NKDEP/IFCC Joint Conference to Address Standardization of Urine Albumin/Creatinine Measurement and Reporting

March 27-28, 2007
The George Washington University, The Marvin Center, Room 308 (Parks Room)
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March 27, 2007

1. **Introductions and conference overview:**
   Dr. Miller welcomed the group and presented introductory comments:
   - The Laboratory Working Group (LWG) has made recommendations for reporting estimated GFR in adults, creatinine standardization, and prescribers of drugs.
   - Other standardization issues the LWG is addressing are standardization of whole blood creatinine, estimated GFR in non-adults, method specificity, urine albumin/creatinine ratio (ACR), and pharmacy practice.
   - Review of the agenda:
     - Presentations by various conference members on the current status of urine albumin measurement.
     - Three specific topic discussion sub-groups meet in the afternoon.
     - Reports from each sub-group with group discussion.
     - Establish the path to move forward with assignments.

   Dr. Panteghini added comments from the IFCC perspective:
   - IFCC represents 37 diagnostic companies, 4 allied scientific societies interested in the practice of laboratory medicine, and 76 national societies of clinical chemistry and laboratory medicine.
   - IFCC mission includes standardization and guidelines for analytes.
   - The achievements of IFCC committees and working groups include implementation of standardization in laboratory medicine.

2. **Clinical reporting considerations - Current decision limits and clinical application in kidney disease**
   - Clinical Applications and Clinical Reporting, Dr. Narva:
     - NKDEP objectives are to improve early detection of CKD, facilitate the identification of patients at risk, and promote evidence-based interventions.
     - Proteinuria is key to the identification of early CKD, is a risk factor for CVD, a modifier for efficacy of ACE inhibitor therapy in both diabetic and non-diabetic kidney disease, and is hypothetically a surrogate outcome for kidney disease and CVD risk reduction.
     - Adherence to guidelines is poor due to lack of available albuminuria testing and non-standard reporting.
     - Improved adherence to guidelines can be achieved through better understanding of albuminuria and more effective use of quantitative albumin testing.
     - The rate of incident reporting ESRD with diabetes is starting to decline reflecting better identification.
   - Additional Issues to Consider in Albumin Creatinine Ratio (ACR) and the Research Implications, Dr. Curhan:
     - Race and gender specific cut-points need to be established.
     - Because ACR is a ratio, things affecting creatinine measurement will also affect ACR, e.g. males and blacks excrete more creatinine than females and non-blacks, respectively.
     - Because females excrete lower amounts of creatinine, thereby resulting in higher ACR, microalbuminuria was originally felt to be more common in women when a single cut-point was used.
     - "Normal" is arbitrary; risk of CVD/death can be increased even in those with higher levels within the "normal" range based on HOPE Study data.
     - In the HOPE Study, for every 0.4 mg/mmol increase in ACR, the adjusted hazard of major CV events increased by 5.9% (95% CI, 4.9%-7.0%).
   - NACB Guidelines on Laboratory Testing in Diabetes (2002), Dr. Bruns:
     - Background: These were developed between1999-2001 and published in 2002.
     - Microalbuminuria was defined as an increased albumin excretion rate (AER) or increased ACR.
     - There are four recommendations:
       1) Annual microalbumin testing of diabetic patients without clinical proteinuria, although the role of testing is not clear in patients with short life expectancy and under treatment with ACE inhibitors.
       2) Analytical imprecision of urine albumin methods should be less than15% CV.

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3) Acceptable sample types for diagnosis are timed urine collections for albumin excretion rates; timed or untimed collections are acceptable for ACR.
4) In order to be useful for screening, semi-quantitative or qualitative tests should be positive in >95% of patients with microalbuminuria.

- The guidelines are now out of date and a new NACB committee is working on draft guidelines to be presented at the Beckman Conference in November 2007, after posting on the NACB web site for comment.
- Issues for the NACB committee are the inappropriate grading system, lack of outcome data supporting the recommendations, no accuracy goals for microalbuminuria testing, need for guideline reviewers that include experts, and acquiring NKDEP and IFCC input.
- Discussion points following Dr. Bruns's presentation:
  1) There is inadequate screening with quantitative total protein. Most of the total protein assays do not have adequate sensitivity to identify a majority of the abnormal patients. However, a protein creatinine ratio may be better. (Note: the topic of quantitative total protein is not on the agenda.)
  2) Urine protein and creatinine testing may be performed more often because it is more available in-house, while urine albumin requires more specialized methods and thus may need to be sent to an outside laboratory.
  3) We need to re-examine the issue of efficacy of ACR testing in patients on ACE inhibitors.

3. Information from EQAS programs on performance attributes of routine methods:

Urine albumin - an international survey - post-analytical external quality assessment, Dr. Sandberg:

- A multi-country survey was initiated by the IFCC Global Campaign of Diabetes Mellitus.
- The purpose of the study was to 1) determine the current status of microalbuminuria testing, diagnosis, and monitoring among physicians, 2) define currently used tests, 3) compare results with international guidelines and reports, and 4) use the results for guideline revisions and physician education.
- The survey involved circulation of a case history to general practitioners in 11 countries, collection of practitioner information, asking for information about their indications for requesting microalbumin testing, asking for information about their routine for first time investigation; then, the case history was presented and they were asked questions about the case history.
- RESULTS:
  - There was a large between country variation with testing performed in-office.
  - Sample type (timed vs. morning vs. random collection) had some variation by country.
  - A large percentage of physicians did not use a confirmatory test for diagnosis of MA.
  - Physicians who had an in-office procedure usually used this procedure for a second, confirmatory test rather than send it out to a larger lab.
  - Most physicians react on critical differences between 2 consecutive results that are much smaller than is analytically justified.
  - There was a large variation between and within countries regarding treatment once microalbuminuria has been diagnosed.
- SUMMARY:
  - There was confusion about the type of sample to collect and reporting units.
  - Guidelines for the diagnosis of microalbuminuria were not followed.
  - Ability to diagnose microalbuminuria in a physician's office is uncertain.
  - Impact of biological variation is not known.
  - There were varied approaches to treatment of microalbuminuria.

EQAS in Scandinavia, Dr. Sandberg:

- Ongoing standardization program to encourage reporting in mg albumin/mmol creatinine.
- In the Norwegian Quality improvement of primary care laboratories (NOKLUS), the sample is fresh urine from patients with microalbuminuria; the program in other Scandinavian countries uses normal urine with added albumin; the urine is pooled, never frozen, and sent fresh.
- There are 2 proficiency samples per year; 700-800 participants; DCA instrument performs well.

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- Grading: “good” if the result is < the target interval ± 7%; “poor” if the result is target interval > ±15%. (Target interval is 0,2 ml /mmol kreatinin)
- Program discourages the use of dipsticks.
- Finland Lab quality program in hospital labs – urine is from humans with added albumin; pools at 2 levels are frozen and thawed in shipping; there are 2 challenges/year.
- EQAS conclusion: Patient samples should be used for proficiency samples and standardization of units should be encouraged.

EQAS in Canada, Dr. Seccombe, “Impact of calibration errors in medical decision making”:

- Calibration error skews test results which skews medical decisions and increases cost of the health care system; physicians tend to have blind faith in lab results assuming that all tests are accurate and there is no lab-to-lab variation.
- Review of world-wide guidelines is very confusing when looking for which tests, which sample, what cut-off points, and how to confirm albuminuria (microalbuminuria); reporting is key to dealing with this problem.
- Regulatory proficiency testing in North America rewards precision, not accuracy because they use peer group means and large ranges for evaluation; there is no incentive to use standardized instrumentation; ACR is often not evaluated.
- Evaluation of labs using dipstick showed wide variability with less than 50% obtaining the correct value; use of dipsticks for albuminuria testing needs to be challenged.
- One manufacturer’s QC material for urine albumin showed an 11-fold difference between the low and high value within the published range of means for 9 different quantitative methods.
- Standardization needs to include measurement, test name, reference intervals to be applied, TE performance goal for field systems, EQA/PT performance criteria, protocol for confirming the result, cut-points to be used in guidelines, and comments to be used with reporting of the test result.

EQAS in USA and Australia, Dr. Miller:

- Experience across the countries is very similar.
- CAP Survey samples are made from human urine, supplemented with albumin and lyophilized; commutability of material is unknown; 3 samples are shipped twice per year and assayed singly.
- Results for albumin show that at the lower range CV variation is more dramatic with some methods having poor performance; at higher levels, the CVs are better and more uniform with CVs generally less than 6% with variation by instrument/method.
- ACR calculated from these proficiency samples yielded a wide variability of results at each level.
- Australian data: Materials are human urine with supplementation and lyophilized – 6 levels, analyzed 4 times/year; within analyzer precision shows similar variability to CAP.
- An Australian/New Zealand practice survey:
  - Sample types recommended by the lab vs. what is received shows variability in the lab recommendation and non-compliance with the recommendation by the physicians.
  - Recommended upper reference limit from 29 labs varied from 15 to 30 mg/L for albumin and 1.0 to 3.6 mg albumin/mmol creatinine.
- CONCLUSIONS: There is variability among labs and methods; variability within albumin methods that needs improvement; and imprecision within creatinine methods seems acceptable, but is open to discussion.
- DISCUSSION: Comment that CV at low concentrations may be deceiving and the SD may meet clinical requirements.

The Japanese Initiative for Standardization - “The Japanese Initiative for Standardization; Preparation of Working Reference Material for Urine Albumin and Total Protein Measurement,” Dr. Itoh:

- Discrepancy of albumin results is caused by lack of international reference material, heterogeneity of the molecular form found in calibrators, presence of degraded or antibody-unreactive albumin in urine, non-

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specific binding of albumin on tubes (negligible now due to improved tubes with hydrophilic coating), and dimeric forms of albumin. Thus, discrepancy in values is an inevitable outcome.

- There is a need for preparations of well-defined reference materials and calibrators.
- Preparation of Prototype I and Prototype II, reference material characteristics: monomeric human serum albumin with a purity of more than 97.5% on HPLC; 0.5 M NaCl, 2% Sucrose, 0.05% NaN3 (1.0 mg/vial) in 20 mM PB; lyophilized; non-biohazard; inter-vial difference is within 3% in the albumin and total protein value by measuring 10 vials 3 times, and precision within 3% CV; stable more than one year stored at 5º C; stable 20 hours at 10º C and 25º C after reconstitution with pure water; preparatory investigations for value assignment from CRM470 according to ISO GUIDE 35 and a BCR report on CRM470.
- Comparison of a tentative reference method with one immunochemical measurement measuring 6 urine pools has excellent correlation as long as the epitope is commonly recognized.
- Application to dipstick test and total protein: reflection ratio measured on a colorimetric analyzer shows similar spectrum using different manufacturer's dipsticks.
- SUMMARY:
  - Working reference material for urine albumin and total protein measurement was prepared, which consists of monomeric albumin of more than 97.5% in purity.
  - The properties almost fulfill the demand for what will be required for a reference material.
  - Value assignment is possible using CRM470 by selected methods with good performance.
  - Primary reference material should be prepared for the establishment of traceability chain in the future.
- Reference material – soon to be: 5,000 vials of a final lot have been prepared in the same manner as prototype II; value assignment will be finished this year and completed in international format, the reference material is to be submitted to JCCLS, international collaboration for this project is welcome.
- DISCUSSION: CRM470 is serum material so a large dilution using pure water is made to use it in a urine method; making a large dilution may have its own set if issues. Dr. Schimmel noted that a new CRM is to be made soon; measurand clarity is needed in order to standardize because materials can only be standardized in terms of a given measurand.

4. Measurement issues for albumin in urine

Sample collection and pre-analytical considerations, Dr. Eckfeldt:

- Literature review yielded representative references and representative examples; there is huge variability in some of these issues which impact the result.
- Albumin in vitro stability: stable for wks at 4º C; a single freezing at -20º C can result in substantial loss of up to 40% although this is variable from patient to patient and dependent on the freezing rate; stability is good if frozen quickly at -80º C.
- Creatinine in vitro stability: Stable for days to 1 week at refrigerated temperature with gradual decrease of about 5-10% over several months; good stability frozen at -20º C and -70º C.
- Stability for both albumin and creatinine: frozen at -80º C for years is very good and is best for epidemiological studies, or liquid at 4º C for up to 1 week is okay for clinical studies; definitely NOT -20º C.
- Biological variability within person is very large; intra-individual variability for albumin excretion rate (AER) is much greater than ACR; daytime variability is greater than night; daytime mean AER and ACR is about 50% greater than in overnight collection or first morning void samples; therefore standardization of collection time is as important as analytical standardization.
- Other factors causing bias in AER or ACR: exercise, hydration and diuresis, inflammation and infections (especially of the urinary tract), blood contamination, and acute stress.
- Conclusion about what to measure and patient preparation:
  - Patient must be at baseline without acute illness, UTI, or inflammation.
  - First morning void seems to have lowest intra-individual variability; possibly screen with randomly collected sample and follow-up with first morning void sample.
  - Data suggests that multiple measurements are needed for diagnosis.

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  - Pre-analytical considerations are MAJOR! We need to worry about these pre-analytical effects which are many-fold higher than the analytical errors of 5 -15%.
  - Postural proteinuria: a small percentage of the population can excrete over 1 gm albumin/g creatinine when standing versus supine, but even for the general population, there is some effect of standing versus supine position on the excretion of protein; the first morning void samples would have minimal increase associated with standing.
- There was a discussion about the total variability versus the biological variability and lab-to-lab variability. Most physicians reviewing the results do not know how much of each of these errors is present; total error between labs could be as large as the quoted biological variation.
- Another point raised is the need to standardize sample handling, e.g. centrifuged vs. uncentrifuged samples and the room temperature versus refrigerated storage of samples that are supersaturated; these factors could affect results if albumin is bound to crystals that precipitate out of solution during refrigeration or centrifugation.

Quantitative urine albumin measurement procedures, Dr. McQueen:

- The HOPE Study used 2 mg/mmol as the cut-point, but Dr. McQueen was unable to find documentation establishing this cut-point; there is a focus on “normal”; more important than a single result is the change in the result between two time points.
- ACR has error in numerator (albumin) and denominator (creatinine).
- Immunoassay is used by most labs but there are many variations of methods, e.g. turbidimetric, nephelometric, RIA, EIA, fluoroimmunoassay, chemiluminescence, and electrochemiluminescence.
- Factors influencing these assays and their detection limits include: different labels; competitive vs. non-competitive assays; homogeneous vs. heterogeneous assays; polyclonal vs. monoclonal antibodies. Yet, all the package inserts quote the same reference ranges.
- Most assays use a turbidimetric method, but some use monoclonal, polyclonal or both antibodies; some are non-competitive assays, while most are competitive.
- Size exclusion HPLC with UV detection has been promoted to detect forms of albumin that are immuno-non-reactive; in diabetics there may be albumin that is “nicked” creating cleavages in the peptide chain, but is held together by its many disulfide bonds and implies that there are higher levels of albumin in early diabetic urine than immunoassays are capable of detecting; size exclusion HPLC consistently yields higher levels of albumin than found in the same urines with immunoassay methods. However, when the HOPE Study urines originally assayed by RIA were reanalyzed using this HPLC method, ROC analysis did not differentiate between the methods as predictors of CV outcomes.
- Chip electrophoresis is an automated system with chip microfluidic separation and fluorescent detection; it has a sensitivity of 5 mg/L and imprecision of 3-13% CV for microalbumin urines up to 200 mg/L.

IDMS candidate reference measurement procedure, Dr. Lieske:

- HPLC assay seemed to detect more microalbumin than did the standard antibody assays, possibly providing an earlier detection of ESRD; therefore, LC-MS method was developed to definitively quantitate urine albumin.
- BSA differs by a couple of amino acids resulting in slightly different fragment sizes, therefore making it useful as an internal standard (IS); concern using BSA as IS so investigated using 15N-labeled recombinant human serum albumin (HSA) which gave comparable results to BSA.
- Standards ranging from 10 - 200 mg/L are made from dilutions of HSA in charcoal stripped human urine which removes most proteins, peptides, and organic compounds, leaving the electrolyte matrix.
- Intra-assay CV is 4 - 6%; inter-assay CV is 12-15% (one at 24%) and may reflect variation due to the daily creation of the standard curve.
- Based on dilution of high samples with charcoal stripped urine, the low limit of detection is 2.5 mg/L and reportable at 10 mg/L.
- CONCLUSIONS: LC-MS measures intact or fragmented albumin; shows great potential to become a reference assay; next steps are to improve precision at the low end sensitivity, quantitate specific fragments, complete comparisons with HPLC and immunoassay methods, complete clinical studies, and protocols to access albumin fragments in different disease states.

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- DISCUSSION: Correlation to immunoassay looks better than with HPLC, possibly due to fragmented albumin; detection limit is determined as a function of the variability, e.g. at 10 mg/L the CVs are about 10 % and down to 2.5 mg/L the CVs are closer to 20%; HSA in standards is quantified by UV measurement.

Urine albumin as a measurand, Dr. Hortin:

- Sources of structural variation in albumin: albumin is a relatively stable structure due to disulfide bonds and globular domains; it is not highly polymorphic.
- There are potential differences between plasma and urine albumin due to increased fragmentation in urine, aggregation, denaturation, chemical modification, and different ligand concentrations; do any of these result in effects on the measurement?
- There have been reports of fragments but there is little information about the proportion of fragments.
- Reports of immuno-non-reactive albumin have been made based on differences in HPLC and immunoassays method; other molecules of about the same size may elute along with fragmented albumin; it is very difficult to make immuno-nonreactive protein; even enzyme digested albumin is still immuno-reactive.
- Western blot shows only small amounts of fragmented albumin in fresh urines while samples frozen at -20º C have a large proportion of fragmented albumin; differences in immunoassay results with large amounts of fragmented albumin are likely related to which epitope of albumin is recognized by the antibody in each assay, i.e. monoclonal antibodies are more susceptible to albumin modification.
- Another important variable in the detection of modified albumin is competitive vs. non-competitive assay.
- Based on studies analyzing fragmented albumin:
  - A turbidimetric assay was found to react nearly equivalently with albumin fragments.
  - Antibody specificity was directed to epitopes distributed across all 3 of the CNBr peptides.
  - Other assays had variable reactivity with fragments. Competitive assay formats or assays that use monoclonal antibodies may have more variable reactivity.
- CONCLUSIONS: Albumin fragments occur but need further quantitative analysis and studies of the mechanism of formation; reactivity of each assay with fragments must be defined as an operating characteristic.
- DISCUSSION: All experiments were done in vitro using chemically modified albumin; fresh samples usually have small amounts of fragments that are usually internally "nicked"; urine from patients with inflammatory kidney disease and glomerular nephritis were assayed and they did not show much fragmentation; what does an epitope look like? Protease digestion cuts between domains leaving conformational and sequential epitopes; there are problems looking at totally reduced and alkylated albumin which would completely break the 3-dimensional structure; this creates precipitation problems.

IVD industry practices for albumin calibration, Dr. Zakowski:

- Information from 7 manufacturers was presented; package inserts for all of the methods are available from Dr. Zakowski on a CD. The list of methods is estimated to account for 90-95% of clinical lab testing in central labs. Most are immunoassays with one representative dipstick, and the methods represent both the competitive and noncompetitive formats. This list does not accurately represent the high volume of Point-of-Care and Physicians-Office environments that use predominantly dipstick technologies.
- Primary reference material used by most manufacturers was CRM470, ERM470, and BCR470 (all are the same material with different names).
- There is no reference method and no urine reference material; generally, CRM470 plasma was diluted and used as the urine reference material; there are likely variations between manufacturers in the protocol, e.g. diluent, dilution, etc.
- Traceability scheme is generally per ISO 17511, but there is reportedly (but questionable) wide variation in results, which is likely due to variation in traceability scheme details, e.g. dilutions, diluents, plasma vs. urine matrix, value transfer protocols.

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- The broad statements about large variability are not supported by data from the CAP LN20-B 2006 U-A Survey, which do not show much variation with the exception of 2 outliers which are 2 methods by the same manufacturer; CAP 2007 UA Survey sample U2 showed a problem with two methods by the same manufacturer (also mentioned by Dr. Miller), but with that exception, overall there is not much variation.
- Just a reminder, high CVs at the low end can be deceiving; we need to look at the SD, e.g. at a level of 10 mg/L with 10% CV, the SD is 1mg/L.
- It is important to determine what difference among methods is clinically significant?
- There are a mix of reference ranges and units listed by the different manufacturers; some refer to the ADA recommendations. Standardizing reporting is an issue.
- Analytical measurement ranges varied by assay system. Most had low ends about 2-5 mg/L and high ends about 200-400 mg/L. Some were able to use automatic sample dilutions to achieve results up to about 10,000 mg/L.
- DISCUSSION: There was a question about the different names for the CRM. It has been known by several names over many years; different names relate to who distributed it and the inserts may be old and not updated to the current name. It is serum based and not plasma based. The bottom line is that it is all the same material. Dr. Curhan commented about what makes a clinically different result, e.g. 2 mg/L vs. 4 mg/L may make a clinical difference. There was concern expressed that using CRM470 for standardization may cause problems related to dilution, matrix effects, and lack of commutability; it is recognized as a poor standard, but it is all that is available at this time.

5. Impact of change in calibration of urine creatinine on the albumin/creatinine ratio

Status of serum creatinine standardization program, Dr. Miller:

- The goal of the creatinine standardization program to improve accuracy and consistency of eGFR by eliminating bias in creatinine assays and recalibration of existing methods is expected to be completed by 2008.
- The approach is to have the IVD manufacturer establish calibration traceability to the highest order reference system with no additional effort by routine laboratories.
- Standardization can be achieved by splitting samples with reference laboratories that perform JCTLM approved IDMS methods and/or using NIST SRM 967 which has just been made available; commutability of SRM 967 with native human sera has been validated.
- In the 2006 CAP Chemistry and Urine Surveys approximately 15% of labs reported that their methods were traceable to IDMS. A comment was made that even though labs are using methods with calibration traceable to IDMS methods, they may not have changed their reference ranges appropriately.
- CAP in collaboration with the NKDEP Working Group, has developed calibration verification material which is fresh frozen serum pools that have commutability documented and values assigned by NIST IDMS method (LN24 Survey). The Dec 2006 survey results illustrate that some manufacturers have introduced IDMS traceable calibration and others have not yet done so.
- Many creatinine methods use the same calibration for serum and urine; but there are differences in viscosity and pH of sample type; also, there are potential differences in method specificity to serum and urine interfering substances.

Traceability considerations specific to urine creatinine, Dr. Greenberg:

- Some manufacturers use the same calibrator and the same assigned values for both serum and urine creatinine standardization. To ensure calibration traceability for IVDs according to the requirements of ISO17511, one must first define the measurand, which includes the sample matrix, and then assign values as appropriate to the particular matrix. With this approach, unique assigned values that are sample matrix specific, even for a single calibrator, are a possible outcome. The key is that assigned values of calibrators need to be appropriately determined independently for each sample type, to ensure trueness in reported values for both urine and serum samples.
- JCTLM lists reference materials available for serum (NIST SRM 909b, IRMM BCR573, 574, 575, and SRM914a [crystalline material]), but nothing is listed for urine creatinine (although the SRM914a

crystalline material should be suitable for high level reference methods for serum or urine). There are no urine matrix reference materials currently available.
- JCTLM lists 3 GC-IDMS reference methods for serum creatinine, and only one method for urine creatinine.
- The metrology infrastructure does not provide tools for creatinine calibration traceability in urine samples; Ortho is using SRM914a and an internal HPLC method that has been validated by a German reference lab using a JCTLM listed GC-IDMS method, but the reference lab is very costly and availability is limited, e.g. results for urine samples sent in January had not been returned as of March 27.
- The pass/fail criteria for PT/EQAS is ± 3 mg/L or ± 15% for AAB and ± 15% for QMPLS.
- CONCLUSIONS: Urine matrix reference material is not available; there is only one lab worldwide with a reference method for urine creatinine; inter-method variation is about 3.5-4% while variation within a single method may be more significant; pass/fail criteria for PT/EQAS of ± 15% may not be sufficient to support a ratiometric application of urine creatinine values in determining ACR ratios.
- COMMENTS: Dr. Schimmel explained that a reference method is determined by assessment based on technical soundness and publication in peer reviewed journal; JCTLM web page defines reference methods by analyte and matrix; a reference laboratory is selected for a specific analyte, by a specific method and application of that method; reference laboratories must obtain accreditation through national organizations (not JCTLM).

March 28, 2007

6. Discussion group reports: Consensus of current status and recommendations for improvement

Group 1 Report: Define the measurand; what molecule(s) or fragments should be measured, Drs. Eckfeldt, Bunk, Hortin, McQueen, and Miller:

- Characterizing fragments found in urine:
  - A better understanding of the cysteine 34 is needed; in vivo, many things attach to cysteine 34; this may have an impact on different methods; cross-linking at this amino acid can yield formation of inter-albumin disulfides.
  - Need a better understanding of the effect on albumin measurement caused by nicked albumin; there are internal cleavages, but the molecule remains structurally intact due to other disulfide bonds.
  - Need a better understanding of the amount of albumin glycation and its impact on the measurement; this has implication for measurement in diabetics versus non-diabetic patients.
  - Monoclonal antibody methods seem to be more sensitive to these issues than polyclonal antibody methods.
- Impact of pH, salt, and other urine molecules has already been discussed.
- Understand storage effects and how many of the changes, e.g. degradation, nibbling off the N terminal, is influenced in vivo versus in vitro; in vivo changes can occur in the bladder based on presence of urinary proteases.
- Proposed experiment to help define analytical performance:
  - Develop a panel of hundreds of clinical urines; characterize patients for drugs and measure various parameters such as pH by dipstick, specific gravity, osmolality, etc.
  - Make multiple aliquots and send to manufacturers to measure albumin by different methodological principles.
  - Also measure diluted CRM470 materials and EQAS materials; include samples with known albumin fragments.
  - If patient samples correlate well, then there is a calibration issue.
  - If patient samples are scattered, then there is a problem of analytical specificity.
  - This experiment would provide information about how many of these issues are important versus simply hypothetical.
  - There are different ways to evaluate the data from this experiment; in a matrix effect study, the x-axis is the reference method and the y-axis is the field method; for this experiment, we could compare field

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method "x" to field method "y", looking at all of the possible comparisons for the methods; or the x-axis could be the median of all methods; alternatively a multivariate approach could be used to look for clustering;
- If the Mayo IDMS method is included this would help determine if the N-terminal 24 peptide fragment is correlated to albumin found in clinical urine samples.
- There should be adequate excess aliquots remaining after the experiment to evaluate outliers.

- DISCUSSION:
  - If we do the experiment, what diseases, racial groups (white, black, Hispanic, Asian), etc should be looked at to get a view of this problem? Depending on how much stratification is done, there may be between 300 -1000 samples. Perhaps initially, we could just record race rather than include a specified number of samples from each race.
  - Qualification study: Before the above experiment can be done, two issues need to be determined,
    1) The affect of sedimentation by centrifugation and
    2) Albumin absorption onto vessel walls (including collection, analyzer, and storage containers). Dr. Itoh stated that hydrophilic coating of the vessel is best.
       - In serum, the actual percent of protein that binds to the vessel is a very insignificant amount of the total, but in the low concentration range found in urine, the amount that binds can be significant.
       - Typical collection cups are polystyrene/polypropylene and the ratio of these compounds varies by manufacturers
  - Keep the initial testing simple; keep additional aliquots in storage to clarify outliers. We want to use clinical samples so that there is access to clinical information.
  - Use only one or two methods for qualification studies; however, evaluation of binding to analyzer containers may have to be done by all participating manufacturers by loading cups and leaving them for a specified amount of time before analysis;
  - The BD website has information about collection containers; Dr. Zakowski volunteered to ask BD about any studies they have related to absorption onto their collection containers.
  - The objective of this experiment is to understand the magnitude of the between-method variability and whether the cause of the variability is a calibration or specificity issue; the samples should be collected from patients with diseases causing moderately elevated urine albumin.
  - Manufacturers may be willing to assay ~100 samples so the experiment needs to target fewer questions. A systematic approach that answers multiple questions requires thousands of samples to be assayed by each method; the simple approach is to take samples as they arrive in the lab and characterize specific things; if we start with a small number of samples, e.g.100, and they all correlate then we are done; more likely it would define certain problem methods that may need a more targeted approach. Dr. Miller commented that we will likely need 200 - 300 samples to get enough information for assessment of the problem; a staged approach will allow us to focus on specific issues.
  - Dr. Bruns asked about the affect that urinary bacteria have on albumin and creatinine measurement. Dr. Eckfeldt stated that the literature discussed adding azides to stabilize urines to bacterial growth.

Group 2 Report: Calibration traceability issues (reference material; reference measurement procedure; traceability and harmonization), Drs. Greenberg, Itoh, Lieske, Myers, Panteghini, Schimmel, and Zakowski:

- The reference system is missing key elements: definition of the measurand, i.e. understanding urine albumin molecular species/epitope and the similarity, or lack of, to serum albumin; definition of appropriate reference material, e.g. appropriateness of CRM 470 (a serum matrix reference material) as surrogate for the measurand, albumin in urine; specific instructions for preparation of the reference material, e.g. dilution, weighing, diluent; lack of a universally agreed upon reference procedure.
- Clinical performance goals: define the amount of bias that can be tolerated, define cut-points relative to age, gender, and race; evaluate performance imprecision and bias in current methods for both urine creatinine and albumin; a statistical simulation is needed to model total error and establish performance criteria.
- Further investigations needed for the Mayo IDMS procedure: N-terminal 24 peptide fragment is potential target; need to determine if this fragment exists in vivo and further characterize the fragmentation pattern both in vivo and in vitro; determine if the measurand is intact albumin or also includes fragmented albumin; determine the relationship between current field method specificity and the N-terminal 24 peptide fragment detected in IDMS method, i.e. IDMS and routine methods must measure the same thing.

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- Further investigation of candidate microalbumin reference materials: need a good definition of the measurand, i.e. intact and/or fragmented albumin; fragmentation can occur in both the patient samples and candidate reference materials; native human albumin spiked into stripped urine matrix vs. CRM 470 dilution protocol; define appropriate matrix for reference materials, i.e. evaluate how the variability of the urine matrix components (pH, protein, osmolality, and viscosity) affect the measurement, and define sample diluent specification.
- Consider implications for field methods based on reference measurement procedure specificity and choices: definition of the measurand; antibody specificity; antibody and assay format specifications; establish field test system with regard to urine matrix.
- Urine creatinine standardization issues: reference method and higher order reference materials need to be established, but encouragement is needed for the expansion of reference lab network to improve access; need to support traceable calibration with appropriate matrix reference material; linking the assignment of values to urine creatinine calibrators from the assigned serum creatinine calibrator value based on a dilution factor may not be appropriate; independent validation of each calibrator for a given sample matrix and sample type is more appropriate.
- RECOMMENDATIONS:
  1) Define clinical measurement goals for both albumin and creatinine in urine; establish goals based on a statistical simulation system and error modeling; compare to state of the art performance. Then re-evaluate needs for other recommendations based on that gap analysis.
  2) Define a full reference system for urine albumin measurement, including agreement on the definition of the measurand, reference materials, and reference procedure; answer critical measurand question about intact versus fragmented albumin; rule out the presence of N-terminal 24 peptide as naturally occurring in patient samples and ensure that there is a constant ratio of N-terminal 24 peptide to “human urine albumin” measurements in field methods; encourage and support further development of the Mayo IDMS method, or others, as a candidate reference procedure.
  3) Define urine albumin reference materials; Dr. Itoh/Japanese project should be completed and published to demonstrate the relationship between urine albumin and serum albumin reference materials, that could validate the application of CRM470 serum reference material for urine albumin measurement; if CRM470 does not prove useful as a urine albumin reference material, determine suitability of JCCLS purified human albumin as a reference material; encourage parallel development by other institutions of candidate human albumin reference materials.
  4) Define specifications for routine methods to ensure robustness to urine matrix variations; impact of pre-analytical centrifugation and sample storage on measurement of albumin and creatinine.
  5) Encourage providers of urine creatinine reference methods to increase capacity and access.
  6) Define and develop matrix reference materials for urine creatinine.
- DISCUSSION: There was much discussion about the amount of analytical error (measurement uncertainty) that is acceptable; this may differ depending on the use of the test, e.g. for diagnosis standardization is more important while for monitoring, precision is key; there are clinical and economic implications of poor performance; acceptable analytical error is relative to (some fraction of) the biological error; the goal is to educate and ensure that generalists and non-physicians who use these results have a better understanding of the variability of the entire system; in reporting ACR, the cumulative/compounded analytical errors of both albumin measurement plus creatinine measurement are important to understand, although using the ratio of the two is intended to remove biological variation; define clinical measurement goals and compare to the state of the art performance, e.g. if 15% CV is needed and the state of the art is 10%, then no further work is needed.

Group 3 Report: Standardization of sample requirement and reporting, Drs. Seccombe, Bruns, Curhan, Fleming, Narva, and Sandberg:

- Clinical, screening, and research applications may have different sample and reporting requirements; one sample type may be better for screening and a different one for diagnosis, management and accessing outcomes; education about reporting requirements should be tailored to each of these specific needs.
- Confirmation of the best sample and conditions for collection is needed; does the first morning void provide better clinical information or have a better predictive/monitoring value than a random collection?
- Studies are needed to evaluate diurnal variation, postural and exercise effects on excretion of albumin.

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- Biological variation: determination of biological variation differences between those with normal versus impaired kidney function and with time of collection; relation of any difference to diagnosis and prediction of clinical outcome.
- Age, gender, and race specific cut-points are required, but need to do this with standardized methods.
- Pregnancy – can ACR replace the use of total protein for clinical decision-making in pre-eclampsia; standardize to one measurement of protein to make clinical decisions.
- Applicable restrictions when ordering this test, e.g. steady state, non-menstruating, free of concurrent infections, supplements, other factors known to adversely impact the test result.
- Identify known methodological interferences (including non-specific binding of protein in the collection container).
- Name of the test is important, e.g. urine albumin or albumin excretion rate; The albumin:creatinine ratio (ACR) is used as a surrogate for albumin excretion rate, which is what we want to know and is a concept that physicians are comfortable with. Proposal was suggested to develop a calculation, analogous to the MDRD equation, to convert ACR to an estimated albumin excretion rate (in mg/day); this would avoid problems of different reference intervals for women and blacks, etc., and would eliminate differences that arise from use of SI vs conventional units. Other similar clinical applications of this type of algorithmic reporting are INR and AFP; clinicians don't have to remember many cut-points for all the variables; need standardized methods before application of this type of algorithm can be investigated.
- Report individual values for albumin, creatinine and the ratio; standardize protocols for screening and confirming a positive result.
- Standardization of reporting units would be ideal, but not practical, e.g. mg/g and mg/mmol/L; both units should be used in any NKDEP materials.
- Emphasize concept of continuously progressive risk through education and in reporting formats used by labs.
- Provide information on the value of the test, e.g. how good is it and how it should be used in the assignment of risk and for monitoring outcomes (CVD, CKD, as a screen for early disease); once the methods are standardized, the discriminating power may be even greater than it is now.
- Conduct a thorough review of the literature and summarize the information that is known about biological variation, pre-clinical variation, etc; identify the gaps that exist and conduct the appropriate studies to fill in the gaps using a standardized methodology.
- Optimize the sampling protocol for the test and then apply it in addressing the baseline studies that are needed for establishing the cut-points and confirming the clinical value of the test.
- DISCUSSION: It was felt that recommendations about collection and reporting can be determined from available data bases, but time and standardized methods are needed to validate use of an algorithm; the paradigm shift of going from urine dipstick to quantitative albumin excretion rate is equivalent to going from serum creatinine to eGFR; reconsideration of using the sample collection with lowest biological variability suggested that it may be more informative to use a sample under more stressed conditions than the first morning void; a study of data sets to look at time of collection and outcomes 5-8 years later may provide an answer to this issue; it was not felt that these answers can be found in a few months; funding sources need to be identified to study some of these issues.
- See also Appendix 1: Additional Discussion from David Bruns.

7. Prioritization of tasks to address the questions raised in discussion groups:
(Note: Names represent interest, not commitment.)

1. Define clinical measurement goals for both albumin and creatinine in urine.
   - Establish goals based on statistical simulations, error modeling, and consultation with experts in Nephrology for clinical needs, including calculated albumin/creatinine ratio. Compare to state of the art performance and to biologic variation literature. G. Curhan, S. Sandberg,

2. Review of literature to understand pre-analytical issues for urinary albumin and creatinine, containers, stability, etc. – J. Fleming, G. Myers

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3. Define specifications for routine method robustness to human clinical sample matrix variations
   - Sample pH, osmolality, viscosity, solids (to centrifuge or not prior to assay?), others.
   - Expert consensus on the range of concentration of various urine components over which albumin needs to be measured. Mine large databases to determine the range of variables observed in clinical samples; then, it is the method manufacturer's job to determine if their method is valid over the ranges defined for the variables. J. Fleming,

4. Develop a panel of native urine samples to assess performance issues by a round robin with routine and IDMS methods; include native urine, candidate RM, EQAS samples, urine containing modified albumin forms, diluted CRM470. M. McQueen, J. Lieske, G. Miller, G. Hortin

   Pre-qualification panel (to be completed before the panel of native samples):
   a) Evaluation of adsorption to collection, storage, and sample containers; J. Zakowski will get information from different manufacturers about storage containers; J. Eckfeldt, D. Bruns to do studies; Y. Itoh volunteered to assist and has experience with hydrophilic containers
   b) Effect of centrifugation: J. Eckfeldt will look at pre/post sediment urine samples with in-house methods for albumin and creatinine.

5. Redefine a reference system for urine creatinine
   - Review requirements to be suitable for urine creatinine to be used in a ratio with albumin
   - Reference measurement procedure exists
   - Encourage increased capacity/access to providers of urine creatinine reference measurement procedures (IFCC WG-GFRA)
   - Define/develop matrix reference materials for urine creatinine - 10 and 200 mg/dL: this is a different issue from urine albumin reference material; NIST could make by spiking in creatinine. D. Bunk, H. Schimmel, G. Miller

6. Define a full reference system for urine albumin.
   - Measurand, reference materials, reference measurement procedures.
   - Answer critical measurand questions re: intact vs. fragments of albumin molecule. J. Lieske, G. Horton
     - Rule out presence of N-terminal 24 peptide epitope as normally occurring fragment in patient samples
     - Ensure constant ratio/relationship of N-terminal 24 peptide to “urine albumin” measurements determined with common field methods.
     - Refer to IFCC nomenclature, properties and units committee for technical measurand name
   - Complete and publish data from the Japanese standardization project to demonstrate that albumin (urine) = albumin (serum) to validate application of dilute CRM470 as reference material. Y. Itoh
   - If CRM470 serum albumin is not suitable, determine suitability of JCCLS candidate reference materials (purified human albumin); confirm commutability with native urine. Y. Itoh
   - Encourage parallel development of alternative candidate urine albumin reference materials.
   - Encourage/support further development of Mayo candidate reference method.

7. Nomenclature for clinical reporting urine albumin (“microalbumin”); collaborate with renal groups. Concept to order “urine albumin,” and report a standardized parameter to be determined. A. Narva

8. Correlative data between albumin/creatinine ratio (ACR) and albumin excretion rate (AER). Develop a new algorithm to convert ACR to AER that incorporates gender, age, and race factors and would not be released until standardization was accomplished. A reference interval for the reported parameter needs to be developed and related to risk assessment. D. Bruns, G. Curhan, J. Lieske

     - Clinically useful values and/or cut-points may be lower (maybe 5-10) than “30” mg/g that is used now for ACR. Risk assessment could go down to zero. If using the parameter to predict risk, then need to measure to see if treatment causes the parameter to decrease.

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8. Immediate next steps:

1. Publish report of this meeting from the NKDEP/IFCC working group. The report will review current practice and address the status of issues and recommendations for addressing the issues. The publication can be linked from web sites NKDEP and IFCC. Could reach clinicians through NKF or American College of Physicians newsletters, and *Endocrine News*.

2. Writing manuscript: G. Miller will take lead for organizing and recruit volunteers for sections based on reports and group discussion summaries.

3. How to tap resources for tasks that will require funding – a group conference call will be scheduled in approximately 6-8 weeks, after minutes and list of tasks are circulated to review the information. Sources of funding to be investigated by G. Miller and A. Narva; applications for funding specific tasks can be made to IFCC.

9. The Lab Working Group (LWG) will meet at AACC in San Diego on July 17, 2007 from 8 am – 12 pm. The LWG will meet for approximately 2.5 hours, followed by an IVD manufacturers' update.

Meeting adjourned at 11:52 am

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Appendix 1. SUMMARY OF DISCUSSION, STANDARDIZATION OF REPORTING OF RESULTS OF URINARY ALBUMIN TESTING

Albumin excretion rate (AER) and albumin:creatinine ratio (ACR)

Proteinuria is classically defined in terms of the rate of urinary excretion of protein, and reference intervals are related to this rate of excretion. The ratio of urinary concentrations of protein and creatinine is often used to avoid the requirement (and errors) of collecting a timed urine specimen to calculate the urinary excretion rate of the protein. The concept underlying this approach is that the rate of excretion of creatinine is reasonably constant and thus the amount excreted reflects the time period of the urine collection.

The amount of a substance excreted in a period of time is calculated as

(concentration of the substance) *times* (volume of urine excreted during that time period).

When the excreted amounts are used in a ratio (a protein and creatinine), the volume terms for both the protein excretion and the creatinine excretion cancel out, and the ratio of their concentrations provides an indicator of the rate of excretion of the protein.

(concentration of protein) *times* (volume of urine excreted during that time period) (concentration of creatinine) *times* (volume of urine excreted during that time period)

= (concentration of protein)
(concentration of creatinine) .

The albumin:creatinine ratio (ACR) is accepted as a surrogate for the albumin excretion rate (AER) because it is highly correlated with AER. Random (non-timed) specimens are collected at any time of day for the ACR, and the usual reference interval is 20-200 mg albumin/g creatinine for both men and women.

Use of ACR as a surrogate for AER involves several assumptions; two key assumptions are not met in practice:

1. To use a single reference interval, the rate of excretion of creatinine is tacitly assumed to be the same in all individuals. By contrast, the excretion of creatinine is well recognized to be affected by a large number of variables, e.g., it is greater in men than in women, greater in blacks than in whites, decreased with muscle wasting, and affected by diet.
2. The excretion rate of creatinine is assumed to be constant throughout the day, but it is not. For example, different rates are associated with first-morning-void specimens, 2-hour (daytime) specimens and 24-hour specimens.

Specific gravity or osmoles of solute are sometimes used in place of creatinine but suffer from similar problems.

Estimated Albumin Excretion Rate as an Alternative to ACR

As an alternative to ACR, we envisioned use of an estimated AER (eAER) based on conversion of the measured ACR to a corresponding AER based on correlation data for ACR and AER in specific demographic groups (such as men and women). Thus the eAER would be determined for patient groups defined by demographic and clinical variables, notably sex and age and time of urine collection (e.g., first morning void vs random daytime sample). This approach has several potential advantages:

1. It allows use of single (and valid) reference interval for males and females and for different times of urine collection.
2. It provides the clinician with a better estimate of the variable of interest (AER) than can be achieved by adjusting the ACR “in one’s head”.

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The key requirement for determining the eAER is knowledge of the regression equations relating ACR and AER in specific demographic and clinical groups. Required data are likely available, but need to be analyzed properly, in ways analogous to the analyses that have been done to allow use of the MDRD equation to estimate GFR. Some additional studies are likely to be needed to extend the available data to demographic groups that have not been studied.

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