

Laboratory Procedure Manual

Analyte: **Urine Albumin**

Matrix: **Urine**

Method: **Fluorescein Immunoassay by Sequoia-Turner Digital Fluorometer, Model 450**

Revised: **May 2019**

as performed by: **University of Minnesota**

Contact: **Blanche Chavers, MD**

Important information for Users

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

Public Release Data Set ALB_CR_K Information

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

File Name	Variable Name	SAS Label
ALB_CR_K	URXUMA	Albumin, urine ($\mu\text{g}/\text{mL}$)
	URXUMS	Albumin, urine (mg/L)

There have been no changes in the urine albumin test methodology or instrumentation to date.

1. SUMMARY OF TEST PRINCIPLE AND CLINICAL RELEVANCE

A fluorescent immunoassay for the measurement of human urine albumin is described by Chavers et al.¹ The methodology involves solid –phase, non-competitive, double-antibody reaction. Urine specimen albumin antigen reacts with albumin antibody that is covalently attached to polyacrylamide beads. This resulting solid-phase antibody complex is then reacted with fluorescein-labeled antibody. Unattached fluorescent antibody and other proteins are removed by centrifugation. The fluorescence of the stable solid-phase double-antibody complex is measured with a fluorometer and is directly proportional to the amount of urine albumin present. The calibration line material is human serum albumin with a range of 0.5 to 20 µg/mL.

Increased microalbuminuria is a sign of renal disease and may be predictive of nephropathy risk in patients with type 1 and type 2 diabetes. It is associated with hypertension and cardiac disease. The fluorescent immunoassay (FIA) has been in service for several years and has proven to be reliable, accurate, precise, and sensitive for the detection of urine albumin excretion. It is especially useful for quantitative analysis of low levels of urine albumin. The FIA resembles the radio-immunoassay (RIA) in technique and sensitivity without the potential health hazards associated with the handling of isotopes in the laboratory.

2. SAFETY PRECAUTIONS

- A. Follow the Laboratory Safety and General Laboratory Practice regulations from the College of American Pathologists (CAP), the Clinical Laboratory Improvement Amendments (CLIA), and Occupational Safety and Health Administration (OSHA). Observe Universal Blood and Body Substance Technique (UBBST) and the Centers for Disease Control (MMWR 36;25;1987) precautions for prevention of HIV transmission in the health care setting.
- B. Wear laboratory coats and disposable gloves when handling urine specimens. Cover the work surface with disposable, absorbent toweling. Place urine tubes, pipet tips, gloves, toweling, etc., and closed residual urine specimens into plastic bags and secure tightly. Urine can be discarded into regular trash. Clean the work surfaces with 0.5% bleach.
- C. Recommend to laboratory personnel performing the assay that they receive the HBV vaccine. Maintain records of vaccination or signed declination forms in the laboratory.
- D. Label all reagents indicating the preparation date, expiration date, formula, lot number if applicable, hazards of the reagent, antidote of contact with hazard, and the initials of the technician.
- E. Note the location for the Material Safety Data Sheets notebook for all chemicals used in the laboratory.
- F. Perform annual Safety Training and Laboratory-Specific Safety Tour noting the location of the chemical spill kit (a solid absorbent material), the location of the fire extinguishers, alarms, fire blankets, eye wash, etc. Dial 911.

3. COMPUTERIZATION; DATA SYSTEM MANAGEMENT

COMPUTERIZATION

Linear regression analysis for the calculation of test results is performed on a Dell OptiPlex 7010 PC computer with Microsoft Excel Software. Format an Excel electronic spreadsheet to be used as a template document for the assay result calculations. Program the formulas for linear regression analysis in order to perform a fill-down of calculations for the unknown specimen values. On this document include sample identification information, (urine collection time and volume, if applicable), the specimen Multistix value, the dilution factor, the duplicate percent relative fluorescence (%RF) values, the specimen values converted to $\mu\text{g/mL}$, and the calculated total $\mu\text{g/mL}$ albumin concentration. Prepare a spreadsheet from the template for each assay.

INFORMATION SECURITY SYSTEM

University computer firewall and password protection are established at startup. All programs and files are backed up monthly to prevent loss due to alteration or destruction. System passwords are used to limit computer access to authorized users. Access codes are never posted and must be memorized and are altered with changes in personnel. University Information Technology policies regarding privacy and security and e-mail are followed. The laboratory reports are verified against hardcopy before results are reported electronically. The laboratory personnel have received HIPPA training. The medical director has approved the content and format of the computer-generated reports.

DATA SYSTEM MANAGEMENT

The integrity of the specimen data is established by routine verification of the transcribed information against the identification on the specimen tube and the hard copy that accompanies all specimens. Urine albumin results with pertinent specimen information are reviewed by the laboratory supervisor and are returned by hard copy or electronic transmission as per study protocols. (For the NHANES, results are returned via internet by File Transfer Protocol (ftp) onto formatted worksheets that were transmitted by the NHANES contract data laboratory, Westat). The data is stored on the computer hard drive and backed-up monthly. The hard copies are organized in notebooks. Worksheets and results are archived indefinitely and located in the laboratory. All sample-person values are linked to analytic batch and date of analysis, quality control, reagent lot numbers and expiration dates. Test results are verified by the technician who performed the assay and by the laboratory supervisor.

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

A. SPECIMEN COLLECTION PROCEDURE

1. Timed or random urine collections are obtained from study subjects as per study protocols. (For the NHANES, random urine specimens are collected at the survey Mobile Examination Centers (MEC).)
2. No special instructions such as fasting or special diets are requested.
3. The optimum specimen tube is a 3- to 5-mL screw-top cryogenic vial. Tubes are selected per study protocols.
4. The optimum sample volume is 3 mL, and the minimum acceptable volume is 1 mL.

5. Patients are prepared according to study protocols that are designed to ensure consistent specimen collection procedures for the variables that affect urine albumin excretion (such as exercise, time of day, water loading, and posture).
6. Female participants are requested to provide a specimen when they are not menstruating to avoid falsely elevated albumin levels.
7. Patient history may include conditions such as urinary tract infection, marked hyperglycemia, severe hypertension, or congestive heart failure, as the interpretation of urine albumin measurements may be confounded by these conditions.
8. Labeling and identification procedures are determined by study protocols. Specimens are identified numerically, not by name.
9. Preservation procedures are determined by study protocols. No chemical preservatives have been used. Temperature preservation is used according to freeze or refrigeration protocol.

B. SPECIMEN STORAGE PROCEDURE

- 1, Urine specimens arrive frozen (on dry ice) or fresh (2-8°C) as per study protocols. (For the NHANES, MEC specimens are shipped on dry ice by overnight courier and refrigerated to thaw upon arrival.) Specimens can remain refrigerated up to 3 weeks until the completion of the analysis.
2. Temperature-sensitive equipment is available for specimen storage. This includes ultra-cold freezers at -80°C, refrigerator at 2-8 °C, and walk-in cold room at 2-8 °C. The university building facilities alarm system provides continuous electronic data on temperatures. Alarms prompt personnel when temperature exceeds set tolerance.
3. Analyzed specimens are returned to frozen storage at -80°C. According to study protocols, specimens are retained for a designated period, then either discarded or returned as described by protocol.
4. Specimen stability at -80°C for at least 1 year has been documented.
5. Specimens are discarded after permission by project director(s).

C. SPECIMEN HANDLING PROCEDURE

1. Handle all urine specimens as if they are capable of transmitting any infectious agent.
2. Return specimens to specified storage as soon as possible to avoid prolonged time at room temperature.
3. Transportation of urine specimens is on dry ice by FedEx. Laboratory personnel are trained on FedEx transport rules and also dry ice handling and other transport rules. Training is documented every 2 years.
4. The university's Advanced Research and Diagnostics Laboratory (ARDL) performs creatinine testing on the same urine specimens. The Creatinine Procedure Manual, the CLIA certificate, quality control data specific to NHANES, and Proficiency Testing records are filed in the Microalbumin Laboratory and signed by the laboratory medical director.
5. Hospital Specimen Receiving manages the transport of specimens between laboratories.
6. Test requests clearly describe the laboratory address and telephone number, the name of the medical director and contact personnel.

D. CRITERIA FOR SPECIMEN ACCEPTABILITY AND REJECTION

1. Corrupted specimen integrity, cracked or leaking tube, unreadable or missing label.
2. Visibly hematuric specimens.
3. Unacceptable temperatures occurred during transport or storage.

4. Sample spilled out. Tube or container empty.

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

Not applicable for this procedure.

6. EQUIPMENT AND INSTRUMENTATION, MATERIALS, REAGENT PREPARATION, CALIBRATORS (STANDARDS), AND CONTROLS

A. EQUIPMENT AND INSTRUMENTATION

1. Fluorometer, Sequoia-Turner model 450, digital (cat. no. 450-000). Excitation energy is provided by a quartz halogen lamp (cat. no. 450-201). Wavelength selection is accomplished with a filter application set for fluorescein, SC 515 and NB 490 Sharp cut emission (cat. no. 450-151) transmits >515 nm and narrow band excitation 490 nm peak, respectively). Adjust the filter at 450-151 for excitation = 485 nm, emission = 525 nm. (Sequoia-Turner Corp., Mountain View, CA).
2. Automatic diluter, Micromedic Systems model 25004, with 50- μ L sampling and 200- μ L dispensing pumps (Micromedic Systems, Division of Rohm and Haas, Horsham, PA).
3. Automatic dispenser, continuous with adjustable volume 0.5- to 20-mL, cat. no. 8885-047004 (Oxford, Division of Sherwood Medical, St. Louis, MO).
4. Water baths, models 180 and 185 (Precision Scientific, Inc., Chicago, IL).
5. Vortex-Genie, model S8223 (Scientific Products, McGow Park, IL).
6. 2-point balance, Mettler P1200, 0.1-1200g (Mettler Instrument Corp., Hightstown, NJ).
7. 4-point balance, Mettler AB54-S, 0.1-50g (Mettler Instrument Corp., Hightstown, NJ).
8. pH meter, Accumet model 15 and pH meter probe part no. 13-620-223A (Fisher Scientific, Fairlawn NJ).
9. Magnetic stir plate, Thermolyne Cimarec 2, model S46725 (Barnstead/Thermolyne, Dubuque, IA).
10. Micro-centrifuges, models 541B and 5424 (Eppendorf, Hamburg, Germany).
11. Centrifuges, model GS-6 series, used at 1500g, swinging buckets cat no 349947 each hold 30X 12-X 75- mm tubes (Beckman Instruments, Palo Alto, CA).
12. Centrifuge, Beckman Avanti JXN-26, used at 1500g, with JLA-16.250 fixed-angle rotor and six 250-mL centrifuge bottles, clear polycarbonate, part no. 356-013 (Beckman Instruments, Palo Alto, CA).

13. Micro-pipet, 0.5- to 10- μ L, Oxford P-7000 series (Oxford, St. Louis, MO) and Dot Scientific Oxford-style tips, cat no. PF2401 (Dot Scientific, Burton, MI).
14. Pipetman, Gilson, P100 and P-200 adjustable (Rainin, San Diego, CA) and yellow tips (USA Scientific, Ocala, FL).
15. Pipetman, Gilson, P-1000 adjustable (Rainin, San Diego, CA) and blue tips (USA Scientific, Ocala, FL).
16. Pipetman, Gilson, P-5000 adjustable (Rainin, San Diego, CA) and tips 500-to 5000- μ L (USA Scientific, Ocala, FL).
17. Repeater pipettor, Eppendorf, cat. no. P-3880-1 and Combitips sizes 500- μ L and 2.5-mL (Brinkman Instruments, Riverview, FL).
18. Traceable big-digit hygrometer/thermometer, cat no 4184 (Control Company, Friendswood, TX).
19. NIST thermometers (through university Biomedical Engineering), FLUKE 52-2 (Tenma Test Equipment, Springboro OH) calibrated and certified annually (Onsite Calibration Service, Louisville, KY).
20. NIST Standard Weights (through university Electronics Instrument Services) ANSI/ASTM Class, calibrated and certified annually (Troemner, Thorofare, NJ).
21. Dell OptiPlex Systems 7010 (Dell Computer Corporation, Round Rock, TX). HP LaserJet Pro 200 (Hewlett Packard Co., Palo Alto, CA).

B. MATERIALS

1. Type 1 water, HPLC Grade, cat no W5, certificate of analysis provided (Fisher Scientific, Fair Lawn, NJ).
2. Type 2 water, distilled, 6 gallons, certificate of analysis provided (Premium Waters, Inc., Minneapolis, MN).
3. Hydrochloric acid, HCl, F.W. 36.46, A.C.S. grade (Fisher Scientific, Fair Lawn, NJ). Store in acid cabinet at 20-25°C up to 2 years. *EXPOSURE HAZARD: Caustic and poisonous, wear safety goggles, mask and gloves.*
4. Potassium hydroxide, KOH, F.W. 56.11, A.C.S. grade (Fisher Scientific, Fair Lawn, NJ). Store at 20-25°C up to 5 years. *EXPOSURE HAZARD: Caustic and poisonous, wear safety goggles, mask and gloves.*
5. Potassium phosphate monobasic, KH_2PO_4 , F.W. 136.09, A.C.S. grade (Mallinckrodt, Baker Inc., Phillipsburg, NJ). Store at 20-25°C up to 5 years.

6. Sodium chloride, NaCl, F.W. 58.44, A.C.S. grade (Avantor Performance Materials, Center Valley PA). Store at 20-25°C up to 5 years.
7. Ovalbumin, chicken egg albumin, average F.W. 42,699, grade V, cat. no. A-5503 (Sigma, St. Louis, MO). Store at 2-8°C, with desiccant according to the package expiration date. *CAUTION: Respiratory dust hazard. Wear mask.*
8. ImmunoBead® Active Matrix, 1-gram vial polyacrylamide beads in 5 mM potassium phosphate, pH 6.3, 0.02% sodium azide, cat. no. 170-5911 Custom (Bio-Rad Laboratories Inc., Hercules, CA). Store at 2-8°C for 5 years.
9. 1-ethyl-3-(3-dimethyl-aminopropyl) carbodiimide HCl (“EDAC”), M.W. 191.7, cat. no. E-6383, protein sequencing grade (Sigma, St. Louis, MO)). Allow for complete return to 20-25°C before opening to avoid condensation. Store at -80°C with desiccant up to 5 years. *CAUTION: Toxic, Wear safety goggles, mask and gloves when handling the dry powder, EPA Code: none.*
10. Goat anti-human albumin (“GAHA”), IgG fraction, 5-mL vial of 40-50 mg lyophilized antibody protein per vial, cat. no. 55028 (MP Biomedicals LLC, Solon, OH). Lyophilized product, store at 2-8°C up to 5 years.
11. Fluorescein-conjugated goat anti-human albumin (“FI-GAHA”), IgG fraction, cat. no. 55162 (MP Biomedicals LLC, Solon, OH). Lyophilized product, store protected from light at 2-8°C up to 5 years.
12. Dialysis tubing, Spectra-Por molecular porous membrane, reorder no. 132678, 2.0 mL/cm, 12-14,000 MWCO. (Spectrum Laboratories Inc., Rancho Dominguez, CA). Store at 2-8°C.
13. Micro-centrifuge tube, 0.50-mL, cat. no. C3259 (ISC BioExpress, Kaysville, UT).
14. Micro-centrifuge tube, 1.5-mL, cat. no. 616-201 (Genesee Scientific, San Diego, CA).
15. Glass test tubes, borosilicate, sizes 12- X 75-mm (Corning Inc., Corning, NY).
16. Filter Unit, 115-mL, 0.2-µm, sterile, disposable, cat no 121-0020 (Thermo Scientific, Rochester, NY).
17. Multistix test strips, cat. No. 2161 (Siemens Healthcare Diagnostics, Inc., Tarrytown, NY). Follow the package insert for explicit directions for use. Store at 20-25°C according to package instructions and expiration date.
18. pH 6.86 Buffer, Certified traceable NIST Standard Reference Material, cat. no. Orion 910686 (Thermo Fisher Scientific, Chelmsford, MA). Store at room temperature until manufacturers expiration date.
19. General laboratory supplies: gloves, lab coats, safety glasses, 5% bleach, 99% isopropyl alcohol, disposable absorbent bench-top toweling, transfer pipets, weighing boats, stir

bars, tubes, parafilm, pH meter solutions, 100-mL wide-mouth Nalgene bottles for immunobead storage, 4-L wide-mouth Nalgene bottles for solution storage, graduated cylinders, 500-mL beakers, 50-mL volumetric flask (University of Minnesota U-Market).

20. Human Serum Albumin (“HSA”), cat. no. A-8763, approximately 99% pure by agarose electrophoresis, essentially globulin-free, derived from Sigma cat. no. A-1653 Cohn Fraction V (Sigma, St. Louis, MO). Store with desiccant at 2-8°C according to the product expiration date. *Caution: Handle as if capable of transmitting infectious agents.*
21. CAP Urine Protein Reference Material, human albumin Cohn Fraction V (College of American Pathologists, Skokie, IL). Store according to product directions. (The calibration material, human serum albumin, was initially checked against this reference protein.) *Caution: Handle as if capable of transmitting infectious agents.*

C. REAGENT PREPARATIONS

1. Sterile distilled water
Filter Type 2 distilled water through sterile, 115-mL, 0.2- μ m, disposable Nalgene Filter Unit under vacuum. Discard unused water.
2. 1 mol/L HCl (pH adjusting solution)
Dilute 8.3 mL in 100 mL Type 2 water and mix well. Store at 20-25°C up to 1 year.
Caution: Prepare in safety hood. Wear safety glasses and gloves. Add acid to water.
3. mol/L KOH (pH adjusting solution)
Dissolve 70.1 g KOH in 250 mL Type 2 water and mix well. Store at 20-25°C up to 1 year.
Caution: Prepare in safety hood. Wear safety glasses and gloves.
4. 0.3 mol/L KH₂PO₄ stock solution
Dissolve 40.8 g of KH₂PO₄ in 1 L of Type 2 water and mix well. Store at 20-25°C up to 1 year.
5. 0.003 mol/L KH₂PO₄ (dialysis buffer for immunobead coupling)
Dilute 20 mL of 0.3 mol/L KH₂PO₄ stock solution to 2 L with Type 2 water and mix well. Adjust the pH to 6.3 with 5 mol/L KOH. Prepare the day before use and store at 2-8°C overnight. Use cold. Discard after use.
6. Phosphate Buffered Saline, stock solution concentrated (20X PBS)
Dissolve 175.4 g NaCl in 666 mL of 0.3 mol/L KH₂PO₄. Dilute to 1 L with Type 2 water and mix well. Store at 20-25°C up to 1 year.
7. Phosphate Buffered Saline, physiologic (1X PBS)
Dilute 200 mL of 20X PBS to 4 L with Type 2 water and mix well. Adjust pH to 7.2 with 5 mol/L KOH. The final concentration is 0.01 mol/L KH₂PO₄, 0.15 mol/L NaCl. Prepare 4 L for assay performance and prepare 8 L for the immunobead coupling procedure. Use cold. Store at 2-8°C up to 3 weeks.
8. 1.4 mol/L NaCl in physiologic (1X) Phosphate Buffered Saline (NaCl-PBS)

Dissolve 245.5 g NaCl in 3 L PBS for immunobead coupling preparation. Mix well. Prepare the day before use and store at 2-8°C overnight. Use cold and discard unused solution.

9. Ovalbumin in physiologic (1X) Phosphate Buffered Saline (oval-PBS)

Dissolve 0.5 g ovalbumin in 100 mL PBS and filter through 0.2-µm sterile Nalgene Filter Unit. Store in this filter unit at 2-8°C up to 3 weeks.

10. Immunobeads: beads coupled to goat anti-human albumin (beads-GAHA)

Day 1 of 2: Prepare 400 mL of beads couples to GAHA. Do not prepare more than a 4-month supply. For an estimated 4,000 specimens per year prepare four grams (400mL) of immunobeads on a quarterly basis. The final concentration of the immunobeads is 10 mg/mL in oval/PBS. (This procedure is adapted from the Bio-Rad package insert².)

GAHA Preparation and Dialysis: Reconstitute each of four vials of GAHA with 5 mL of sterile Type 2 water and incubate at 2-8°C for 1 hour. Combine the vials into approximately 25 cm of dialysis tubing. Dialyze against 2 L of 0.003 mol/L KH₂PO₄, pH 6.3, for 4 hours at 2-8°C.

Beads Preparation: Transfer 4X (four 1-g, 100-mL vials) of beads into 2X centrifuge bottles and spin 14 minutes at 3500 rpm in JLA-16.250 rotor in the Beckman Avanti JXN-26. Decant and combine the 4 grams of beads in a 500-mL beaker with a 400 mL mark and use a total volume of 400 mL 0.003M KH₂PO₄, pH 6.3 to rinse out the centrifuge bottles. Check that the pH is 6.3, adjust if needed, with 1 mol/L HCl. Cover and incubate at 2-8°C for 1 hour.

Combine GAHA with Beads: Add GAHA from the dialysis tubing to the beads, adjust the pH to 6.3 with 1 mol/L HCl or 5 mol/L KOH as needed and incubate at 2-8°C for 1 hour. This final concentration is 40- to 50-mg of antibody protein per 1 gram of beads.

Perform the bead-antibody coupling procedure: Set the pH meter probe in the solution, stirring. Have ready the pH solutions 1 mol/L HCL and 5 mol/L KOH. Quickly add 800 mg of EDAC while stirring. The pH will oscillate rapidly. Use the pH solutions as needed to maintain the pH at 6.3 for 30 min during this equilibration process. Initially, 1 mol/L HCl will be required to maintain pH 6.3. After 30 minutes the pH will stabilize. Divide the immunobeads equally into each of six 250-mL centrifuge bottles. Incubate at 2-8°C overnight.

Prepare for Day 2 centrifugation/washing procedure: Place the JLA 16.250 rotor (Beckman Avanti JXN-26 centrifuge) in the cold room to cool overnight. Prepare the immunobead washing buffers (8L of PBS and 3 L of 1.4 mol/L NaCl-PBS) and store at 2-8°C overnight.

Day 2 of 2:

Perform six washing procedures with cold buffers and centrifuge set at 4°C. For each wash, fill the bottle with buffer, centrifuge at 3100 rpm (1500 x g) for 11 minutes with

the brake set on high, decant, blot, and re-suspend the immunobeads with a small volume of the next wash solution with a gentle rolling motion. Perform the wash procedure 2 times with PBS and then wash 2 times with 1.4 mol/L NaCl-PBS. Wash 2 times more with PBS.

Re-suspend the contents of each of the six bottles with 100 mL of PBS and incubate at 2-8°C for 3 hours.

Fill the bottles with PBS and do a final centrifugation. Decant.

Re-suspend the contents of each bottle with a small volume of oval/PBS. Combine the six bottles and adjust the total volume to 400 mL in a 500-mL beaker. Divide into four 100-mL portions in the 100 mL Nalgene bottles. Store at 2-8°C for 4 months, never allowing beads to freeze.

11. Fluorescein goat anti-human albumin, stock solution concentrated, FI-GAHA (10X)
Reconstitute each vial of lyophilized product with 2 mL of sterile Type 2 water. Combine all vials together and place them at 2-8°C, protected from light, for 1 hour. Prepare aliquots of 10 mL into 10-mL screw-top tubes. Store the vials at -80°C protected from light up to 2 years.
12. Fluorescein goat anti-human albumin, working solution, FI-GAHA (1X)
 - a. Prepare a 1:10 dilution of the 10X stock. Add 90 mL of oval/PBS to the 10 mL aliquot of FI-GAHA stock solution (or other proportions as determined by the limiting curve data, see below) by mixing in a 200-mL beaker. Transfer 10 mL each of the working solution into ten of the 10-mL Nalgene blue top tubes. Before use on each assay day, use the IEC centrifuge at 2500 rpm (1500 x g) for 5 min to remove cryo-precipitates. Return the excess to refrigeration, at 2-8°C up to 1 month.
 - b. Test new lots of FI-GAHA if the manufacturer changes the fluorescein/protein ratio (F/P) in order to assure the optimum concentration in the assay system. Plot a limiting curve: analyze the "20 µg/mL HSA" of the calibration line with serial dilutions of FI-GAHA and graph the data. Select the dilution that is 2 times the concentration at the point (or "limit") where the graph detours from a straight line and begins to plateau.

D. CALIBRATORS (Standards)

Calibration line material: Human Serum Albumin (HSA) 1.0 mg/mL

Prepare frozen aliquots of a 1 mg/mL solution of HSA in order to thaw and use one for every assay. Weigh 50 mg of lyophilized HSA on a balance of 0.1 mg sensitivity and dissolve in 50 mL of sterile Type 1 water using a 50-mL volumetric flask. Pipet 0.3 mL into 0.50-mL micro-centrifuge tubes: use the repeater pipet 2 times set on #3 with the 2.5-mL Combipip to deliver 0.3 mL. Label each aliquot "HSA." Prepare adequate numbers of aliquots in order that one set of materials could last for an expected 2-year period. When these materials change, perform a comparability study of at least 100 specimens to demonstrate the relationship of the values before and after the change, testing the old and the new HSA in the same assay. Acceptable tolerance is defined by the laboratory's verification of linearity >0.9900 (R²) and quality control

urine pools within the expected 95 % limits as described by Westat Rules³. *CAUTION: Handle as if capable of transmitting infectious agents.*

Primary standard reference material

The primary calibration standard for the fluorescent immunoassay is “CAP Urine Protein Reference Material, human albumin Cohn Fraction V”. Reconstitute the material as described in the package insert and devise a dilution schematic that correlates with the concentrations of the standard line calibration material for comparison. (This was used initially prior to putting the FIA into service). *CAUTION: Handle as if capable of transmitting infectious agents.*

E. CONTROLS

Quality control material: control pool urine, three levels

Prepare frozen aliquots of three control pools in order to thaw and use one of each for every assay. The three levels of control represent low, middle, and high positions on the standard line. Pool and screen urine from a number of healthy volunteers to obtain albumin concentrations of approximately 2, 7, and 15 µg/mL. (You may need to add a strong solution, 1 mg/mL, of Human Serum Albumin, Sigma 8763, to urine control qc high in order to achieve 15 µg/mL.) Filter each urine pool through sterile 0.2-µm Nalgene Filter Unit under vacuum. Mix well. Pipet 0.4 mL into 0.50-mL micro-centrifuge tubes with a pool ID label for each concentration level. Use the repeater pipet 2 times set on #4 with the 2.5-mL Combitip to deliver 0.4 mL. Label each aliquot. Prepare adequate numbers of aliquots in order that one set of materials could last for the duration of the study. When these materials change, perform a comparability study to demonstrate the relationship of the values before and after the change in the controls. Store at -80°C for up to 4 years. *CAUTION: Handle as if capable of transmitting infectious agents.*

7. CALIBRATION AND CALIBRATION VERIFICATION PROCEDURES

CALIBRATION PROCEDURE

- a. For each assay prepare a calibration line from the results of the dilutions of standard line material, 0 µg/mL to 20 µg/mL HSA, as described in Table 1.
- b. For each assay calibrate the fluorometer instrument. Adjust the fluorometer to read 0% relative fluorescence (RF) and 100% RF for the HSA standard line calibration limits 0 µg/mL and 20 µg/mL, respectively. This will establish a known relationship between the instrument measurement and the true value of the albumin analyte.

CALIBRATION VERIFICATION PROCEDURE

- a. Verify that the instrument remains in calibration during the fluorometry procedure by periodically re-checking that the 0 µg/mL and the 20 µg/mL HSA samples remain in adjustment at 0% RF and 100% RF, respectively.
- b. Verify linearity with linear regression analysis and accept test results when the correlation coefficient (R^2) is >0.9900 with a y-intercept near zero.
- c. Verify that the three quality control urine specimens are within the expected 95% limits as described by the Westgard Rules³. Check this and other rules regarding “out of control” criteria as listed in the QC section.
- d. Verify the concentration of the “1.0 mg/mL HSA” standard line calibration material by checking it against the primary standard, “CAP Urine Protein Reference Material, human

albumin Cohn Fraction V", a reference material of known value. (This was done initially prior to putting the FIA into service).

8. PROCEDURE OPERATING INSTRUCTIONS: CALCULATIONS; INTERPRETATION OF RESULTS

PROCEDURE OPERATING INSTRUCTIONS

a. Preliminaries prior to the assay day

1. Number single 1.5-mL micro-centrifuge tubes to correspond with each urine specimen.
2. Number duplicate 12- X 75-mm tubes (use a waterproof marker) for each of the three quality control urine samples, the standard line samples (labeled from 0 to 20 µg/mL, described on Table 2), and for each urine specimen. Perform in triplicate any specimen that requires immediate reporting.
3. Prepare a worksheet to compile the information corresponding with the information on the Excel spreadsheet described in section 3. On the worksheet place one of the 3 quality control urine samples in the 0 position before specimen number 1, one near the middle, and the third after the specimens. Rotate these positions in subsequent assays.
4. Adjust the water bath for optimum volume and maintain at 37°C temperature.

b. Sample preparation on the assay day

1. Urine specimen preparation for the assay day
 - a. Thaw and mix the urine specimens by inverting the tubes a minimum of eight times. Number the specimen tubes to correspond with the specimen identification numbers on the original accompanying test request. Prepare the FIA worksheet with the same corresponding numbers. Transfer 200 µL of urine specimen to the corresponding numbered 1.5-mL micro-centrifuge tube using the P-200 pipet. Centrifuge the tubes for 2 min in the micro-centrifuge. Perform specimen analysis with the same conditions as the standard line material and controls.
 - b. As directed in the Multistix package insert, perform the dipstick on each specimen to measure and record the albumin value. This estimated albumin value determines the specimen dilution factor and the settings for auto-diluter (Micromedic) sampling syringe as described in Table 1. Knowledge of the approximate albumin concentration of the specimen enables the technician to prepare the specimen so that it will fall within the standard curve, 0 µg/mL to 20 µg/mL. Record the dipstick albumin values and the corresponding dilution factors onto the worksheet as described in Table 1. Analyze each specimen at the specified dilution and corresponding setting for the sampling syringe:

Urine specimens with very low Multistix albumin values

Specimens with "negative" or "non-detect" (negative and colorless) values may require analysis at 2X the concentration (i.e. dilution factor

of “2”). This is adjusted with the Micromedic syringe set at 80%. Note: Specimens that fail to fall on the standard curve at this setting are considered < LOD.

Urine specimens with low to moderate Multistix albumin values
Specimens with values of “negative”, or “negative-trace” have dilution factors “1”, and “0.5”, respectively. They are tested directly on the Micromedic without hand-dilution, with the Micromedic sampling syringe set at 40% and 20%, respectively.

Urine specimens with high Multistix albumin values
Specimens with high values of “trace”, “+”, “++”, or “+++” must be diluted. Dilute with oval/PBS as directed in Table 1. The dilution factors will be “0.1”, “0.02”, “0.01”, and “0.001”, respectively, and the Micromedic sampling syringe is set at 40%.

Table 1: Prepare dilutions of the urine samples

Multistix value	Dilution factor	Hand Dilution		Micromedic Automatic Diluter		
		Sample volume (µL)	Oval/PBS buffer (µL)	Micromedic sampling syringe setting (%)	Sample volume (µL)	Final volume with addition of immunobeads (µL)
Non-detect	2	-	-	80	40	75
Negative	1	-	-	40	20	75
Neg/Trace	0.5	-	-	20	10	75
Trace	0.1	50	450	40	20	75
+	0.02	20	980	40	20	75
++	0.01	10	990	40	20	75
+++	0.001	2	1998	40	20	75

1. Control pool urine preparation for the assay day
Thaw and vortex one aliquot of each of the three control pools on the day of an assay. Transfer each to a 1.5-mL micro-centrifuge tube with the P-200 pipet. Centrifuge the tubes for 2 min in the micro-centrifuge. The dilution factor is 1. Use the Micromedic sampling syringe set at 40%. Perform sampling in duplicate with the same conditions as the urine specimens.
2. Calibration line material preparation for the assay day
Thaw and vortex one aliquot of the “1.0 mg/mL HSA” on the day of the assay. Transfer to a 12- X 75-mm tube. Prepare the HSA calibration line by following the schematics in Table 2. Use the Oxford micro-pipet and 12- X 75-mm tubes. Vortex. The dilution factor is 1. Use the Micromedic sampling syringe set at 40%. Perform sampling in duplicate with the same conditions as the urine specimens.

Table 2: Prepare Calibration Line using Human Serum Albumin (HSA), 1 mg/mL

HSA line [$\mu\text{g}/\text{mL}$]	Volume of HSA (μL)	Volume of oval/PBS (mL)
0	0	1
0.5	1	2
1	2	2
2	4	2
5	5	1
10	10	1
15	15	1
20	20	1

c. Instrument Setup for the assay day

Water bath

Preset and maintain at 37°C. Adjust the volume with tap deionized water. Check and record the temperature on the assay day prior to the incubation. Verify temperature at the end of the assay incubation.

Micromedic auto-diluter

1. Immerse the tubing end into constantly stirring immunobeads and prime the Micromedic by washing through the bead solution with the dials set for maximum volume dispensing, just enough to fill the lines without the presence of bubbles. Prevent the beads from settling in the tubing while the instrument is not in continuous running operation.
2. Set the instrument for automatic sampling and dispensing. The number dialed on each syringe pump equals the percentage of the syringe volume. The maximum volume of the left (sampling) syringe is 50 μL ; the maximum volume of the right (dispensing) syringe is 200 μL .
 - a. To Prime – push both (“set” and “run”) buttons down.
 - b. To Set – put the “set” button down and the “run” button up to dial in percentages. Set the left (sampling) syringe at 20%, 40% or 80% according to the dilution factor schematic in Table 2. Keep the right (dispensing) syringe at 38.5% to deliver 75 μL of immunobeads to all samples.
 - c. To Run – push the “run” button down and “set” button up. Insert the delivery tip into the specimen. Press the foot pedal to siphon the sample. Avoid disturbing the pellet and remove the drop adhering to the delivery tip. Press the foot pedal to eject the sample combined with the beads into the 12- X 75-mm tubes. The final volume of sample combined with the immunobeads is 75 μL .
 - d. Clean the Micromedic when finished by running several cycles of Type 2 water (approximately 5 mL). Store the Micromedic with Type 2 distilled water-filled tubing. Record the cleaning onto worksheet.

Oxford continuous automatic dispenser

Prime with PBS to fill the lines. Adjust settings for dispensing the 4-mL volume or the 2-mL volume as required in the procedure. When finished, flush the PBS out and leave the lines dry. Record the cleaning onto worksheet.

Beckman GS-6 Centrifuge

Set at 3000 rpm (1500 x g) and 10 min for two washes. Use the swinging bucket rotor with carriers that fit the 12- X 75-mm tubes. Perform centrifugation at 20-25°C with the brake set at maximum.

Fluorometer

Turn the instrument on and allow it to warm up for a minimum of 30 minutes prior to use. Set the GAIN dial at "5". The raw data is recorded from the digital readout as percent relative fluorescence (% RF). The 12- X 75-mm glass tubes function as disposable cuvettes. Calibrate the fluorometer with the ZERO and SCAN dials as described in the following.

d. Operation of the assay procedure

1. Place the bottle of immunobeads in a beaker of ice. Stir gently on a magnetic stir plate.
2. Use the Micromedic auto-diluter to combine the samples with immunobeads as directed in Table 1 in the sample preparation section.
 - a. Dispense into duplicate 12- X 75-mm tubes according to the dilutions as listed on the worksheet for the standard line tubes, the three quality control urines, and the urine specimens.
 - b. Dispense in order: pipet the standard line first, followed by one control, then one-half of the specimens, then the second control, then the last half of the specimens, ending with the third control.
 - c. Dispense in triplicate any sample that has a "rush" status or was problematic on previous analysis.
3. Mix by gently shaking the rack of tubes X3, cover, and incubate for 1.5 hours in the 37°C water bath.
4. Prepare sufficient quantity of the FI-GAHA working solution. Add 50-µL of FI-GAHA working solution to each tube. Use the repeater pipet set on #5 with the 500-µL Combitip. The pipet will deliver eight 50-µL aliquots for each fill.
5. Mix by gently shaking the rack of tubes X3, cover, and incubate in the 37°C water bath for 2 hours minimum (incubation overnight is acceptable).
6. Perform two washing procedures to remove the unbound FI-GAHA from the immunobead-antigen complex. Use the automatic dispenser to add 4 mL cold PBS to quench the incubation reaction. Operate the Beckman GS-6 centrifuge as described in 'c. Instrument Set-up...'. For each of two washes, add 4 mL of cold PBS, centrifuge, decant the supernatant and blot, then vortex the tube to re-suspend the pellet in the residual buffer. Add final volume of 2 mL cold PBS after the washing procedure.
7. Perform fluorometry:
 - a. Vortex and wipe the outside of each tube until clean and dry. Insert each tube into the fluorometer and read and record the displayed %

RF immediately while the beads are in suspension to prevent potential deterioration of fluorescein.

- b. Calibrate the 0 µg/mL (blank) tube to 0% RF: insert the 0 µg/mL tube and set the ZERO dial to "0". Calibrate the 20 µg/mL tube to 100% RF: insert the 20 µg/mL tube and set the SCAN dial to "100".
- c. Record the % RF values onto the worksheet for the remaining standard line tubes, controls, and the unknown specimens.
- d. Verify the continued accuracy of the test method by demonstrating that the fluorometer dials remain in adjustment: periodically, and at the end of the run, re-check that the 0 µg/mL and the 20 µg/mL samples are at 0% RF and 100% RF, respectively.
- e. Turn off the fluorometer. Decant tube contents into sink and then discard tubes into glass waste.
- f. Perform Micromedic daily maintenance and record this onto the FIA worksheet. Clean the laboratory work area with bleach according to safety guidelines.

e. Replacement and periodic maintenance of key components

1. Fluorometer

Clean the filter windows with Type 2 water and lens paper monthly. Replace the lamp as needed. Perform twice annual comparisons of test results with the current fluorometer and the back-up fluorometer using the defined relationship criteria of absolute difference and % difference.

2. Balances, Pipets, and Diluter/Pipettor

Calibrate and clean every 6 months. Replace parts as needed. (Professional service is used.)

3. Centrifuges

Calibrate and perform parts and maintenance service annually. (Professional service is used.)

4. Computer

Verify the linear regression calculations on the Excel software assay template annually.

5. pH meter

Calibrate annually using certified traceable NIST Standard Reference Material and set tolerance limits for acceptance criteria. Perform regular calibration with pH solutions before each use. Maintain adequate electrode solution volume.

6. Temperature-sensitive equipment

Test temperatures using NIST thermometers annually. Calibration is performed as needed and batteries are replaced as needed.

CALCULATIONS AND INTERPRETATION OF RESULTS

1. Enter the % RF values of the standard line, controls, and specimens onto the formatted Excel spreadsheet.
2. Apply the equation for a straight line, $y=mx + b$ and perform linear regression analysis on the standard line results to obtain slope (m), y-intercept (b), R^2 and fitted line. The % RF value is directly proportional to the µg/mL of the standard line.

3. Convert the % RF values of the controls and specimens into $\mu\text{g}/\text{mL}$ albumin by performing a “fill-down” of the regression analysis equation. The dilution factor is multiplied by the converted $\mu\text{g}/\text{mL}$ to obtain the total $\mu\text{g}/\text{mL}$ albumin concentration.
4. Print the Excel document and verify that every value (i.e., specimen identification number, dilution factor, and % RF) matches those on the worksheet.
5. Record the values for the three quality control urine samples on the Levy-Jennings charts to determine satisfactory analytic performance.
6. Evaluate the performance of each assay before sending out results. Acceptable limits for linearity and quality control tolerance must be met for all reportable results.
 - a.) **Acceptable Fluorometry Measurement Range**

Any sample that is out of range, must be re-tested at the appropriate dilution.
The acceptable % relative fluorescence (%RF) range is 0 to 100 %RF.
 - b.) **Acceptable Fluorometry Duplicate Sample Range**

All samples are tested in duplicate and the mean value is used.
The acceptable % relative fluorescence (%RF) duplicate sample range is:
4 units for 0 to 20 %RF values
5 units for 21 to 50 %RF values
8 units for 51 to 100 %RF values
 - c.) **Acceptable Linear Regression Analysis**

The correlation coefficient (R^2) must be >0.9900 , with the y-intercept near zero
 - d.) **Acceptable Quality Control Pool Values**

The three QC pools (low, mid, high) are expected to be within tolerance limits as described by Westard Rule. QC is “out of control” if any of these events occur:
*A single run value for one or more pools falls outside the upper or lower 99% limit.
*The run values for two or more pools fall either above or below the 95% limit.
*Two successive run values for a single pool fall either above or below the 95% limit.
 - e.) **Acceptable CV% for the 2% quality control repeat (2% qcr) samples**
10%.

9. REPORTABLE RANGE OF TEST RESULTS

- a. The range of test values for the fluorometer instrument is the 0 to 100 % RF which corresponds with the range of linearity for the standard line, 0 to 20 $\mu\text{g}/\text{mL}$ HSA.
- b. Specimens with fluorometry values that are below the % RF of the standard line value 0.5 $\mu\text{g}/\text{mL}$ are not accepted. Specimens with unacceptable low % RF values at dilution factor “1” are re-analyzed at 2X the concentration (i.e. the dilution factor “2” setting on the Micromedic) in a subsequent assay. The lowest calculated concentration with dilution factor “2” is 0.25 $\mu\text{g}/\text{mL}$ which “rounds” to 0.3 $\mu\text{g}/\text{mL}$. Specimens with unacceptable low values at dilution factor “2” are designated <LOD.
- c. Specimens with fluorometry values that are above the % RF of the standard line value 20 $\mu\text{g}/\text{mL}$ are not accepted and are re-analyzed after dilution in a subsequent assay.
- d. The lower limit of detection for the assay is 0.3 $\mu\text{g}/\text{mL}$; there is no upper limit of detection. Therefore, the “reportable range of results” is “0.3 $\mu\text{g}/\text{mL}$ and up” or “ $\geq 0.3 \mu\text{g}/\text{mL}$ ” or “ $\geq 0.3 \text{ mg}/\text{L}$ ”.

10. QUALITY CONTROL (QC) PROCEDURE

- a. Quality Control System

Internal QC procedures monitor analytical performance relative to medical goals and alert analysts to unsatisfactory analytical performance. Estimates of imprecision and

permanent confidence limits are generated from 100-pair data (100 assay days) of three control urine pool levels. Calculated from the 100-pair data are the % CV (percent correlation of variance), the SDo (standard deviation overall), and the duplicate range (average of the differences between duplicates, average R). Tolerance limits for controls are established by using the Westgard Rules³ as guidelines. The tolerance limits are defined as the mean ± 2 SDo, or 95% warning limit, and the mean ± 3 SDo, or 99% action limit. When new control urine pools are initiated into the assay, the new mean is established from 20-pair data (20 assay days) and analyzed with the older controls during this time period. Calculations from these 20-pair (% CV, SDo, and duplicate range) are used only to monitor the 100-pair permanent confidence limits. Low, medium, and high range bench quality control urine pool samples are analyzed in duplicate in each analytical run (a set of assays performed on a given day) so that Judgements can be made on the day of analysis. The three levels of control urine pools are the same in all assays. Supplemental methods of quality control such as blind controls or split duplicate samples are included according to study protocols. (Note: the urine albumin test method has no relationship to the urine creatinine test method.)

Prepare a Levy-Jennings Means Chart for each of the three urine pool levels. Chart the means and tolerance limits on the graph paper. Plot the duplicate values for each control as a pair of dots for each assay. On this chart highlight unacceptable control values and action taken, document reagent changes and other pertinent information. Prepare a cumulative table of duplicate values and the difference between duplicates for the three control urine samples. Highlight unacceptable duplicates according to the within batch duplicate range (average R) tolerance limits and make notation on the Levy-Jennings charts. If the assay is determined to be out of range, perform function checks on the reagents and instrumentation. Provide assay results only after problems are corrected. The Levy-Jennings charts are reviewed and signed monthly by the medical director. For the NHANES, quality control data are sent monthly to Westat and are also reported in the quarterly reports.

The criteria to reject a run are as follows and the quality control system is declared “out-of-control” if any of the following events occur on the Means Chart:

- *A single run value for one or more pools falls outside the upper or lower 99% limit.
- *The run values for two or more pools fall either above or below the 95% limit.
- *Two successive run values for a single pool fall either above or below the 95% limit.

b. Quality Assurance System

General Quality Assurance systems are in operation to detect errors, monitor tolerance limits on temperatures and equipment, and ensure proper reagent labeling and equipment maintenance. NIST probes are used to determine within-range thresholds for acceptance criteria. The laboratory ensures that samples are collected, handled, shipped, preserved and stored correctly, and rejected if the criteria are not met. A “Continuous Quality Improvement Plan and Documentation” program is in effect for the laboratory.

c. External Quality Assessment

The laboratory is enrolled in a Proficiency Testing Program, as well as other external quality assessment activities to evaluate the accuracy of the test performance.

1. Proficiency Test, Accutest, One-World Accuracy, three test events annually.

2. Accuracy-Based Urine, ABU, College of American Pathologists, two test events annually.
3. Inter-assay performance is evaluated by repeat testing of 2% of the specimens from previous assays.
4. Intra-assay performance is monitored by study protocols which include blind split duplicates in the regular flow of analysis.

The laboratory has periodic on-site inspections by project directors, the regulatory agency CLIA, and university safety officers.

d. Accreditation

The laboratory is certified by the Clinical Laboratory Improvement Amendment, CLIA.

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

- a. Retest specimens at the appropriate dilution, that are out of range of the acceptable test values for the fluorometer instrument, the % RF of 0.5 µg/mL to 20 µg/mL HSA, at the appropriate dilution.
- b. Retest specimens with discordant duplicate % RF values. The acceptable duplicate ranges are as follows: 4 units for 0 to 20 % RF, 5 units for 21 to 50 % RF, 8 units for 51 to 100 % RF.
- c. Retest the entire assay if the R² value is <0.9900.
- d. Retest the entire assay if the quality control urine samples are outside of the limits as described by the Westgard Rules³.
- e. Replace reagents or recalibrate instrumentation as necessary for corrective action. Introduce reagents with new lot numbers and/or preparation dates into the assay one at a time to facilitate identifying problems.
- f. Report results on affected specimens only after corrective action and documentation of statistical control.

12. LIMITATIONS OF METHOD; INTERFERING SUBSTANCES AND CONDITIONS

LIMITATIONS OF METHOD

The lower limit of detection for the assay is 0.3 µg/mL. The lowest reportable value is also 0.3 µg/mL. Specimens that are below 0.3 µg/mL are designated “<LOD”. There is no upper limit of detection for the assay because the specimens can be diluted for analysis.

INTERFERING SUBSTANCES

Female participants who are menstruating may have contaminated urine specimens resulting in falsely elevated albumin levels. Unusually high albumin values, >1000 µg/mL, may reflect hematuria and are routinely tested by dipstick for hemoglobin. There is no interference from the fluorescein excreted into the urine following retinal angiography.

INTERFERING CONDITIONS

The interpretation of urine albumin measurements may be confounded by conditions such as urinary tract infection, marked hyperglycemia, severe hypertension, or congestive heart failure. Conditions that may affect urine albumin excretion are exercise, time of day, water loading, and posture.

13. REFERENCE RANGES (NORMAL VALUES)

Table 3: Reference Values of Urine Albumin in apparently healthy subjects

Number of Subjects	Author	Method	µg/mL	µg/min	mg/24 h
100 adults	Chavers ⁽⁴⁾	FIA	0.2-17.3*	0.8-14.6	1.1-21.0
20 adults	Mogenson ⁽⁵⁾	RIA		5-20	3.6-23
11 adults	Howey ⁽⁶⁾	RIA	8.13+	7.1**, +	10.2 ⁺
20 Children	Fielding ⁽⁷⁾	ELISA		1.2-15.9**	1.7-22.9
NHANES III	Jones ⁽⁸⁾	FIA	<30	2.9-4.4	
646 adolescents	Rademacher ⁽⁹⁾	FIA			

* obtained from subject data in the published manuscript

** calculated from published units

+ mean values

FIA - fluorescent immunoassay, RIA – radioimmunoassay, ELISA – enzyme-linked immunoassay

14. CRITICAL CALL RESULTS (“PANIC VALUES”)

There are no “panic values” which indicate immediate medical intervention for unusual urine albumin values. Study-specific critical values are defined with a code as determined by the study coordinators. Nephropathy can be defined by increased urine albumin values when measured by urine albumin excretion rate or urine albumin/creatinine ratio.

15. SPECIMEN STORAGE AND HANDLING DURING TESTING

Specimens are stored at 2-8°C during preparation of testing. Specimens may reach room temperature during the following procedures: pipetting, micro-centrifugation, and during the Micromedic procedure. A critical temperature during the testing procedure is the 37°C incubation. When incubation ends, cold buffer is introduced to quench the reaction and to begin the centrifugation/washing procedure. During fluorometry, the samples will approach room temperature.

16. ALTERNATIVE METHODS FOR PERFORMING TEST OR STORING SPECIMENS IF TEST SYSTEM BECOMES INOPERABLE

- We do not have an alternative method for performing the test if the test system fails.
- Manual pipets can be substituted for the automatic dispensers. An identical fluorometer is ready for backup.
- Return specimens to -80°C if the test system is out of operation for >3 weeks.

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

TEST RESULTS REPORTING SYSTEM

- Test results are reported by hard copy or by electronic transmission as designated by individual study protocols.
- The albumin test result is reported as µg/mL with one decimal point.

- c. Unusual albumin values that are designated “< LOD” or are > 1000 µg/mL are flagged for attention.
- d. Other test result reporting systems for albumin are designated by individual study protocols. Examples: albumin excretion rate is calculated from timed (overnight or 24-hour) urine collections and reported as µg/min or mg/24 h and albumin-to-creatinine ratio is calculated from random urine spot collections and is reported as mg albumin/g creatinine, µg albumin/mg creatinine, or mg albumin/mmol creatinine.

PROTOCOL FOR REPORTING CRITICAL CALLS

Not applicable for this procedure.

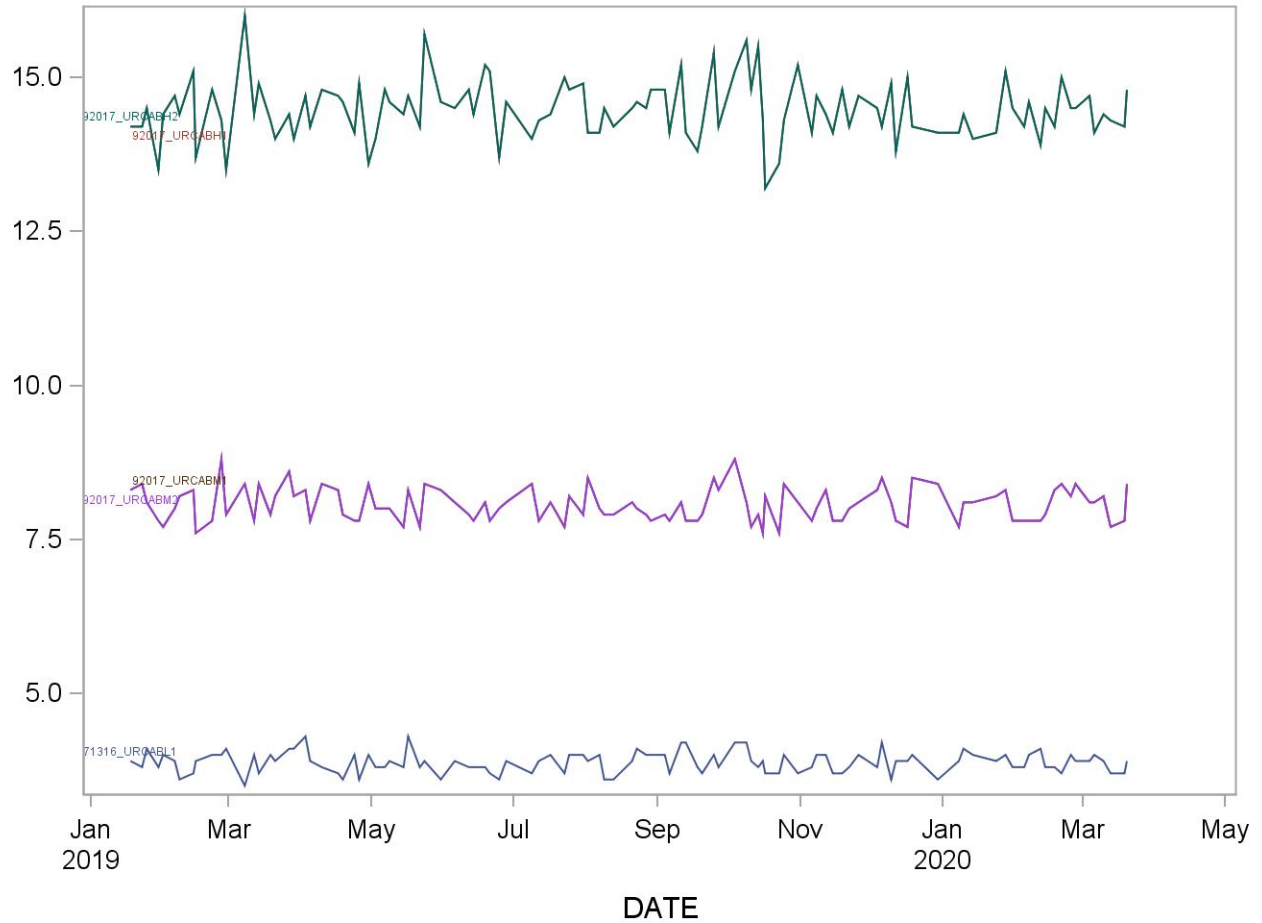
18. TRANSFER OR REFERRAL OF SPECIMENS; PROCEDURES FOR SPECIMEN ACCOUNTABILITY AND TRACKING

Specimens are logged into data books by the laboratory upon receipt. They are received by the laboratory in batches or as single specimens, frozen or fresh, arriving by overnight courier or hand-carried by study personnel from coordinating centers. For the NHANES, batches of frozen specimens are received by FedEx. Completed specimens are re-frozen in the original tubes at -80°C. Specimens are discarded after permission from the project director(s). All communications, notebooks, discs, and files containing raw data, final data, QC information, etc. are retained as documentation for specimen accountability and tracking.

19. SUMMARY STATISTICS AND GRAPHS

**2019-2020 Summary Statistics and QC Chart
URXUMA (Albumin, urine (ug/mL))**

Lot	N	Start Date	End Date	Mean	Standard Deviation	Coefficient of Variation
92017_URCABH1	107	18JAN19	20MAR20	14.468	0.482	3.3
92017_URCABH2	107	18JAN19	20MAR20	14.468	0.482	3.3
71316_URCABL1	107	18JAN19	20MAR20	3.880	0.170	4.4
92017_URCABM1	107	18JAN19	20MAR20	8.058	0.272	3.4
92017_URCABM2	107	18JAN19	20MAR20	8.058	0.272	3.4



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