Laboratory Procedure Manual

Analyte: N-terminal hemoglobin adducts of Formaldehyde

Matrix: Red Blood Cells

Method: Liquid Chromatography Tandem Mass Spectrometry

Method No: 1017

Revised:

As performed by:

Clinical Chemistry Branch
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Public Release Data Set FORMAL_I Information

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

File Name	Variable Name	SAS Label
FORMAL_I	LBXFOR	Formaldehyde (nmol/g)

1 SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

1.1 Clinical relevance

Formaldehyde (FA) is an environmental chemical occurring in tobacco smoke, building materials, and furniture, among other sources [1]. FA was classified as carcinogenic to humans by the International Agency for Research in Cancer (IARC) [2, 3]. The National Toxicology Program classifies it as “known to be a human carcinogen”. People exposed to high levels of FA are at increased risks of nasopharyngeal cancer and lymphohematopoietic cancer, specifically myeloid leukemia. FA is also produced by most living organisms as part of regular metabolic activities.

FA is highly reactive towards biomolecules and can react with proteins to form so called “adducts” [4]. Adducts with hemoglobin have successfully been used as biomarkers of exposure for a range of environmental chemicals [5].

1.2 Test principle

This procedure describes a method to measure hemoglobin adducts of FA in human erythrocytes. It consists of 5 parts (see also Appendix 1).

1. Preparation of the specimen for measurement
2. Total hemoglobin measurement in the sample solution used for hemoglobin adduct measurements
3. Enzymatic digestion of the sample solution
4. Analysis of digested samples by HPLC-MS/MS
5. HPLC-MS/MS data processing and evaluation

Because results are reported in nanomol adduct per gram of hemoglobin used in the measurement, the amount of hemoglobin used for this analysis needs to be determined. Therefore, this procedure includes a commercial method for measuring total hemoglobin in clinical samples [6].

Assessment of FA-hemoglobin adducts is performed by quantitation of the N-terminal peptide of the hemoglobin beta-chain after digestion with trypsin. This peptide has FA attached at the N-terminal valine (FA-Val-His-Leu-Thr-Pro-Glu-Glu-Lys-OH) [7]. The corresponding stable isotope labeled peptide (FA-Val($^{13}C_5$,^{15}N)- His-Leu-Thr-Pro-Glu-Glu-Lys-OH) is used as internal standard (IS).

1.3 Scope

The measurement procedure described in this document is intended for quantitatively measuring the N-terminal FA peptides described in section 1.2 of this document. This method is not suitable for measuring hemoglobin adducts in animals. It addresses all aspects related to the measurement process (specimen collection, storage, processing, analysis, and reporting). This document is not intended to provide information on data interpretation.

2 SAFETY PRECAUTIONS

2.1 General Safety

All blood specimens must be considered potentially positive for infectious agents including HIV, Hepatitis B, and Hepatitis C. Hepatitis B vaccination series are required for all analysts performing this measurement procedure.

Universal precautions must be observed: protective gloves, laboratory coat, and safety glasses must be worn at all times during all tasks of this measurement procedure. Additional information can be found in the laboratory Chemical Hygiene Plan (CHP).

Disposable bench covers must be used during sample preparation and sample handling and must be discarded after use. All work surfaces must be wiped with 10% bleach solution when work is finished.

Extra caution is required when removing external screw caps of sample cryovials. Always place a Kimwipe over the screw cap to prevent any blood exposure. A face shield may also be worn.

Operators of this method must meet CDC and Division of Laboratory Sciences (DLS) safety policies before performing this procedure.

2.2 Chemical Hazards

All acids, bases, other reagents, and organic solvents used in this measurement procedure must be handled with extreme care. The chemicals are caustic, flammable, and/or toxic and must be handled only in a well-ventilated area or, as required, under a chemical fume hood. Appropriate personal protective equipment (gloves, safety glasses, and lab coats) must be worn at all times while handling the following chemicals:

Ammonium Bicarbonate- Hygroscopic. May cause eye and skin irritation. Keep lid closed tightly when not in use. Store it in desiccator to prevent adsorption of moisture.

Formic Acid- Corrosive liquid. It will cause severe burns to all body tissue. It may be fatal if swallowed. Always handle in fume hood, do not leave bottle open when not in use.

Methanol- A flammable solvent and may form explosive vapors. The vapor may be irritating to the eyes, nose, and throat. Methanol is poisonous and may be absorbed through the skin. Any exposed skin areas should be immediately flushed with water. Keep container tightly closed and sealed in the designated flammable cabinet until ready for use.

Hemoglobin Reagent Set- Irritating to eyes, respiratory system, gastrointestinal system, and skin. Handle in well-ventilated areas or as required under a fume hood. Contains cyanide. Avoid inhalation.

2,2,2-Trifluoroethanol- Flammable and toxic by inhalation and through skin absorption. Handle in well-ventilated areas or as required under a fume hood.

Glacial Acetic Acid- Flammable liquid and its vapors are corrosive. Inhalation may cause lung and tooth damage. Liquid and mist cause severe burns to all body tissue and may be fatal if swallowed.

Trypsin- May cause sensitization by inhalation. It is Irritating to eyes, respiratory system, and skin.

Safety data sheets (SDSs) for all chemicals are readily available as hard copies in the laboratory. They can also be viewed at http://www.ilpi.com/msds/index.html or at http://intranet.cdc.gov/ohs.

2.3 Radioactive Hazards

There are no radioactive hazards associated with this measurement procedure.

2.4 Mechanical Hazards

There are only minimal mechanical hazards when performing this procedure using standard safety practices. Analysts must read and follow the manufacturer's information regarding safe operation of the equipment. Avoid direct contact with the mechanical and electronic components of analytical equipment and instrumentation unless all power is off. Generally, mechanical and electronic maintenance and repair must be performed by qualified technicians only.

Manufacturer's operating instructions are located in the FA Project area of the Protein Biomarker Laboratory.

2.5 Waste Disposal

All waste needs to be labeled and processed in accordance with CDC policies.

All solid waste used in the sample preparation process (e.g., disposable pipette tips, well plates, gloves) and any residual sample materials in contact with blood or blood products must be autoclaved.

All glass and sharps (e.g., broken glass) must be placed in appropriate sharps containers.

All liquid waste must be labeled and processed using the CDC waste management and chemical tracking systems.

2.6 Training

Analysts performing this measurement procedure must successfully complete all safety trainings mandated by CDC and DLS policies.

Further, the analyst must have received training on specific instrumentation used with this measurement procedure from qualified PBL staff or qualified staff from the manufacturer.

3 COMPUTERIZATION AND DATA – SYSTEM MANAGEMENT

3.1 Software and Knowledge Requirements

This measurement procedure requires handling of different software operated instruments such as Thermo Fisher TSQ Vantage (using Xcalibur Software) and automated workstation Tecan Freedom Evo 200 (using EVOware) or Hamilton MicroLab STARLet Liquid Handler (using MicroLab STAR Vector Software). Only staff who received specific training are authorized to operate these instruments.

Calculated results obtained with the Xcalibur software are performed by the analysts using procedures developed and established by the Protein Biomarker Laboratory and DLS. Assessment of bench quality control (QC) results is performed using SAS software and maintained by the DLS. The database activities and QC calculations are performed by dedicated and specially trained staff.

3.2 Sample Information

All samples must be labeled as described in the DLS Policies and Procedures Manual. No personal identifiers are used, and all samples are referenced to coded sample identifiers.

3.3 Data Maintenance

Information about samples and related analytical data are evaluated prior to being entered into the DLS database (STARLIMS) for transcription errors and overall validity. Filing of electronic and physical files and their maintenance are the responsibility of designated staff in the Protein Biomarker Laboratory and are described in work instructions. The STARLIMS database is maintained by DLS staff and is routinely backed up by CDC Information Technology Services Office (ITSO).

3.4 Information Security

Information security is managed at multiple levels. The information management systems that contain the final reportable results are restricted through user ID and password security access. The computers and instrument systems that contain the raw and processed data files require specific knowledge of software manipulation techniques and physical location. Site security is provided through restricted access to the individual laboratories, buildings, and offices. Confidentiality of results is protected by referencing results to coded sample IDs.

4 PROCEDURE FOR COLLECTING, STORING, AND HANDLING SPECIMENS; CRITERIA FOR SPECIMEN REJECTION

4.1 General Specimen Requirements

No fasting or special time of day for specimen collection is required. There are no special requirements such as adherence to special diets for this assay.

Blood samples are obtained by venipuncture. Whole blood specimens for FA hemoglobin adduct analysis are collected in vacutainer tubes containing K_3-EDTA as anticoagulant.

This analysis has been evaluated for erythrocytes obtained from K_3-EDTA whole blood. The applicability of this method for other coagulants was not tested.

At least 200 µL of EDTA-whole blood is needed. Alternatively, a sample volume of 400 µL red blood cells diluted 1/1 (v/v) with DI water is required per analysis. Additional sample is needed if blood clots are present in the vial.

The sample can be dispensed and transported in cryogenic vials with external screw caps. These cryovials should be labeled in accordance to CDC and NCEH/DLS policies.

Other specimen handling conditions are outlined in the DLS Policies and Procedures Manual.

4.2 Specimen Storage

Collected specimens can be shipped refrigerated at 2-8 ºC (whole blood) and kept refrigerated up to 3 days until separation of RBC's from plasma is performed.

Once the RBC's are isolated, they can be stored at -70 ºC. Samples are stable for 5 years, if stored at -70 ºC.

Multiple freeze-thaw cycles of blood samples possibly increases the formation of blood clots and oxidation of hemoglobin. Therefore, diluted samples should not undergo more than 3 freeze-thaw cycles.

4.3 Unacceptable Specimens

Specimens that do not meet the above mentioned criteria, were transported at room temperature, or have evidence of leakage are not acceptable.

5 PREPARATION OF REAGENTS, CALIBRATION MATERIALS, CONTROL MATERIALS, AND ALL OTHER MATERIALS; EQUIPMENT AND INSTRUMENTATION

5.1 Equipment, Chemicals, and Consumables

The chemicals, equipment, and other materials described below or equivalents can be used in this measurement procedure.

5.1.1 Equipment, Chemicals, and Consumables Used for Reagent Preparation

1. Accumet AR 20 pH meter (Fisher Scientific, Suwanee, GA) with Orion Micro Combination pH electrode, pH range 0-14, temperature range 0-100 ºC. (Thermo Electron Corp., Bellefonte, PA)
2. Sartorius Analytical Balance (Accurate "0.00 mg" Digital Scales and Electronic Balances, Edgewood, NY)
3. Sartorius Chemical Balance (Electronic "0.000 g" Balances, Edgewood, NY)
4. Fisherbrand Desiccator Cabinets, Tall (Fisher Scientific, Suwanee, GA)
5. Desiccant, 6-8 Mesh 100% (EMD Chemicals, Gibbstown, NJ)
6. Glass beakers, 2L volumetric flask, 500mL, 100mL graduated cylinders (Fisher Scientific, Suwanee, GA)
7. Class A silanized volumetric flasks, and silanized volumetric glass pipettes 1 mL, 2mL, 3mL, and 4mL (Fisher Scientific, Suwanee, GA)
8. Falcon tubes, 15 ml, 50 mL plastic (KSE, Durham, NC)
9. Hemoglobin Reagent Set (HRS), (Teco Diagnostis, Anaheim, CA)
10. Linearity Total Hemoglobin Control, HC-110LIN, (Analytical Control Systems, Inc, Fishers, IN)
11. Glacial Acetic Acid ACS grade (J. T.Baker, Phillipsburg, NJ)
12. Hemoglobin Controls Low, Mid, and High Levels (Pointe Scientific, Canton, MI)
13. Sodium chloride solution, 0.9%, 1000 mL (Hospira, Inc., Lake Forest, IL)

5.1.2 Equipment, Chemicals, and Consumables Used for Sample Processing

1. Tecan Freedom Evo-2 200 (Tecan US., Research Triangle Park, NC)
2. Eppendorf Centrifuge 5810 R V4.2 with A-4-62 rotor (GMI, Ramsey, MN)

3. Filtered conductive tips for Freedom Evo, 200 µL, 1000 µL (Tecan US., Research Triangle Park, NC)
4. Eppendorf repeater pipette (Eppendorf, Ramsey, MN)
5. Hand held scanner (Symbol, Technologies. Inc, Bohemia, NY)
6. Transfer pipettes (Samco scientific, San Fernando, CA).
7. Boekel Orbitron rotator, model II (Fisher scientific, Suwanee, GA)
8. Eppendorf Thermomixer R with MTP (Eppendorf, Westbury, NY)
9. Thermo Fisher ALPS 50V Thermal Plate Sealer (Thermo Fisher, Waltham, MA)
10. Biohit Proline 50-200 µl pipette (Biohit, Neptune, NJ)
11. Rainin RT-200 pipette, 1000 µl (Rainin, Oakland, CA)
12. Tecan mca sbs 150 µL filter tips
13. Genevac Elite EZ-2 sample evaporator (GeneVac Inc., Valley Cottage, NY)
14. 96 well- 2 mL, rectangular well, pyramid bottom, storage plate (Seahorse Bio, Billerica, MA)
15. 96 well- 1ml, round well collections plates (Waters, Milford, MA)
16. 96 well- 350 µl flat bottom MTP plate, Non-binding surface (Corning, NY)
17. 2 mL PE 96-well pattern sealing film (Bio Tech Solutions, Mt. Laurel, NJ)
18. Aluminum Pierce Seal (Biosero, Newhall, CA)
19. Nalgene 2 mL cryovials with external-thread (Fisher Scientific, Suwanee, GA)
20. Hamilton glass syringe 10 µL (Hamilton, Reno, NV)
21. Formic Acid, High Purity (Fluka, St. Louis, MO)
22. 2,2,2-Trifluoroethanol (Acros Organics, NJ)
23. Ammonium bicarbonate (Fisher scientific, Suwanee, GA)
24. Trypsin Gold Mass Spectrometry grade (Promega, Madison, WI)
25. Glacial Acetic Acid, 99.8% (Acros Organics, NJ)

5.1.3 Equipment, Chemicals, and Consumables Used for Sample Measurement

1. EON Spectrophotometer (Bio-Tek Instruments, Winooski, VT)
2. HemoCue Hb 201+ (Hemocue Inc., Lake, Forest, CA)
3. RAPID Slit Seal 96 Well Sealing Tape (Eicom USA, Inc., Bethesda, MD)
4. Thermo TSQ Vantage with ESI Source (Thermo Electron, San Jose, CA)
5. Thermo Accela UPLC with Open Autosampler and Photodiode Array Detector (Thermo Electron, San Jose, CA)
6. Luna C18, 100 x 2.1mm, 3 µ, (Phenomenex, Torrance, CA)
7. SecurityGuard Column Cartridge (Phenomenex, Torrance, CA)
8. Methanol HPLC grade 99.9 % (Fisher Scientific, Suwanee, GA)
9. Deionized water with a resistivity ≥ 18 megaOhm-cm and filtered before use, using 0.45 µm nylon filters or Water OPTIMA grade from Fisher Scientific (Fisher, Suwanee, GA)
10. Formic Acid, High Purity (Fluka, St. Louis, MO)

5.2 Preparation of Reagents Used for Sample Preparation

All reagents are prepared using deionized water with a resistivity ≧18 MegaOhm-cm, and filtered before use, using 0.45 µm nylon filters.

5.2.1 Preparation of Hemoglobin Reagent Solution (HRS)

The HRS solution is prepared following the manufacturer instructions. This solution is prepared from a reagent powder by reconstituting 1 pack of Hemoglobin Reagent Powder in 1-L volumetric flask using 18 MegaOhm-cm DI-water.

HRS is stored in a light protected bottle (amber glass or bottle wrapped in aluminum foil) at room temperature (18-26 ºC) for no longer than 2 years. 1L HRS solution is sufficient for approximately 1,000 samples.

Note: The HRS solution contains 0.5 mM of potassium ferricyanide, 0.7 mM potassium cyanide, buffers, and stabilizers. Clean surface after handling. Remove contaminated clothing and wash before reuse. Minimize dust generation and accumulation. Do not get in eyes, on skin, or on clothing. Do not ingest or inhale. Acids should not be used around sodium cyanide or potassium cyanide unless absolutely necessary and then only after careful planning. Use only with adequate ventilation or respiratory protection.

5.2.2 Reagents Used for Red Blood Cells Digestion

1. 200 mM ammonium bicarbonate, pH 8.5
   Prepare the 200 mM ammonium bicarbonate solution by weighing 31.6 g of NH_4HCO_3, transferring it to a 2,000 ml beaker, and adding DI water (≥18 MegaOhm-cm resistivity) to a total volume of about 1,800 ml. Adjust pH to 8.5 using a pH meter. Transfer to 2L volumetric flask and fill to the mark with DI Water. Store in 10 ml portions at -70 °C in 15 mL falcon tubes. Thaw and check the pH before use.

2. 50 mM acetic acid solution
   Transfer 143 µL of glacial acetic acid (use a 250 µL Hamilton glass syringe) into 50 ml volumetric flask and fill with DI-water to mark. Store the solution at 2-8 °C in a 50 mL falcon tube.

3. Trypsin solution preparation
   Add 120 µL of 50 mM acetic acid to vial containing the lyophilized enzyme (each vial of trypsin from Promega contains 100 µg of enzyme). One vial of enzyme is enough to digest 5 patient samples. If more than one vial of trypsin is required, pool the contents of multiple vials into a cryovial. Prepare a fresh solution immediately before use.

5.2.3 Reagents Used for Analysis of Digested Samples by HPLC/MS/MS

Solvent 1: Methanol with 1% formic acid
Solvent 2: Water with 1% formic acid
Wash Solvent 1: Methanol
Wash Solvent 2: Water
The HPLC mobile solvents are prepared as needed.

5.3 Calibration Materials

5.3.1 Calibrators and Internal Standard Materials

Peptide Calibrators and internal standards, FA-VHLTPEEK and FA-V*HLTPEEK, were custom synthesized and procured from CPC Scientific (Sunnyvale, CA). See Appendix 2 for the structure of peptide analyzed by this method. The peptide purity and peptide content as indicated from the HPLC analysis and amino acid analysis are listed below:

FA Octapeptide (FA-VHLTPEEK)

FW: 963.5 g/mol,
Chemical formula: $C_{43}H_{69}N_{11}O_{14}$
Peptide Purity: 98.6%
Peptide Content: 70%

Labeled FA Octapeptide (FA-V($^{13}C_5$,^{15}N)HLTPEEK):
FW: 969.5 g/mol,
Chemical formula: $C_{38}{}^{13}C_5H69N_{10}{}^{15}N\ O_{14}$
Peptide Purity: 98.6%
Peptide Content: 76%

Protein calibrators may be used in lieu of peptide calibrators to compensate trypsin lot to lots variability. Protein calibrators are made by custom dilutions of a blood pool that was characterized using peptide calibrators for 20 days.

5.3.2 Preparation of Calibrators for FA Adduct Measurement

All glassware in contact with the calibrators must be silanized to avoid loss of analytes by adsorption to the glass.

Pre-screen RBC pools to have an FA Octapeptide concentration measured around 11.5 µmol/L.

Pull desired amount of the pre-screened RBC blood pool from the -70°C freezer and thaw on the rotator. Sonicate volumetric flasks and stir bars in 100% DI water followed by 100% methanol. Then rinse materials with methanol and DI water.

Prepare 300mL of CC0 using volumetric flasks. Prepare calibrator solution for level 1 through 8 using the FA intermediate calibrator CC0 using pre-screen RBC blood pool according to the dilution scheme outlined in Table 1.

Table 1: Example of dilution scheme for FA protein calibrator solutions

Calibrator Lot Number	Amount of RBC from blood pool (mL)	Volume of Saline Added (mL)	Total Volume Prepared (mL)	Final Concentration (nM)
CC0 (intermediate)	120	180	300	4611
A17C01L04	10	190	200	231
A17C02L04	10	90	100	412
A17C03L04	20	80	100	628
A17C04L04	30	70	100	810
A17C05L04	40	60	100	957
A17C06L04	50	50	100	1102
A17C07L04	60	40	100	1240
A17C08L04	80	20	100	1629

Place in the 4°C fridge for short term storage or proceed to aliquot. Aliquot 400 µL of each calibrator level into 2.0mL cryovials and store calibrators at -70°C.

Calibrators are stable for at least 5 years when stored at -70ºC and should not be reused. FA adduct levels for each levels of protein calibrator must be verified by peptide calibrators over 20 days before use for sample analysis.

5.3.3 Preparation of Internal Standard Solutions for Formaldehyde Adducts Measurement

All glassware in contact with the peptide standard must be silanized. Otherwise, losses due to adsorption of the peptides to the glass are observed resulting in inaccurate calibrators.

The internal standard working solution is prepared from an internal standard stock solution which is created from custom synthesized material. If different solutions are used, the preparation procedures need to be adjusted

accordingly. This procedure provides 2000 mL of internal standard working solution sufficient for approx. 38,400 analytical samples (approx. 400 96-well plates).

The internal standard (IS) working solution is prepared from a IS stock solution:

Preparation of Internal Standard Stock Solution "A"

Using an analytical balance, weigh out 2.19 (±0.001) mg of stable isotope-labeled FA-VHLTPEEK onto a clean weighing paper and transfer to a 10 mL volumetric flask. Add ≥18 megaOhm-cm DI water to just below the fill line of the volumetric flasks.

Place flask in 20 ºC water bath for at least 15 minutes to reach 20 ºC and add 18 megaOhm-cm DI water to the fill line. Mix solution. Aliquot solution to a 15-mL falcon tube, label the tube appropriately and store them in the -70 ºC freezer.

Preparation of Internal Standard Working Solution

Transfer 5,550 µL of internal standard stock solution "A" into a 2,000 mL volumetric flask.

Place flask in the 20 ºC water bath for at least 15 minutes to reach 20 ºC and add 18 megaOhm-cm DI water to the fill line. Mix solution. Aliquot solution in 10 mL aliquots (in 15-mL falcon tubes), label tubes appropriately and store them in the -70 ºC freezer.

5.3.4 Preparation of Calibrators for Hemoglobin Measurement

Calibrator solutions are prepared using commercial materials (ACS, Inc). The material is diluted with DI-water following the dilution scheme outlined in Table 2. The volumes need to be adjusted if the undiluted material has a different total hemoglobin concentration than outlined in Table 2.
These calibrators are prepared weekly.

Add 2.5 mL 18 megaOhm-cm DI water to one vial of "Linearity Control". Place on Hematology Mixer for mixing. Dilute the "Linearity Control" with 18 megaOhm-cm DI water, using the dilution scheme shown in Table 2. Label vials appropriately with date, initials, and contents. Note that the total hemoglobin calibration material expires seven days after it is made. Place calibrators in the refrigerator until use.

Table 2: Dilution Scheme for Total Hemoglobin Calibrator Solutions

Calibration Curve Level	Final Concentration of Hb Calibrators Solutions (g/dL)	Volume of Hb Stock Calibrator Solution (µL)	Volume of Water (µL)	Total Volume (µL)
1	20.0 g/dL	800 µL	0 µL	0.800 mL
2	16.0 g/dL	700 µL	175 µL	0.875 mL
3	12.0 g/dL	600 µL	400 µL	1.000 mL
4	8.0 g/dL	400 µL	600 µL	1.000 mL
5	4.0 g/dL	200 µL	800 µL	1.000 mL
6	2.0 g/dL	100 µL	900 µL	1.000 mL
7	1.0 g/dL	50 µL	950 µL	1.000 mL
8	0.5 g/dL	100 µL (of 4.0 g/L Stock)	700 µL	0.800 mL

6 PROCEDURE OPERATION INSTRUCTIONS; CALCULATIONS; INTERPRETATION OF RESULTS

All instruments are assessed for correct function using the manufacturer's acceptance criteria before use. Specific details related to the operation instructions such as file names used in the execution are documented in work instructions.

6.1 Specimen Storage and Handling during Testing

All vials are labeled according to DLS Policies and Procedures Manual and barcodes are scanned during the process of sample preparation, sample transfer, and analysis in order to ensure that individual samples can be tracked throughout the process.

Samples are stored frozen at -70 °C until they are analyzed. Specimens are allowed to reach room temperature by thawing and are mixed on hematology mixer for at least 30 minutes immediately prior to sampling. Residual samples can be reused.

Processed samples ready for analysis by HPLC-MS/MS are stored in the auto-sampler at 4°C and can be stored refrigerated for up to a month.

6.2 Preparation of Samples for Analysis

Each batch of patient samples run with protein calibrators includes the following samples:

- Bench QC samples (low, medium, and high)
- Reagent blank (reagent with IS)
- Matrix blank (without IS)
- A set of protein calibrators (8 levels)

1. Assess all samples for acceptability using the criteria described in previous section.
2. Frozen red blood cell samples, QC samples, internal standard working solutions, and calibrator working solutions (protein) are allowed to reach room temperature on a rotator and are homogenized by placing them on the hematology mixer for 30 minutes.
3. Place all patient samples, QC samples, internal working solution, and calibrator working solution (protein) on the Tecan Freedom Evo-2 Liquid Handler (or Hamiliton MicroLab STARLet) instrument in the designated locations in a manner that allows the instrument's barcode scanner to read all barcodes. Place all additional reagents on the instrument at the designated positions. See work instruction for specific details. Appendix 3 lists reagent volumes needed.
4. Scan all sample vials. When a barcode cannot be read, the instrument software will prompt and will allow manual entry of the barcode information.
5. Pipette 50 µL red blood cell samples and QC samples from cryovials to 96-well 2 mL plates ("*Dilution Plate*") using the Tecan (or Hamilton) instrument. The program will pause with a wait prompt to allow assessing of samples for blood clots. If a blood clot is observed, it is removed by the operator using a wooden application stick. If the blood clot is large, sample is removed from the cell and the same sample is pipetted manually to the plate without blood clots.

After the scanning process is successfully completed, the Tecan (or Hamilton) instrument generates a file with information containing the barcode information, location of the particular sample, calibrator, and reagents on the instrument, and date/time when the scan was performed. This file is transferred to a defined location on the CDC

network and its information is used to create a run sequence for the HPLC-MS/MS instrument and to verify run log sheets.

6.3 Measurement of Total Hemoglobin by Spectrophotometry

Three plates are prepared; all steps are performed on the Tecan automated pipettor (or Hamilton).

- Dilution Plate (50 µL RBC into 950 µL 0.9% Saline solution)
- HRS Plate (50 µL RBC into 950 µL HRS solution)
- Microtiter Plate (*MTP)* (200 µL from "HRS Plate" for measurement)

1. Transfer 950 µL 0.9% saline into "*Dilution Plate*" and mix all sample wells (1:20 DF).
2. Transfer 50 µL diluted sample solution from "*Dilution Plate*" to the "*HRS Plate*".
3. Pipette 50 µL of diluted ACS calibrators and hemoglobin QC controls *Sample Racks* to the "*HRS Plate*".
4. Transfer 950 µL HRS solution into "*HRS Plate*" and mix all wells.
5. Let "HRS Plate" sit for three minutes to complete reaction.
6. Blank Microtiter plate (*MTP).*
7. Transfer 200 µL from *"HRS Plate"* to MTP.
8. Insert the 96-well "*MTP"* with the 200 µL sample solutions in plate reader.
9. Measure the absorbance at 540 nm of the sample solution.
10. Save absorbance reading and calculate total hemoglobin concentration by importing readings in calculation template and by using the calibration curve data obtained with this measurement.

6.4 Calibrators, Internal Standard and Trypsin Digestion

1. Aspirate 50 µL of the diluted samples from "Dilution Plate" into "Digestion Plate".
2. Transfer 50 µL of protein calibrators to the calibrator wells of "Digestion Plate".
3. Transfer 80 µL of 2,2,2-trifluoroethanol (TFE) to all wells of "Digestion Plate".
4. Seal plate with Biosero plate seal using the VITL thermal plate sealer set at 168°C.
5. Incubate the samples for 1 hour in the Thermomixer instrument at 700 rpm at 70 °C.
6. Move plate to Genevac solvent evaporator and evaporate samples for 20 min to remove TFE.
7. Move plate to Tecan instrument and transfer 50 µL of internal standard to all wells except Matrix Blank.
8. Add 80 µL of saline to all samples.
9. Add 80 µL of 200 mM ammonium bicarbonate buffer to all wells.
10. Add 20 µL of trypsin to all wells.
11. Centrifuge the plate to spin down trypsin and mix samples.
12. Cover plate with Biosero plate seal using the VITL thermal plate sealer set at 168°C.
13. Incubate the samples for 48 hours at 48 °C, 700 rpm in a Thermomixer instrument.
14. After incubation, add 10 µL of formic acid to all wells using a glass syringe to stop the digestion.
15. Proceed with HPLC-MS/MS analysis. If samples are not analyzed immediately do not add formic acid. Instead, place plate in -70 °C freezer until ready for analysis. Thaw samples and add 10 µL of formic acid using a glass syringe to all wells immediately prior to analysis.

6.5 Analysis of N-terminal Peptides by HPLC/MS/MS

6.5.1 Creating a Run Sequence

The run sequence is created using the scan file generated by the Tecan instrument (or Hamilton) and includes instrument and other HPLC-MS/MS parameters. The run sequence is then uploaded on the HPLC-MS/MS system. An example of sequence can be found in Appendix 4.

6.5.2 Chromatographic and Mass Spectrometry Conditions

The following HPLC-MS/MS parameters are used:

Chromatography Conditions:

LC System:	Accela Pump 1250 (Thermo Fisher Scientific)
Column:	Luna, 100 x 2.1 mm, 3 µm (Phenomenex)
Column Temperature:	30°C
Sample temperature:	4°C
Injection volume:	10 µl
Wash solvent 1:	Methanol 100
Wash solvent 1 volume:	600 µL
Wash solvent 2:	Water/Methanol 50:50
Wash solvent 2 volume:	200 µL
Solvent A:	Water, 1% formic acid
Solvent B:	Methanol, 1% formic acid
Solvent C:	Not used
Solvent D:	Not used
Gradient:	See table 3

Table 3. HPLC Gradient Pump A

Time (min)	%A	%B	Flow rate ul/min
0	90	10	400
5	60	40	400
6	0	100	600
9	0	100	600
9.1	90	10	400
12	90	10	400

Mass Spectrometry Conditions:

MS Instrument:	TSQ Vantage
Acquisition mode:	Single Reaction Monitoring (SRM)
Acquisition Delay:	1 min
Ionization Mode:	ESI in positive ion mode
Spray Voltage:	4,100 V
ESI Vaporization Temperature:	450 °C
Capillary Temperature:	270 °C
Sheath Gas:	Nitrogen 40 psi
Aux Gas:	Nitrogen12 psi
Q2 Gas Pressure:	1.2 m Torr
Chrom filter:	10
Segment:	1
Scan Width:	0.010
Scan Time:	0.100
Q1 PW:	0.30
Q3 PW:	0.70

S-Lens value: 103
HESI probe position: C
Single Reaction Monitoring (SRM) Conditions; see condition in Table 4.

Table 4: FA SRM Masses (m/z):

Compound	Transition	Q1 mass (m/z)	Q3 mass (m/z)	Collision Energy (V)
FA-VHLTPEEK	Quantitation	482.80	220.99	30
	Confirmation	482.80	716.29	17
FA-V($^{13}C_5$,^{15}N)HLTPEEK	Quantitation	485.80	227.10	28
	Confirmation	485.80	716.29	17

6.5.3 Processing of HPLC-MS/MS Data

1. Transfer data files generated by the HPLC-MS/MS system to the dedicated place on the CDC network.
2. Use a dedicated data processing method within the Xcalibur software to identify relevant chromatographic peaks based on their retention time and integrate the area under the curve. Manual integration may be required if automatic processing fails to integrate the peaks properly.
3. Document integrated peaks as electronic files (in "pdf" format") and save integration results. See Appendix 5 for a typical chromatogram.
4. Import the integration results text file into an MS Excel template where final results are calculated.
5. Review integrations and integration results by the project lead or a designee. Correct detected errors. Only consider data that passed this review process for further processing.

6.5.4 Data Calculations

1. For quality control, calculate area ratios from the quantitation ion and the confirmation ion "Confirmation Ion Ratio". Consider analytes only with a Confirmation Ion Ratio ±20% of the target value for further processing.
2. Calculate area ratios for calculating analyte concentration using the analyte and internal standard area counts.
3. Generate calibration curves with the area ratios from the calibrator samples and their assigned values using unweighted linear regression.
4. Do not process further for sample batches with calibration curves not meeting DLS and laboratory specific quality criteria.
5. Calculate the analyte concentration using the area ratio calculated for the unknown sample and the regression parameters of the corresponding calibration curve.
6. Do not use area ratios for analytes outside the established linear range or to calculate the reportable results. Reanalyze these samples after appropriate dilution or concentration.
7. Normalize the analyte concentration for the amount of total hemoglobin present in the sample solution.

7 CALIBRATION AND CALIBRATION VERIFICATION

7.1 Calibration

7.1.1 Calibration of Instruments and Equipment

All volumetric pipettes are calibrated annually following procedures recommended by the manufacturers and calibration is verified 6 months after calibration. Mass spectrometry instruments are calibrated for mass accuracy regularly as recommended by the manufacturer and following the manufacturer's procedures. Accuracy of other

equipment such as pH-meters are verified regularly according to the manufacturer's recommendation or using established references (i.e., commercial buffer solutions, external thermometers).

7.1.2 Calibration of Measurement

Peptide calibrators used in this measurement procedure were specifically synthesized for this project. Calibration solutions using peptide calibrators are prepared starting with gravimetric measurements as indicated in section 5.5. For Metrological traceability according to ISO 17511 see Appendix 9. Protein calibrators were analyzed and characterized for 20 days using peptide calibrators. Calibrators are analyzed together with each set of samples.

7.2 Calibration Verification

Calibration verification of equipment is performed every six months or earlier when recommended by the manufacturer or as indicated in CLIA '88 §493.1255(b) and ISO 17511 [8].

Mass spectrometry instruments are calibrated for mass accuracy regularly by following the manufacturers' instructions using calibration materials specified by the manufacturer.

Higher order standards or reference materials are currently not available for this analyte. Method accuracy is assessed with method known as accuracy by mixing. This is performed by mixing low and high pools at different volume ratios (25%, 50% and 75%) and recovery is calculated from the expected values versus the measured value.

With each set of samples, 8 levels of calibration material including a blank with IS, a reagent and a matrix blank, along with a low, medium, and high quality control material covering the reported range of FA adducts are analyzed in compliance to DLS Policies and Procedures Manual Section 6.6. Possible shifts in calibration are assessed by comparing bench QC material data against predefined acceptance limits using a SAS software program used and maintained by DLS.

Calibration is further verified by analyzing standards every 6 months and comparing the results obtained against predefined acceptance limits (+/- 15% from target value).

8 METHOD PERFORMANCE CHARACTERISTICS

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

8.1 Analytical Measurement Range and Linearity Limits

Linearity of the method was determined according to Clinical and Laboratory Standard Institute (CLSI) guideline EP6-A [10]. The linear range of the standard calibration curves and the method limit of detection (LOD) determine the reportable range of results. Samples with values exceeding the highest reportable limit may be diluted and reanalyzed so that the measured value will be within the range of the calibration. The low end of the linear range is limited by the method LOD. Samples whose concentrations are below the method LOD are reported as non-detectable.

This method calibration curve is linear for FA-VHLTPEEK in the range 103.1 to 1,091.3 nmol/L. The calibration curve for FA adducts is in the range of 8.25 pmol to 87.30 pmol (0.00829 nmol to 0.0873 nmol). The total hemoglobin is linear in the range of 0.4 g/dL to 20.3 g/dL (0.0002 g to 0.01015 g when using 50 uL sample). The analytical measurement range would translate to 0.82 to 436.6 nmol/g hemoglobin for formaldehyde adducts.

8.2 Limit of Detection

The limit of detection (LOD) was determined using the Taylor Method by diluting the low QC material with bovine hemoglobin. The detection limits determined for FA-VHLTPEEK is 0.67 nmol/g Hb.

8.3 Analytical Specificity

The specificity of the measurement is achieved through the following:

- Comparison of the chromatographic retention times of the analyte with the structurally identical internal standard. The analyte is identified as such when it has the same chromatographic retention time as the internal standard.
- Tandem mass spectrometry is used to identify the quantitation and confirmation ions, which are specific mass-to-charge ratios of the analyte precursor ion and analyte-specific product ions. If the confirmation ion is not present, the compound is considered not identified.
- Assess the ratio of the results using the quantitation and the confirmation ion.
- Measuring the ratio of the confirmation to the quantitation ion area in the calibration curve and comparing this number to those obtained in the analyzed samples.

8.4 Accuracy and Precision

The precision of the method was determined according to CLSI guideline EP5-A2 [10] by analyzing 3 levels of QC materials in duplicate on 20 different days, and in addition, according to the DLS Policy and Procedure Manual by analyzing 3 levels of bench QC materials in duplicate on 10 different days. The evaluation includes within-run and between-run and total precision assessments (see Table 5). Total precision using the DLS method for three levels of material did not exceed 15% relative standard deviation (coefficient of variation (CV). Please refer to Appendix A for more details. The precision of the method is reflected in the variance of QC samples with three different concentration levels analyzed over time. Records of the method precision are maintained in the quality assurance (QA) binder. The evaluation of within- and among-run imprecision using both methods are similar. The concentration of the QC ranges are applicable to the method.

Table 5: Method Precision according to DLS Policy and Procedure Manual

	QC Low	QC Medium	QC High
Within Run (Std Dev)	6.68	9.81	9.19
Between Run Precision N=20 (Std Dev)	6.04	4.32	2.51
Total Rel Std Dev (%)	13.42	11.23	9.23

The accuracy of the measurement was determined according to CLSI guideline EP15-A2 [12]. Accuracy can be assessed through a method known as accuracy by mixing. This is performed by mixing low and high pools at different volume ratios (25%, 50% and 75%) and recovery is calculated from the expected values versus the measured value. Recovery of the analyte is calculated as percent recovery and compared to acceptable levels as outlined FDA guideline for biomedical tests (15% recovery, 20% recovery at LLOQ).

Percent recoveries ranged from 95.5% to 99.5% over two days for an average of 97.0%. The average coefficient of variation was 6.1% (see Table 6).

Table 6: Method Accuracy by Mixing

FA-VHLPEEK	Mean Recovery (%)	CV (%)
Day 1	94.5	5.7
Day 2	99.5	6.6
Average	97.0	6.1

8.5 Limitations of Method; Interfering Substances and Conditions

No known interference exist for this analyte measured with the described method.

8.6 Method Ruggedness

Variability of the digest buffer pH and concentration, digestion time, amount of total hemoglobin used for digestion, and the amount of enzyme used for digestion were assessed. The following results were obtained:

1. pH of the Buffer Determination: The pH of the ammonium bicarbonate buffer as described in Section 5.2.2 should be within the range of pH 8.0 -8.5.
2. Buffer Concentration Determination: The concentration of ammonium bicarbonate as described in Section 5.2.2 should be 200 mM.
3. Hemoglobin Digestion Time Determination: 48 $\pm$ 2 hour digestion is necessary to reach consistent digestion of Hb samples.
4. Hemoglobin Digest Concentration Determination: hemoglobin concentration at 1:10 dilution should be avoided and Hemoglobin concentration at 1:20 dilution can be used.
5. Enzyme Amount Determination: Trypsin amount should be 12.5-20.0 µg of trypsin per sample.

8.7 Stability of the Analyte

The stability of the analytes was evaluated by measuring low and high QC pools and protein calibrator stock solutions at time of preparation and after storage at different temperatures (-70 °C for 2 years, 20 °C for 4 hours, and 4 °C for 48 hours). The stability of the analytes in processed samples at 4 °C was evaluated at time of preparation and storage up to 20 days. Data for stability are shown in Appendix A Table 10.

9 QUALITY ASSESSMENT AND PROFICIENCY TESTING

9.1 Quality Control Procedures

9.1.1 Quality Control Materials

Bench QC Materials

The bench QC pools used in this method comprise three levels of concentration spanning the “low-normal” (64.70 nmol/g hemoglobin), medium (90.86 nmol/g hemoglobin) and “high-normal” (104.88 nmol/g hemoglobin ranges) of the analyte of interest:

The QC pool is prepared by selecting red blood cells from individuals with adduct values with the desired concentration. Bench QC samples are aliquoted into 2.0 mL cryovials and stored at -70 ºC.

The quality control materials for total hemoglobin measurements provided by the manufacturer are used and assessed according to the manufacturer’s recommendations.

9.1.2 Assessment of Runs Using QC Materials

The results from the QC pools are assessed after each run. Acceptance criteria for values obtained with the bench QC materials "QC limits" are established according to the procedure described by Caudill et al. [13] and DLS PM Section 6.

The measurements are declared “in control” if all three QC results are within 2s_i limits.
If one of the three QC results is outside the 2S_i limits then apply the rules below and reject if any condition is met - the run is then declared “out of control”:

(a) 1 3S Rule—Run mean is outside a 3 S_i limit
(b) 2 2S Rule—Two or more of the three run results are outside the same 2 S_i limit
(c) 10 Xbar Rule—Current and previous nine run results are on the same side of the characterization mean
(d) R 4S Rule—two consecutive standardized* run results differ by more than 4 S_i (i.e. 95 percent range limit).

* Standardized results are used because different SLQIDs have different means.

Note: Since runs have a single results per SLQCID for 3 SLQCIDs, comparison of results for the R 4S rule will be with the previous result within the current run or with the last result of the previous run.

A QC program written in SAS is available from the DLS Quality Assurance Officer and should be used to apply these rules to QC data and generate Shewhart QC charts. No results for a given analyte are to be reported from an analytical run that has been declared “out of control” for that analyte as assessed by internal (bench) QC.

The initial limits of the QC materials are established by analyzing pool material in 20 consecutive runs. When necessary, limits are updated to include more runs.

While a study is in progress, electronic copies of the QC results from each run are stored in the analyte-specific folder. Electronic copies of the tracking of the QC results over time are stored in the analyte-specific folder. A hardcopy of the QC results from each run is also kept by the person responsible for data checking.

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

If the calibration or QC assessments fails, all operations are suspended until the source or cause of failure is identified and corrected. Analytical results are not reported. After calibration and /or quality control have been reestablished, analytical runs may be resumed.

9.2 Proficiency Testing

There are no external proficiency testing (PT) materials available for the analysis of FA-VHLTPEEK. Therefore, the PT scheme for this method is administered by an in-house PT Coordinator as described in the guideline of the Clinical Laboratory Standards Institute GP29-P [9]. In this procedure, five PT pools spanning the full range of analyte values likely to be encountered in human specimens are prepared in-house and characterized by measuring 20 separate vials from each pool in at least 10 different runs. The means and standard deviations are obtained from these measurements.

PT challenges will be performed twice per year (once every 6 months) where the PT Coordinator will randomly select 5 vials for use in a particular proficiency testing challenge.

When these PT samples have been analyzed, the results will be given to the PT Coordinator, who will assess the data to see if at least 4 of the 5 results for each analyte are within the set limits (80% is considered passing as described in the CLIA regulations). If fewer than 4 of the 5 proficiency testing samples are within the limits for a given analyte, the challenge is considered as failed: no patient samples are to be analyzed and appropriate actions to correct this problem need to be initiated. Analysis of patient samples can resume after the problem was corrected and another PT challenge passed successfully.

The QC materials for tHb measurements provided by the assay manufacturer are used and assessed according to the manufacturer's recommendations. An in-house characterization is also performed to assess the manufacture's values. Duplicate measurements for at least 20 consecutive runs are established and means and plus ranges are calculated. If results vary from the manufacture's values, the in-house characterized values are assigned as total Hb limits.

10 REFERENCE RANGES (NORMAL VALUES)

Population-based reference ranges have not been established. An in-house assessment using convenience samples from 135 individuals (adult men and women) found FA adducts in all samples with the following values:

Mean (range): 104.8 nmol/g Hb (59.27- 130.57 nmol/g Hb)

11 TEST RESULT REPORTING SYSTEM

The test reporting system as described in the most recent version of the DLS Policies and Procedures Manual is used when reporting test results. The system consists of review steps at multiple levels such, as results verification by a DLS statistician and DLS management.

Results are reported to 3 significant digits based on assay sensitivity calculations. Data are reported in nmol/g Hb.

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

Because the analysis of hemoglobin adducts of FA is inherently complex and challenging, there are no acceptable alternative methods of analysis in the laboratory. If the analytical system fails or if the QC systems or the calibrations fails to meet acceptable criteria, all operations are suspended until the source or cause of failure is identified and corrected. We recommend that the specimens be stored at -70°C until the system is restored to functionality and checked for any irregularities (i.e., low calibration curve regression, change in slope or intercept, high blank concentration, low internal standard sensitivity).

If the source of failure is easily identifiable, for instance, a pipetting error, the problem is immediately corrected. Otherwise, fresh reagents are prepared and the mass spectrometer is cleaned. Before beginning another analytical run, several QC materials (in the case of QC failure) or calibration standards (in the case of calibration failure) are reanalyzed. After system, calibration, or quality control has been reestablished, analytical runs may be resumed.

13 PROCEDURE FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Following successful completion of analysis, remaining samples will be retained until all results have been reported and sufficient time has passed for review of the results. After this time, samples are either returned to the contact person who requested the analysis or are treated according to DLS and CDC policy.

Standard record keeping (e.g., database, notebooks, and data files) is used to track specimens. Records (including related QA/QC data) are maintained for 3 years, and duplicate records are kept off-site in electronic format. Study subject confidentiality is protected by providing personal identifiers only to the medical officer if needed or remain with the contact person who requested the analyses.

14 TRANSFER OR REFERRAL OF SPECIMENS

Transfer or referral of specimens will follow the procedures outlined in the most recent version of the DLS Policies and Procedures Manual.

15 CRITICAL CALL RESULTS (“PANIC VALUES”); PROTOCOL FOR REPORTING CRITICAL CALLS

Currently, no information is available linking hemoglobin FA adduct data to certain health outcomes.

16 PROCEDURES FOR MICROSCOPIC EXAMINATIONS; CRITERIA FOR REJECTING INADEQUATELY PREPARED SLIDES

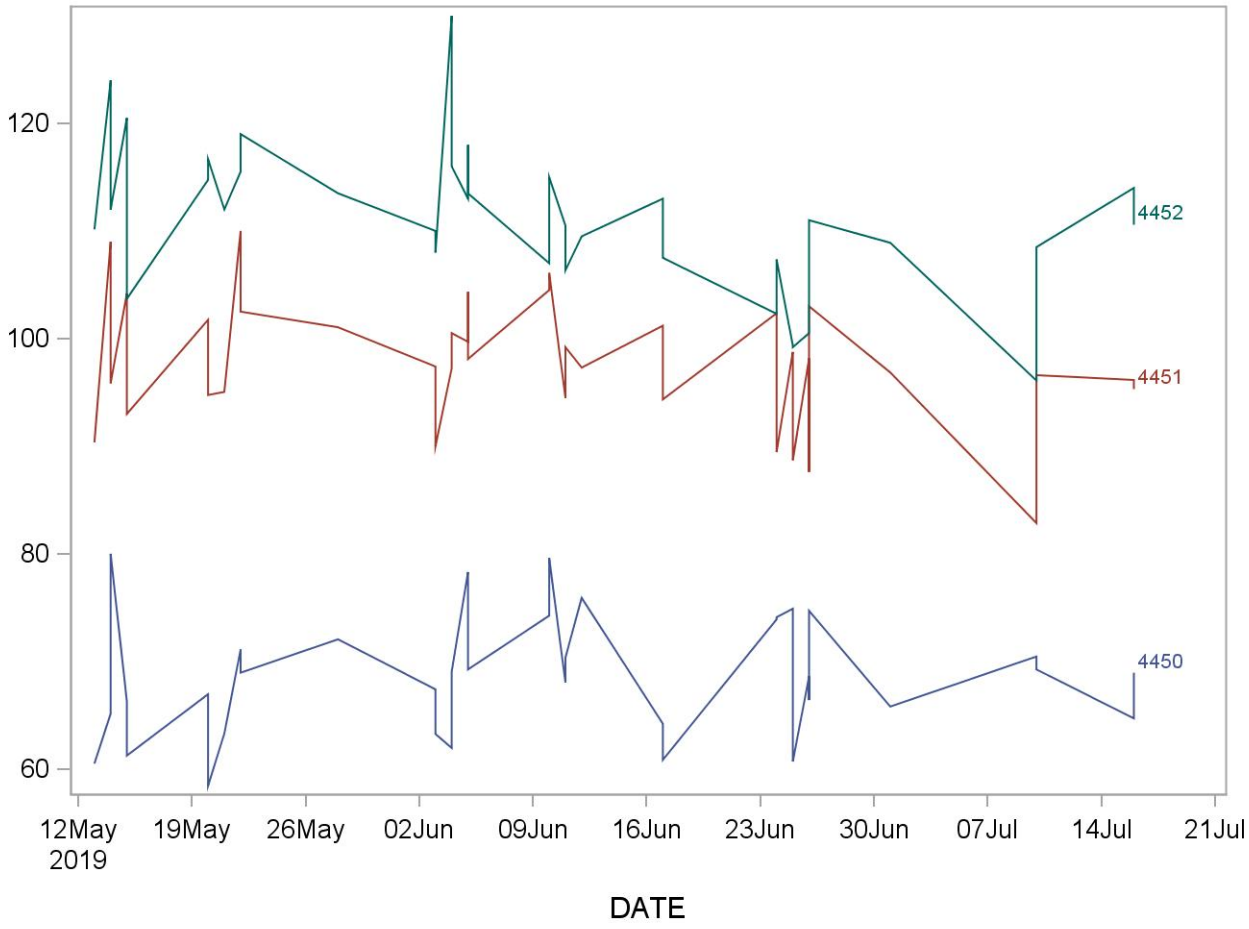
Not applicable for this procedure.

17 SUMMARY STATISTICS AND QC GRAPHS

See following pages

2015-2016 Summary Statistics and QC Chart for Formaldehyde (nmol/g HB)

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
4450	37	13MAY19	16JUL19	68.654	5.564	8.1
4451	37	13MAY19	16JUL19	97.776	5.936	6.1
4452	37	13MAY19	16JUL19	110.834	6.959	6.3



18 REFERENCES

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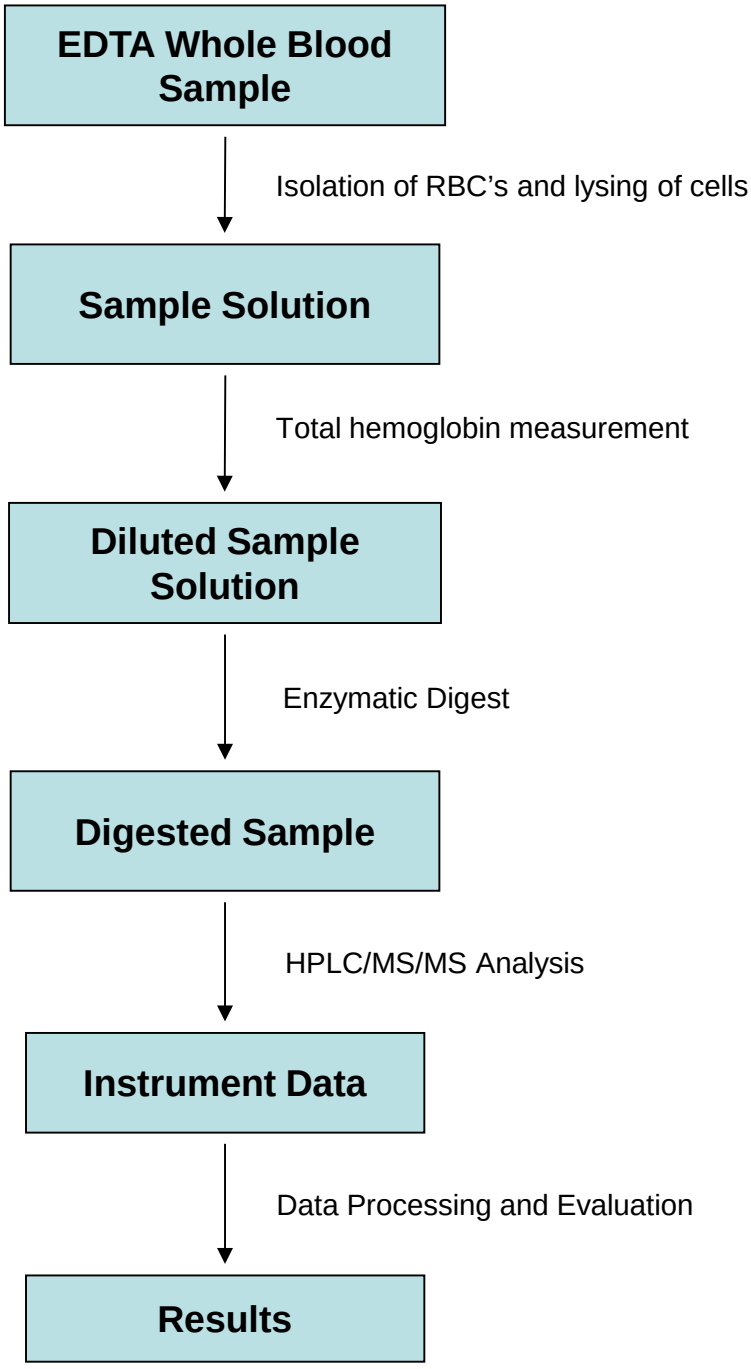
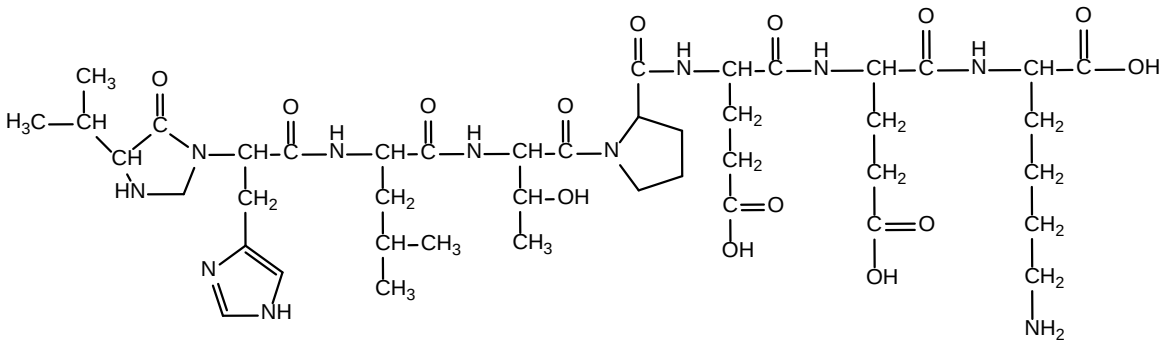
19 APPENDICES

Appendix 1: Flow Chart Describing Measurement Procedure for FA Hemoglobin Adducts



Appendix 2. Structure of Peptide Analyzed by this Method



FA-VHLTPEEK
$C_{43}H_{69}N_{11}O_{14}$
Exact Mass: 963.50
Mol. Wt.: 964.07

Appendix 3. Approximate amount of reagents needed for one plate

Reagent	Amount (using Peptide Calibrators)	Amount (using Protein Calibrators)
HRS Solution	96 mL	96 mL
Internal Standard	5 mL	5 mL
Calibrators	80 µL each calibrator level	50 µL each calibrator level
Ammonium Bicarbonate	8 mL	8 mL
Trypsin	20 vials of 100 µg	20 vials of 100 µg
2,2,2-trifluoroethanol (TFE)	8 mL	8 mL
Formic acid	1 mL	1 mL

Appendix 4. Example of Analytical Sequence

Bracket Type=4								
Sample Type	File Name	Sample Name	Sample ID	Position	Path	Instrument Method	Process Method	Inj Vol
Unknown	001_20150120_P_0041	20150120_Water1	20150120_Water1	CStk1-03:96	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	003_20150120_P_0041	20150120_MSTest_338	20150120_MSTest_338	CStk1-03:84	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	005_20150120_P_0041	20150120_Water2	20150120_Water2	CStk1-03:96	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	007_20150120_P_0041	20150120_Blank	20150120_CStk1-04:1	CStk1-04:1	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	009_20150120_P_0041	20150120_Cal A	20150120_CStk1-04:13	CStk1-04:13	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	011_20150120_P_0041	20150120_Cal B	20150120_CStk1-04:25	CStk1-04:25	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	013_20150120_P_0041	20150120_Cal C	20150120_CStk1-04:37	CStk1-04:37	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	015_20150120_P_0041	20150120_Cal D	20150120_CStk1-04:49	CStk1-04:49	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	017_20150120_P_0041	20150120_Cal E	20150120_CStk1-04:61	CStk1-04:61	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	019_20150120_P_0041	20150120_Cal F	20150120_CStk1-04:73	CStk1-04:73	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	021_20150120_P_0041	20150120_Cal G	20150120_CStk1-04:85	CStk1-04:85	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	023_20150120_P_0041	20150120_Water3	20150120_Water3	CStk1-03:96	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	025_20150120_P_0041	20150120_Cal H	20150120_CStk1-04:2	CStk1-04:2	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	027_20150120_P_0041	20150120_Reagent Blank	20150120_CStk1-04:14	CStk1-04:14	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	029_20150120_P_0041	20150120_Matrix Blank	20150120_CStk1-04:26	CStk1-04:26	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	031_20150120_P_0041	20150120_Bovine RBC	20150120_CStk1-04:38	CStk1-04:38	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	033_20150120_P_0041	20150120_8320001RA	20150120_CStk1-04:50	CStk1-04:50	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	035_20150120_P_0041	20150120_8320002RA	20150120_CStk1-04:62	CStk1-04:62	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	037_20150120_P_0041	20150120_8390174RA	20150120_CStk1-04:74	CStk1-04:74	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	039_20150120_P_0041	20150120_8390175RA	20150120_CStk1-04:86	CStk1-04:86	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	041_20150120_P_0041	20150120_Water4	20150120_Water4	CStk1-03:96	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	043_20150120_P_0041	20150120_8400003RA	20150120_CStk1-04:3	CStk1-04:3	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	045_20150120_P_0041	20150120_8400004RA	20150120_CStk1-04:15	CStk1-04:15	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	047_20150120_P_0041	20150120_2229852RD	20150120_CStk1-04:27	CStk1-04:27	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	049_20150120_P_0041	20150120_2229852RD	20150120_CStk1-04:39	CStk1-04:39	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	051_20150120_P_0041	20150120_2229858RD	20150120_CStk1-04:51	CStk1-04:51	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	053_20150120_P_0041	20150120_2229858RD	20150120_CStk1-04:63	CStk1-04:63	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	055_20150120_P_0041	20150120_2229861RD	20150120_CStk1-04:75	CStk1-04:75	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	057_20150120_P_0041	20150120_2229861RD	20150120_CStk1-04:87	CStk1-04:87	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	059_20150120_P_0041	20150120_Water5	20150120_Water5	CStk1-03:96	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	061_20150120_P_0041	20150120_2229872RD	20150120_CStk1-04:4	CStk1-04:4	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	063_20150120_P_0041	20150120_2229872RD	20150120_CStk1-04:16	CStk1-04:16	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	065_20150120_P_0041	20150120_2229874RD	20150120_CStk1-04:28	CStk1-04:28	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	067_20150120_P_0041	20150120_2229874RD	20150120_CStk1-04:40	CStk1-04:40	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	069_20150120_P_0041	20150120_2229101RD	20150120_CStk1-04:52	CStk1-04:52	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	071_20150120_P_0041	20150120_2229101RD	20150120_CStk1-04:64	CStk1-04:64	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	073_20150120_P_0041	20150120_2229202RD	20150120_CStk1-04:76	CStk1-04:76	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	075_20150120_P_0041	20150120_2229202RD	20150120_CStk1-04:88	CStk1-04:88	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	077_20150120_P_0041	20150120_Water6	20150120_Water6	CStk1-03:96	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	079_20150120_P_0041	20150120_2229303RD	20150120_CStk1-04:5	CStk1-04:5	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	081_20150120_P_0041	20150120_2229303RD	20150120_CStk1-04:17	CStk1-04:17	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	083_20150120_P_0041	20150120_2229404RD	20150120_CStk1-04:29	CStk1-04:29	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	085_20150120_P_0041	20150120_2229404RD	20150120_CStk1-04:41	CStk1-04:41	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	087_20150120_P_0041	20150120_2229505RD	20150120_CStk1-04:53	CStk1-04:53	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	089_20150120_P_0041	20150120_2229505RD	20150120_CStk1-04:65	CStk1-04:65	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	091_20150120_P_0041	20150120_2229606RD	20150120_CStk1-04:77	CStk1-04:77	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	093_20150120_P_0041	20150120_2229606RD	20150120_CStk1-04:89	CStk1-04:89	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	095_20150120_P_0041	20150120_Water7	20150120_Water7	CStk1-03:96	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	097_20150120_P_0041	20150120_+019213RD	20150120_CStk1-04:6	CStk1-04:6	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	099_20150120_P_0041	20150120_+019213RD	20150120_CStk1-04:18	CStk1-04:18	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	101_20150120_P_0041	20150120_BQC2	20150120_CStk1-04:30	CStk1-04:30	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	103_20150120_P_0041	20150120_BQC2	20150120_CStk1-04:42	CStk1-04:42	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	105_20150120_P_0041	20150120_+037215RA	20150120_CStk1-04:54	CStk1-04:54	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	107_20150120_P_0041	20150120_+037215RA	20150120_CStk1-04:66	CStk1-04:66	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	221_20150120_P_0041	20150120_Water14	20150120_Water14	CStk1-03:96	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	223_20150120_P_0041	20150120_MSTest_338	20150120_MSTest_338	CStk1-03:84	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	225_20150120_P_0041	20150120_Water15	20150120_Water15	CStk1-03:96	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	227_20150120_P_0041	20150120_Blank	20150120_CStk1-04:1	CStk1-04:1	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	229_20150120_P_0041	20150120_Cal A	20150120_CStk1-04:13	CStk1-04:13	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
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Unknown	233_20150120_P_0041	20150120_Cal C	20150120_CStk1-04:37	CStk1-04:37	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	235_20150120_P_0041	20150120_Cal D	20150120_CStk1-04:49	CStk1-04:49	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
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Unknown	241_20150120_P_0041	20150120_Cal G	20150120_CStk1-04:85	CStk1-04:85	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
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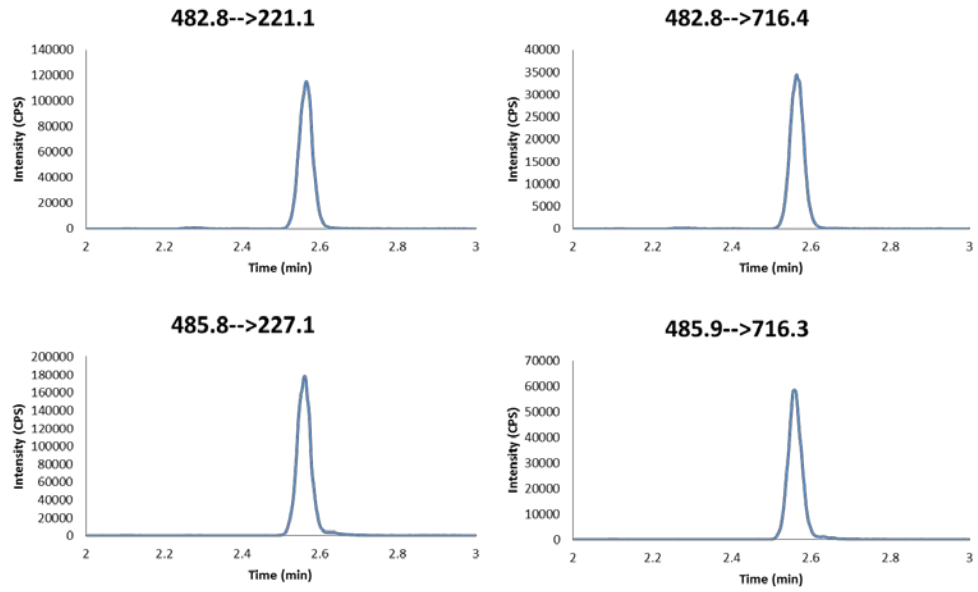
Unknown	249_20150120_P_0041	20150120_MSTest_338	20150120_MSTest_338	CStk1-03:84	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10
Unknown	251_20150120_P_0041	20150120_Water15	20150120_Water15	CStk1-03:96	Z:_FA METHOD\LAB\DATA\2015\01_15\20150120_P_0041_PT_CT_TF	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\FA_method_Luna_opti_newtran_fa-vhltpeek_082814	Z:_FA Method\Lab\Data\2015\01_15\20150120_P_0041_PT_CT_TF\20140829_FA_FAIS_CT	10

Appendix 5. Typical Chromatograms

Low QC



Appendix 6. Related Documents

1. DLS Policies and Procedures Manual:

   http://intranet.cdc.gov/nceh-atsdr/dls/pdf/05/DLS_Policies_and_Procedures_Manual.pdf

2. NCEH/DLS Online Safety Manual:

   http://intranet.cdc.gov/nceh-atsdr/dls/safety_manual/index.html

3. Clinical Laboratory Improvement Amendments of 1988 (CLIA). 42CFR493 from February 28, 1992.

Appendix 7. Document Compliance Tables

CLIA	DLS P&PM Sec 8.2	This document
Requirements for patient preparation specimen collection, labeling, storage, preservation, transportation, processing, and referral Criteria for specimen acceptability and rejection	requirements for specimen collection and processing, including criteria for specimen rejection specimen storage criteria	Sec 4 4.1 General Specimen Requirements 4.2 Specimen Storage 4.3 Unacceptable Specimens
Microscopic examination, including the detection of inadequately prepared slides	N/A	N/A
Step-by-step performance of the procedure, including test calculations and interpretation of results	step-by-step performance of the procedure, including test calculations and interpretation of results	Sec 6
Preparation of solutions, calibrators, controls, reagents, stains, and other materials used in testing.	preparation of reagents, calibrators, controls, solutions and other materials used in testing	Sec 5
Calibration and calibration verification procedures	calibration and calibration verification procedures	Sec 7
Reportable range for test results for the test system as established or verified	the reportable range for patient test results	Sec 8
Control procedures	quality control procedures, including PT materials and programs/procedures used	Sec 9 9.1 Quality Control Procedures 9.2 Proficiency Testing
Corrective action to take when calibration or control results fail to meet the laboratory's criteria for acceptability	remedial action to be taken when calibration or control results are outside acceptable limits	Sec 9 9.1.3 Remedial Action if Calibration or QC Systems Fail to Meet Acceptable Criteria
Limitations in the test methodology, including interfering substances.	limitation in methods, including interfering substances	Sec 8 8.5 Limitations of Method, Interfering Substances and Conditions
Reference intervals (normal values)	reference range (normal values)	Sec 10
Imminently life-threatening test results or panic or alert values.	life-threatening or "panic values" protocol for reporting panic values	Sec 15
Pertinent literature references	pertinent literature references	Sec 17
	course of action if test system becomes inoperable	Sec. 12
	criteria for referral of specimens (usually not needed)	N/A
	safety considerations for performing the method	Sec 2.
		Sec 1 Summary test principle and clinical relevance

Appendix 8. Symbols, Abbreviations, Terminology

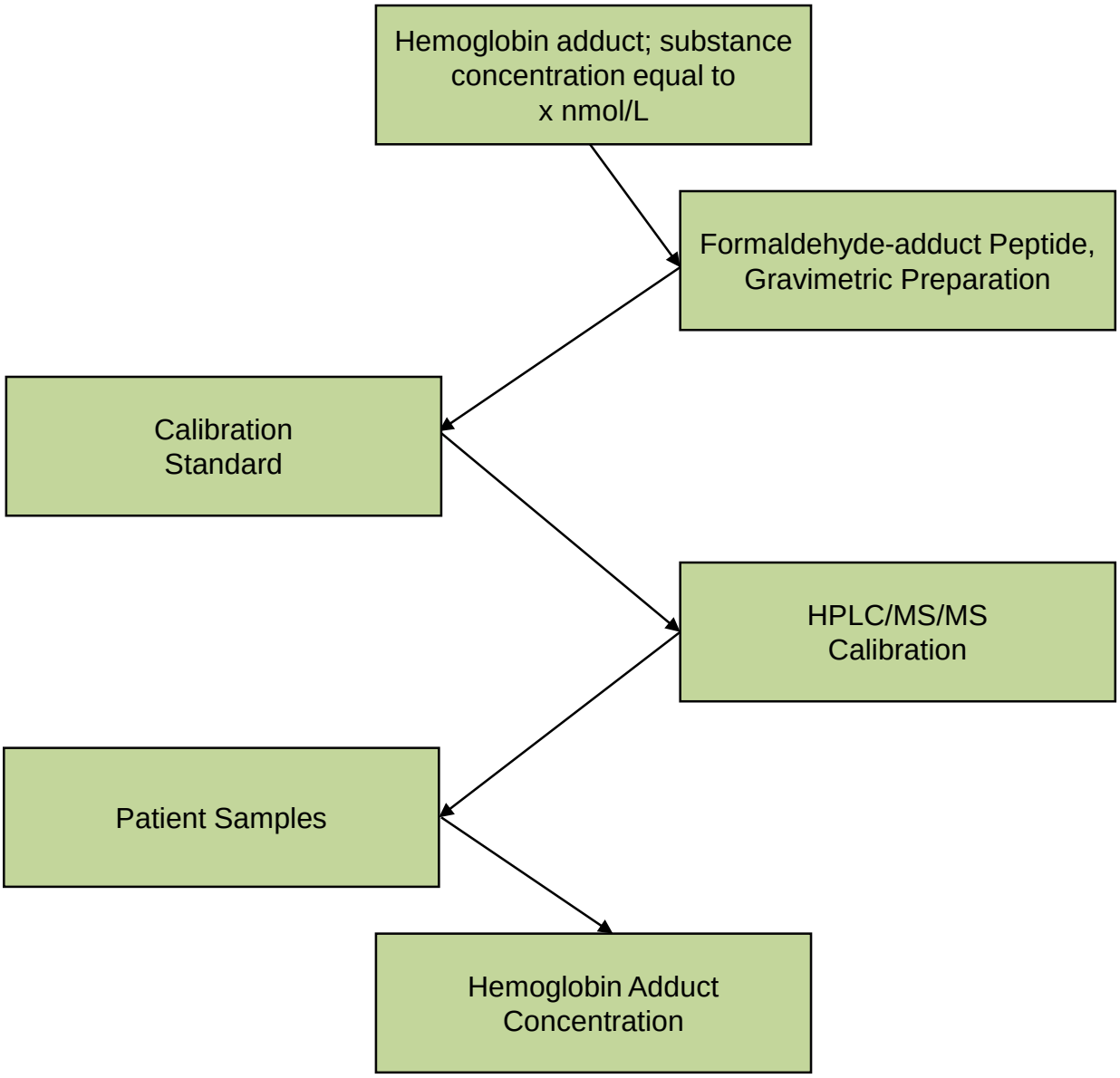
^{13}C	Labeled carbon (5) isotope
^{15}N	Labeled nitrogen (1) isotope
CC	Calibration Curve
CCB	Clinical Chemistry Branch
CDC	Centers for Disease Control and Prevention
CHP	Chemical Hygiene Plan
CLIA	Clinical Laboratory Improvement Act/Amendment
CLSI	Clinical and Laboratory Standards Institute
CV	Coefficient of Variant
DI	De-ionized
dL	Deciliter
DLS	Division of Laboratory Sciences
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
FA	Formaldehyde
FDA	Food and Drug Administration
g	Grams
Hb	Hemoglobin
HC	HemoCue
HIV	*Human Immunodeficiency Virus*
HPLC	*High Performance Liquid Chromatography*
HRS	Hemoglobin Reagent Set
Hz	Hertz
IARC	International Agency for Research on Cancer
ID	Identifier
IS	Internal Standards
ISO	International Organization for Standardization
ITSO	Information Technology Service Office
L	Liter
LC/MS/MS	Liquid Chromatography/Tandem Mass Spectrometry
LOD	Limit of Detection
LOQ	Limit of Quantitation
mg	Milligram
mL	Milliliter
mm	Millimeter
MSDS	Material Safety Data Sheets
MTP	Microtiter Plate
N/A	Not Applicable
nmol	Nanomol
NCEH	National Center of Environmental Health
NHANES	National Health and Nutrition Examination Survey
nm	Nanometer
OHS	Occupational Health and Safety
OSHA	Occupational Safety and Health Administration
PBL	Protein Biomarker Laboratory
pH	Negative Logarithm of the Molar Concentration of Dissolved Hydronium Ions
PM	Preventative Maintenance
pmol	Picomole
psi	Pounds Per Square Inch
PT	Proficiency Testing
Q0-Q3	Quadrupole Mass Analyzers
QA	Quality Assurance
QC	Quality Control
RBC	Red Blood Cells
RPM	Revolutions Per Minute

SAS	Statistical Analysis Software
SD	Standard Deviation
SRM	Selected Reaction Monitoring
tHB	Total Hemoglobin
TFE	2,2,2-trifluoroethanol
µmol	Micromole
v/v	Volume concentration
VHLTPEEK	Peptide (Valine, Histidine, Leucine, Threonine, Proline, Glutamic Acid, Glutamic Acid, Lysine)
WI	Work Instructions

Terminology

The terminology defined in CLIA '88 (57 FR 7139 Subpart A Sec. 493.2) is used in this document. Otherwise the terminology described in the Clinical and Laboratory Standards Institute's terminology database was used. The database can be accessed at:
http://clsi.org/standards/harmonized-database/

Appendix 9: Metrological Traceability of FA Measurements



Appendix 10: List of Tables

Appendix **A: Performance of the Method**

Table 7: LOD, specificity and fit for intended use

Analytes	Limit of Detection (LOD)	Interferences successfully checked in at least 50 human samples	Accuracy, precision, LOD, specificity and stability meet performance specifications for intended use
FA-VHLTPEEK	0.67 nmol/g	yes	yes

Table 8: Accuracy by Mixing

FA-VHLTPEEK	Replicate	Expected nmol/g	Day 1 nmol/g	Mean nmol/g	Recovery (%)	Expected nmol/g	Day 2 nmol/g	Mean nmol/g	Recovery (%)
Low QC Pool	1	N/A	38.19	37.7	N/A	N/A	35.75	40.5	N/A
	2		35.65				39.42		
	3		38.43				40.09		
	4		38.06				43.80		
	5		38.01				43.26		
High QC Pool	1	N/A	60.08	65.9	N/A	N/A	67.89	71.0	N/A
	2		63.58				68.48		
	3		65.99				70.82		
	4		70.59				71.25		
	5		69.32				76.81		
Mix 1 75% Low Pool 25% High Pool	1	44.73	37.60	42.5	95.02	48.11	40.79	45.4	94.40
	2		42.30				45.53		
	3		43.53				45.97		
	4		45.43				46.86		
	5		43.65				47.92		
Mix 2 50% Low Pool 50% High Pool	1	51.79	43.72	48.1	92.96	55.76	48.97	55.1	98.86
	2		48.54				57.25		
	3		50.08				56.20		
	4		48.33				55.38		
	5		50.05				57.80		
Mix 3 25% Low Pool 75% High Pool	1	58.85	51.89	56.2	95.58	63.40	61.41	66.7	105.28
	2		55.70				64.26		
	3		57.13				67.36		
	4		57.74				66.43		
	5		58.78				74.27		
				Day 1 Mean Recovery (%)	94.5	Day 2 Mean Recovery (%)	99.5	Total Mean Recovery (%)	97.0

Table 9: Formaldehyde Method Precision according to the DLS Policy and Procedure Manual

FA-VHLTPEEK						
Quality material 1						
Run	Result 1 nmol/g	Result 2 nmol/g	Mean nmol/g	SS 1	SS 2	2*mean^2
1	59.36	54.19	56.77	6.68	6.68	6446.68
2	61.14	56.20	58.67	6.09	6.09	6884.26
3	74.55	53.32	63.94	112.66	112.66	8176.37
4	77.15	81.98	79.57	5.83	5.83	12661.49
5	70.17	69.90	70.03	0.02	0.02	9808.83
6	62.35	59.01	60.68	2.78	2.78	7364.49
7	63.94	66.96	65.45	2.28	2.28	8566.74
8	85.14	66.57	75.85	86.24	86.24	11507.04
9	74.83	74.08	74.45	0.14	0.14	11086.17
10	66.05	65.02	65.53	0.27	0.27	8589.18
Grand sum	1341.89	Grand mean	67.09			
			Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
		Within Run	445.99	44.60	6.68	9.95
		Between Run	1057.41	117.49	6.04	9.00
		Total	1503.40		9.00	13.42
Quality material 2						
Run	Result 1 nmol/g	Result 2 nmol/g	Mean nmol/g	SS 1	SS 2	2*mean^2
1	78.40	88.87	83.64	27.40	27.40	13990.98
2	82.74	84.65	83.69	0.92	0.92	14009.25
3	113.37	94.82	104.09	86.10	86.10	21671.48
4	96.91	93.35	95.13	3.16	3.16	18099.05
5	104.05	82.88	93.47	112.02	112.02	17472.84
6	102.48	84.35	93.41	82.11	82.11	17452.59
7	114.88	103.98	109.43	29.68	29.68	23949.57
8	109.30	85.85	97.57	137.45	137.45	19041.72
9	100.71	101.38	101.04	0.11	0.11	20419.02
10	93.78	91.11	92.44	1.78	1.78	17091.29
Grand sum	1907.86	Grand mean	95.39			
			Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
		Within Run	961.46	96.15	9.81	10.28
		Between Run	1201.67	133.52	4.32	4.53
		Total	2163.13		10.72	11.23
Quality material 3						
Run	Result 1 nmol/g	Result 2 nmol/g	Mean nmol/g	SS 1	SS 2	2*mean^2
1	92.71	99.10	95.90	10.22	10.22	18394.67
2	92.08	88.11	90.10	3.96	3.96	16234.65
3	115.15	112.27	113.71	2.07	2.07	25859.79
4	108.19	100.56	104.38	14.54	14.54	21788.34
5	121.55	96.35	108.95	158.76	158.76	23740.95
6	111.37	99.54	105.46	34.98	34.98	22241.90
7	116.18	103.69	109.94	39.06	39.06	24171.48
8	113.37	89.56	101.46	141.72	141.72	20588.57
9	97.44	101.72	99.58	4.58	4.58	19830.80
10	106.23	99.28	102.76	12.08	12.08	21117.95
Grand sum	2064.44	Grand mean	103.22			
			Sum squares	Mean Sq Error	Std Dev	Rel Std Dev (%)
		Within Run	843.94	84.39	9.19	8.90
		Between Run	873.35	97.04	2.51	2.44
		Total	1717.29		9.52	9.23

Table 10: Formaldehyde Quality Control Sample Stability

FA-VHLTPEEK nmol/g tHb						
Quality Material 1	A17QC1L02	Initial measurement	Freeze/Thaw Cycles	Bench-top stability	Short-Term Storage	Long-Term Storage
Replicate 1		60.87	55.79	67.73	67.73	61.86
Replicate 2		59.87	59.99	64.67	64.67	56.64
Replicate 3		59.53	56.94	66.64	66.64	54.88
QC Char Fixed Mean	60.33	60.09	57.57	66.35	66.35	57.79
% difference from QC Char Fixed Mean		0.4	4.6	10.0	10.0	4.2
% difference from the initial measurement			4.2	10.4	10.4	3.8
Quality Material 2	A17QC2L02	Initial measurement	Freeze/Thaw Cycles	Bench-top stability	Short-Term Storage	Long-Term Storage
Replicate 1		80.53	84.59	92.11	92.11	75.93
Replicate 2		86.29	83.18	93.44	93.44	82.10
Replicate 3		77.25	85.61	94.08	94.08	77.27
QC Char Fixed Mean	84.74	81.36	84.46	93.21	93.21	78.43
% difference from QC Char Fixed Mean		4.0	0.3	10.0	10.0	7.4
% difference from the initial measurement			3.8	14.6	14.6	3.6
Quality Material 3	A17QC3L02	Initial measurement	Freeze/Thaw Cycles	Bench-top stability	Short-Term Storage	Long-Term Storage
Replicate 1		96.27	97.56	110.19	110.19	95.45
Replicate 2		97.16	97.79	104.96	104.96	81.18
Replicate 3		96.23	97.35	109.28	109.28	93.40
QC Char Fixed Mean	105.64	96.55	97.57	108.15	108.15	90.01
% difference from QC Char Fixed Mean		8.6	7.6	2.4	2.4	14.8
% difference from the initial measurement			1.1	12.0	12.0	6.8

FA-VHLTPEEK nmol/g tHb (Processed Samples)			
Quality Material 1	A17QC1L02	Initial measurement	Processed sample stability
Replicate 1		60.87	61.79
Replicate 2		59.87	57.80
Replicate 3		59.53	57.61
QC Char Fixed Mean	60.33	60.09	59.06
% difference from QC Char Fixed Mean		0.4	2.1
% difference from the initial measurement			1.7
Quality Material 2	A17QC2L02	Initial measurement	Processed sample stability
Replicate 1		80.53	76.91
Replicate 2		86.29	81.44
Replicate 3		77.25	75.35
QC Char Fixed Mean	84.74	81.36	77.90
% difference from QC Char Fixed Mean		4.0	8.1
% difference from the initial measurement			4.2
Quality Material 3	A17QC3L02	Initial measurement	Processed sample stability
Replicate 1		96.27	96.88
Replicate 2		97.16	91.09
Replicate 3		96.23	-
QC Char Fixed Mean	105.64	96.55	93.99
% difference from QC Char Fixed Mean		8.6	11.0
% difference from the initial measurement			2.7