



Laboratory Procedure Manual

Analytes: Chromium and Cobalt

Matrix: Whole blood

Method: Inductively Coupled Plasma Mass Spectrometry (ICP-MS)

Method No: 3030.1

Revised:

As performed by:

Inorganic and Radiation Analytical Toxicology Branch
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.

Data File Name	Variable Name	SAS Label
CRCO_I	LBXBCR	Chromium (µg/L)
	LBXBCO	Cobalt (µg/L)

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1. CLINICAL RELEVANCE AND TEST PRINCIPLE

a. Clinical Relevance

Chromium (Cr) is a naturally occurring element whose nutritional bioavailability and toxicity depends on its oxidation state. Trivalent chromium is considered an essential nutrient while hexavalent chromium is a human carcinogen and a commonly encountered occupational hazard for humans [1, 2]. Cobalt (Co) is considered essential because it is part of the B₁₂ vitamin which is important for human brain and nervous center functioning and cell metabolism [3, 4]. While it is essential at certain lower levels, exposures to high levels of cobalt can affect the heart and/or lungs. Elevated exposures in animals have been shown to affect the liver and kidneys. The Agency for Toxic Substances and Disease Registry (ATSDR) lists cobalt as a possible carcinogen to animals due to research performed by the International Agency for Research on Cancer where direct contact with cobalt occurred [3]. It is uncertain whether or not the effects seen in animals will also be seen in humans, and this uncertainty adds additional concerns with a problem seen with failed metal-on-metal (MoM) hip implants.

Once degradation of a MoM hip implant occurs, elevated levels of chromium and cobalt are seen in the bloodstream. The increase can be up to ten times the levels seen in individuals without MoM implants [5]. Inflammation, carcinogenic and teratogenic effects, and allergic reactions have been linked to the accumulation of these metals in the body [6]. In order to gain information about the effects of higher chromium and cobalt concentrations in the bloodstream due to failed MoM implants, biomonitoring data for typical levels of these analytes in the population needs to be available to be used as a comparison. The reference values provided in the literature for these metals in whole blood are based on smaller scale studies which typically target a small population for a short period of time within a narrow geographic area. As a result, the analytical results obtained are not reflective of typical values for the U.S. population as a whole. One avenue for gaining such data is through the analysis of specimens collected in the National Health and Nutrition Examination Survey (NHANES). NHANES is used to assess the health and nutritional status of the United States population. Biomonitoring data about environmental exposures to numerous chemicals is obtained through this survey and is made publicly available in the year following the end of each two-year NHANES cycle. The MoM implant degradation issues spurred the incorporation of chromium and cobalt data collection into the 2015 to 2016 NHANES survey with data being publicly available as early as 2017. Once baseline population exposure values are established through NHANES, it can be determined whether or not the levels of cobalt and chromium in a person, particularly someone with a MoM hip implant, exceed what is considered “normal” or baseline for the population targeted (i.e. people older than 40). Researchers can then begin to more accurately correlate certain adverse health effects with certain levels of exposure.

Before data collection could begin, an analytical method had to be developed that could accurately quantify these two metals in whole blood. Previous methods of analyzing chromium in whole blood included the usage of atomic absorption spectrometry (AAS) or sector field inductively coupled plasma mass spectrometry (ICP-MS) [7]. However, both of these techniques have their limitations and drawbacks. AAS does not provide the detection limits needed to analyze these metals at the trace levels needed for biomonitoring purposes, and it does not allow for the measurement of multiple analytes simultaneously [8]. Sector field ICP-MS is not a preferred technique to handle the sample throughput demands of a biomonitoring method due to the complexity and cost of the instrumentation. The Inorganic and Radiation Analytical Toxicology branch (IRAT) developed a biomonitoring method which uses quadrupole ICP-MS to rapidly and accurately quantify cobalt and chromium in human whole blood samples while eliminating the potential interferences that exist with the analysis of samples of this nature. To ensure the accuracy of the measurements reported, our laboratory also focuses on lot screening of the devices used in the sample collection, storage, and preparation processes to ensure that background levels of the analytes, particularly chromium, are not present in the items used for sample collection, storage, preparation or analysis.

b. Test Principle

The concentrations of chromium (^{52}Cr) and cobalt (^{59}Co) in whole blood specimens are directly measured using inductively coupled mass spectrometry (ICP-MS). This analytical technique is based on analyte detection using quadrupole ICP-MS technology including Kinetic Energy Discrimination (KED) technology which minimizes or eliminates many argon-based polyatomic interferences [9]. Although it is unnecessary to measure cobalt in KED mode, both cobalt and chromium are measured in KED mode to reduce the stabilization time between modes [10]. The sample goes through a nebulizer where it is converted into an aerosol upon entering the spray chamber. Carried by a stream of argon gas, a portion of the aerosol is transported through the spray chamber and then through the central channel of the plasma where it is heated to temperatures of approximately 6000-8000°K. This thermal energy atomizes and ionizes the sample. The ions and the argon enter the mass spectrometer through an interface that separates the ICP (operating at atmospheric pressure of approximately 760 torr), from the mass spectrometer (operating at approximately 10^{-5} torr). Once inside the mass spectrometer, the ions pass through the ion optics which uses an electrical field to focus the ion beam into the collision cell (QCell™). The QCell™ is pressurized with an appropriate reaction gas (in this case helium) and contains a flatpole quadrupole system. Elimination or reduction of argon-based polyatomic interferences takes place through the interaction of the reaction gas with the interfering polyatomic species in the incoming ion beam. The ions go from the collision cell to the mass-analyzing quadrupole before striking the surface of the detector. Once ions pass through the cell and are electrically selected for passage through the analytical quadrupole, electrical signals resulting from the ions striking the detector are processed into digital information that is used to indicate the intensity of the ions. The intensity of ions detected while aspirating an unknown sample is translated into an elemental concentration through comparison of the analyte to internal standard signal ratio of the unknown with the ratio obtained when aspirating calibration standards. This method is a variation of IRAT's method used to analyze lead, cadmium, mercury, manganese, and selenium in whole blood which was originally based on the method by Lutz et al [11].

Anticoagulants such as ethylenediaminetetraacetic acid (EDTA) have to be added to the vessel that the sample is collected in because without the anti-coagulant present, the specimen will clot immediately after it is taken from the body and drawn into the collection tube. Whole blood specimens are prepared for analysis through a simple dilution procedure. The patient sample is thoroughly mixed via vortexing to ensure that all cellular components in the specimen are uniformly distributed. Due to the propensity of certain metals to bind to certain components of the blood, the sample has to be homogenized prior to an aliquot being removed for sample preparation. This mixing step is critical to ensure that the concentrations of the analytes of interest accurately reflect the average concentration in the entire specimen. After proper mixing, the sample is diluted in the ratio of one part sample + one part water + 18 parts diluent resulting in a 20x dilution. Tetramethylammonium hydroxide (TMAH) and Triton X-100 are used in the diluent to solubilize the blood components. The addition of Triton X-100 gives the added benefits of elimination of biological deposits on the sample introduction components and reduction of air bubbles on the inner surfaces of the sample tubing. Ammonium pyrrolidine dithiocarbamate (APDC) in the sample diluent aids in solubilizing metals released from the blood. One percent ethyl alcohol in the sample diluent aids in the solubility of the blood components and aids in aerosol generation by reduction of the surface tension of the solution. The internal standard concentration is the same for all blanks, calibrators, quality control specimens (QC), and unknowns. Monitoring the ratio of the instrument signal of the analyte to the instrument signal of its internal standard allows for correction for instrument noise and drift as well as sample-to-sample matrix differences.

The analysis of chromium and cobalt in blood matrices by ICP-MS can be problematic due to polyatomic interferences. The interferences come from a number of different sources such as the sample matrix itself, the matrix of the reagents used for analysis, or the argon plasma [12]. Since the interferences are the same mass as the analyte being measured, proper interference removal is necessary to ensure that only the analyte of interest is being quantified. A list of the most common interferences for cobalt and chromium are presented in **TABLE 1-1**. As previously mentioned, elimination or reduction of argon-based polyatomic interferences takes place in the collision cell by KED using helium gas as the collision

gas. The collision cell is pressurized with helium gas which collides with the ions. Such collisions will occur more frequently with larger polyatomic ions than smaller atomic analyte ions, hence, lowering the kinetic energy of the polyatomic ions relative to the atomic ions. The cell is maintained at a voltage more negative than the analyzing quadrupole creating the KED. The faster moving analyte ions are able to overcome it while the larger polyatomic ions are not. As a result, the analyte ions are separated from the interference ions. With this analytical method, it was determined that using the KED mode with helium at a flow rate of 5.0 mL/min was sufficient for the removal of these interferences.

TABLE 1-1: POTENTIAL INTERFERENCES FOR CR AND CO IN ICP-MS^{13, 14}

Analyte	Potential Interferences
⁵² Cr	³⁵ Cl ¹⁶ O ¹ H ⁺ , ⁴⁰ Ar ¹² C ⁺ , ³⁶ Ar ¹⁶ O ⁺ , ³⁷ Cl ¹⁵ N ⁺ , ³⁴ S ¹⁸ O ⁺ , ³⁶ S ¹⁶ O ⁺ , ³⁸ Ar ¹⁴ N ⁺ , ³⁶ Ar ¹⁵ N ¹ H ⁺ , ³⁵ Cl ¹⁷ O ⁺ , ¹ H ⁵¹ V ⁺ , ¹⁰³ Rh ⁺⁺
⁵³ Cr	³⁷ Cl ¹⁶ O ⁺ , ³⁸ Ar ¹⁵ N ⁺ , ³⁸ Ar ¹⁴ N ¹ H ⁺ , ³⁶ Ar ¹⁷ O ⁺ , ³⁶ Ar ¹⁶ O ¹ H ⁺ , ³⁵ Cl ¹⁷ O ¹ H ⁺ , ³⁵ Cl ¹⁸ O ⁺ , ³⁶ S ¹⁷ O ⁺ , ⁴⁰ Ar ¹³ C ⁺ , ¹⁴ N ³⁹ K ⁺
⁵⁹ Co	⁴³ Ca ¹⁶ O ⁺ , ⁴² Ca ¹⁶ O ¹ H ⁺ , ²⁴ Mg ³⁵ Cl ⁺ , ¹⁴ N ⁴⁵ Sc ⁺ , ¹ H ⁵⁸ Ni ⁺ , ³⁶ Ar ²³ Na ⁺ , ⁴⁰ Ar ¹⁸ O ¹ H ⁺ , ⁴⁰ Ar ¹⁹ F ⁺

2. SAFETY PRECAUTIONS

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

Obtaining the proper training and usage of personal protective equipment (PPE) is mandatory for individuals within DLS that work with the analytical method. Follow universal precautions; every patient sample must be handled as potentially infectious. Wear gloves, a lab coat, and safety glasses while handling human blood, plasma, serum, urine, or other bodily fluid or tissue. CDC operators of this method must take the Hazardous Chemical Waste Management for CDC Workers and Bloodborne Pathogens courses upon initial hire and yearly refreshers thereafter. Place disposable plastic, glass, and paper (e.g., pipette tips, autosampler tubes, and gloves) that come into contact with human biological fluids in a biohazard autoclave bag. Keep these bags in appropriate containers (i.e. autoclave pans or autoclave step cans) until they are sealed and autoclaved. When laboratory work is finished, wipe down

all work surfaces where human biological fluids were handled with either a 10% (v/v) sodium hypochlorite solution or a broad spectrum disinfectant. Dispose of all diluted biological specimens in a biohazard autoclave bag at the end of the analysis following guidelines found in the CDC/DLS guidelines for disposal of hazardous waste.

Thermo Fisher Scientific provides safety information that must be read prior to operating the iCAP Q ICP-MS. This information is found in the Thermo® Fisher Scientific iCAP™ Q Operating Manual (Chapters 4 and 9). Possible hazards encountered while using this instrument include toxic gases (with inappropriate exhaust), electrical hazards, ultraviolet radiation, high voltages, radio-frequency radiation, sharp edges, pinch point hazard, and high temperatures.

Handle acids and bases with extreme care because they are caustic and toxic. Reagents used in this study include those listed in Section 5 of this procedure. 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 at <http://intranet.cdc.gov/CHATS/OHS/chem/search.asp>.

3. DATA SYSTEM MANAGEMENT

To maintain the integrity of analytical data generated by this method, eliminate hand entry of specimen identifiers or analytical results whenever possible. Proofread all transcribed data and regularly back up critical files on the ICP-MS computer's hard drive. It is recommended that a defragmentation program be run on the computer's hard drive on a periodic basis.

a. Data Entry and Transfer

Use bar code scanners to enter sample identifiers into the ICP-MS computer software whenever possible to avoid transcription errors and to speed up sample processing. When bar code scanners cannot be used, proofread transcribed data after entry. Handle or transfer data electronically when reporting or moving data to other computerized data-handling software programs or storage locations.

b. Routine Computer Hard Drive Maintenance

Defragment the instrument computer's hard drive by using software such as Microsoft Windows® Disk Defragmenter (located in Start > All Programs > Accessories > System Tools) or an equivalent program to maximize computer performance and maintain data integrity for files on the hard drive. An entry will automatically be logged in the Windows™ system event log when this process is performed providing documentation of this step.

c. Data Backup

(1) Schedule of Data Backups

Sample results generated by this analytical method are stored long-term on compact discs and on the CDC network shared drive. The stored results must include at least the analysis date, quality-control (QC) results for the run, and the results of specimen analysis by specimen identifier (ID).

Whenever making a backup (daily or weekly) include the directories and subdirectories:

- ✓ C:\ProgramData\Thermo\Qtegra_ApplicationData\Workspace\LabBooks (include all subdirectories)

- ✓ C:\ProgramData\Thermo\Qtegra_ApplicationData\Workspace\Reports (include all subdirectories)
- ✓ C:\Event Log Backup
- ✓ Any other relevant files or folders.

Daily. Full data backups onto an encrypted, external hard drive (if equipped to do so).

Weekly. Full data backups onto one or more recordable compact discs (CD-R) or digital video discs (DVD). These backups are made in duplicate.

(2) Backup Procedures

Before making a CD or DVD backup, the information from the Windows Event Viewer needs to be saved. Access the Event Viewer by either clicking on the desktop icon or by clicking on Start in Windows. The Event Viewer will appear (see **Figure 2-1**).

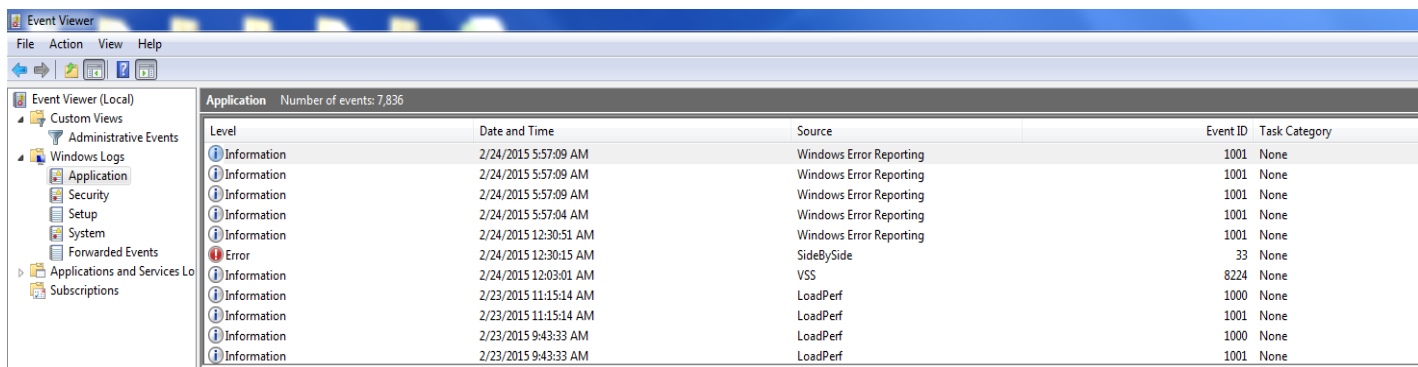


Figure 2-1: Event Viewer

Right click on Application. Choose “Save All Events As...” Save the file in the folder “C:/Event Log Backup”. Name the file “Application iCAP Qa-I MM-DD-YY” where I is the instrument name (either A or B) and “MM” is the month, “DD” is the day, and “YY” is the last two digits of the year. The following window will pop up.

Display Information

To enable viewing this log correctly on other computers, you may need to include display information.

No display information

Display information for these languages:

English (United States)

Show all available languages

Note: Not all languages may be available for all the selected sources

OK Cancel

Select English as the language and click

OK

(a) Secondary (External) Hard Drive Backups

- If the computer is equipped with an encrypted external hard drive, configure Microsoft Windows® Backup™ (located in Start > Programs > Accessories > System Tools) or other applicable program to do a daily backup which includes the Thermo Qtegra™ LabBooks and computer data directories (see *Backup Procedures* above). If possible, this backup should be scheduled for a time when the computer will most likely be idle (such as 4 AM each day).

(b) Compact Disc Backups

- Use CD-R disks only (recordable compact disks), *not* CD-RW disks (rewritable compact disks) so that the compact disk cannot be over-written after creation. DVD discs are also acceptable when transferring large amounts of data (or working with large databases).
- Use Roxio Creator or an equivalent software program to make CD/DVD backups.
- Back up the most recent data on the hard drive in duplicate onto two disks. Manually select each dataset folder (subdirectories under "C:\ProgramData\Thermo\Qtegra_ApplicationData\Workspace\LabBooks"), the reports folder, the event log backup, and other relevant files (i.e., optimization, tuning, and sample files) that are to be included on these backups.
- Keep one copy of the CD-R disk in a building other than the laboratory (in case of fire). Keep the other copy in or near the ICP-MS laboratory for easy retrieval, if necessary.

(c) Deleting Data from the Hard Drive

- If data needs to be deleted from the hard drive (i.e. for computer replacement or upgrade, to free up hard drive space, etc.) backup all of the data on the hard drive in duplicated using one of more disks.
- Verify that the data can be retrieved from the disk(s) before actually deleting the data.

- To verify that the data was backed up properly, open any file on the disk by using the appropriate computer software program such as Qtegra.
- After verifying that all backups are operational, delete the original data from the hard drive.

d. Documentation of System Maintenance

Computer Maintenance:

Record any maintenance of computer hardware or ICP-MS software in the instrument logbook. This includes disk defragmentation, software upgrades, software patches, etc.

Instrument Maintenance:

Document instrument maintenance in hard copies of data records (i.e., daily maintenance checklists, service records logs, and instrument lab notebooks). Whenever a service call is placed, store copies of any documents related to the service call in the instrument logbook.

4. COLLECTING, STORING, AND HANDLING SPECIMENS; CRITERIA FOR REJECTING SPECIMENS

a. Specimen Type

The specimen type is human whole blood. No special instructions for fasting or special diets are required of patient or study subjects.

b. Specimen Collection, Handling, and Storage

The optimal amount of specimen is 2.0 mL; the minimum is 0.60 mL. Use sterile specimen containers for specimen acquisition. Acceptable containers for the storage of whole blood for this method include 2 mL polypropylene cryogenic vials (e.g. Nalgene, item # 500-0020) or equivalent storage containers.

To ensure the validity of the analytical results that we produce, our laboratory is heavily involved in the lot screening of all materials used for sample collection, storage, and analysis. We want to ensure that background levels of the analytes of interest are not present in concentrations above the thresholds that we have established. For this method, we screen manufacturing lots of needles, vacutainers, cryovials, and other items for both cobalt and chromium contamination prior to use. Chromium is a widespread contaminant, and it has been shown that the stainless steel needles used to draw blood samples contain chromium in small amounts. While it is recommended that a Teflon catheter be used to draw the blood for chromium and other metals such as titanium, that is not always practical [10]. To eliminate possible chromium contamination of the patient samples from the use of stainless steel needles, we recommend that the blood vial used for this assay be taken from the second vial collected. If contamination is present in the needle, the first draw of the sample will flush out the surface contamination leaving the second draw free from contaminants. The first vial collected can be used for analytical measurements of other elements.

Specimen handling conditions are outlined in the current version of the Division of Laboratory Sciences Policies and Procedures Manual. 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. This includes, but is not limited to, recognized proper sampling technique, storage in pre-screened containers, packaging and transport in suitably labeled containers that will maintain a temperature of approximately 4°C or a lower temperature throughout transport. Upon receipt, store the specimens at approximately -70°C or lower temperature until the samples will be analyzed. After thawing the samples and preparing them for analysis, refreeze the remaining portions at the same approximate storage temperature.

c. Criteria for an Unacceptable Specimen

A specimen is deemed unacceptable when there are low sample volumes (< 0.600 mL), suspected contamination due to improper collection procedures or collection devices, and/or contamination during sample preparation/analysis. Specimen contact with dust or dirt may compromise test results. It is important that prior to or during sample preparation the analyst identify any sample having clots or micro-clots (small clots). Do not analyze clotted samples by this method due to the inhomogeneity issues. Process the sample as “not reportable” in STARLIMS. Any errors encountered in the laboratory that result in the sample being compromised must be reported to the supervisor immediately. In all of these instances, request a second blood specimen, if possible.

5. CHEMICALS AND REAGENTS, STANDARDS, QUALITY CONTROL MATERIALS

a. Chemicals and Reagents

1. Water, high purity ($\geq 18 \text{ M}\Omega\cdot\text{cm}$ resistivity), Aqua Solutions, or equivalent
2. ICAP™ Q calibration solution, Thermo Fisher Scientific, Item # 1323760, (Item # Thermo-5A for Inorganic Ventures), or equivalent
3. ICAP™ Q tune solution (daily performance solution), Thermo Fisher Scientific, Item #

- 1323770, (Item # Thermo-4AREV for Inorganic Ventures), or equivalent
4. Tetramethylammonium hydroxide, (CAS# 75-59-2), 25 wt. % in H₂O, Alfa Aesar, Item # 20932, or equivalent
 5. Triton X-100™ BioXtra, Sigma-Aldrich, Item # T9284, or equivalent
 6. Ammonium pyrrolidine dithiocarbamate (APDC), (CAS# 5108-96-3), laboratory grade, Fisher Scientific, Item # A182-10, or equivalent
 7. Ethanol (CAS# 64-17-5) M.W. 46.07, USP dehydrated 200 proof, Fisher Scientific, Item # 111000200CSPP, or equivalent
 8. Veritas™ double-distilled nitric acid, GFS Chemicals, Item # 621, or equivalent
 9. Veritas™ double-distilled hydrochloric acid, GFS Chemicals, Item # 2185, or equivalent
 10. Liquid argon, Airgas South, Inc., or equivalent
 11. High purity helium, Airgas South, Inc., or equivalent
 12. Bleach (10% sodium hypochlorite solution), Fisher Scientific, Item # 23-640-501, or equivalent disinfecting agent
 13. Human whole blood with EDTA, Tennessee Blood Services, or equivalent

b. Standards

The following is a list of possible sources of stock materials for elemental chromium, elemental cobalt, and the internal standards used for this analytical method (gallium and scandium). To make calibrator sets, while it is preferred to have at least three different lots of materials from three different vendors on hand at any given time, different lots of the same material from one vendor will count as two separate sources. The sources listed are to be used as a guide; other National Institute of Standards and Technology (NIST) traceable sources can be used.

1. 1000 µg/mL Cobalt in 2% HNO₃ (CAS # 7440-48-4), High-Purity Standards, Item # 100013-1, or equivalent
2. 1000 ppm (µg/mL) Cobalt for ICP (CAS # 7440-48-4), Inorganic Ventures, Item # CGCO1, or equivalent
3. 1000 µg/mL Claritas PPT Grade Cobalt Standard (CAS # 7440-48-4), SPEX CertiPrep, Item # CLCO2-2Y, or equivalent
4. SRM 3113 – Cobalt (Co) Standard Solution, NIST, Item # SRM 3113, or NIST traceable equivalent
5. 1,000 µg/mL Chromium⁺³ in 2% HCl (CAS # 7440-47-3), High-Purity Standards, Item # 100012-6, or equivalent
6. 1,000 (µg/mL) Chromium⁺³ for ICP (CAS # 7440-47-3), Inorganic Ventures, Item # CGCR(3)1, or equivalent
7. 1,000 µg/mL Claritas PPT Grade Chromium Standard (CAS # 7440-47-3), SPEX CertiPrep, Item # CLCR2-2Y, or equivalent
8. SRM 3112a – Chromium (Cr) Standard Solution, NIST, Item # SRM 3112a, or NIST traceable equivalent
9. 1,000 ppm (µg/mL) Gallium (Ga) for ICP (CAS # 74440-55-3), Inorganic Ventures, Item # CGGA1-1, or equivalent
10. 1,000 µg/mL Gallium in 2% HNO₃ (CAS # 74440-55-3), High-Purity Standards, Item

#100019-1, or equivalent

11. 1,000 ppm ($\mu\text{g/mL}$) Scandium (Sc) for ICP (CAS # 12060-08-1), Inorganic Ventures, Item # CGSC1-1, or equivalent
12. 1,000 $\mu\text{g/mL}$ Scandium in 2% HNO_3 (CAS # 12060-08-1), High-Purity Standards, Item # 100048-1, or equivalent

c. Quality Control Materials

Quality control (QC) materials are prepared from pooled human whole blood collected from several anonymous donors. The donor blood can be purchased commercially. QC materials designated for bench quality control purposes will need to be “characterized” as described in the section titled *Establish QC limits for each QC pool*. The blood QC pools made for this method are named as:

QC Level	QC Designation ID
low pool	LB-yy###
high pool	HB-yy###
elevated 1 pool	EB-yy###
elevated 2 pool	EB-yy###

where “yy” is the last two digits of the production year and “###” is the assigned pool identification number. See the “Preparation of Quality Control Material” section of this method for details on preparation.

6. INSTRUMENTATION, EQUIPMENT, SOFTWARE, AND SUPPLIES

a. Instrumentation

(1) ICP-MS System

All components listed below used for this analytical method were obtained from Thermo Fisher Scientific.

1. Inductively coupled plasma mass spectrometer, specifically the Thermo® iCAP™ Qa (upgraded to an iCAP Qc) ICP-MS with collision cell (QCell™) capability, or equivalent
2. Qtegra™ Intelligent Scientific Data Solution™ (ISDS) Software instrument control and data handling software, or equivalent
3. TF-2500 IPR, 208 V, 60 Hz Chiller - water recirculator, Item # 121123010000008, or

equivalent

4. Electron Multiplier (SEM) iCAP™ Q, Item # 1299710, or equivalent
5. iCAP™ Q Autosampler Housing, Item # 1322190, or equivalent



Figure 6-1: iCAP Q ICP-MS with integrated autosampler housing

(2) ESI SC-FAST Autosampler Choices

1. SC-4DX FAST autosampler, P/N 4DXF-73A, or equivalent
2. SC-4DQ FAST autosampler, Thermo Fisher Scientific, P/N 1317000, or equivalent

(3) ICP-MS Consumables

Unless otherwise indicated, part numbers (P/N) listed below are Thermo Fisher Scientific part numbers from the June 2013 *iCAP Q Consumables and Parts Catalog*. Equivalent high quality parts from other suppliers may be used.

1. PVC peristaltic pump tubing (ID 0.762 mm – black-black (3 bridges, flared end), P/N 1320060, or equivalent
2. Santoprene™ tube- ID 1.295 mm – gray-gray (3 bridges, straight end), P/N 1320110, or equivalent
3. Platinum sampler cone, P/N 3601289, or equivalent
4. Platinum skimmer cone – insert version, P/N 1324540, or equivalent
5. Skimmer cone insert 3.5 (High Matrix Tolerance), P/N 1318480, or equivalent
6. Sampling cone gasket, P/N 1310900, or equivalent
7. Extraction lens 2 – ICAP Q, P/N 1310730, or equivalent
8. iCAP™ Q quartz torch, P/N1230790, or equivalent
9. iCAP™ Q torch holder, P/N 1231000, or equivalent
10. Injector 2.0 mm ID quartz, P/N 1305640, or equivalent
11. Quartz cyclonic spray chamber, P/N 1317080, or equivalent

12. PFA adapter elbow for spray chamber, P/N 1320270, or equivalent
13. Spray chamber drain line for iCAP Q, P/N 1320280, or equivalent
14. PTFE coated O-ring for torch holder gas inlet, P/N 430122820891, or equivalent
15. FFKM 2 mm OD (skimmer cone mount), P/N 1326830, or equivalent
16. O-ring 104 x 2.5 Viton™ OD (Interface block to source chamber), P/N 1231640, or equivalent
17. O-ring 60 x 2.5 Viton™ OD (Interface block to source chamber), P/N 1231650, or equivalent
18. Load coil assembly iCAP™ Q, Item # 1305260, or equivalent
19. Inline water filter 5 µm, P/N 1600550, or equivalent
20. 100 micron filter kit without grill, P/N 598000000003, or equivalent
21. Air filter iCAP™ Q, P/N 1294680, or equivalent
22. Pump oil PFPE, P/N 1319310, or equivalent
23. PFA-ST MicroFlow Nebulizer, ESI, P/N ES-2040-73, or equivalent
24. Tubr Teflon™ 1/16 OD 0.2 ID 10 ft, P/N 1600061, or equivalent

(4) ESI SC-FAST Autosampler Consumables

Part numbers listed below are Elemental Scientific Inc. (www.elementalscientific.com) part numbers, unless otherwise indicated.

1. P6 CTFE High-flow 6 port valve head, P/N PF-P6, or equivalent
2. CTFE Stator for 6 port valve head, P/N PF-P6S, or equivalent
3. PFA Rotor for 6 port valve head, P/N PF-P6R, or equivalent
4. Sample Probe, P/N SC-5037-3995-150, or equivalent
5. Carrier solution probe, P/N SC-5037-3501, or equivalent
6. Tubing for carrier solution, P/N SC-0316-0500-30, or equivalent
7. Tubing for nebulizer, P/N SC-0317-0250-30, or equivalent
8. Tubing for rinse station, P/N SC-0302-0500, or equivalent
9. Bottle for rinse solution, P/N SC-0305-1, or equivalent
10. Rinse line, P/N SC-0302-1001, or equivalent
11. Large rack 60 positions, P/N LR-60-16, or equivalent
12. Standard rack 10 positions, P/N ST 10-28, or equivalent
13. 2 mL sample loop, P/N SC-0315-20, or equivalent
14. Vacuum line for SC-FAST, P/N SC-0321-32-20, or equivalent
15. Waste line for SC-FAST, P/N SC-0323-0002-215, or equivalent

b. Equipment

1. Water purification system for providing ultrapure water with a resistivity $\geq 18 \text{ M}\Omega\cdot\text{cm}$, Aqua Solutions, or equivalent
2. High-precision analytical balance capable of accurately weighing milligram amounts of

- material to the tenth of a milligram or better, Sartorius™ Cubis™ MSA Precision balance, Item # MSA1203S100, Fisher Scientific, or equivalent
3. Analytical balance for routine weighing of material to the nearest hundredth of a gram and with a loading capacity of at least 200 g, Sartorius™ Cubis™ MSA Analytical balance, Item # MSA324S100DI, Fisher Scientific, or equivalent
 4. Gilson 402™ Programmable Diluter-Dispenser (or equivalent) equipped with 10-mL dispensing syringe and a 500 µL sampling syringe, Gilson Inc, or equivalent
 5. Calibrated hand-held adjustable pipetters that cover the range of accurate liquid delivery from 50 µL to 5000 µL. Eppendorf™ Xplorer™ electronic programmable pipetters, Fisher Scientific, Item # 14-286-552, 14-286-553, 14-286-554, and 14-286-555, or equivalent
 6. Honeywell Xenon 1902 USB 2D barcode reader, Computech International, Item # 1902HHD-0USB-5F, or equivalent
 7. Eppendorf™ Repeater™ pipetting device(s) capable of accurately dispensing multiple microliter aliquots of liquid, Fisher Scientific, or equivalent
 8. Vortex mixer, VWR, Item # 945303, or equivalent
 9. Gas regulator for helium, Matheson Tri-Gas Products, or equivalent
 10. Gas regulator for argon gas, Matheson Tri-Gas Products, or equivalent
 11. BRADY IP-600 or BP-PR 600 Plus barcode printer, Brady, or equivalent

c. Computer Software

1. Qtegra™ Intelligent Scientific Data Solution™ (ISDS) instrument control and data handling software, Thermo Scientific®
2. pdfFactory Pro 5.0 or later version, FinePrint Software, LLC, www.fineprint.com or equivalent. This product is used for creating electronic Portable Document Files (PDF) directly from Microsoft® Windows print dialog box
3. Teklynx CodeSoft 2014 barcode label software, Brady, Item # CS14ENTF, or equivalent
4. Microsoft Office (MS Word and MS Excel)

d. Supplies

1. 2-200 µL Eppendorf pipette tips, 960 tips per case, Fisher Scientific, Item # 05-403-66, or equivalent
2. 20-300 µL Eppendorf pipette tips, 960 tips per case, Fisher Scientific, Item # 05-403-67, or equivalent
3. 50-1000 µL Eppendorf pipette tips, 960 tips per case, Fisher Scientific, Item # 05-403-68, or equivalent
4. 5 mL Eppendorf pipette tips, 500 tips per case, Fisher Scientific, Item # 05-403-117, or equivalent
5. Combitips Plus 5 mL, Fisher Scientific, Item # 21-381-330, or equivalent
6. Kay-Dry™ paper towels and Kim-Wipe™ tissues, Kimberly-Clark Corp., Item # 06-666-11, or equivalent
7. Cotton swabs (Hardwood Products Co. ME), or equivalent

8. Nitrile, powder-free examination gloves, VWR, Item # 32934, or equivalent
9. Biohazard autoclave bags, Fisher Scientific, Item # 01826D, or equivalent
10. 15 mL conical polypropylene centrifuge tube, VWR, Item # 82050-276, or equivalent
11. 50 mL polypropylene centrifuge tubes, Fisher Scientific, Item # 14-432-22, or equivalent
12. Thermal transfer printable labels, Brady, Item # THT-126-499-3, or equivalent
13. Black 4300 series thermal transfer printer ribbon, Item # R4302, or equivalent

7. STANDARDS AND SAMPLE PREPARATION PROCEDURES

a. Preparation of Stock Solutions

ATTENTION!

Acid wash all containers. Rinse at least three times with 2% (v/v) HNO₃ and at least three times with approximately ≥ 18 M Ω ·cm water. Verify cleanliness of the containers by analyzing the rinsate (deionized water) at the end of the acid washing procedure.

- 1. Internal standard intermediate mixture (20 mg/L gallium and 20 mg/L scandium in 1% (v/v) HNO₃)**
Partially fill a 50 mL volumetric flask with 1% (v/v) HNO₃ and add 1 mL of the 1,000 μ g/mL Ga stock standard and 1 mL of the 1,000 μ g/mL Sc stock standard. Dilute to volume (50 mL) with 1% (v/v) HNO₃, and mix thoroughly. Prepare ahead of time or as needed. Store at room temperature, and label the container appropriately. The expiration date of the mixture is one year from the date of preparation.
- 2. 20% Intermediate Triton X-100® solution**
Add 200 mL of Triton X-100® to a one liter container that is partially filled with approximately ≥ 18 M Ω ·cm water. Dilute to volume (1 L) with deionized water, add a acid washed Teflon-coated stir bar to the bottle to stir the solution on a magnetic stir plate overnight. The overnight stirring is needed to completely dissolve the Triton X-100. Store at room temperature and label the container appropriately. The expiration date of the solution is one year from the date of preparation.
- 3. Sample diluent/carrier solution (20 μ g/L Ga, 20 μ g/L Sc, 0.01% APDC, 0.4% (v/v) TMAH, 1% ethanol, and 0.05% Triton X-100)**
Add 0.4 g of APDC, 16 mL of 25% (v/v) TMAH, 40 mL of ethanol, and 10 mL of 20% Triton X-100® solution to a 4 liter container partially filled with approximately 18 M Ω ·cm water. Add 4 mL of 20 mg/L of the internal standard intermediate mixture and dilute to volume (4 L) with approximately ≥ 18 M Ω ·cm water. Mix thoroughly. Prepare ahead of time or as needed. Store at room temperature and label the container appropriately. The expiration date of the solution is one year from the date of preparation.
- 4. Rinse solution (0.01% APDC, 0.4% (v/v) TMAH, 1% ethanol, and 0.05% Triton X-100)**
Add 0.4 g of APDC, 16 mL of 25% (v/v) TMAH, 40 mL of ethanol, and 10 mL of 20% Triton X-100® to a 4 liter container partially filled with approximately ≥ 18 M Ω ·cm water. Dilute to volume (4 L) with approximately ≥ 18 M Ω ·cm water. Mix thoroughly. Store at room temperature, and label the container appropriately. The expiration date of the solution is one year from the date of preparation.

b. Preparation of Matrix-Matched Material ("Base Blood")

Blood collected from anonymous donors is mixed together to provide a blood sample (base blood) that will give a rough approximation of the blood typically found in the United States population. Several bags of whole blood are purchase from Tennessee Blood Services or

another equivalent vendor. Each individual bag of blood is screened for concentrations of the analytes of interest. Once screened, the blood bags that contain blood with the lowest chromium and cobalt concentrations are pooled together in a larger acid washed polypropylene (PP), polymethylpentene (PMP), or Teflon™ container. The pooled blood is stirred for at least 30 minutes on a large stir plate. Make sure that the stir bar used has been acid washed prior to use. Dispense the pooled blood into individual 2 mL cryovials (approximately two milliliter aliquots) for long term storage.

c. Preparation of Quality Control Materials

In efforts to more accurately matrix match quality control materials to patient samples, human whole blood, as opposed to caprine or bovine blood, is used for the preparation of quality control materials. Bags of whole blood containing Ethylenediaminetetraacetic acid (EDTA) are purchased from Tennessee Blood Services, or equivalent vendor. Each individual bag of blood is screened for concentrations of Cr and Co. Once screened, the bags are assigned to the “low”, “high”, “elevated 1”, or “elevated 2” pool based on the target concentration values desired for Cr and Co in each pool. Each bag contains approximately 500 mL of blood, but it is impossible to obtain exactly 500 mL of blood from each bag. Once it is determined which bags will be used for each pool, the blood used from the bags is combined and measured using volumetric flasks so that the final volume of the pooled blood can be determined. The aliquots from the volumetric flasks are transferred into a larger acid washed container (acid washed PP, PMP, or Teflon™) until the desired final volume of pooled blood is obtained. This process is completed for each of the four pools. A clean large stir bar is added to each container, and the pooled blood is mixed on a stir plate for at least 30 minutes.

Before spiking each pool, analyze an aliquot from each unspiked blood pool to obtain the background levels of chromium and cobalt. Use these results to determine the amount of each analyte that needs to be spiked into each pool on top of the concentrations of each analyte that already exist in the pooled blood. Once the spike volumes have been determined, use single element stock standards (preferably NIST traceable standards) to spike each pool to bring it to the desired concentration. After spiking, stir the pools overnight. Keep the blood refrigerated whenever possible. Remove an aliquot from the pool before dispensing and analyze it for chromium and cobalt to determine if the values in the pool are actually close to the target concentration. If problems are identified with the pools, address and correct all problems before aliquotting the pooled blood into separate 2 mL cryovials.

Dispensing can be accomplished most easily using a Digiflex automatic pipetter in continuous cycling dispense mode. Dispense the pools in a clean room environment to reduce the possibility of introducing contamination to the pool. Replace the tubing attached to the dispensing syringe (left when looking at the front of Digiflex) with a length of clean Teflon™ tubing long enough to reach into the bottom of the container while it is sitting on the stir plate. Check the cleanliness of the Digiflex before use by analyzing 1-2% (v/v) HNO₃ which has been flushed through the Digiflex. With a large stir plate, continue stirring the blood pool to be dispensed. While the pool is stirring, recirculate the spiked blood pool through the Digiflex using the “Prime” function. Be sure to secure the tubing in the container using Parafilm, or equivalent product, so that both ends of the tubes will remain in the spiked blood during the priming process. After the blood has been priming for at least 20 minutes, stop the Digiflex. Remove the dispensing end of the tubing from the blood. Dispense approximately 1.8 mL of the pool into a 2 mL cryovial. Test this aliquot for chromium and cobalt to make sure that the spiked values have remained the same. If the values are close to target, the aliquotting can begin.

While maintaining constant stirring of each pool, aliquot approximately 1.5 mL to 1.8 mL of blood into a sufficient number of pre-labeled, serialized 2 mL cryovials to provide QC material for 1000 or more runs. Store the aliquots of the QC material at approximately -70°C or a lower

temperature. The expiration date of the material is set at 5 years from the date made. If results from future stability studies prove that the shelf life is lower, this information will be updated.

d. Preparation of Calibration Standards

For this analytical method, two calibrations are used. The normal calibration curve is the standard curve used. It covers samples with analyte concentrations up to 15 µg/L. The majority of the samples analyzed will most likely fall within this range. The elevated calibration curve is used to cover samples with analyte concentrations above 15 µg/L but below 100 µg/L. The instructions for making both will be covered in this section.

CAUTION!

Chromium and cobalt at high levels are treated as toxic and carcinogenic substances! Take extra care to avoid accidental ingestion or inhalation of these materials. **Wear appropriate personal protective equipment (PPE).** Clean up any spills that occur according to applicable hazardous material spill procedures.

***Note:** All preparations will be performed gravimetrically (wt/wt) unless otherwise noted. All gravimetric measurements assume the density of the substance weighed is equal to the density of water (1g/cm³).*

Definitions:

- **Stock Standard:**
These are the commercially available stock solutions with concentrations of approximately 1,000 or 10,000 mg/L.
- **Intermediate Standard:**
This is a 10,000 µg/L solution prepared from the dilution of the commercially available stock standards.
- **Working Calibrator:**
This is a dilution of the intermediate standard prepared in base blood. A working calibrator is used in this method to build a calibration curve.

Use a high precision analytical balance capable of accurately weighing material to the tenth of a milligram or better. To the fullest extent possible, it is important to use the balance in an environment that is vibration-free, free of air drafts, and away from direct sun light. Use a balance safety hood, if available.

(1) Preparation of Intermediate Calibration Standards

Gravimetrically prepare the intermediate calibration standards (one for each analyte) in 2% HNO₃ /1% HCl:

1. Using the volumes listed in **TABLE 7-1** pipette the appropriate volume of the chromium stock standard into a labeled 50 mL PP centrifuge tube.
2. Record the weight of each volume pipetted. Calculate the expected concentration in µg/L of each solution. Record this information in the laboratory notebook.

3. Mix the solution thoroughly.
4. Repeat this process for cobalt.
5. Store the centrifuge tubes at room temperature. The expiration date is one year from the date of preparation.

TABLE 7-1: PREPARATION OF INTERMEDIATE CALIBRATION STANDARDS

Stock standard	2% HNO ₃ / 1% HCl mL	Stock standard volume mL	Target Concentration µg/L
Cr	49.95	0.050	~1000
Co	49.95	0.050	~1000

(2) Preparation of Intermediate “Mixed elements” Calibration Solutions

Gravimetrically prepare the intermediate mixed elements calibration solutions.

1. Using the volumes listed in **TABLE 7-2** pipette the appropriate volume of each intermediate calibration standard into each of the 50 mL PP centrifuge tubes (8 tubes for eight calibration standards).
2. Record the weight of each volume pipetted. Calculate the expected concentration in µg/L of each solution. Record this information in the laboratory notebook.
3. Mix each solution thoroughly.
4. Store at room temperature and label appropriately. The expiration date is one year from the date of preparation.

TABLE 7-2: PREPARATION OF INTERMEDIATE “MIXED ELEMENTS” CALIBRATION SOLUTIONS

Intermediate Mixed Elements Calibrator Level	Used for Working Calibrator	Volume			Target Cr and Co Concentration µg/L
		Water (mL)	Cr (µL)	Co (µL)	
S1	STD001	24.167	833*		0.50
S2	STD002	22.50	2500*		1.5
S3	STD003	24.75	125	125	5.0
S4	STD004	24.625	187.5	187.5	7.5
S5	STD005 and ESTD001	24.25	375	375	15.0
S6	ESTD002	22.75	1125	1125	45.0
S7	ESTD003	21.25	1875	1875	75.0
S8	ESTD004	20	2500	2500	100.0

*Use the S5 calibrator to make S1 and S2.

(3) Working Calibrators

At any given time, it is necessary to have at least three sets of working calibrators on hand which are made from three independent preparations of stock standards. The original materials must be from different lots, preferably from different vendors. Alternate between different sets of working calibrators from analytical run to analytical run. Prepare the working calibrators as outlined in the next section.

- e. Preparation of Working Calibrators, Patient Specimens, and QC Materials for Analysis

CAUTION!

Work with open vials or tubes containing biological samples in a biological safety cabinet (BSC). Wear appropriate personal protective equipment (gloves, lab coat and safety glasses). Refer to SOP *DLS 3520 (Operation of Benchtop Automatic Pipettes with Hazardous Chemicals)* for additional safety precautions.

One batch ("run") is defined as the analysis of a contiguous set of samples (typically 30) bracketed by low and high bench QC material. Each bench QC level (low and high or elevated level 1 and elevated level 2) needs to be analyzed at the beginning and end of a batch run in separate tubes/vials. *Sharing of even a single prepared QC tube or vial for more than one analysis is disallowed.* This means that the autosampler cannot be programmed to analyze QC more than once out of the same tube. It is permissible to "piggyback" two runs in succession following a single calibration such as for an overnight analysis as long as each analytical batch run is bracketed by its own uniquely prepared bench QC material. Under a single calibration, the total analysis time cannot exceed 24 hours. For sample preparation, proceed with step 1 below.

1. Identify, gather, and thaw the necessary specimen vials containing the blood samples for the batch ("run") to be analyzed.
2. For each batch run, thaw one tube each of low and high bench QC materials "LB-yyxxx" and "HB-yyxxx" (for explanation of nomenclature, see the *Quality Control Materials* section).
3. Label 15 mL PP conical centrifuge tubes as follows: "AQBLK", "S0", "S1", "S2", "S3", "S4", "S5", and "BldBlk" for the regular calibration curve. In addition, label tubes for the QC samples (two LB and two HB). If the elevated calibration curve is required then label tubes as follow: "AQBLK", "S0", "S5", "S6", "S7", "S8", "BldBlk" and the tubes for the two QC samples (EB1 and EB2). Arrange these tubes in order and place in a test tube rack. For patient samples, use a barcode printer program to print labels for the centrifuge tubes. The individual sample IDs can be scanned into the Excel template used for the Codesoft barcode label program or taken directly from an Excel file containing the sample IDs taken from the laboratory information management system. Do not hand enter sample IDs.
4. Inspect a Gilson 402™ Dilutor-Dispenser to ensure that it is equipped with a clean 10 mL dispensing syringe and a clean 500 µL sample syringe. If possible, dedicate a dilutor-dispenser to this method to reduce the likelihood of it getting contaminated from other processes. Examine the PTFE piston nose on the syringe piston for precipitates and/or debris, and if either is present, replace the syringe with a new one. Dedicate a separate set of syringes to calibrator preparation.
5. Attach a bottle of the diluent solution to the dilutor-dispenser diluent reservoir line. To minimize evaporation and decrease the possibility of contamination, use a capped bottle with a small hole in the cap that is slightly larger than the outer diameter of the reservoir line. Insert the reservoir line through the hole in the cap. Make sure that the end of the reservoir line is completely submersed in the diluent. It is important that the end of the line remain submersed throughout sample preparation in order to prevent air bubbles.
6. Place a waste beaker in position to collect the effluent liquid from the tip of the

sample/dispense tubing. Prime the liquid path by pressing the “Prime” button on the Gilson. Allow the syringes to cycle at least three or four times before stopping the prime function by pressing the “Start/Stop” button.

7. If not done at a previous date, create two sample preparation methods on the Gilson 402™ Dilutor-Dispenser that will be used during the preparation of all samples. Once programmed, the first method (A) will complete the following step in a sequential user controlled fashion:

- a) Uptake 10 µL of air into the sample uptake line.
- b) Uptake 250 µL of deionized water into the sample uptake line.
- c) Dispense 250 µL of deionized water and 2250 µL of diluent into the centrifuge tube.

Repeat steps (a) – (c) until the program is manually stopped.

Name and save this method in the keypad so that it can be recalled when needed. This method only needs to be programmed once.

Once programmed, the second method (B) will accomplish the following:

- a) Uptake 10 µL of air into the sample uptake line.
- b) Uptake 250 µL of sample (blood or intermediate mixed calibration solution) into the sample draw-line.
- c) Dispense 250 µL of sample and 2250 µL of diluent into the centrifuge tube.
- d) Dispense 10000 µL of diluent (for flushing to prevent contamination).

Repeat steps (a) – (d) until the program is manually stopped

Name and save this method in the keypad so that it can be recalled when needed. This method only needs to be programmed once. Both methods must be set up such that no step shall execute until the user has pressed the black button on the black dispenser control device. *For detailed instructions on programming, please consult the Gilson 402™ Dilutor Dispenser User's Guide.*

8. Recall the sample preparation methods created in step 7. Using method A, follow the steps (including the 10 µL air uptake) to uptake 250 µL of deionized water into the probe and uptake 2250 µL of diluent from the reservoir. Dispense both into the “AQBLK” centrifuge tube. Repeat this for the “S0”, “BldBlk”, and unknown samples centrifuge tubes.
9. Vortex the base blood. Using method B, uptake 250 µL of base blood into the probe and 2250 µL of diluent from the reservoir. Dispense both into the “S0” labeled centrifuge tube. Repeat this for the “BldBlk” centrifuge tubes. The final volume in each tube will be 5 mL.
10. After each sample/diluent mix is transferred, wipe the dispensing tip with a clean Kim Wipe or rinse with deionized (DI) water to avoid sample to sample contamination.
11. For the calibrators, use method B twice. Vortex each calibrator intermediate and the base blood before use for approximately three to five seconds. During the first cycle uptake 250 µL of base blood into the probe and 2250 µL of diluent from the reservoir. Dispense this into the centrifuge tubes labeled “S1”, “S2”, “S3”, “S4”, and “S5”. During the second cycle uptake 250 µL of the respective intermediate mixed calibration solution into the probe and 2250 µL of diluent from the reservoir. Dispense this into the respective centrifuge tube. The final volume in each tube will be 5 mL.
12. For unknown samples, use method B once. Vortex the unknown blood sample for approximately three to five seconds. Uptake 250 µL of the unknown sample and 2250 µL of diluent from the reservoir. Dispense this into the barcode-labeled tube that corresponds to the sample being prepared.

13. After capping all 15 mL conical centrifuge tubes containing sample/diluent mix, vortex each tube for at least 3 to 5 seconds.
14. After sample preparation is complete, use bleach solution or an equivalent disinfecting agent to clean up the laboratory prep areas used as well as any spills.

8. INITIAL ICP-MS INSTRUMENT AND AUTOSAMPLER SOFTWARE SETUP

a. ICP-MS Instrument Setup

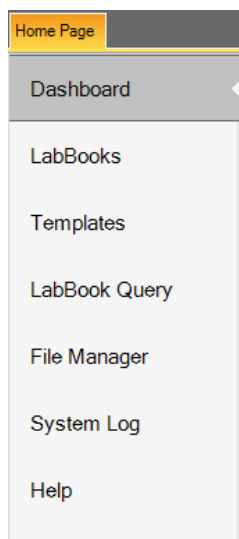
Programming the ICP-MS and autosampler software as outlined in this section will eliminate the need to go through lengthy programming before each analytical run. The templates created in this section can be modified as needed over time.

(1) Configuration

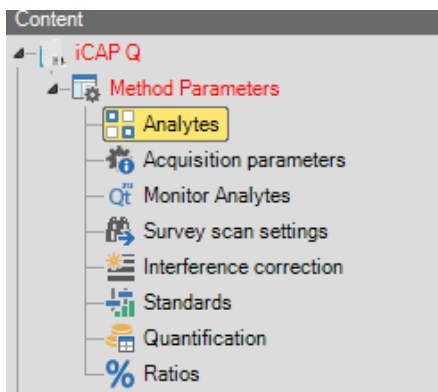
Upon installation of the ICP-MS, the “Configurator” is set up by the service engineer performing the installation. For more information on what is done during this process, refer to the Thermo Fisher Scientific ICAP Q™ Software Manual.

(2) Programming the iCAP Q™ method parameters in the Qtegra software

1. Launch Qtegra. The screen will default to the “Dashboard” and the menu below will appear on the left side of the screen.



2. Go through the procedure below to get started.
 - Click “Templates”.
 - Select the radio button for “Create a blank Template”.
 - For “NAME” fill in an appropriate template name.
 - For “Evaluation” select “eQuant” from the drop down box.
 - Click “Create Template”.
3. A new window opens with a cascading series of folders on the left side of the screen.



If all of the folders under **Method Parameters** are not shown, click on the black triangle to the left of **Method Parameters** to expand the folder. Items (a) through (h) will cover the parameters that need to (or can) be defined in the template that is being created.

(a) Analytes

Click on each analyte (Cr and Co) and each internal standard (Sc and Ga) to select it. Right click on Cr and check the box for the “53Cr” isotope to select it in addition to “52Cr”. “52Cr” is the most abundant isotope and is the one that is used for the quantification of analytical results for chromium. “53Cr” is monitored for informational purposes only.

(b) Acquisition parameters

Use the information in **TABLE 8-1** to input the acquisition parameters.

TABLE 8-1: ACQUISITION PARAMETERS

Identifier	Dwell time (s)	Channels	Spacing (u)	Measurement mode	Resolution
45Sc(KED)	0.1	1	0.1	KED	Normal
71Ga(KED)	0.1	1	0.1	KED	Normal
52Cr(KED)	0.2	1	0.1	KED	Normal
53Cr(KED)	0.2	1	0.1	KED	Normal
59Co(KED)	0.25	1	0.1	KED	Normal

Note: To enter the dwell time, the leading zero before the decimal point has to be entered. The “fill down” option can be used for the measurement mode column.

(c) Advanced parameters

These are located at the bottom of the page within the “Acquisition Parameters” section. Update the information in the software to match the following.

- (i) Number of sweeps = 20
- (ii) Measurement order = KED
- (iii) External Input Trigger = Digital IN 1
- (iv) Level Trigger = None; Edge Trigger = None
- (v) External Output Trigger = Digital OUT1
- (vi) Trigger step = Never

(d) Monitor analytes

Monitored Analytes		
	Uptake	Wash
Minimum Delay (s)	30	30
Maximum Delay (s)	300	300

(e) Survey scan settings

The parameters for the survey scan settings are listed in **TABLE 8-2**.

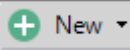
TABLE 8-2: SURVEY SCAN PARAMETERS

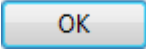

Start mass (u)	End mass (u)	Dwell Time (s)	Spacing (u)	Measurement Mode	Resolution
4.60	245.00	0.001	0.2	Normal	KED

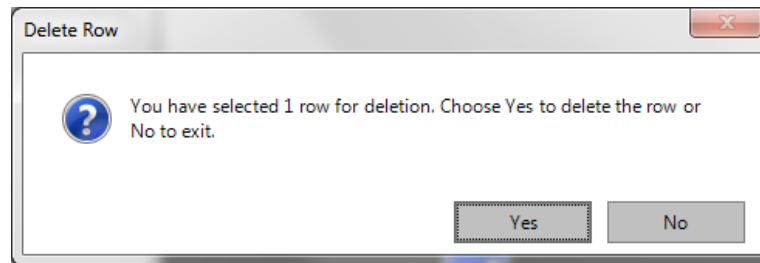
(f) Interference correction

The information in this section is not altered for this method.

(g) Standards

Click on the drop down box next to  and select "Elemental Standard". Fill in the "Standard Name" with "Standard 1" or similar designation. Filling in the "Standard Description" field is optional.

Check the box for "Create standard using analyte list" to bring all of the analytes over. Click . Click on the line for Sc. Click  to delete that line. The following window will pop up.




Click "Yes". Repeat this procedure for Ga.

The standard concentrations are listed in **TABLE 7-2**. For "Standard 1" change the concentration to 0.5 for both analytes. In the "Unit" field select "µg/l" from the drop box selections. Repeat the procedure used for the addition of Standard 1 for Standards 2 through 5.

An entry also has to be made for the internal standards. Click on the drop



down box next to  and select “Internal Standard (Isotopical)”. Fill in the “Standard Name” with “Internal Standards” or similar designation. Select Sc and Ga from the periodic table that appears. Change the concentration to 20 for each internal standard and select “µg/l” for the units.

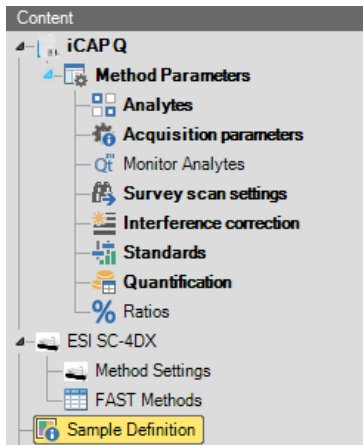
(h) Quantification

- (i) Click on Quantification. For the template being created, the quantification parameters are listed in **TABLE 8-3**.
- (ii) In the “Quantification” section internal standard (IS) recovery limits are set. Low and high warning limits have been set to 80% and 120% respectively while the low and high failure limits have been set to 75% and 125%, respectively.
- (iii) The “Use Quality Control” option can be used to monitor the QC material results for failures. The usage of this feature is optional, but if this box is checked, a new section (“Quality Control”) will appear. In the Quality Control section, ranges for QC can be filled in. Several options are available when the QC do not fall within the user specified limits including recalibrating the instrument or ending the analysis.

TABLE 8-3: QUANTIFICATION

Analyte	Measurement Mode	Quantify	Internal Standard	Fit Type	Weighting	Forcing	Use for SemiQuant
45Sc	KED	No	Use as internal standard	Linear	Absolute SD	No	Yes
71Ga	KED	No	Use as Internal standard	Linear	Absolute SD	No	Yes
52Cr	KED	Yes	Use Interpolation	Linear	Absolute SD	No	Yes
53Cr	KED	Yes	Use Interpolation	Linear	Absolute SD	No	Yes
59Co	KED	Yes	Use Interpolation	Linear	Absolute SD	No	Yes

4. After the **Method Parameters** are set up, update the **Sample Definition** section.



Click on **Sample Definition**. Three sections will appear – Initial Actions, Continuing Actions, and End Actions.

(i) Initial Actions

This section includes all of the samples that are analyzed at the beginning of every analytical run.

- 10 aqueous blanks
- 10 blood banks
- aq Blk
- 3030 AQBLK
- bb Blk
- 3030 STD000
- 3030 STD001
- 3030 STD002
- 3030 STD003
- 3030 STD004
- 3030 STD005
- 30303 BldBlkChk
- AQ Blk
- LB-14112 (or current low bench QC)
- HB-14114 (or current high bench QC)
- blk

(ii) Continuing Actions

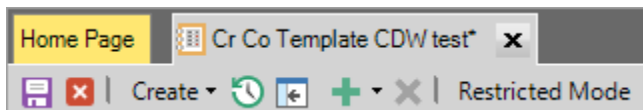
This is the section where the unknown sample IDs will be entered after the LabBook for the day is created. The number of survey runs, the number of main runs, sample type, and internal standard selection can be entered in the template.

(iii) End Actions

This section includes the samples that are analyzed at the end of each analytical run.

- AQ Blk
- LB-yyxxx (current low bench QC)
- HB-yyxxx (current high bench QC)
- Blk

Start with the “Initial Actions” section and click the green “+” sign to add rows.



Fill in the information listed below:

- *Label* = sample ID (or QC/standard/blank name) Fill in the names for the stability samples, standards and blanks as listed in 8.4.(i).
- *Survey Runs* = 1
- *Main Runs* = 3. This is the number of replicates.
- *Comment* = This is optional.
- *Sample Type* = "STD" for standards 1 through 5 and "UNKNOWN" for everything else including the calibration blank
- *Internal Standard* = select "internal Standard" to indicate that an internal standard will be used
- *Standard* = Choose the level from the drop down box for Standards 1 through 5.
- *Dilution Factor* = 1
- *Amount* = leave blank
- *Final Quantity* = leave blank
- *Total Dilution Factor* = 1
- *Rack Number* = the rack number on the ESI autosampler
- *Vial Number* = the position on the ESI autosampler tray

With the "Continuing Actions" section, fill in the fields as noted above. The *Rack Number* and *Vial Number* can be left at 1. These locations will be updated when the sample IDs are entered before each analysis.

With the "Ending Actions" section, fill in the fields for the four samples listed in section 8.4.(iii) in the same manner as the information is filled in for the "Initial Actions" samples. The *Sample Type* is "UNKNOWN" for all of the entries in this section.

5. The ICP-MS portion of the template is now complete with the exception of one update to the template that will have to be made in terms of the ESI autosampler. The instructions for that update are in the next section (b).
6. A separate template will need to be made for the elevated calibration curve. This can be done in either one or two ways. Steps one through 4 above can be used to create a new template from scratch or the existing template can be copied and modified to reflect the new calibration standards (standards 5 through 8).

b. ESI SC-4DX or ESI SC-4Q FAST autosampler setup

1. The "Instrument Control" is not launched with the launching of the Qtegra software. There is a shortcut for it on the desktop, or it can be accessed from the Windows Start menu ("Start" -> "All programs" -> "Thermo Qtegra" -> "Instrument Control"). Launch "Instrument Control". Click on the name of the autosampler ("ESI SC-4DX" or "ESI SC-4Q"). At the bottom of the screen, there are three tabs – Autosampler, FAST Method, and Valves & Pumps.
2. Click "Settings". Three sections will appear: Racks, Rinse, and Motors. Fill in the

information listed in **TABLE 8-4** for Racks. These values are subject to change if the conditions have to be re-optimized.

3. In the “Motors” section, set the parameters to the values listed below:

(a) Horizontal

- (i) Start Speed = 3
- (ii) Max Speed = 4
- (iii) Accel / Decel = 4

(b) Rotational

- (i) Start Speed = 2
- (ii) Max Speed = 3
- (iii) Accel / Decel = 3
- (iv) Enable RAF = 0

(c) Vertical

- (i) Start Speed = 3
- (ii) Max Speed = 3

TABLE 8-4: ESI SC-4DX AND ESI SC-4Q FAST RACK SETTINGS

	ESI-SC-4DX	ESI-SC-4'Q
RACKS		
Tray		
Layout	2X2	2X2
Rack		
Region	Rack1*	Rack1*
Type	LR60	LR60
Probe Depth [mm]	140	134
Autotune		
Rack	Rack1	Rack1
Vial	10	10

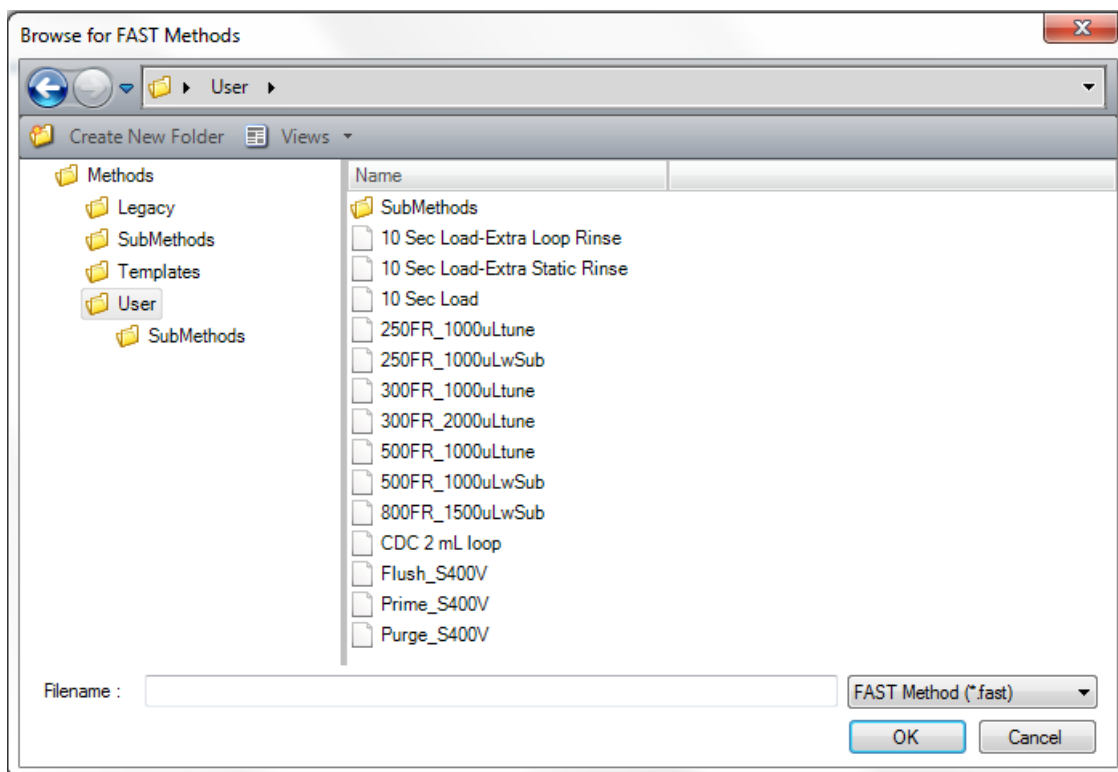
*Racks 2, 3, and 4 have the same information as Rack1. For Rack0, the only option is “Standard10_Q” for the ESI SC-4Q. Choose “Standard10” for the ESI SC-4DX.

- (iii) Accel / Decel = 3

4. Click on “FAST Method” and click the green “+” symbol. Three options will appear – “Open a user Method”, “New user method from a SC FAST template”, and “New blank method”. Choose the top option.



Click on the box next to the drop down box. The following window appears.



Double click on “User” to select a method such as “CDC 2 mL Loop”. Click on the floppy disk to “Save as” a new method name. Change the parameters to the parameters listed in **TABLE 8-5** for the SC-4DX autosampler or **TABLE 8-6** for the SC-4Q autosampler. Make sure to save the method after making any changes.

TABLE 8-5: ESI SC-4DX FAST METHOD PARAMETERS

Events	Actions
On Probe Down	FAST Vacuum 1 - On
On Probe Down	FAST Valve 1 Load
Probe in Sample at 4.0s	FAST Valve 1 Inject
Probe in Sample at 3.0s	Move Rinse: R1=2s & R2=2s
Rinse Completed	Probe Up
On Rinse	FAST Valve 1 Load
On Rinse at 0.5s	Probe Down
On Rinse at 10.0s	FAST Valve 1 Inject
On Rinse at 11.0s	FAST Valve 1 Load
On Rinse at 12.0s	Move To Next

TABLE 8-6: ESI SC-4Q FAST METHOD PARAMETERS

Events	Actions
On Probe Down	FAST Vacuum 1 - On
On Probe Down	FAST Valve 1 Load
Probe in Sample at 4s	FAST Valve 1 Inject
Probe in Sample at 3s	Move Rinse: R1=2s & R2=2s
Rinse Completed	Probe Up
On Rinse	FAST Valve 1 Load
On Rinse at 0.5s	Move Rinse: R1=0s & R2=2s
On Rinse at 12.0s	FAST Valve 1 Inject
On Rinse at 13.0s	FAST Valve 1 Load
Rinse completed	Move To Next

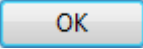
- If needed, you can manually toggle the autosampler valve between load and inject or turn the autosampler pump on and off and pumps on and off. This is done on the “Valves & Pumps tab at the bottom of the “ESI SC-4DX” tab.
- Return to the “LabBooks” section of Qtegra of the template you created and click on “FAST Methods”. The ESI SC-4DX or ESI SC-4Q FAST method created above can be uploaded by clicking the option next to the drop down box under “open a user method”. Double click on the user folder and select the FAST method that was created and saved in “Instrument Control”. Click the purple disk and  to save the LabBook template.
- Click on “Method Settings” and under settings select “Analysis”. Fill in the information listed in **TABLE 8-7** for Sample, Rinse 1, and Rinse 2.
- All of the software programming is now complete.

TABLE 8-7: ESI SC-4DX AND ESI SC-4Q FAST METHOD SETTINGS

	ESI SC-4DX	ESI SC-4Q
SAMPLE		
Uptake Time (s)	15	15
Wash Time (s)	4	4
RINSE 1		
Wash time [s]	2	2
Max Pump On [s]	unchecked, 1	unchecked, 1
Max Pump Off [s]	unchecked, 0	unchecked, 0
Additional Flush [s]	2	10
RINSE 2		
Wash time [s]	2	2
Max Pump On [s]	checked, 1	checked, 1
Max Pump Off [s]	unchecked, 1	unchecked, 1
Additional Flush [s]	2	10

9. INSTRUMENT START-UP PROCEDURES AND SAMPLE ANALYSIS

a. Start-Up Actions

1. Perform the necessary daily checks. Record any maintenance procedures in the instrument logbook.
 - (a) waste container – Empty if necessary. Waste can only be poured in the sink if the pH is in the range of $2 > \text{pH} < 12.5$. Although, the pH of this waste stream is typically 8.5 or 9, check the pH before discarding the waste.
 - (b) carrier and rinse solutions – Check to see if there is a sufficient amount of solution in each and that both are properly connected.
 - (c) helium supply- Check to see if the cylinder needs changing.
2. While the manufacturer's recommendation may be to change the peristaltic pump tubing when flat spots begin to develop, our laboratory practice is to change the peristaltic pump tubing each day that the instrument is utilized. Make sure that the tubing is clamped to the peristaltic pump before igniting the instrument.
3. Once these preliminary checks have been taken care of, launch the "Instrument Control" program. In the top left corner of the instrument software, click the "ON" tab to ignite the plasma. In the bottom of the open window, the ignition sequence will be displayed. Ignition will occur suddenly with a single audible "pop" and white light from the plasma will emanate from the injector. The light may flicker initially, but it will establish a steady intensity after 5 to 10 seconds unless a problem exists with the system. The external peristaltic pump will automatically start.
4. On occasion, the plasma may extinguish a few seconds after ignition. Promptly reignite by clicking "ON". Usually, the plasma will remain ignited after the second try. If not, investigate the cause of the plasma instability (refer to the *iCAP Q Operating Manual*).
5. After successful plasma ignition, record the base vacuum pressure in the instrument logbook. Place the carrier probe into the diluent or a container of deionized water. Monitor the tubing that drains the spray chamber for approximately 30 seconds. If you see liquid filling the spray chamber without being drained immediately remove the carrier probe from the diluent or water. Determine whether or not the peristaltic pump is rotating in the proper direction. If not, reverse the direction of the rotation of the peristaltic pump. Ensure that the peristaltic pump tubing is installed correctly. Put the probe back into the liquid. If the spray chamber is still not draining, adjust the tension on the tubing via the screws on the peristaltic pump clamps until the flow is continuous and smooth and moving in the right direction.
6. Click on the "Experiment Configuration" tab and click on the name for the ESI autosampler. The "Autosampler" tab is the default selection. If either the instrument or autosampler has been turned off, click on "Tools" and select "Initialize" to initialize the autosampler. If the autosampler loses the calibration, select "Calibrate" and follow the steps to calibrate the probe.
7. In the "iCAP Q" tab, select standard "STD" mode. Let the instrument warm up for approximately 15 minutes. Scroll through the tabs on the "Control Panel" (bottom left corner) to make sure all graphical indicators of instrument readiness are green. If not, take the appropriate actions described in the Operating Manual.
8. On the left side of the "Instrument Control" software, click on "Control Panel. The main iCAP Q parameters can be seen which are similar to the parameters listed in **TABLE 9-1**. Please note that these parameters are subject to change with each autotune or instrument optimization. They are provided to be used as a reference.

TABLE 9-1: ICAP Q TYPICAL PARAMETERS

Tune Settings STD mode

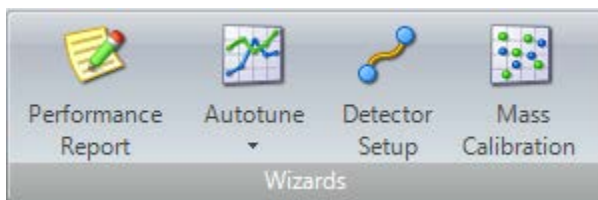
CCT Entry Lens (V)	-71.98
Angular Deflection (V)	-376.99
Deflection Entry Lens (V)	-35.01
Spray Chamber Temperature (°C)	2.70
Peristaltic Pump Speed (rpm)	32.00
Cool Flow (L/min)	14.00
Sampling Depth (mm)	5.00
Plasma Power (W)	1550.00
Auxiliary Flow (L/min)	0.80
Nebulizer Flow (L/min)	0.95
Torch Horizontal Position (mm)	0.05
Torch Vertical Position (mm)	-0.70
Extraction Lens 2 (V)	-191.33
CCT Focus Lens (V)	0.24
D1 Lens (V)	-200.05
D2 Lens (V)	-90.01
CCT Bias (V)	-2.01
CCT Exit Lens (V)	-160.01
Focus Lens (V)	20.20
Quad Entry Lens (V)	-33.60
Pole Bias (V)	-1.00
Virtual CCT Mass to Dac Factor	130.00
Virtual CCT Mass to Dac Offset	20.00
Virtual CCT Mass parameter b	0.65
Virtual CCT Mass Maximum Dac Limit	4095.00

Tune Settings KED mode

CCT Bias (V)	-21.00
CCT Exit Lens (V)	-40.00
Focus Lens (V)	-8.00
Quad Entry Lens (V)	-58.00
Pole Bias (V)	-18.00
CCT1 Flow (mL/min)	5.00
CCT1 Shut-Off Valve	1.00
Virtual CCT Mass to Dac Factor	60.00
Virtual CCT Mass to Dac Offset	140.00
Virtual CCT Mass parameter b	1.00
Virtual CCT Mass Maximum Dac Limit	4095.00

b. Performance Check

1. After ICP-MS warm up, place the carrier probe in the daily performance check solution (THERMO-4AREV). Click on the "Performance Report" part of the "Wizards" tab.



Select the radio button next to “Run an existing Performance report” and click

Next >

Double-click on the method “STD_5MIN” (or similar name). Click

Next >

Next >

2. If the daily performance check report passes, check the box for “Open Report”. Click “Finish”. Print a copy of the report for the instrument logbook and record the information on the daily maintenance checklist page. Click “Export” followed by “OK”. Create the folder for the current date. Save the standard mode daily performance check PDF results in the folder that was just created. The software automatically saves the raw data for the daily performance check in the following folder:

C:\ProgramData\Thermo\Qtegra_ApplicationData\Workspace\Reports.

3. If the report fails, perform the following troubleshooting steps:

- Turn the instrument off and check the ICP-MS consumables (cones, lens, etc.) if they were not checked at the beginning of the day. Replace any components that need to be replaced. Notate any maintenance procedures performed in the instrument logbook. If components were changed, re-ignite the plasma, wait for the instrument to stabilize and reanalyze the standard mode daily performance check.

- If there were no issues with the consumables, after plasma re-ignition and instrument stabilization, perform an autotune using the daily performance check solution. Click “Autotune” on the “Wizards” tab and select “Autotune Wizard”. Under “Run an existing Autotune sequence”, select the method named “SourceTune High Matrix”. Press

Next >

to start the analysis. If the autotune is successful, select

Save

To view the report after completion, click on “Autotune” in “Views”. Analyze a daily performance check in STD mode again. If it passes, proceed to KED mode.

- If the daily performance check still does not pass specifications, place the probe in the higher concentration tuning solution (THERMO-5A). Allow the solution to aspirate and reach the detector. Perform a mass calibration by clicking on the “Mass Calibration” part of the “Wizards” tab. Check the box next to “Execute a Coarse Mass Calibration”. Step through the prompts to complete the mass calibration. If the coarse calibration does not pass, perform a full mass calibration by clicking on “Mass Calibration” but leaving the box next to “Execute a Coarse Mass Calibration” unchecked. If a successful mass calibration is obtained, select “Save”. Aspirate the daily performance check solution again, and perform an Autotune followed by a standard mode daily performance check. If they both pass, proceed to KED mode.
- If either the autotune or standard daily performance check fails, switch back to the higher concentration tuning solution and perform a detector cross calibration, click on “Detector Setup” in “the Wizards” tab then select method cross calibration. If the detector cross calibration is not successful, follow the iCAP Q troubleshooting procedures listed in the iCAP Q Operating Manual then contact technical support. If it is successful, perform another mass calibration, autotune, and a daily performance check using the appropriate

solutions for each. Make sure that the correct solutions are aspirated for each one. If a passing daily performance check is obtained, fill in the *Daily Maintenance Checklist* in the instrument logbook according to the completed optimization procedures. If not, contact technical support.

4. Once the daily performance check passes in STD mode, switch to KED mode by selecting "KED" from the drop down list on the "iCAP Q" tab. Let the instrument stabilize for approximately five minutes. Perform a daily performance check in KED mode by following the same steps used for the standard mode daily performance check. The only difference is that the method "KED_5min" (or equivalent) is chosen instead of "STD_5MIN" (or equivalent).
5. If the KED mode daily performance report passes, record the counts per second for cobalt on the daily maintenance checklist of the instrument logbook. Save the PDF of the KED daily performance report in the same folder that the standard mode report was saved in. The KED performance report does not have to be printed.
6. If the report "FAILS" switch back to STD mode. Allow the instrument to stabilize in STD mode for approximately five minutes. Perform an autotune and a new standard mode daily performance. Switch back to KED mode, allow it to stabilize, and perform another KED mode performance check. If it still fails, contact the manufacturer's technical support.
7. To look up, save, or print previous daily performance reports select "Performance Report" on the "Views" window.

c. Starting the Run

Bench QC, reference materials and calibration verification:

Bench "QC": Analysis of bench QC permits assessment of methodological imprecision, determination of whether the analytical system is 'in control' during the run, and assessment of time-associated trends. Before QC materials can be used in the QC process, they must be characterized by at least twenty (20) analytical runs to determine appropriate QC parameters.

Bench QC pool analyte concentrations in this method span the analyte concentration range of the calibrators including "low-normal" ('Low QC') and "high-normal" ('High QC') concentrations.

In each analytical run the analyst will test each of the two bench QC samples two times, subjecting them to the complete analytical process. Bench QC pool samples are analyzed first in the run after the calibration standards but before any patient samples are analyzed. This permits making judgments on calibration linearity and blank levels prior to analysis of patient samples. The second analysis of the bench QC pools is done after analysis of all patient samples in the run (typically 20-30 patient samples total) to ensure analytical performance has not degraded across the time of the run. If more patient samples are analyzed on the same calibration curve after the second run of the bench QC, all bench QC must be reanalyzed before and after the additional samples.

Reference materials: Historical challenge samples from proficiency testing programs or commercially-produced reference materials may be useful when NIST SRMs are unavailable.

Calibration verification: The test system is calibrated as part of each analytical run with NIST-traceable calibration standards. These calibrators, along with the QCs and blanks, are used to verify that the test system is performing properly.

1. By clicking on the Dashboard section of Qtegra, a summary of the main iCAP Q parameters can be seen. All green squares indicate that everything is working properly. If some of the indicators are not green, click "Get Ready".

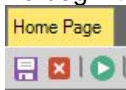


Follow the prompts to correct the issues. The mode will revert back to STD mode if the “Get Ready” button is clicked; therefore, place the instrument back in KED mode after a new standard mode daily performance check is successfully obtained.

2. Create a new LabBook from an existing template or a previous LabBook. Fill in the “NAME” with “CrCo_mmdyyy_short description of analysis” then select the radio button for “Create a new LabBook from an existing template” or “Create a new LabBook either from an existing LabBook”. If creating a LabBook from an existing template, select the appropriate template and enter the number of patient samples that are scheduled to be analyzed. Through the carryover study performed as a component of the method development process, it was decided that no more than 30 unknown samples will be in an analytical run (i.e. between a set of bracketing QC).

Note: If the decision is made to analyze more than 30 samples by using bracketing sets of QC, the template will not be affected. The additional QC and samples will have to be appended to the list).

3. With the LabBook that was just created, select “Sample List” to create/modify the sample list. If the template was used to create the LabBook, only the unknown sample IDs and autosampler locations will have to be entered. For unknown samples, either copy the IDs from an Excel file and paste them into the “Continuous Actions” section or use a barcode scanner to input the IDs. To add a sample right click on the row above the row where the sample(s) will be inserted. Select “Insert rows”. Choose from available names or select “blk UNKNOWN” under “Continuing Actions”. A box will appear where you will enter the number of rows that need to be added. To delete rows (samples) from a LabBook right click on the sample(s) and select “Delete selected rows”. If the LabBook was not created from a template, add the sample IDs for stability samples, blanks, calibrators, QC, and unknowns using the information in section 8.4 of this document.
4. The “Status” column shows whether or not the sample has been analyzed. Green indicates that the sample has been analyzed and gray indicates that the sample has not been analyzed. The “Rack Number” and “Vial Number” are filled in to show the location of the sample on the ESI autosampler. If a sample has been diluted beyond the 20x dilution used for preparation, enter the dilution factor into the “Dilution Factor” column. “Special Blank” indicates which “blank” will be subtracted from the sample or standard. In this method, “3030 STD000” (the base blood blank) is subtracted from standards 001 through 008, and “3030 AQBLK” (aqueous blank) is subtracted from all other samples (everything after the calibrator).
5. Keep the following in mind while filling out the “Sample list” table. No more than 30 samples are to be analyzed between bracketing QC. Samples must always be bracketed by two levels of QC (either low / high or elevated level I / elevated level II). Both QC are analyzed before the patient samples are analyzed, and again after patient samples have been analyzed. No more than 24 hours can elapse between the time that the actual analytical run starts (the analysis of the “3030 STD000”) and the analysis of the last vial.
6. To begin the analysis of the stability samples, click the play symbol (green and white)



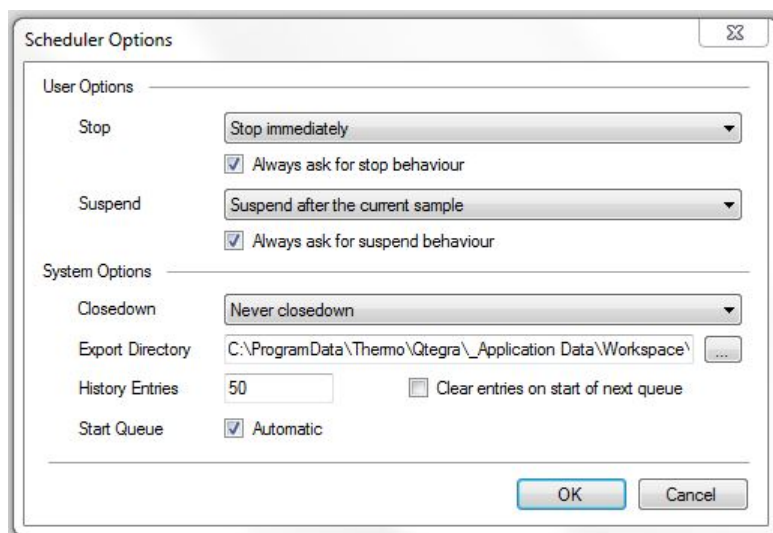
located in the upper left hand corner of the screen.

7. Monitor the operation of the autosampler to ensure that it is working properly (i.e. the

autosampler loop is filling properly, the sample is reaching the nebulizer at the correct time, the autosampler is going to the correct vial, etc.).

8. Right click on the first sample after the stability samples to choose the option to “Suspend before the measurement of the sample”. This will stop the LabBook from analyzing the remaining samples in the list (standards, QCs and samples) until prompted to do so. This will allow time to assess the results from the stability samples. If the concentrations of Cr and Co and internal standard recoveries in the stability sample base blood blanks are consistent, start the analysis of the remainder of the run by clicking “Play”.
9. Monitor the analysis in real-time as much as possible to ensure that everything is working. Confirm the following:
 - (a) Linear calibration curve is obtained. The correlation coefficient (R^2) should be a minimum of 0.98.
 - (b) The initial bench QC results are within the acceptable limits. Results for both analytes should be within $\pm 2SD$ of the characterized mean.
10. If an analyte result for the beginning QC material(s) falls outside of $\pm 2SD$ of the characterized mean, the following steps are recommended:
 - (a) Evaluate the blank results.
 - (b) Determine if the three replicates fail the rep delta rules (See APPENDIX B).
 - (c) Evaluate the consistency of the internal standard across the measurements (esp. the calibrators).
 - (d) Evaluate calibration curves. If a particular calibration standard is obviously in error, it can be re-analyzed as a sample (old or new dilution) and incorporated into the curve through data reprocessing as a calibrator. As a last resort, a single calibration point per analyte between or including STD002 and STD004 for the normal calibration curve or ESTD002 and ESTD003 for the elevated calibration curve can be removed from the curve. Do not drop STD000, STD001 or STD005 or ESTD001 or ESTD004). Follow up problems with calibration standards with appropriate corrective actions (e.g. re-preparation of intermediate working standards or troubleshooting instrument parameters).
 - (e) Prepare a fresh dilution of the failing QC material (same vial) and reanalyze it to see if the QC dilution was not properly made.
 - (f) Prepare a fresh dilution of the failing QC material (unused vial) and analyze it to see if the QC vial had become compromised.
 - (g) Prepare and analyze new working calibrators.
 - (h) Test a different preparation of intermediate working calibration standards.

If these steps do not result in correction of the out-of-control values for QC materials, consult the supervisor for other appropriate corrective actions.
11. At any time, to suspend or temporarily stop the run, click on the “Scheduler” tab in the bottom left corner. Select “Pause” and select the radio button for the appropriate to either pause “After current sample” or pause “After current experiment”. Click “OK” to resume analysis. Click “Play”.
12. To set up the instrument to automatically turn off after the samples are analyzed, select “Options” (the gear symbols) on the “Scheduler” tab. Select the appropriate stopping method.



d. Instrument Shut Down

1. In the “LabBook” in the “Sample list” section, browse through the list to see whether or not all of the samples that were selected to be analyzed have a green circle in the “status” column (which means all the samples were successfully analyzed). If not, determine why samples were skipped, correct the problem, and reanalyze the affected samples.
2. If the instrument is not set up to automatically shut down after sample analysis is complete, go to “Instrument Control” and click “OFF” to extinguish the plasma. The peristaltic pump turns off automatically whenever the plasma is extinguished. Release the tension on the peristaltic pump tubing by unclamping it.
3. Remove the calibrators, QC, and sample centrifuge tubes from the ESI SC-4DX or ESI SC-4Q FAST autosampler. Discard them according to CDC’s biohazard waste disposal guidelines.

e. Weekly Maintenance

To ensure that the instrumentation remains in proper working order, weekly maintenance of the iCAP Q ICP-MS and ESI autosampler is strongly encouraged. The maintenance regimen includes cleaning and/or replacement of key ICP-MS and autosampler consumables. All maintenance activities will be documented in the instrument logbook. Weekly, the analyst will examine the following:

1. **Capillary tubing:**

Examine all capillary tubing connected to the sample probe, carrier probe, nebulizer, autosampler waste, and rinse to check for any signs of damage (crimping, stretching, etc.). Replace if needed.

2. **Sample introduction components:**

Inspect the following sample introduction system components for wear, damage, and cleanliness. One practice used by our laboratory is having at least one set of clean spares on hand so that the items removed from the instrument can go through a more rigorous cleaning.

(a) Teflon torch holder – inspect; replace as needed

(b) Quartz torch – clean or replace

(c) Quartz injector – clean or replace



(e) Load coil – inspect for holes and damage

(f) PFA adapter elbow for spray chamber – inspect; replace as needed

(g) PFA-ST MicroFlow Nebulizer - clean or replace

3. Interface components:

Replace the platinum sampler cone, sampling cone gasket, platinum skimmer cone, and 3.5" skimmer cone insert. Make sure that the sampling extraction lens 2 is in good shape. Clean the components after they are removed from the instrument. Replace with a spare or new item.

Please note that after replacing the cones they need to be conditioned. To condition the cones, prepare approximately 1.5 L of 100x base blood in DI water in a 2 L Nalgene bottle and leave the sample probe in the bottle overnight with the instrument running.

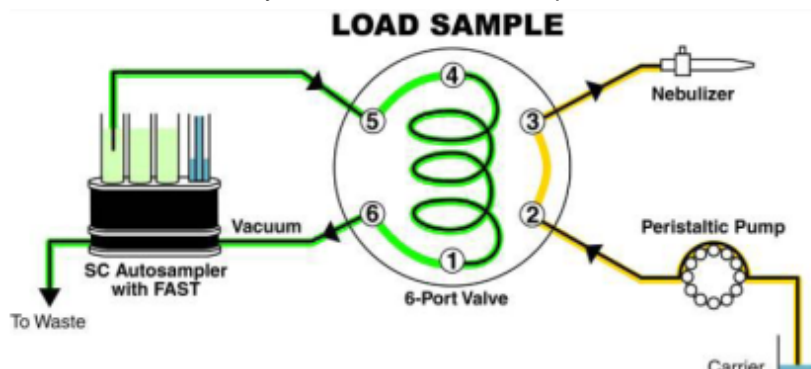


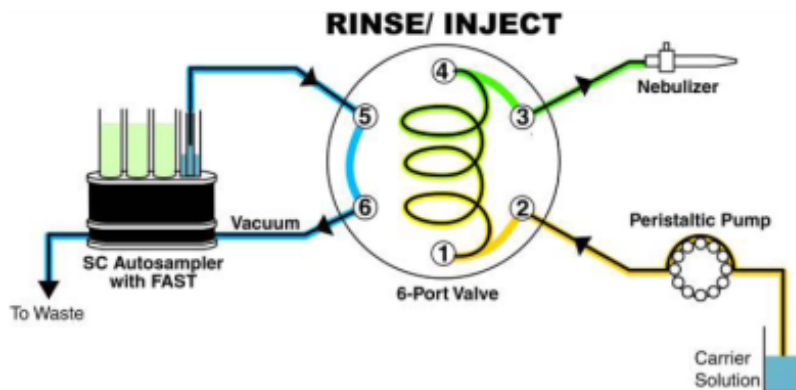
4. Autosampler system:

Examine the valve of the SC-4DX FAST or SC-4Q FAST for visible wear or leaks. Make sure all lines to the port of the valve are secure and intact. Periodically (at least once per month), the rotor seal of the valve needs to be checked for build-up and wear. Replace if wear is evident (plastic shavings inside the valve) or there is build-up in the seal. When reassembling the valve, it is imperative that the lines are re-connected to the proper port (see **Figure 9-1**). Replace the 2 mL sample loop, if blood residue has built up in the loop. After the autosampler check or maintenance is complete, watch the autosampler perform for the first few stability samples to ensure that the components are functioning properly (the sample is loading, the loop is being filled, the rinse is filling and draining properly, etc.).

Figure 9-1: Configuration of tubing and devices for liquid handling using FAST sample introduction.

Below shows the correct connections to the 6-port FAST valve. The two diagrams show the differences in liquid flow directions when the valve changes from “Load” to “Inject”. This change is internal to the valve. The shift of the valve cannot be seen, but it can be heard, and felt (with hand on the valve). The light indicators on the actuator body also indicate the valve position.





Carrier solution pushes sample into nebulizer
at the same time sample line is rinsed



- a. Port 1: sample loop (white nut).
- b. Port 2: 0.5 mm ID probe (red nut) for carrier solution.
- c. Port 3: nebulizer line (green nut) for transfer of liquid to nebulizer.
- d. Port 4: sample loop (white nut).
- e. Port 5: 0.8 mm ID probe (blue nut) for diluted samples.
- f. Port 6: vacuum line (black nut).

10. POST-RUN DATA ANALYSIS

a. Data Processing and Analysis

1. In the LabBook's "Evaluation Results" section (**Figure 10-1**) click on "Concentrations". Clicking on the "+" beside each sample allows you to expand the window to show the three replicates, mean, RSD, and standard deviation (SD). If you select all lines first, when you right click on the "+" you can select the option for "Expand all levels". This shows this information for all analyzed samples.
2. Scroll through the samples to see if there are any noticeable intensity "spikes" in replicates (i.e. if one replicate is significantly higher than the other two). The spike may be a different

color than the other two replicates, indicating that the software recognizes that this is actually a spike. If you notice what may be a potential spike, check the acceptability of the replicates using the replicate delta procedure (**Appendix B**). This will determine whether or not the sample needs to be reanalyzed.

3. If there is a sample that requires reanalysis but the closing QC has already been analyzed, the sample will have to be analyzed in either a separate batch run with bracketing QC or with a new calibration (depending upon how much time has lapsed since calibration).
4. If any of the samples have concentrations between 15 µg/L and 100 µg/L, they need to be prepared again and reanalyzed with the elevated calibration curve. The preparation, analysis, and data processing process is the same as with the normal calibration curve. If an analyte requires analysis with this calibration curve, report the result for that analyte from this calibration. Report all analytes that fell within the normal calibration range from the analysis of the analyte with that calibration curve.
5. Samples with concentrations between 100 µg/L and 5000 µg/L need to be diluted. A 1:50 dilution is the maximum dilution that can be used. Dilute the samples with the same base blood that is used to make the elevated calibration curve. Analyze the diluted sample(s) using this elevated curve.
6. The STARLIMS database is set up to flag a sample analyte for the following situations: failure for the replicate delta rules, is above the highest calibration standard, or needs a dilution. Therefore, even if the analyst does not notice the need for reanalysis immediately during analysis or immediately post-analysis, the need for reanalysis will be known prior to setting the results final in STARLIMS.
7. To export results into an Excel file, go to “Query” (**Figure 10-2**). In order to create a file which can be imported into the STARLIMS database, the following selections have to be made and saved as a preset by clicking on the “floppy disk” icon located in the middle of the top tool bar:
 - Under “Category” check Concentration average, Concentration RSD, and Concentration per Run.
 - Under “Columns” check “Results”. Expand the “Results” section by clicking on “+” and check “Analytes”. Select analytes of interest (Cr, Co) and the internal standards (Ga and Sc).
 - Also under “Columns” check “Sample list”. Expand the folder by clicking on “+”. Select “Index”, “Start time”, “Label”, and “Dilution factor”.
 - Press the Refresh  button on the top middle of the icon menu to update the calculations and reflect new selections.
 - Press the Export icon  in the top left corner. The document will appear showing the same columns shown in **Figure 10-2** in Excel format. Save the file as an Excel workbook (.xls) in the LabBooks folder under the date when the samples were analyzed. The file name contains the date and description of the samples analyzed plus the calibration standards lot used “CrCo mmddyyyy_name_Lot_”.
8. Click on the “Reports” under the “Query” section (**Figure 10-2**). This will produce the calibration curves used in calculating the concentrations for 59Cr and 52Cr plus tabulated standards concentrations and internal standards recoveries. This file is saved into the LabBooks folder along with analytical run results. If the report format needs to be changed click on the “Edit report” tab in the middle of the toolbar at the top of the screen and select the desired options for the new report layout.

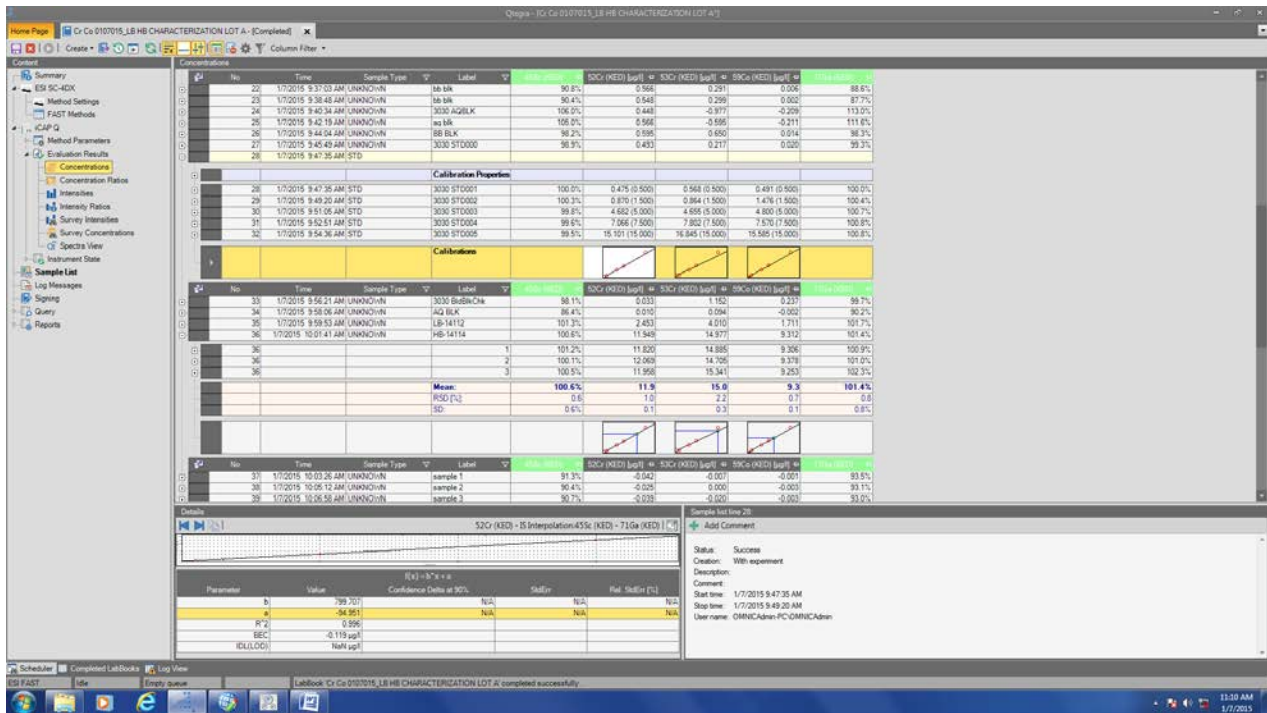


Figure 10-1: LabBook “Evaluate results” example

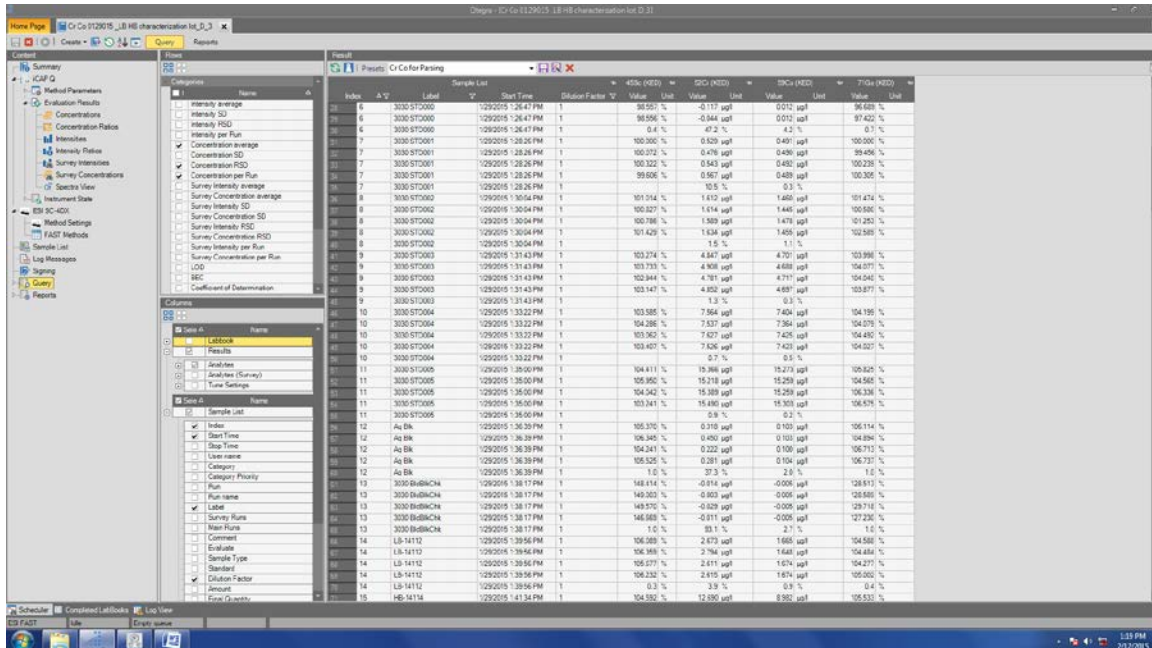


Figure 10-2: LabBook “Query” example

11. RECORDING OF SAMPLE AND QC DATA

Transferring the Data to STARLIMS

1. Transfer the “CrCo mmddyyy_name_Lot_”.xls file(s) via encrypted USB drive or CD or DVD to the appropriate subdirectory on the network drive where exported data is stored. The directories are named according to instrument\year\month\ and study name or ID, for example,

“\.....\Nutritional\Instruments\iCAPQ\iCAPQa_A\2015\2\02172015\CrCo mmddyyy_name_Lot_”

2. In addition to the “CrCo mmddyyy_name_Lot_”.xls file, save the STD and KED mode daily performance reports (autotune, detector and mass calibration reports if performed), calibration curve reports, and actual instrument LabBook for the day of analysis on the shared network drive in the location mentioned above in 11.1.
3. The “CrCo mmddyyy_name_Lot_”.xls file containing results from the instrument has to be “parsed” using a specially designed macro-enabled file developed to get the data into the appropriate format to be uploaded to the STARLIMS database. This file is developed by the CDC DLS STARLIMS support team and is tailored to capture the necessary information from the output file from this instrument. The imported file includes the instrument ID, analyst ID, analysis date and time, sample ID, sample results, calibration standards lot number, etc. The macro-enabled parsing file is located in the instrument folder on the shared drive.
4. Import the parsed instrument file into the STARLIMS system.

12. FINAL REVIEW OF THE DATA

a. Run Data

To conserve resources, run data is printed to PDF instead of making paper copies. If the samples analyzed are part of a study that has an associated study folder, notate the shared drive location of the electronically stored data on the cover of the study folder.

b. Plotting QC Results

Importing data into DLS STARLIMS allows for the capture of quality control data that can be monitored through the generation of statistical quality control plots using SAS. Both the analyst and the supervisor must monitor these plots regularly for any trends in the bench QC data. If trends are observed by the analyst, contact the laboratory supervisor. Investigate the cause of the trend(s) and work with the supervisor to rectify the problem.

c. Supervisor Review

DLS STARLIMS allows a person with supervisory authority to review the QC and sample results in the system (electronically). After the supervisor reviews the data, he or she may release the data in STARLIMS to be further reviewed for reporting externally.

13. QUALITY CONTROL PROCEDURES

IRAT uses the method described in this protocol for environmental and occupational health screening studies.

The analyst inserts each level of the bench QC into each batch run twice 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 used to estimate methodological imprecision and to assess the magnitude of any time-associated trends. The standard bench QC pools used in this method comprise two levels of concentrations spanning the “low-normal” and “high-normal” ranges for each analyte. Both of these pools are 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. If a second batch of samples is 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 evaluated and treated independent of the first run. In the instance where patient samples are above 15 µg/L, an elevated calibration curve is to be used. This calibration curve has its own set of QC samples (elevated level 1 and elevated level 2) that are to be treated and evaluated in the same manner as the low-normal and high-normal bench QC.

a. Establish QC limits for each QC pool.

Before a newly prepared QC pool is put into use, a run to assess the homogeneity of the pool is performed. Vials are randomly chosen and randomly analyzed; however, the first and last vials dispensed are always included in the homogeneity study. Unlike the characterization of the QC, the homogeneity study can be completed in a single run. Once analysis is complete, the data is statistically evaluated to determine whether or not trends exist in QC recovery at different dispensing points in the pool. If the pool does not vary from beginning to end (i.e. is homogenous) or if problem vials can be identified and eliminated, the characterization of the QC pool is the next step. If inhomogeneity exists, the source(s) of the problem has to be identified and the pool has to be re-made and dispensed again.

To complete the characterization that will allow you to assess limits for each pool, analyze QC from each pool (low and high) on 20 different days, preferably among all of the instruments that will be used to analyze this method. Bracketing QC must be analyzed with each batch run. During the 20 characterization runs, previously characterized QC materials or pools with target values assigned by outside laboratories are also analyzed if available to evaluate each run’s QC. Blanks can be inserted as filler in the middle of the run. Import the results from each characterization run into STARLIMS. When a sufficient number of characterization runs have been performed, use SAS to calculate the mean and standard deviation for the analytes in each pool. These values will be used as the official limits for the QC pool.

b. Precision and Accuracy

QC Results Evaluation. After completing a run, refer to the consult the QC limits to determine whether the run is “in control” for each analyte. *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 limit, the run will be accepted. This can be determined post analysis.

2. If either the beginning or closing QC result is outside the 2s limits, the run will be evaluated. The run will be rejected (fails) if any of the following four situations exist:
 - **1_{3s}** – Average of both low QCs OR average of both high QCs is outside of a 3s limit.
 - **2_{2s}** – Average of both low QCs AND average of both high QCs is outside of 2s limit *on the same side of the mean*.
 - **R_{4s} sequential** – Average of both low QCs AND average of both high QCs is outside of 2s limit *on opposite sides of the mean*.
 - **10_x sequential** – The previous nine mean QC results for the previous nine runs were *on the same side of the mean* for either the low OR high QC.

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

c. Remedial Action If Calibration or QC Systems Fail to Meet Acceptable Criteria

If an analyte fails to pass QC according to the report generated by SAS, the following steps must be taken if possible:

- Check the data for each blank, calibrator, QC, and sample for any abnormalities that may have been missed during post run visual results review.
- Check the ICP-MS stability during the run by examining the degree of variability and drift in internal standard intensities over the course of the run. Irreproducibility that exceeds 15%, drift greater than 20%, or sudden large changes in internal standard recoveries likely indicate a problem with plasma stability.
- Set up a new run for the reanalysis of the patient samples affected by the previous failed run. All samples will have to be prepared again.
- If these three steps do not result in correction of the out-of-control values for QC materials, consult the supervisor for other appropriate corrective actions. No analytical results will be reported for runs that are not within statistical control.

14. LIMIT OF DETECTION AND LINEAR RANGE TESTED

The limits of detection (LOD) for chromium and cobalt in whole blood were calculated in accordance to guidelines established by the Division of Laboratory Sciences. The method used to calculate the LODs is based on the recommendations of the Clinical and Laboratory Standards Institute (CLSI) and take both Type I and Type II errors into consideration. According to the guidelines established by our division, at least four concentration levels are to be analyzed in each run with one level having analyte concentrations below the projected LOD. For this method, the matrix blank was used to satisfy the

criterion of having a level below the LOD while the first three calibrators of the standard calibration curve were used for the other three levels. All samples used for LOD calculations were human whole blood specimens. Two analysts were used for the measurements which covered over 60 runs over a three month time span.

According to division protocol, for linear relationships the following equation is used to calculate the LOD: $conc_{LOD} = [\text{mean}_b + 1.645 \cdot (s_b + B)] / (1 - 1.645 \cdot A)$ where mean_b is the mean of the blank, s_b is the standard deviation of the blank, B is the y-intercept, and A is the slope. For quadratic relationships ($y = Ax^2 + Bx + C$), the following equation is used to calculate the LOD: $conc_{LOD} = [-b \pm \sqrt{b^2 - 4ac}] / 2a$ where $a = 1.645 \cdot A$, $b = (1.645 \cdot B) - 1$, and $c = \text{mean}_b + 1.645 \cdot (s_b + C)$. With both linear and quadratic relationships, the resulting calculated LOD has a 5% false negative rate. A linear relationship was the best fit with this analytical method, and the limits of detection for chromium and cobalt in whole blood were determined to be 0.41 µg/L and 0.06 µg/L, respectively.

As stated in the DLS Policies and Procedures Manual “uncertainty increases as values approach the limit of detection.” Division policy prohibits reporting results lower than the lowest calibration curve point. Report results below the detection limit as “< LOD” (where “LOD” is the calculated lowest detection limit). The LOD calculation is reevaluated whenever major changes to the method occur.

It was established that cobalt is linear to 500 µg/L while chromium is linear to 1500 µg/L using the elevated calibration curve and analyzing the samples undiluted. This information is for reference only because anything above 100 µg/L (but less than 5000 µg/L) is diluted and reported from the dilution.

TABLE 14-1: LIMITS OF DETECTION (LOD) AND LINEAR RANGE TESTED (LRT)

Species Chemical Name	Abbreviated Name	Limit of Detection, µg/L	Highest Concentration for Linear Range Tested, µg/L
Chromium	Cr	0.41	1500
Cobalt	Co	0.06	500

15. REPORTABLE RANGE OF RESULTS

Blood chromium and cobalt results are reportable in the range of greater than the calculated limit of detection up to 5000 µg/L. When a sample result for any analyte is greater than the highest calibrator within the run (15 µg/L) but less than 100 µg/L, the sample needs to be reanalyzed using the elevated calibration curve. Bracketing elevated QC needs to be analyzed within the elevated calibration run along with the patient sample that falls within that range. The sample result will be reported from that run. The maximum dilution allowed for each analyte is a 1:50 dilution making the reportable range 5,000 µg/L for this analytical method.

16. SPECIAL PROCEDURE NOTES – CDC MODIFICATIONS

None applicable for this method.

17. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

All potential interferences on chromium and cobalt are eliminated by the operation of the collision cell (KED mode) using helium gas and optimized instrument parameters. This is discussed in detail in section 1.b.

18. REFERENCE RANGES

The reference ranges for chromium and cobalt (see **TABLE 18-1**) are based on literature reports of data collected during the analysis of blood samples representing a normal, healthy population or population of interest.

TABLE 18-1: REFERENCE RANGES FOR CHROMIUM AND COBALT

Analyte, µg/L	Reference Range
Chromium	0.7 to 28.0 µg/L [4]
Cobalt	(N=64) 9 with no implants Range 0.224 to 0.565 µg/L [8]
	0.8 µg/L according to National Institute for Research and Safety (INRS) [15]
	(N=130) Germany Mean =0.19 µg/L, Range is 0.04-8 µg/L 95 th percentile = 0.41 µg/L [16]
	French study healthy individuals (N=100) Median = 0.25 µg/L Range of 5 th to 95 th percentile (0.04 – 0.64 µg/L) [17]

19. ACTION-LEVEL RESULTS

Concentrations for chromium and cobalt that are observed to be greater than the “first upper boundary” (defined in DLS STARLIMS as the “1UB”) must be either reanalyzed with the elevated calibration curve or diluted. The 1UB for both analytes is 15 µg/L. If the concentration is between 15 µg/L and 100 µg/L, the sample needs to be reanalyzed with the elevated calibration curve. If the concentration is between 100 µg/L and 5000 µg/L, the sample needs to be diluted and reanalyzed.

20. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens may reach and maintain ambient temperature during sample preparation. Take stringent precautions to avoid external contamination. After the samples are prepared for analysis, return unused portions in the original containers to approximately -70°C freezer storage as soon as possible.

21. ALTERNATE METHODS FOR PERFORMING TEST AND STORING SPECIMENS IF TEST SYSTEM FAILS

If the ICP-MS malfunctions to the point where analysis of the samples is impossible, prepared specimens can be stored at approximately 4°C or a lower temperature until the analytical system is restored to full functionality. The unused portion of the original specimen must be returned to a -70°C or lower temperature freezer after the sample preparation is complete.

22. TEST-RESULT REPORTING SYSTEM; PROTOCOL FOR REPORTING CRITICAL CALLS (IF APPLICABLE)

Report test results as outlined in the *DLS Policies and Procedures Manual*. For critical calls, the supervisor must notify the supervising physician or principal investigator as soon as possible. The most expeditious means must be used (e.g., telephone, FAX, or E-mail).

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

For select studies, a specimen folder is set up. A tracking form is initiated and placed in the folder to be given to the analyst performing the analysis. The form tracks location, status, and final disposition of the specimens. When sample analysis is completed, update and place the tracking form in the specimen folder.

If possible, use standard electronic record keeping means such as STARLIMS to track specimens. Locations of specimens can be stored electronically via spreadsheets and saved on the shared network drive. Use only numerical identifiers (e.g., case ID numbers); all personal identifiers are available only to the medical supervisor or project coordinator to safeguard confidentiality. Refer to the *DLS Policies and Procedures Manual* for up-to-date record retention guidelines.

24. BI-ANNUAL INSTRUMENT-TO-INSTRUMENT COMPARISON

Per CLIA requirements, if an analytical method is performed on more than one instrument an instrument-to-instrument comparison has to be performed twice per year. Five samples are chosen that cover the analytical range. QC samples and SRMs can be used to satisfy this requirement. The same sample IDs are prepared and analyzed on each instrument. The Pearson product-MoMent correlation coefficient for the results for each analyte has to be greater than 0.95. If the coefficient is not greater than 0.95, remedial action has to be taken.

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26. APPENDIX

a. APPENDIX A: Ruggedness Testing

Critical Parameter Test 1: This test evaluated the significance of the gas flow rate of helium in KED mode on the recovery of the analytes. The KED gas flow used in the established method is 5 mL/min. Gas flows of 4 mL/min (20% lower than the typical gas flow) and 6 mL/min (20% higher than the typical gas flow) were evaluated.

Test Details:

1. Twelve samples (four individual samples from three HB-14114 vials), calibrators, blanks, and QC were prepared and analyzed at one of the KED gas flow rates.
2. At the end of the analysis, the KED gas flow rate was changed. Another set of samples prepared in the same manner as item number 1 above were analyzed.
3. This was repeated for the third gas flow rate.
4. For this test, all method parameters except for the KED gas flow rate were held constant.

Critical Test Parameter 1. Ruggedness Testing Results: Evaluating the significance of the gas flow rate of helium in KED mode on the recovery of the analytes. Analyses were performed on December 19, 2014. Results below are the average of the 12 replicates of high bench QC (HB-14114) analyzed as samples in each run. The least squares mean and the 95% confidence intervals are given for each analyte at each condition. The characterized QC mean and the acceptance levels at $\pm 2SD$ are given as well. All concentrations are in $\mu\text{g/L}$.

Analyte	Target Mean and 2SD Range	4 mL/min (decreased)	5 mL/min. (per method)	6 mL/min. (increased)
BCR	12.26 11.05 – 13.46	13.02 12.89 – 13.16	12.25 12.12 – 12.39	12.08 11.94 – 12.21
BCO	8.89 7.95 – 9.83	8.99 8.92 – 9.05	8.99 8.92 – 9.06	9.11 9.04 – 9.18

Results of Statistical Analysis:

For both analytes, statistical evaluation of the results showed a statistical significance ($p < 0.05$) between the normal gas flow rate and the higher gas flow rate. There was also a statistically significant difference between the normal gas flow rate and the lower gas flow rate for chromium but not cobalt. When comparing the least squares means to the characterized QC results (target mean and 2SD range), the results at all conditions were within our QC acceptance limits. The presence of a statistical significance

does not necessarily translate into scientific significance in this case. A statistically significant difference was not found between the normal KED gas flow rate and the lower gas flow rate for cobalt.

The conclusion was drawn that the method is rugged enough to withstand changes of the KED gas flow rate. However, a comparison of the least squares mean for the established method flow rate to the target mean (which correlates with what was spiked into the quality control pool) shows that the 5 mL/min KED gas flow rate is the optimum condition for this parameter.

Critical Parameter Test 2: This test evaluated the significance of the rinse time used on the recovery of the analytes. The rinse time used in the established method is 33 seconds. Rinse times of 24 seconds (approximately 27% lower than the typical rinse time) and 39 seconds (approximately 18% higher than the typical rinse time) were evaluated.

Test Details:

1. Twelve samples (four individual samples from three HB-14114 vials), calibrators, blanks, and QC were prepared and analyzed at one of the sample rinse times.
2. At the end of the analysis, the rinse time was changed. Another set of samples prepared in the same manner as item number 1 above were analyzed.
3. This was repeated for the third rinse time.
4. For this test, all method parameters except for the sample rinse time were held constant.

Critical Test Parameter 2. Ruggedness Testing Results: Evaluating the significance of the rinse time used on the recovery of the analytes. Analyses were performed on December 22, 2014. Results below are the average of the 12 replicates of high bench QC (HB-14114) analyzed as samples in each run. The least squares mean and the 95% confidence intervals are given for each analyte at each parameter. The characterized QC mean and the acceptance levels at $\pm 2SD$ are given as well. All concentrations are in $\mu\text{g/L}$.				
Analyte	Target Mean and 2SD Range	24 s (decreased)	33 s (per method)	39 s (increased)
BCR	12.26 11.05 – 13.46	11.64 11.51 – 11.78	11.90 11.76 – 12.03	11.72 11.59 – 11.86
BCO	8.89 7.95 – 9.83	8.80 8.66 – 8.94	8.67 8.53 – 8.81	8.71 8.57 – 8.85

Results of Statistical Analysis:

For both analytes, statistical evaluation of the results showed a statistical significance ($p < 0.05$) between the normal rinse time and the shorter rinse time. When comparing the least squares means to the characterized QC results (target mean and 2SD range), the results using a 24 s rinse time were well within our QC acceptance limits. The presence of a statistical significance does not translate into scientific significance in this case. A statistically significant difference was not found between the normal rinse time and the longer rinse time. The conclusion with this test was that the method is rugged enough to withstand fluctuations in the rinse time.

Critical Parameter Test 3: This test evaluated the significance of the percentage of TMAH used in the diluent on the recovery of the analytes. The percentage of TMAH in the diluent in the established method is 0.4%. Diluent TMAH percentages of 0.3% (25% lower than the typical TMAH percentage in the diluent) and 0.5% (25% higher than the typical TMAH percentage in the diluent) were evaluated.

Test Details:

1. Two additional diluents were made (one with 0.3% TMAH and one with 0.5% TMAH).
2. For each diluent used, twelve samples (four individual samples from three HB-14114 vials), calibrators, blanks, and QC were prepared.
3. The three sets of samples were analyzed by ICP-MS using the optimized analytical method.
4. All method parameters except for the percentage of TMAH in the diluent were held constant.

Critical Test Parameter 3. Ruggedness Testing Results: Evaluating the significance of the percentage of TMAH in the diluent on the recovery of the analytes. Analyses were performed on December 31, 2014. Results below are the average of the 12 replicates of high bench QC (HB-14114) analyzed as samples in each run. The least squares mean and the 95% confidence intervals are given for each analyte at each parameter. The characterized QC mean and the acceptance levels at $\pm 2SD$ are given as well. All concentrations are in $\mu\text{g/L}$.

Analyte	Target Mean and 2SD Range	0.3% TMAH (decreased)	0.4% TMAH (per method)	0.5% TMAH (increased)
BCR	12.26 11.05 – 13.46	12.00 11.82 – 12.17	12.48 12.31 – 12.66	12.49 12.31 – 12.66
BCO	8.89 7.95 – 9.83	9.32 9.18 – 9.45	9.12 8.99 – 9.26	9.40 9.26 – 9.53

Results of Statistical Analysis:

For both analytes, statistical evaluation of the results showed a statistical significance ($p < 0.05$) between the normal and lower diluent TMAH percentages for chromium, the normal and lower TMAH percentages for cobalt, and the normal and higher TMAH percentages for cobalt. When comparing the least squares means to the characterized QC results (target mean and 2SD range), the results were well within our QC acceptance limits. In this test, the presence of a statistical significance does not translate into scientific significance. A statistically significant difference was not found between the results from the diluents with normal and higher TMAH percentages for chromium. The conclusion with this test was that the method is rugged enough to withstand fluctuations in the percentage of TMAH in the diluent.

Critical Parameter Test 4: This test evaluated the significance of the dilution factor used for sample preparation. The dilution factor used for sample preparation is 20x. Dilution factors of 15x (25% lower than the typical dilution factor) and 25x (25% higher than the typical dilution) were evaluated. The results are presented in the table below.

Test Details:

1. For each sample preparation dilution factor examined, twelve samples (four individual samples from three HB-14114 vials), calibrators, blanks, and QC were prepared using that dilution factor.
2. The three sets of samples were analyzed by ICP-MS using the optimized analytical method.
3. All method parameters except for the sample preparation dilution factor were held constant.

Critical Test Parameter 4. Ruggedness Testing Results: Evaluating the significance of sample preparation dilution factor on analyte recovery. Analyses were performed on January 02, 2015. Results below are the average of the 12 replicates* of high bench QC (HB-14114) analyzed as samples in each run. The least squares mean and the 95% confidence intervals are given for each analyte at each condition. The characterized QC mean and the acceptance levels at $\pm 2SD$ are given as well. All concentrations are in $\mu\text{g/L}$.				
Analyte	Target Mean and 2SD Range	15x* (decreased)	20x (per method)	25x (increased)
BCR	12.26 11.05 – 13.46	18.49 18.01 – 18.96	11.69 11.21 – 12.09	10.68 10.25 – 11.11
BCO	8.89 7.95 – 9.83	12.71 12.37 – 13.04	8.58 8.28 – 8.88	7.60 7.30 – 7.90

Results of Statistical Analysis:

For both analytes, statistical evaluation of the results showed a statistical significance ($p < 0.05$) between samples prepared with the normal dilution factor and samples prepared with the smaller dilution factor. The same applies to the comparison of results from samples prepared at the normal dilution level and samples that were more dilute (larger dilution factor). When comparing the results to the characterized QC results (target mean and 2SD range), the results at 15x and 25x dilutions were not within our QC acceptance limits. The statistical significance seen is valid. It was concluded that the method does not provide acceptable results at dilution factors outside of the established level for this method. This corresponds to what was seen during the method development process in selection of the final dilution factor used for sample preparation.

*For the 15x dilution factor test, only nine replicates were available.

Critical Parameter Test 5: This test evaluated the significance of the percentage of ethanol used in the diluent on the recovery of the analytes. The percentage of ethanol in the diluent in the established method is 1%. Diluent ethanol percentages of 0.8% (20% lower than the typical ethanol percentage in the diluent) and 1.2% (20% higher than the typical ethanol percentage in the diluent) were evaluated. The results are presented in the table below.

Test Details:

1. Two additional diluents were made (one with 0.8% ethanol and one with 1.2% ethanol).
2. For each diluent used, twelve samples (four individual samples from three HB-14114 vials), calibrators, blanks, and QC were prepared.
3. The three sets of samples were analyzed by ICP-MS using the optimized analytical method.
4. All method parameters except for the percentage of ethanol in the diluent were held constant.

Critical Test Parameter 5. Ruggedness Testing Results: Evaluating the significance of the percentage of ethanol in the diluent on the recovery of the analytes. Analyses were performed on February 09, 2015. Results below are the average of the 12 replicates of high bench QC (HB-14114) analyzed as samples in each run. The least squares mean and the 95% confidence intervals are given for each analyte at each condition. The characterized QC mean and the acceptance levels at $\pm 2SD$ are given as well. All concentrations are in (in $\mu\text{g/L}$).				
Analyte	Target Mean and 2SD Range	0.8% ethanol (decreased)	1% ethanol (per method)	1.2% ethanol (increased)
BCR	12.26 11.05 – 13.46	11.25 11.09 – 11.41	11.28 11.12 – 11.43	11.68 11.52 – 11.84
BCO	8.89 7.95 – 9.83	8.51 8.42 – 8.61	8.58 8.46 – 8.65	7.60 8.27 – 8.47

Results of Statistical Analysis:

For both analytes, statistical evaluation of the results showed a statistical significance ($p < 0.05$) between the normal and increased ethanol amounts for chromium and the normal and increased ethanol amounts for cobalt. When comparing the least squares means to the characterized QC results (target mean and 2SD range), the results were well within our characterized QC acceptance limits. In this test, the presence of a statistical significance does not translate into scientific significance. A statistically significant difference was not found between results from the diluents with normal and lower ethanol percentages for either analyte. The conclusion with this test was that the method is rugged enough to withstand fluctuations in the percentage of TMAH in the diluent.

b. APPENDIX B: Replicate Delta Determination Rules

The replicate delta (rep delta) is the difference between the largest replicate and the smallest replicate. The rep delta limit for Cr and Co is set to 1.

The rep delta rules for chromium and cobalt for this method are interpreted as follows:

1. If the concentration of a sample is greater than or equal to 10 µg/L, the rep delta for that sample will be compared against 10% of the mean.
 - (a) If the rep delta is greater than 10% of the mean, the sample will have to be reanalyzed.
 - (b) If the rep delta is less than 10% of the mean, the sample QC will pass.

Example 1:

Replicates: 10.02, 12.04, 15.06

Mean: 12.37

10% of Mean: 1.24

Rep delta: 5.04

Rep delta limit: 1

5.04 (rep delta) is greater than 1.24 (10% of mean), so the sample QC fails, and the sample needs to be reanalyzed.

Example 2:

Replicates: 97.80, 98.44, 97.69

Mean: 97.98

10% of Mean: 9.798

Rep delta: 0.75

Rep delta limit: 1

0.75 (rep delta) is less than 9.798 (10% of mean), so the sample QC passes in terms of rep delta.

2. If the concentration of a sample is less than 10 µg/L, the rep delta for that sample will be compared against 1 (the rep delta limit).
 - (a) If the rep delta is less than 1, the sample QC will pass.
 - (b) If the rep delta is greater than 1, the sample QC will fail.

Example 1:

Replicates: 7.80, 8.44, 7.69

Mean: 7.98

10% of Mean: 0.7977

Rep delta: 0.75

Rep delta limit: 1

0.75 (rep delta) is less than 1 (rep delta limit), so the sample QC passes in terms of rep delta.

Example 2:

Replicates: 1.80, 3.44, 5.69

Mean: 3.64

10% of Mean: 0.364

Rep delta: 3.89

Rep delta limit: 1

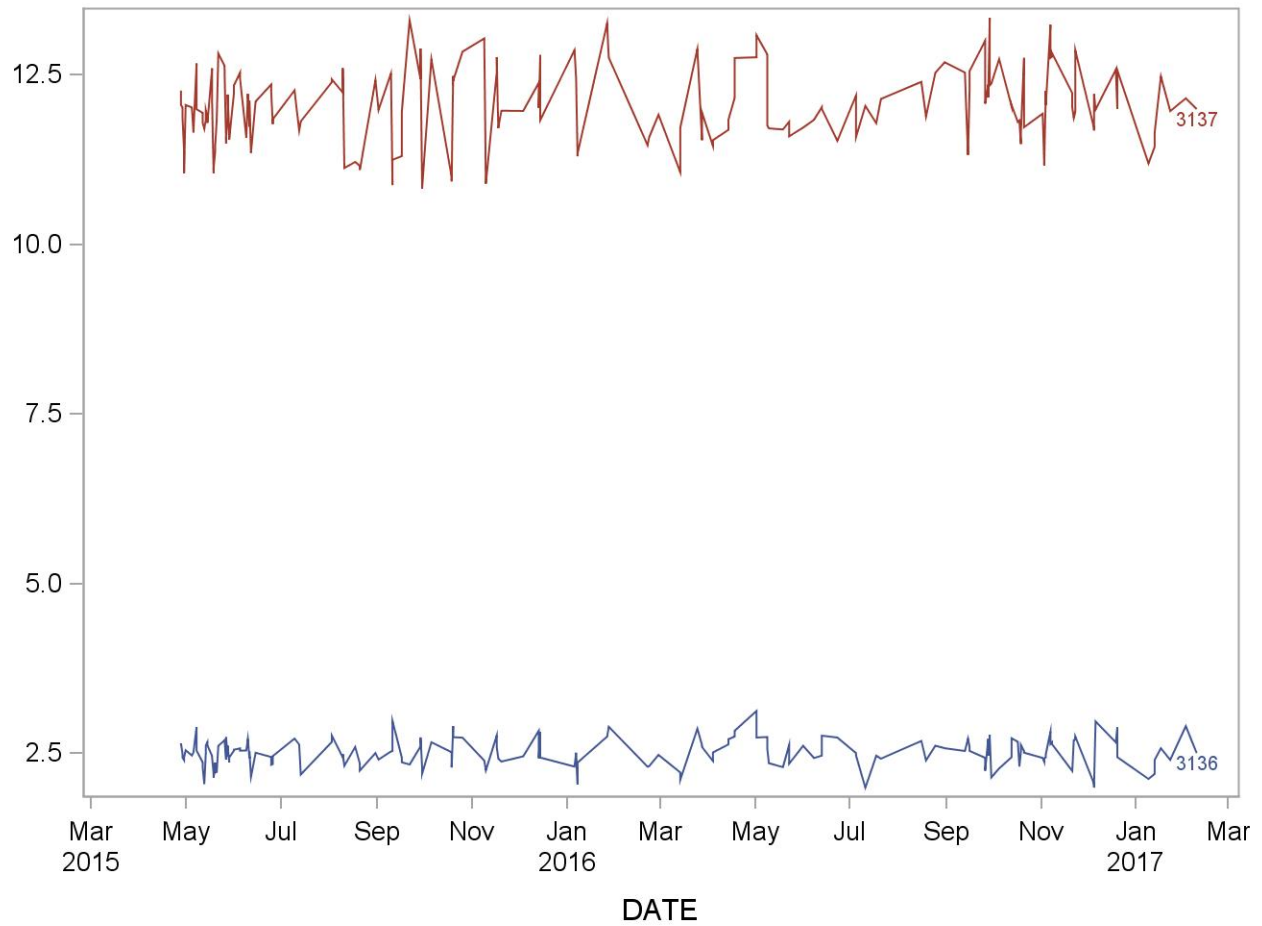
3.89 (rep delta) is greater than 1 (rep delta limit), so the sample QC fails, and the sample needs to be reanalyzed.

27. SUMMARY STATISTICS AND QC GRAPHS

See next pages.

Summary Statistics and QC Chart for Chromium, Blood (mg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
3137	177	28APR15	09FEB17	12.019	0.554	4.6
3136	177	28APR15	09FEB17	2.504	0.210	8.4



Summary Statistics and QC Chart for Cobalt, Blood (mg/L)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
3137	169	28APR15	09FEB17	8.744	0.368	4.2
3136	169	28APR15	09FEB17	1.632	0.071	4.3

