QC.

7. Calibration and calibration verification procedures

The SevenExcellence pH/Ion meter is calibrated before each analytical run. Prior calibration data cannot be used. If more than one meter is used within the lab, an instrument comparison is competed twice a year. Details on how to perform instrument calibration can be found in Section 8.B.i (ISE control) and 8.C.i (LabX control).

8. Procedure operating instructions, calculation, interpretation of results

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. Pre-Analytical Steps

i. Turn the ion meter on if it is powered off.

ii. Remove the protective cap from the electrode and set the cap aside.

iii. Add filling solution (Ion electrode A) each day before using the electrode.

iv. Check the waste container. If it is full or near the full line, prepare the full container for disposal and replace the waste container with an empty one.

v. Identify and gather the vials of urine samples for the batch ("run") to be analyzed. 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 single run cannot exceed 24 hours. See Table 3 for an example of a 20-sample run.

vi. For each batch run, remove an adequate number of low and high bench QC vials "LU-yyxxx" and "HU-yyxxx" from the refrigerator. For an explanation of the nomenclature, see Table 1 in Section 5.C.

vii. Prior to analysis, allow all samples and QC materials to reach room temperature for a minimum of 20 minutes after thawing.

viii. Arrange the samples in order of analysis starting with deionized water, beginning QC, patient samples, and ending QC.

ix. If data will be collected with a computer, make sure computer is on and LabX is running. If the computer and LabX software will not be use, make sure the printer is connected and powered on, and configured to print automatically.

Table 3 Example of samples analyzed in a run for 20 samples

Run block	Sample order	Sample name(s)
Calibration	1 - 5	Standard 1 to Standard 5
Blank check	6	3049-wtrblk
Low QC	7	LU-yy###
High QC	8	HU-yy###
Samples	9 - 28	Use sample IDs on the sample label
Low QC	29	LU-yy###
High QC	30	HU-yy###

B. Analytical Run: ISE control

i. Calibration: ISE control

1) On the home screen (Figure 1) of the SevenExcellence Ion meter touch "pH/Ion"

2) The module settings will appear on the screen (Figure 2).

3) Select “Calibration settings” to review the calibration settings before starting the direct calibration. (See Figure 3, Figure 4 and Table 4)

Table 4 Calibration settings on SevenExcellence ISE meter for DLS 3049

Calibration setting	Value on ISE
Measurement type	Ion
Calibration standard group	URINE FLUORIDE
Unit	mg/L
Calibration mode	Linear
Number of standards	5
Standard 1	0.25 mg/L
Standard 2	1 mg/L
Standard 3	2 mg/L
Standard 4	10 mg/L
Standard 5	20 mg/L

4) Touch “Back” to return to the module settings screen.

5) Touch “Calibrate” on the module settings screen (bottom right corner) to start the direct calibration.

6) The ISE will prompt the user for the correct calibration standard, e.g. the first message will say “Immerse sensor in buffer/standard 0.25 mg/L.”

7) Place the correct 30 mL HDPE solution bottle on the magnetic stirrer. Thoroughly mix the prepared calibrator sample before placing it on the magnetic stirrer.

8) Immerse the sensor in the solution and touch “OK” on the LCD screen.

9) Wait until the analysis of the standard is complete (300 seconds).

10) Remove the sensor from the solution and rinse it well with deionized water, collecting the wash water in a secondary container. Blot the sensor dry using Kimwipes or other appropriate wipe.

11) The ISE will prompt the user for the next calibration standard. Repeat steps 7-10 until all five calibrators are tested.

12) Review the slope information (Figure 5) before proceeding with sample analysis. A slope of 95%-105% is required to continue with sample analysis.

13) If the slope does not meet the requirements, prepare calibrators from a different lot of calibrators and repeat the calibration test. Proceed with analysis only after requirements are met.

14) Touch “Back” on the LCD to return to the module settings screen.

15) When acceptable calibration is complete, the calibration report will be printed.

16) Scan printed out calibration report and save as a PDF document.

17) The 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

18) Affix printed calibration report inside the lab notebook.

ii. Analyzing Samples: ISE control

1) Transfer waste in secondary container into the waste collection carboy if needed. Check the volume level in the secondary container throughout the run and empty it as needed.

2) Touch “Measurement settings” on the module settings screen (See Figure 2).

3) Touch the sample ID field as shown in Figure 6 to update the sample name.

4) Enter the sample ID of the next sample to be analyzed.

5) Touch “OK” (Figure 7) after the name has been entered by typing in the Sample ID from the meter.

6) Touch “Save” (Figure 8).

7) Place the 30 mL HDPE bottle with the sample (prepared and well mixed) on the magnetic stirrer.

8) Immerse sensor in the sample and touch “OK” on the LCD screen. The instrument will begin taking a measurement.

9) Wait until the analysis of the sample is complete (300 seconds).

10) When the measurement is completed the fluoride concentration of the sample will appear on the screen. The sample result will print automatically. Record the result in the laboratory notebook. Save this printout to scan to .pdf when the run is complete.

11) Remove the sensor from the solution and rinse it well with deionized water, collecting the wash water in a secondary container. Blot the sensor dry using Kimwipes or other appropriate wipe.

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 7-12 for all samples in the run.

C. Analytical run: LabX control

i. Calibration: LabX control

1) In LabX click on “Show workbench”. Select the ISE meter name of the meter that will be calibrated for the analytical run.

2) 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.

3) The LabX software and the ISE meter will prompt the user for the correct calibration standard, e.g. the first message will say “Immerse sensor in buffer/standard 0.25 mg/L.”

4) Place the correct 30 mL HDPE solution bottle on the magnetic stirrer. Thoroughly mix the prepared sample before placing it on the magnetic stirrer.

5) Immerse sensor in the solution and touch "OK" on the LCD screen or in LabX.

6) Wait until the analysis of the standard is complete (300 seconds).

7) Remove the sensor from the solution and rinse it well with deionized water, collecting the wash water in a secondary container. Blot the sensor dry using Kimwipes or other appropriate wipe.

8) The LabX software and the ISE meter will prompt the user for the next calibration standard. Repeat steps 4-7 until all five calibrators are tested.

9) Review the slope information (Figure 5) before proceeding with sample analysis. A slope of 95% - 105% is required to continue with sample analysis.

10) If the slope does not meet the requirements, prepare calibrators from a different lot and repeat the calibration test. Proceed with analysis only after requirements are met.

11) In LabX, click on “Results sets” then click on “SevenExcellence”. When the list of tasks appears on the screen, click on the calibration task completed to create the Calibration report.

12) Open the results, select the “Raw data” tab, and click on “Print Data Tab” to open the Calibration Raw results report.

13) The results report window will appear on the screen.

14) Click on “Export as PDF” to save the calibration report on the instrument computer.

15) Transfer the calibration report with the analytical run data in this location:

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

ii. Analyzing Samples: LabX control

1) Transfer waste in secondary container into the waste collection carboy if needed. Check the volume level in the secondary container throughout the run and empty it as needed.

2) In LabX click on “Show workbench”. Select the ISE meter name of the meter that will be calibrated for the analytical run (ISE-C or ISE-D).

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.

4) Place the 30 mL HDPE bottle with the sample (prepared and well mixed) on the magnetic stirrer.

5) Enter the sample ID of the next sample to be analyzed in the prompt shown in Figure 10. **Once the “OK” button has been pressed, the meter will immediately begin reading the sample.**

6) Wait until the analysis of the sample is complete (300 seconds).

7) The sample ID prompt screen will appear upon the completion of each sample. Enter the sample ID only when ready to begin analysis of the next fully mixed sample.

8) After all samples have been analyzed, press the “end sample scope” button on the prompt to end the run.

D. Data Processing

i. Data processing: LabX control

1) In LabX click on “Results sets” then click on “SevenExcellence”. When the list of tasks appears on the screen, click on the task completed to export the results from the analytical run. (See Figure 11 and Appendix D: Data Processing – Saving Calibration Curves and Run Data).

2) Save the export file with run results in the C drive on the instrument computer using the following nomenclature: "UFYYMMDDRR_ISEX ” where "YY" is the last two digits of the year, "MM" is the month, "DD" is the day, “RR” is the run number of the day, and “X” is the ISE meter.

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) Upload the results to STARLIMS. This process is detailed in Appendix C: STARLIMS Upload Procedure

ii. Data processing: ISE control

1) All analytical data must be transcribed to an Excel template and saved as an Excel workbook file. The template (Results template_ISE-Urine-Fluoride.xlsx) is located in the network folder:

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

2) Scan all pages of the laboratory notebook with data for the analytical run and save as a .pdf file.

3) Save the PDF in the run folder for that date on the shared drive using the following nomenclature: “UFYYMMDDRR_ISEX” where "YY" is the last two digits of the year, "MM" is the month, "DD" is the day, “RR” is the run number of the day, and “X” is the ISE meter. This will also be attached as a document to the STARLIMS run.

4) Open the Excel template for data entry. Transcribe the data and name the .xslx file using the “UFYYMMDDRR_ISEX” naming convention. Store the file in the shared drive run folder for that date.

5) 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.

6) After the team lead or lab chief approves the data in the Excel file through email confirmation, upload the results to STARLIMS. This process is detailed in Appendix C: STARLIMS Upload Procedure

E. System Maintenance

i. 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.

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

9. Reportable range of results

The limit of detection for this method is found in Appendix A. The reportable range for this analytical method is LOD-20.0 mg/L.

10. Quality control procedures

The analytical data from QC materials are used to estimate methodological imprecision and to assess the magnitude of any time-associated trends. The two bench QC pools used in this method have different concentration levels which span the “low-normal” and “high-normal” ranges for fluoride in urine. A sample from both pools is analyzed after the calibration

standards are analyzed but before any patient samples are analyzed. A sample from each pool is analyzed again at the end of the run after the last patient sample is analyzed. If a second analytical run is completed using the same calibration, the patient samples in that run needs to have another set of QC samples bracketing those patient samples. If two runs are completed under the same calibration, 8 QC samples will be analyzed – 2 low and 2 high for run 1 and 2 low and 2 high for the second run. The QC samples from the second run are evaluated independently from the QC samples of the first run.

A. Definitions

i. Si = Standard deviation of individual results

ii. Sm = Standard deviation of the run means

iii. Sw = Within-run standard deviation

B. QC Results Evaluation

After completing an analytical run, compare the QC results against the QC limits to obtain an initial, informal determination of whether or not the run is potentially "in control". Please note that the results will still have to be evaluated using SAS; this only gives you an idea of the probable pass/fail status. If results are outside of two standard deviations (2s), the pass/fail criteria of the run can only be determined by using SAS.

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:

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

ii. If one of the QC means is outside the 2s limits, apply the rules below and reject the run if any condition is met.

1) Extreme Outlier – Run mean is beyond the characterization mean +/- 4Sm.

2) 2S Rule – Both run means are outside the same 2Sm limit.

3) 3S Rule – Run mean is outside a 3Sm limit.

4) 10 X-bar Rule – Current and previous 9 run means are on same side of the characterization mean.

iii. If one of the 4 QC individual results is outside of the 2Si limits, apply the rules below. Reject the run if any of the conditions below are met:

1) Extreme Outlier – One individual result is beyond the characterization mean +/- 4Si.

2) R 4S Rule – Within-run ranges for all pools in the same run exceed 4Sw (i.e., 95% range limit).

3) Note: Since runs have multiple results per pool for 2 pools, the R 4S rule is applied within runs only.

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

11. 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 (3049-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 statistical control limits.

12. Limitations of method; interfering substances and conditions

No known limitations of the method or interfering substances.

13. 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. We would therefore expect that most human urine samples would contain a fluoride concentration near this range.

14. Critical call results (panic values)

Not applicable

15. Specimen storage and handling during testing

Samples are allowed to reach room temperature during preparation. If there is enough remaining sample for a subsequent analysis, return sample to its storage location, otherwise pour remainder into the waste container.

If sample is QNS – report as QNS

If sample is tested, QC fails, but now sample is QNS, report it as QNS

Return unused samples to proper storage location.

16. Alternate methods for performing test or storing specimens if test system fails

Not applicable

17. Test results reporting system; protocol for reporting critical calls (if applicable)

Not applicable

18. Transfer or referral of specimens; 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 for 4 weeks or less otherwise frozen at ≤20°C. 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.

19. Method performance documentation

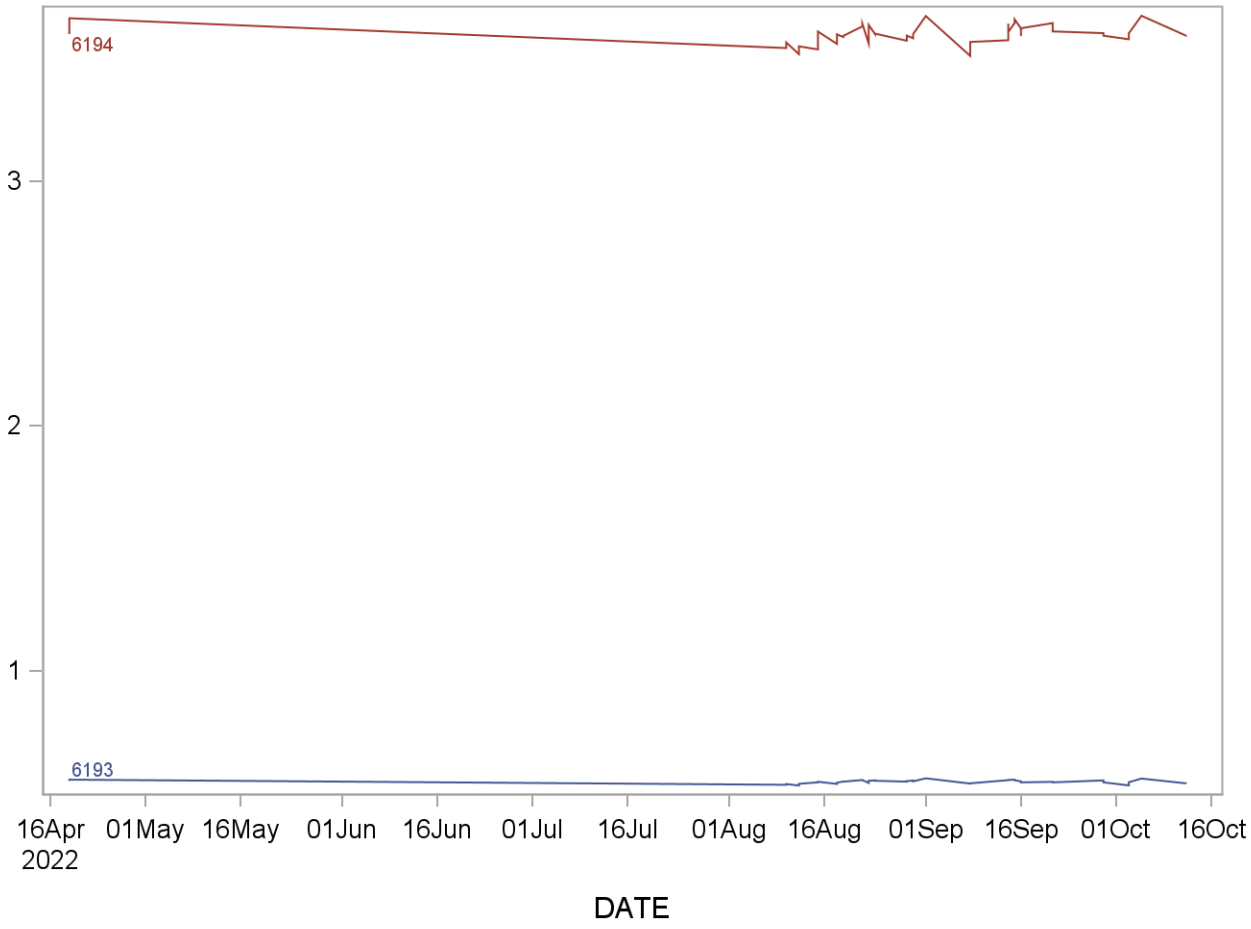
Method performance documentation for this method including accuracy, precision, sensitivity, specificity, and stability is provided in Appendix A of this method documentation. **The signatures of the Branch Chief and Director of the Division of Laboratory Sciences on the first page of this procedure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

20. Summary Statistics and QC Graph

Please see following pages.

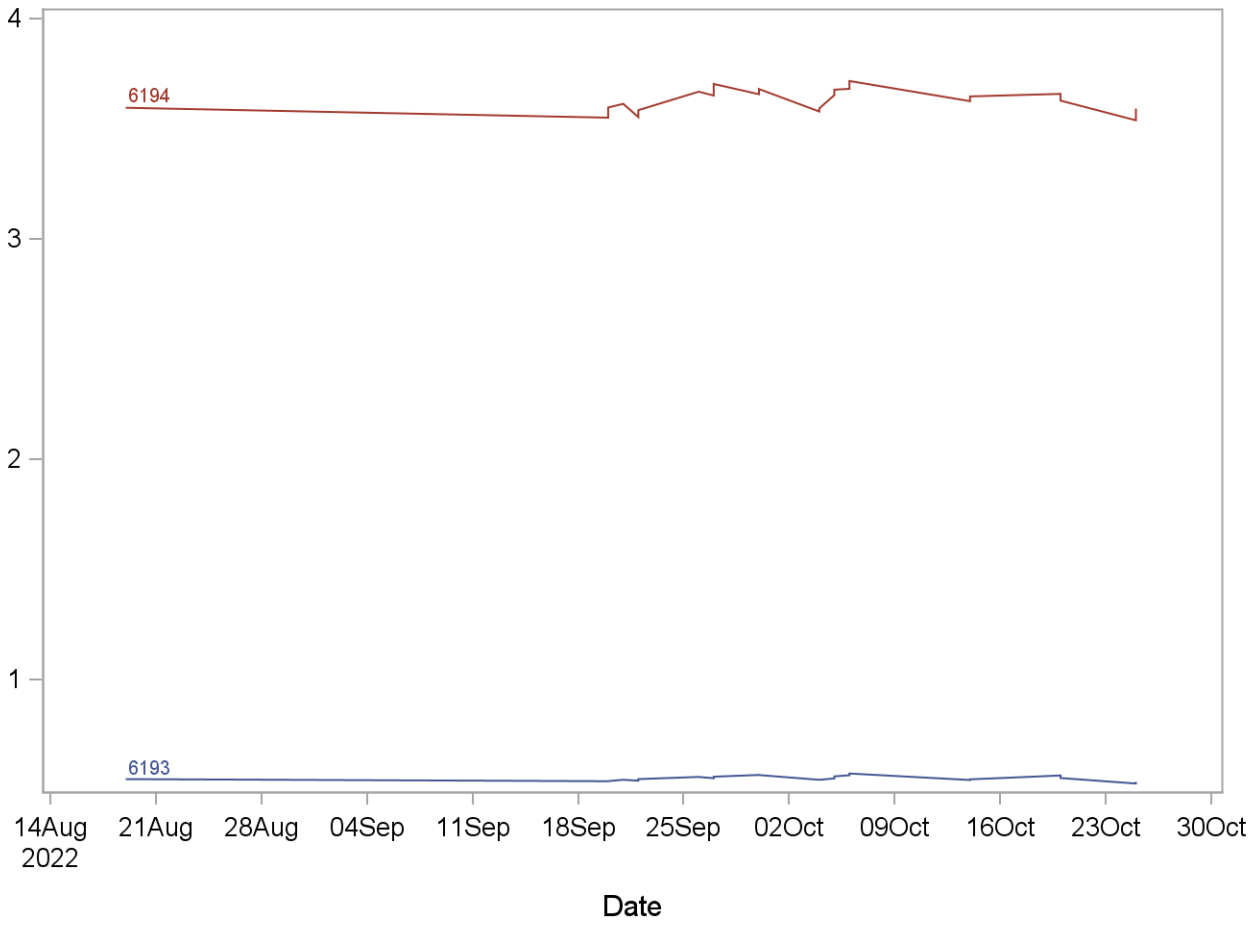
2014 Summary Statistics and QC Chart
UR1FL (Fluoride, Urine 1st collection (mg/L))

Lot	n	Start Date	End Date	mean	Standard Deviation	Coefficient of Variation
6194	44	19APR22	12OCT22	3.6024	0.0384	1.1
6193	44	19APR22	12OCT22	0.5457	0.0071	1.3



2014 Summary Statistics and QC Chart
UR2FL (Fluoride, Urine 2nd collection (mg/L))

Lot	N	Start Date	End Date	MEAN	Standard Deviation	Coefficient of Variation
6193	25	19AUG22	25OCT22	0.5474	0.0125	2.3
6194	25	19AUG22	25OCT22	3.6217	0.0521	1.4



21. References

1. Palmer C, W.S., *Position of the American Dietetic Association: The impact of fluoride on health.* Journal of the American Dietetic Association, 2005. **105**(10): p. 1620-1628.
2. EPA, U.S., *Fluoride relative source contribution analysis, EPA 820-R-10-0* 2010, EPA Health and Ecological Criteria Divsiion, Office of Water: Washington DC, USA.
3. Gooch, B.F., *U.S. public health service recommendation for fluoride concentration in drinking water for the prevention of dental caries.* Public Health Reports, 2015. **130**(4): p. 318-331.
4. Council, N.R., *Effects of Ingested Fluoride on Renal, Gastrointestinal, and Immune Systems*, in *Health Effects of Ingested Fluoride*. 1993, The National Academies Press: Washington, DC. p. 206.

22. Figures

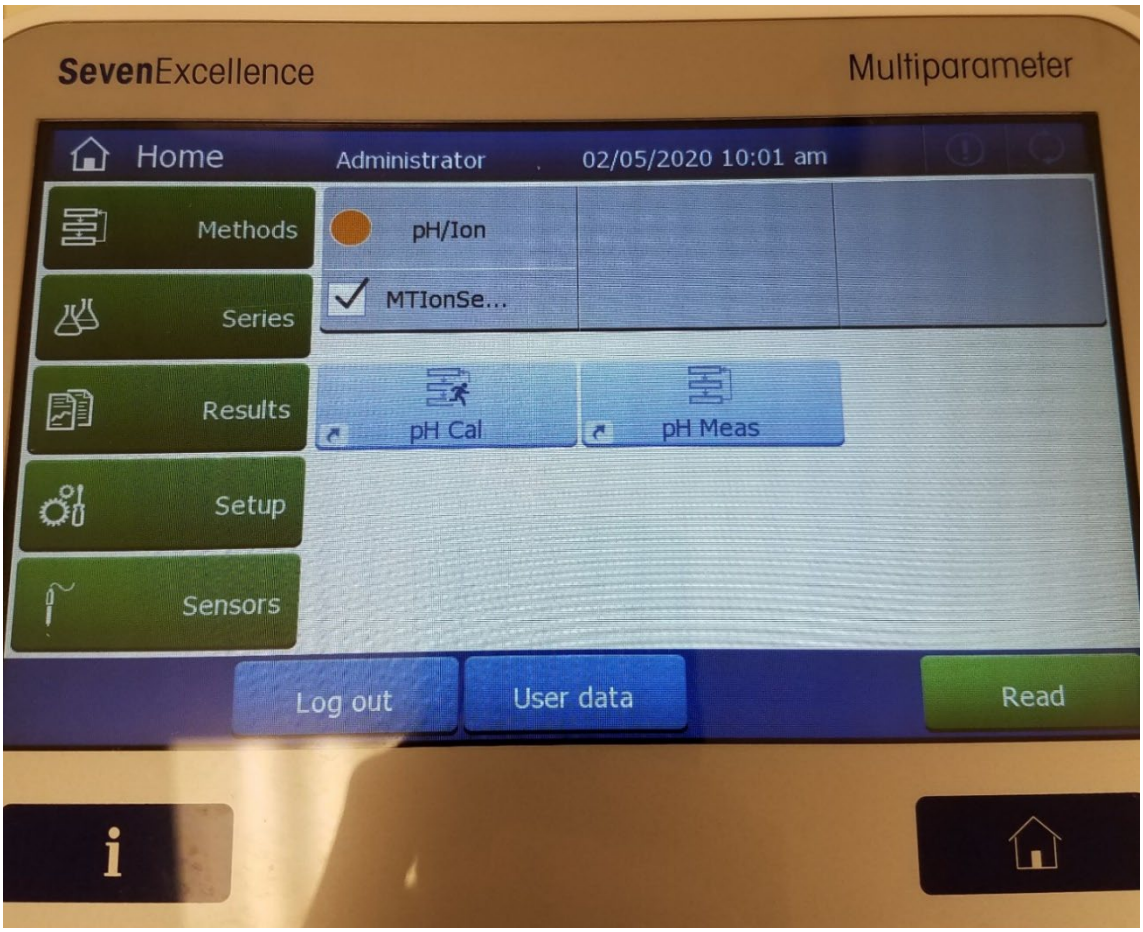


Figure 1 SevenExcellence LCD Home Screen

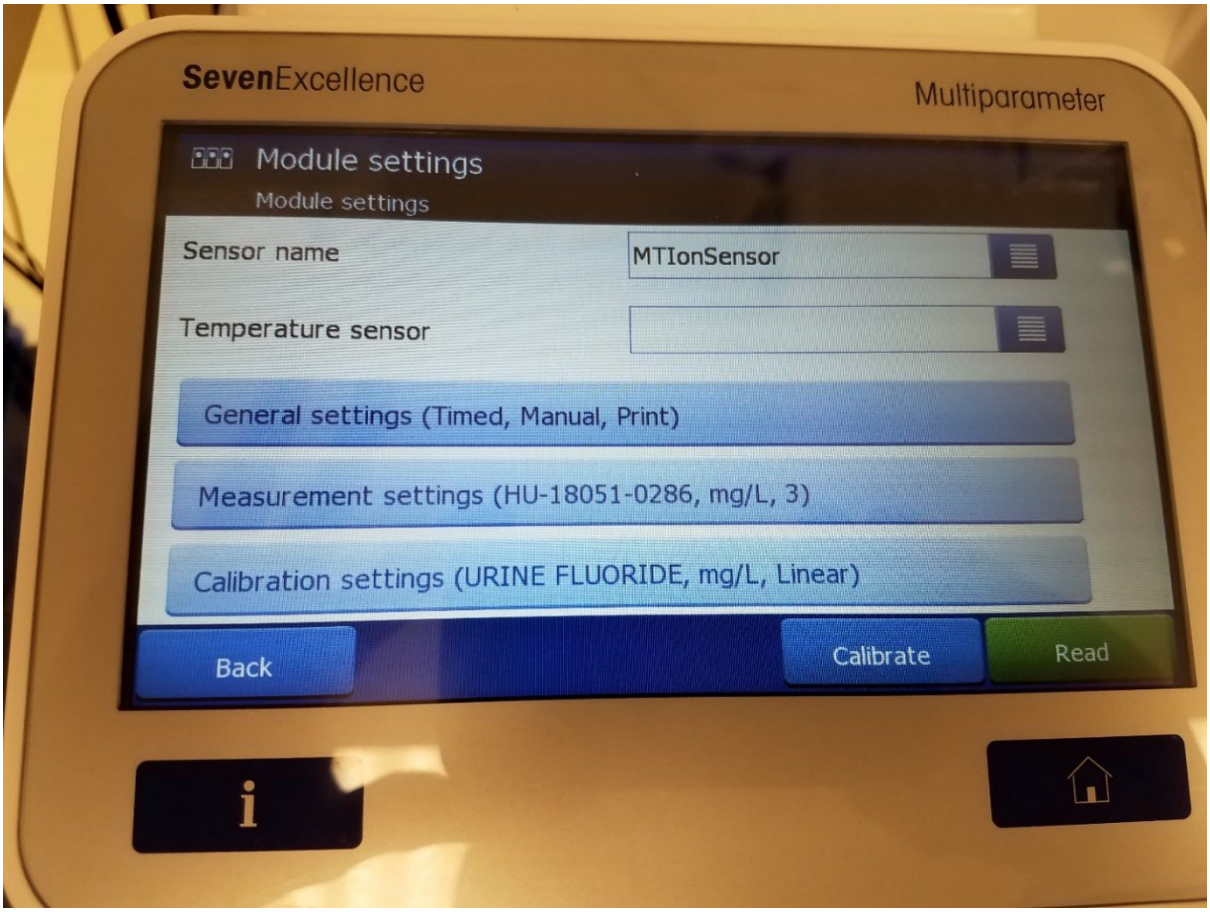


Figure 2 Module Settings Screen

Figure 3 Calibration settings A

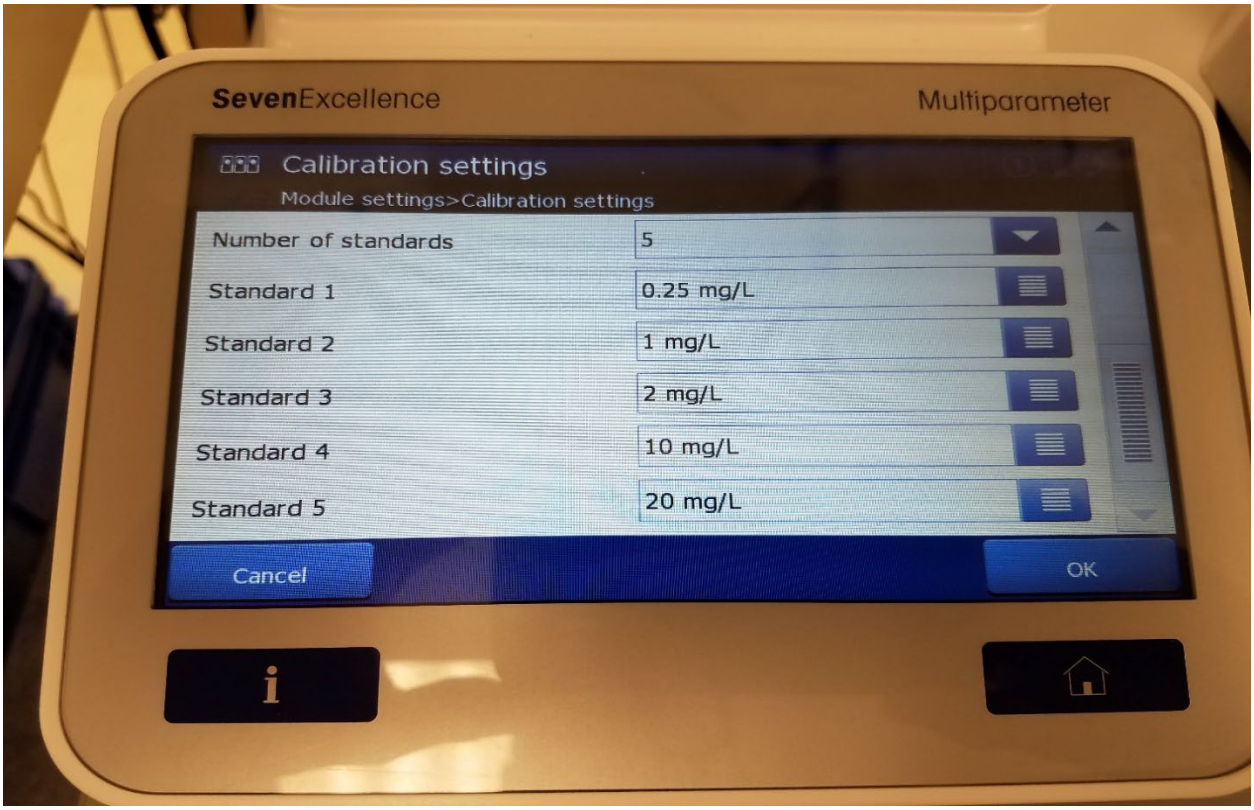


Figure 4 Calibration Settings B

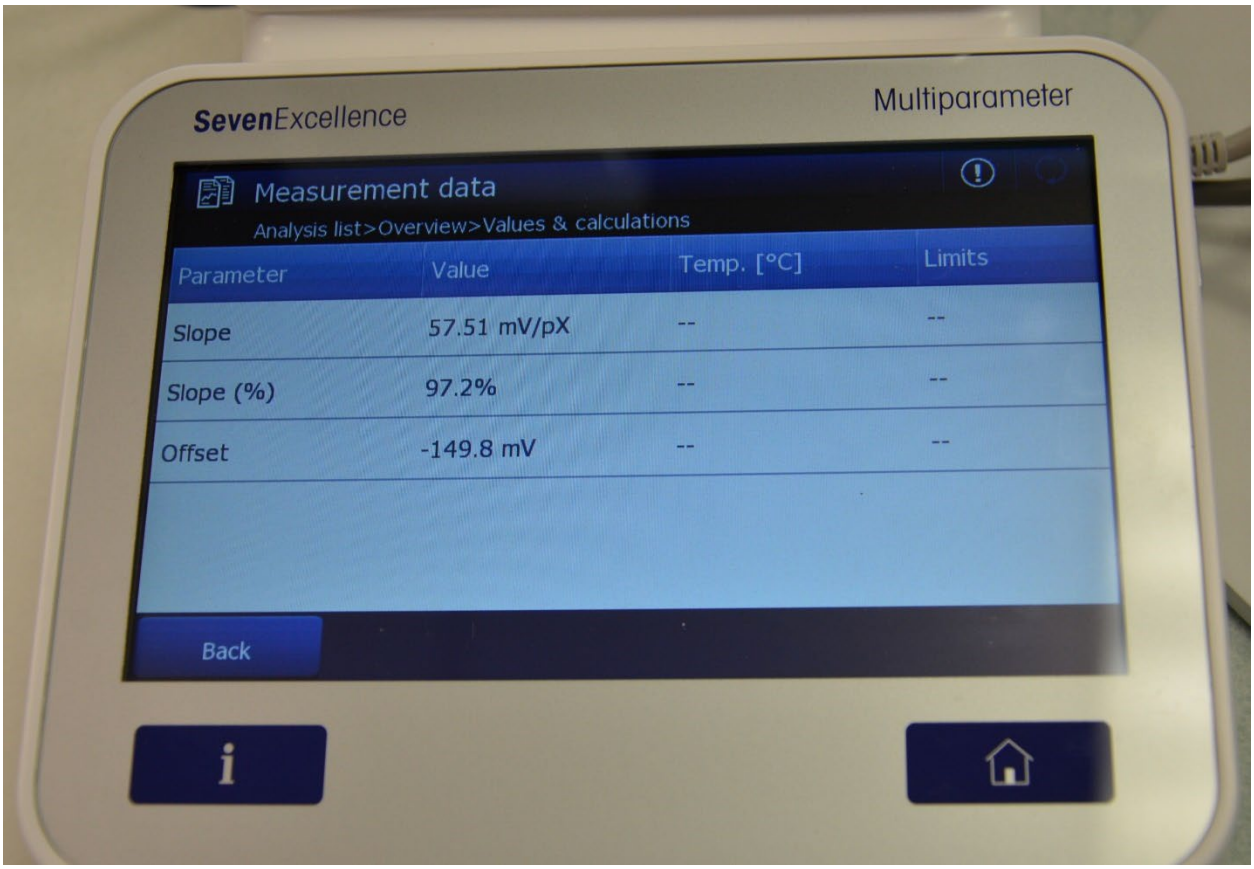


Figure 5 Calibration results displayed on the SevenExcellence ISE meter

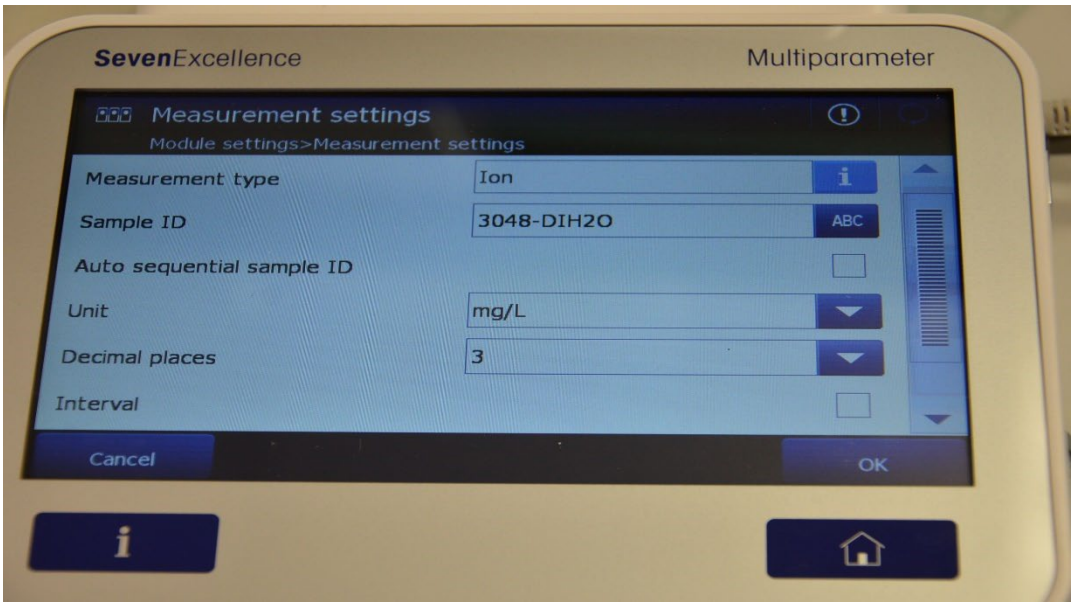


Figure 6 Sample ID Entry

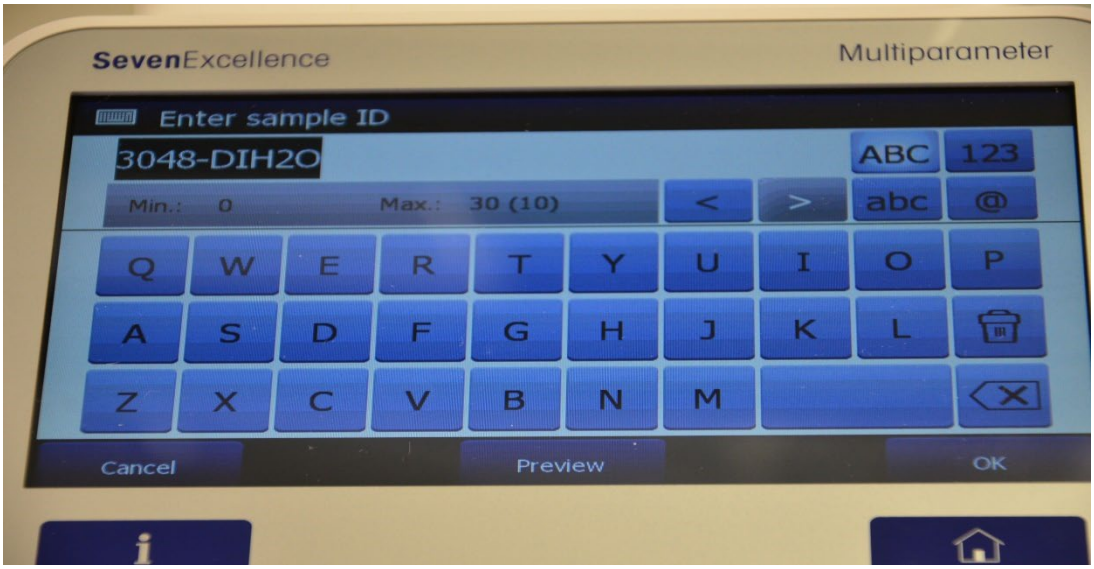


Figure 7 Sample ID Entry

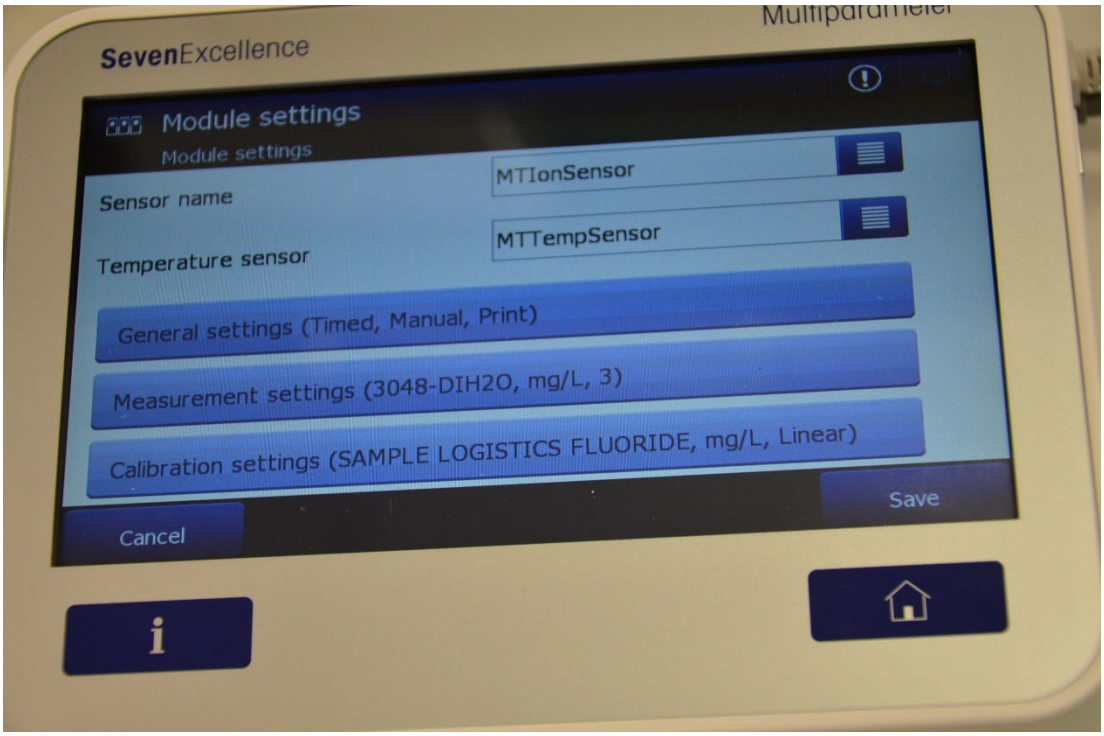


Figure 8 Module Settings

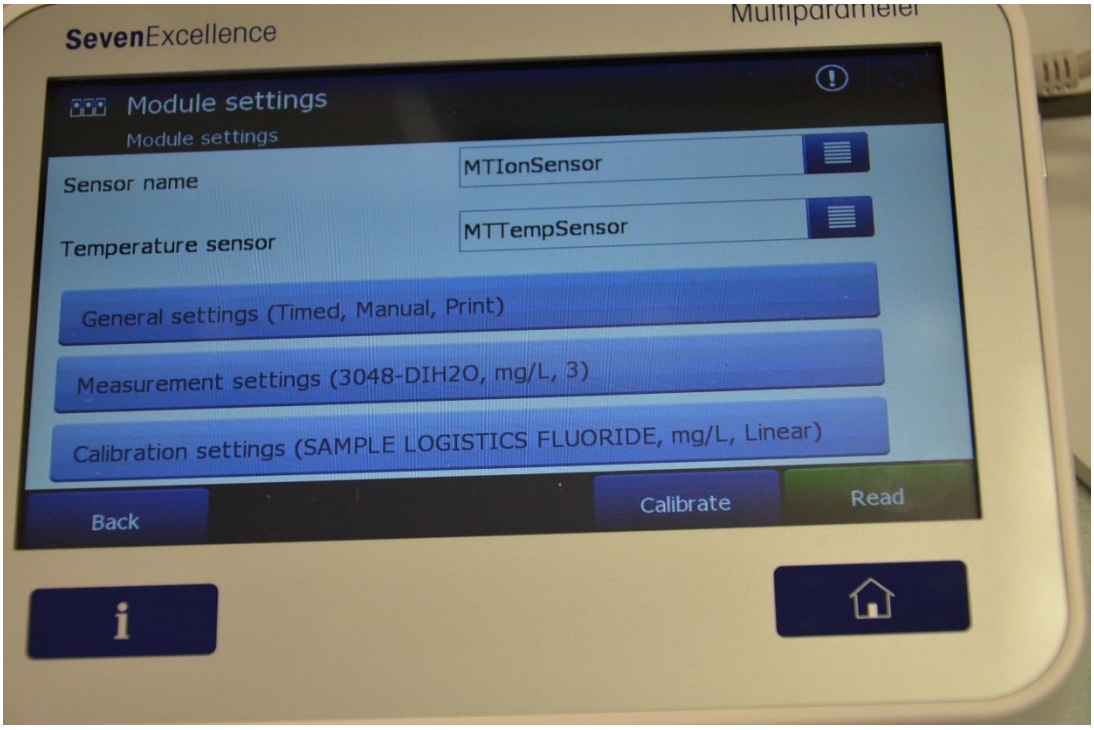


Figure 9 Module settings screen "Read" button bottom right corner

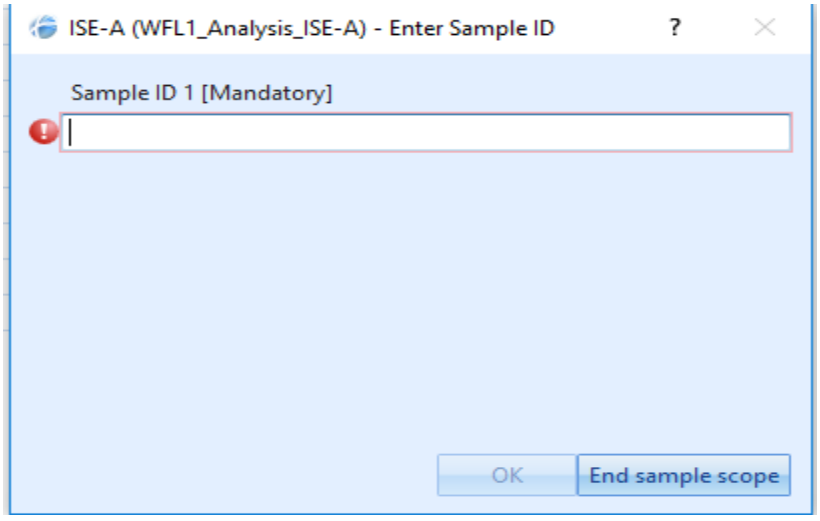


Figure 10 Sample ID prompt screen

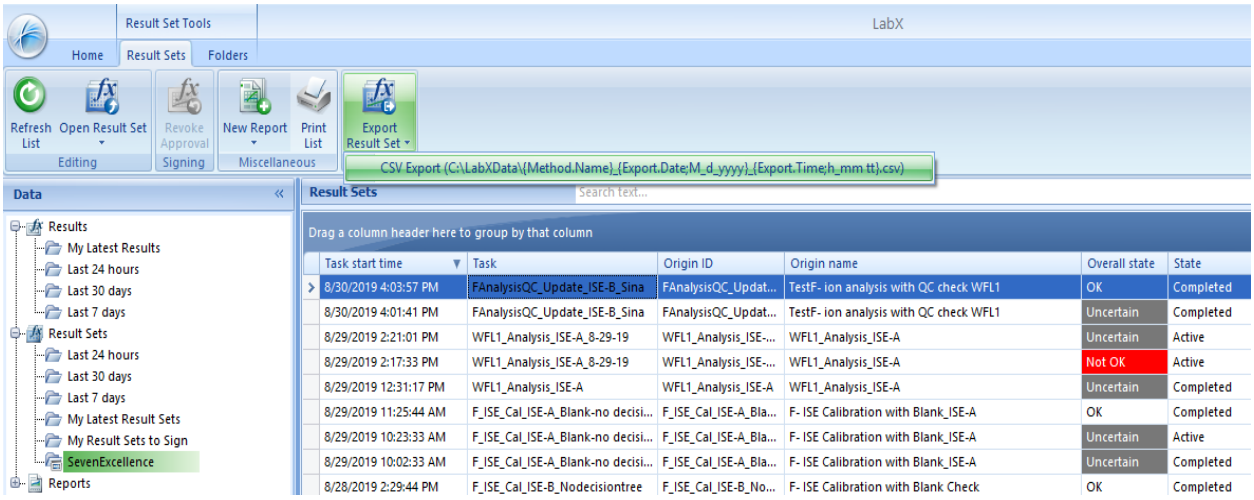


Figure 11 LabX export report from analytical run

23. Appendix A: Method Performance Documentation

A. Accuracy

Accuracy using Spike Recovery

Recovery = (final concentration – initial concentration)/added concentration
Recovery should be 85-115% except at 3*LOD where can be 80-120%

Method name: Urine Fluoride
Method #: 3049
Matrix: Urine
Units: mg/L
Analyte: **Fluoride**

		Sample 1					Sample 2					Mean recovery (%)	SD (%)
	Replicate	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)	Spike concentration	Measured concentration Day 1	Day 2	Mean	Recovery (%)		
Sample	1	0	0.12	0.119	0.1		0	0.123	0.119	0.1		92.0	0.5
	2		0.13	0.119				0.12	0.117				
	3		0.119	0.119				0.119	0.117				
Sample + Spike 1	1	1.0	1.041	1.047	1.0	92.5	3.0	2.886	2.87	2.9	92.2		
	2		1.045	1.047				2.897	2.87				
	3		1.049	1.047				2.897	2.882				
Sample + Spike 2	1	2.0	1.973	1.97	2.0	92.7	4.0	3.801	3.795	3.8	92.0		
	2		1.973	1.978				3.801	3.795				
	3		1.973	1.978				3.816	3.795				
Sample + Spike 3	1	5.0	4.704	4.698	5	91.4	6.0	5.602	5.616	6	91.4		
	2		4.686	4.698				5.623	5.594				
	3		4.704	4.644				5.602	5.594				

Figure 12 DLS PPM Method Performance Documentation: Accuracy

B. Precision

Precision

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

Method name: Urine Fluoride
Method #: 3049
Matrix: Urine
Units: mg/L
Analyte: **Fluoride**

Quality material 1

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	SL run#	run date	Instrument
1	0.513	0.523	0.52	0.000025	0.000025	0.536648	80866	10/23/2019	ISE-D
2	0.521	0.523	0.52	0.000001	0.000001	0.544968	80867	10/24/2019	ISE-C
3	0.510	0.522	0.52	3.6E-05	3.6E-05	0.532512	80868	10/25/2019	ISE-D
4	0.513	0.519	0.52	9E-06	9E-06	0.532512	80980	10/28/2019	ISE-C
5	0.517	0.515	0.52	0.000001	0.000001	0.532512	80982	10/29/2019	ISE-C
6	**0.514**	**0.524**	0.52	0.000025	0.000025	0.538722	81143	10/30/2019	ISE-D
7	0.512	0.520	0.52	0.000016	0.000016	0.532512	81145	10/31/2019	ISE-D
8	0.510	0.512	0.51	0.000001	0.000001	0.522242	81146	11/1/2019	ISE-D
9	0.512	0.520	0.52	0.000016	0.000016	0.532512	81174	11/5/2019	ISE-D
10	0.515	0.526	0.52	3.025E-05	3.025E-05	0.5418405	81364	11/6/2019	ISE-C

Grand sum 10.341 **Grand mean** 0.51705

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
Within Run	0.0003205	3.205E-05	0.005661272	1.09
Between Run	0.00016645	1.84944E-05	0	0.00
Total	0.00048695		0.005661272	**1.09**

Quality material 2

Run	Result 1	Result 2	Mean	SS 1	SS 2	2*mean^2	SL run#	run date	Instrument
1	3.321	3.360	3.34	0.00038025	0.00038025	22.3178805	80866	10/23/2019	ISE-D
2	3.313	3.339	3.33	0.000169	0.000169	22.124552	80867	10/24/2019	ISE-C
3	3.380	3.434	3.41	0.000729	0.000729	23.215298	80868	10/25/2019	ISE-D
4	3.400	3.427	3.41	0.00018225	0.00018225	23.3039645	80980	10/28/2019	ISE-C
5	3.403	3.417	3.41	4.9E-05	4.9E-05	23.2562	80982	10/29/2019	ISE-C
6	3.420	3.447	3.43	0.00018225	0.00018225	23.5778445	81143	10/30/2019	ISE-D
7	3.429	3.388	3.41	0.00042025	0.00042025	23.2357445	81145	10/31/2019	ISE-D
8	3.430	3.390	3.41	0.0004	0.0004	23.2562	81146	11/1/2019	ISE-D
9	3.425	3.439	3.43	4.9E-05	4.9E-05	23.557248	81174	11/5/2019	ISE-D
10	3.439	3.452	3.45	4.225E-05	4.225E-05	23.7429405	81364	11/6/2019	ISE-C

Grand sum 68.053 **Grand mean** 3.40265

	Sum squares	Mean Sq Error	Std Dev	Rel Std Dev
Within Run	0.0052065	0.00052065	0.022817756	0.67
Between Run	0.02733205	0.003036894	0.035470019	1.04
Total	0.03253855		0.042175493	**1.24**

Figure 13 DLS PPM Method Performance Documentation: Precision

C. Stability

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 LU-18050 and HU-18051 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 LU-18050 and HU-18051 were used for the measurements. Samples were left in the vials for 72 hours.
Processed sample stability = Assess short-term stability of processed samples, including resident time in autosampler
Describe condition: QC pools LU-18050 and HU-18051 were used for the measurements. Samples were prepared with TISAB II and left on the bench for
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 LU-18050 and HU-18051 will be used for measurements. This will be completed in November of 2020.

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

Method name:	Fluoride in Urine
Method #:	3049
Matrix:	Urine
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	0.519	0.500	0.519	0.499	0.519	0.509	0.519	0.500
Replicate 2	0.526	0.502	0.526	0.503	0.526	0.509	0.526	0.504
Replicate 3	0.526	0.504	0.526	0.507	0.526	0.509	0.526	0.494
Mean	0.523666667	0.502	0.523666667	0.503	0.523666667	0.509	0.523666667	0.5
% difference from initial measurement	--	**-4.1**	--	**-3.9**	--	**-2.8**	--	**-4.6**

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	3.425	3.286	3.425	3.34	3.425	3.367	3.425	3.207
Replicate 2	3.479	3.325	3.479	3.42	3.479	3.38	3.479	3.323
Replicate 3	3.493	3.392	3.493	3.407	3.493	3.38	3.493	3.363
Mean	3.465666667	3.33	3.465666667	3.39	3.465666667	3.38	3.465666667	3.3
% difference from initial measurement	--	**-3.8**	--	**-2.2**	--	**-2.6**	--	**-4.8**

Figure 14 DLS PPM Method Performance Documentation: Stability

D. LOD, specificity and fit for intended use

Method name:	Fluoride in Urine
Method #:	3049
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	0.144	N/A	yes

Figure 15 DLS PPM Method Performance Documentation: LOD, specificity and fit for intended use

24. Appendix B: Ruggedness Testing

Parameter Test #1: Effect of amount of TISAB II added to sample

Evaluating the significance of ratio of TISAB II to patient 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% HU-18051
b. 60% TISAB II to 40% HU-18051
c. 50% TISAB II to 50% HU-18051
d. 45% TISAB II to 55% HU-18051
e. 55% TISAB II to 45% HU-18051

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

Analyses were performed on December 13, 2019. Results below are the average of 3 replicates of high bench QC (HU-18051) analyzed as samples in each run under 6 varying ratios of TISAB II to HU-18051. All concentrations are in mg/L.

Table 5 Ruggedness test: Effect of amount to TISAB II added to sample

Percentage of TISAB II to Percentage of HU-18051	Replicate 1	Replicate 2	Replicate 3	STARLIMS Run #	Average of Replicates	HU-18051 Target Mean	% Difference
40% TISAB II 60% HU-18051	3049_Rugged_40a	3049_Rugged_40b	3049_Rugged_40c	82975	4.113	3.298	22.0%
	4.124	4.108	4.108				
60% TISAB II 40% HU-18051	3049_Rugged_60a	3049_Rugged_60b	3049_Rugged_60c	82975	2.689	3.298	20.3%
	2.689	2.689	2.689				
50% TISAB II 50% HU-18051	3049_Rugged_50a	3049_Rugged_50b	3049_Rugged_50c	82975	3.392	3.298	2.8%
	3.357	3.41	3.41				
45% TISAB II 55% HU-18051	3049_Rugged_45-5a	3049_Rugged_45-5b	3049_Rugged_45-5c	82975	3.755	3.298	13.0%
	3.735	3.765	3.765				
55% TISAB II 45% HU-18051	3049_Rugged_55-5a	3049_Rugged_55-5b	3049_Rugged_55-5c	82975	3.053	3.298	7.7%
	3.053	3.053	3.053				

Conclusion:

The results obtained for the samples prepared with 50% TISAB II and 50% sample (HU-18051) showed that this is the ideal ratio for this method. Varying the TISAB II to sample ratio, had a noticeable impact on recovery of the spiked analyte; therefore, it is critical to maintain the appropriate TISAB II to sample ratio and use quality control materials that are taken through the same sample preparation process.

Ruggedness testing note:

There are no other alterable parameters with this ISE method.

25. Appendix C: STARLIMS Upload Procedure

1) Open the STARLIMS application and select “Pending Runs Assigned to My Labs”.

2) From the Test drop down menu select “3049 (Fluoride in urine by ISE)”.

3) 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".

4) Select “[2] Mark Null Results”. This will mark any QC sample that does not have a result.

5) 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.

6) Select “[4] Evaluate Run QC”. Choose “Proceed to next step” on the QC Evaluation box.

7) 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.”

8) Click “[5] Set Run QC Statuses”. Select “Proceed” if the QC passes; otherwise change the status to fail before proceeding.

9) Click “[6] Attach SAS QC File”. Select the PDF version of the SAS output previously saved.

10) 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.

11) 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.

12) Fill in “User Field 3” with the study ID and “User Field 4” with the group number.

13) 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.

14) Notify the appropriate personnel via email that the run is ready to be reviewed.

26. Appendix D: Data Processing – Saving calibration and run data

1) Select the 'data' tab in the bottom left of the LabX software. Click on last 7 days in the 'results sets' section. This will tabulate all measurements done using the same method ID. (See Figure 16)

2) Open the calibration curve data set and select 'Raw Data', then click the 'Print Data Tab'. (See Figure 17)

3) Use the 'export to PDF' function to then save the file to the appropriate folder. (See Figure 18)

4) When exporting results for samples, select the 'Result Details' tab and copy paste the results into an excel worksheet. Then save the excel sheet into the Lab Data folder. (See Figure 19)

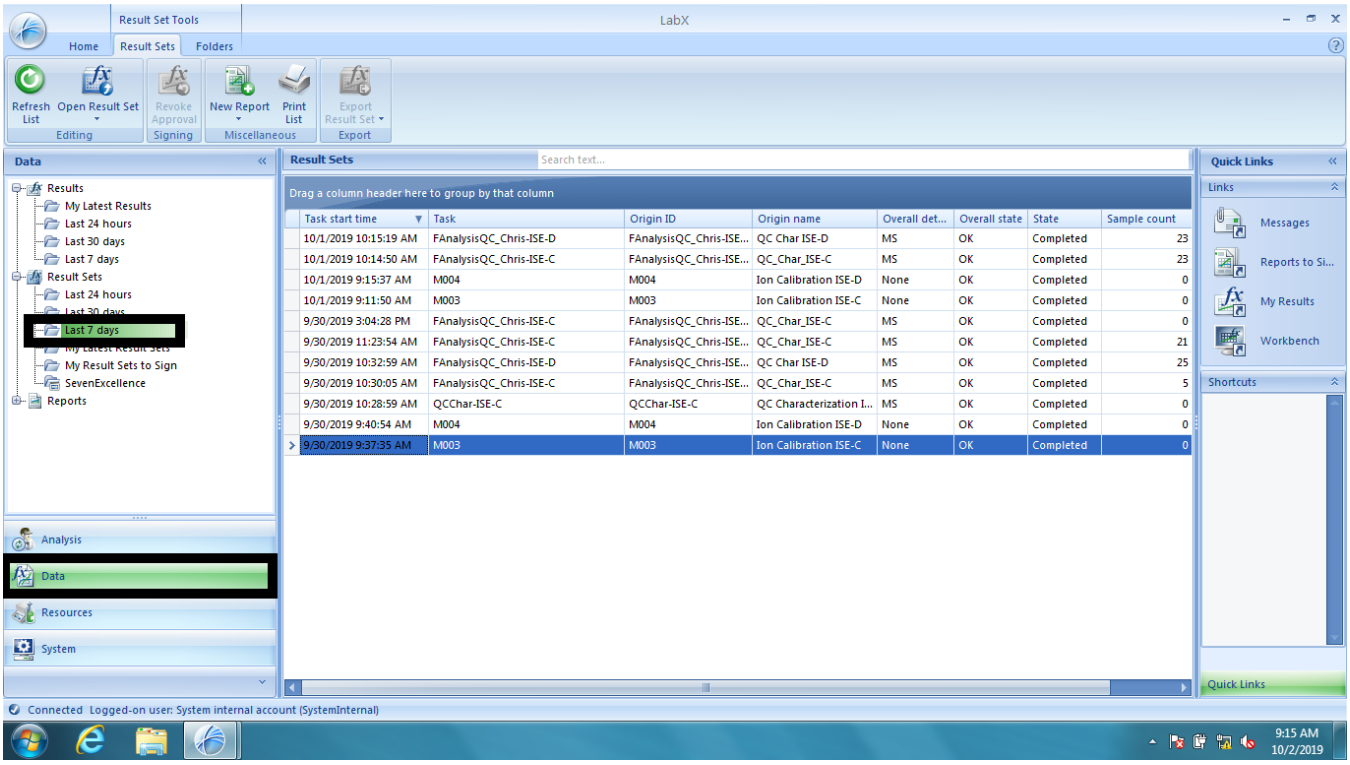


Figure 16 LabX Data processing: location of data

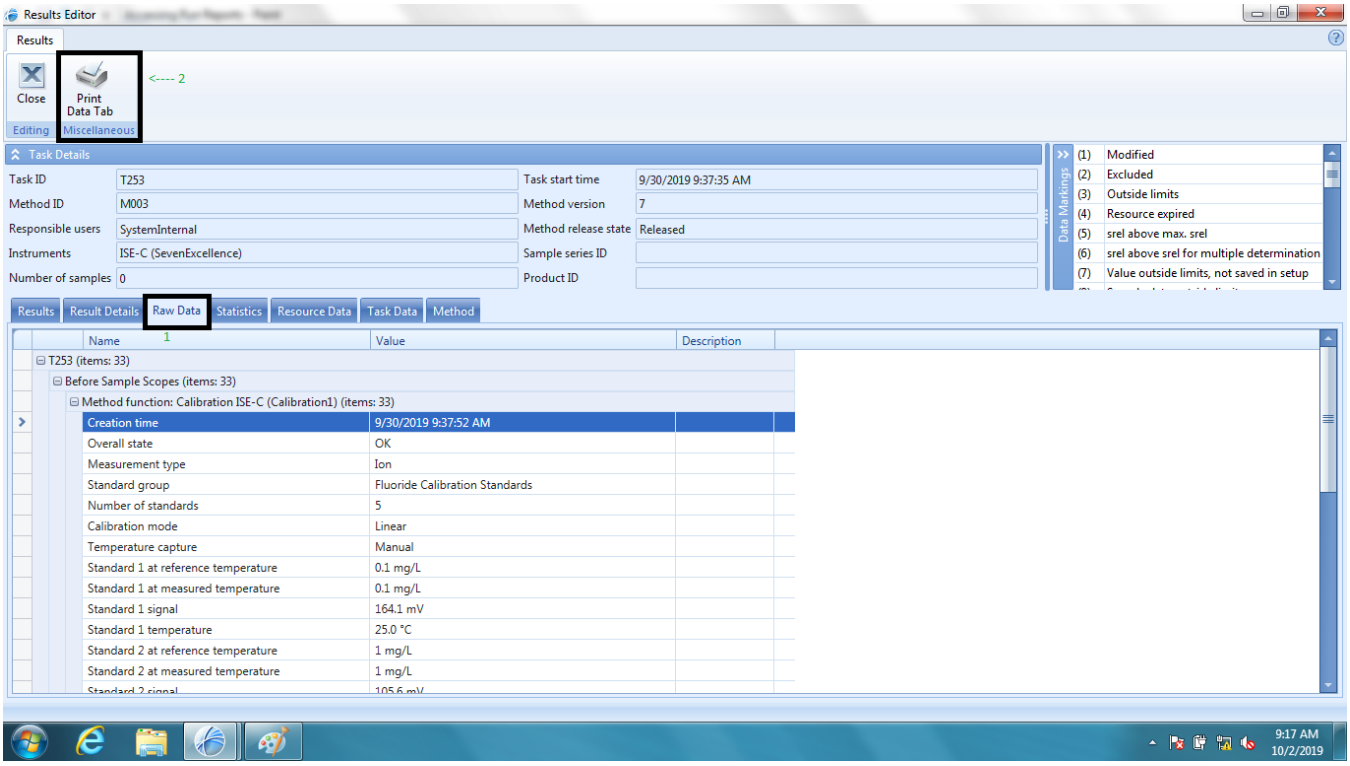


Figure 17 LabX Data processing: calibration curve

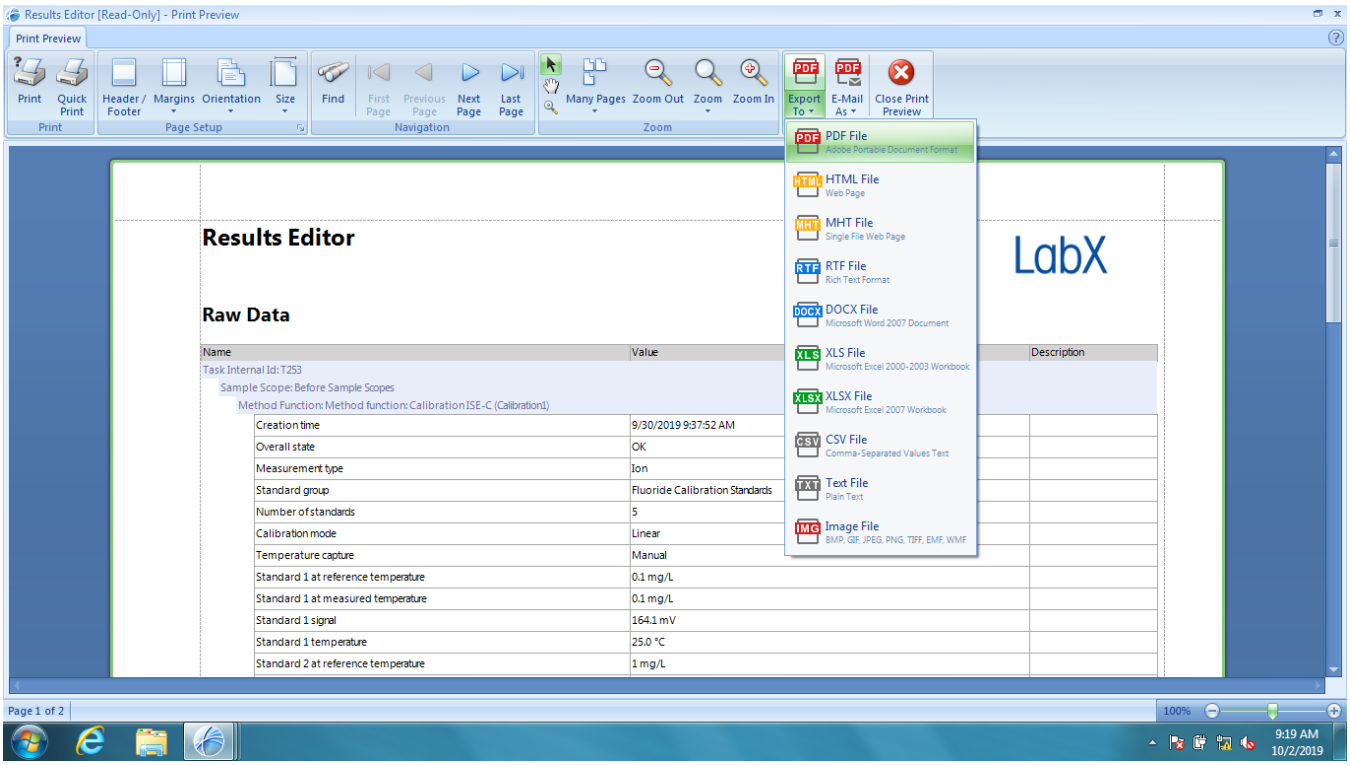


Figure 18 LabX Data processing: export to .pdf

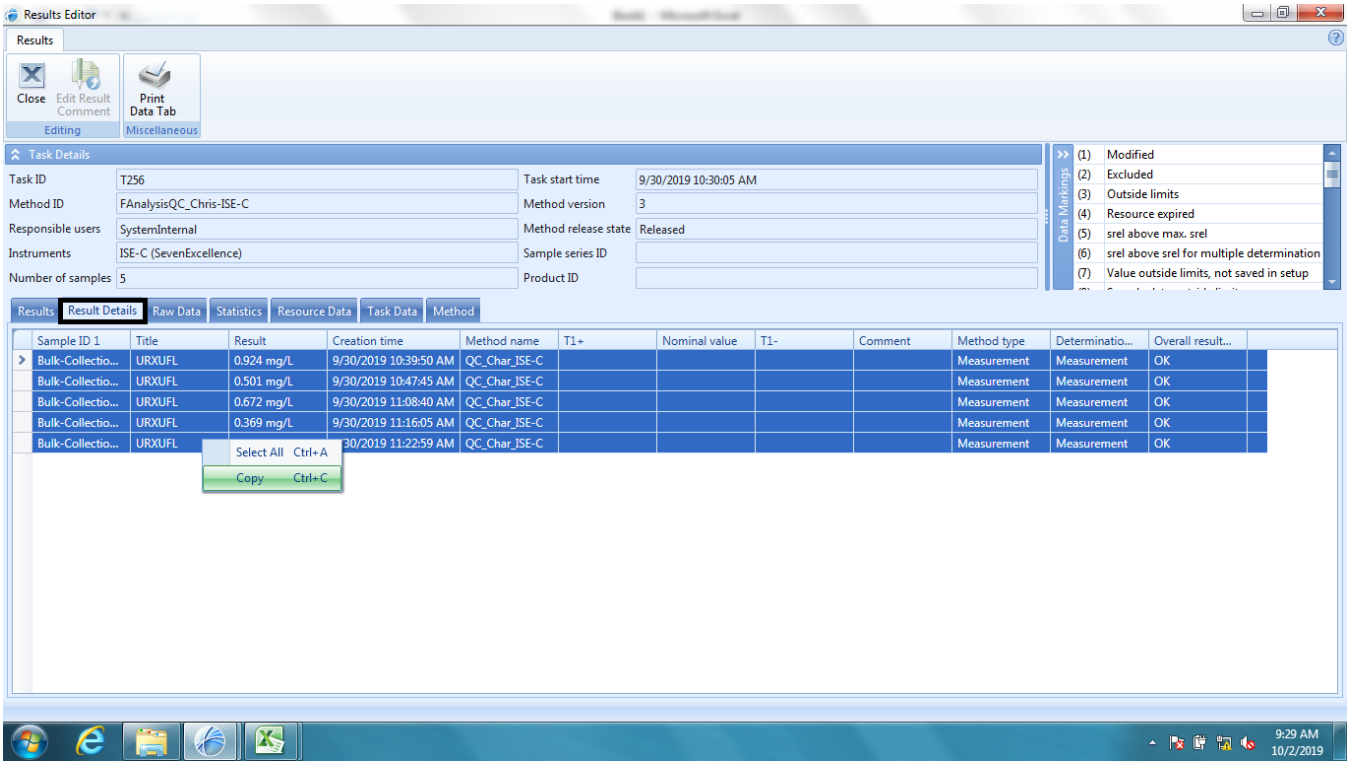


Figure 19 LabX Data processing: select and copy data