Development and Performance of an Albumin-Creatinine Ratio Assay on the Afinion AS100 Analyzer

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Background: Diabetic nephropathy is one of the most serious and most frequent secondary complications of diabetes mellitus, resulting in increased morbidity and mortality rates. Microalbuminuria is the earliest stage of diabetic nephropathy and is characterized by a persistent and significant elevation in urinary albumin excretion. When quantifying urine proteins, creatinine measurements are used to correct for varying diuresis because creatinine is produced at an approximately constant rate.

Methods: The Afinion AS100 Analyzer is a compact, benchtop, multiassay analyzer for point-of-care testing. The Afinion ACR assay presented here analyzes both the albumin and creatinine levels in a urine sample simultaneously within a single device. Albumin is quantified using an immunometric membrane flow through assay, using monoclonal antibody-coated membrane and monoclonal antibodies conjugated to colloidal gold. Creatinine is quantified using an enzymatic colorimetric test involving 4 enzymatic steps. At analysis completion, the concentrations of albumin, creatinine, and albumin-creatinine ratio (ACR) are shown on the Afinion AS100 Analyzer display screen.

Results: Measurement ranges are 5 to 200 mg/L (albumin) and 16 to 340 mg/dL (creatinine). Both assays are linear over the whole dynamic range. Comparison of Afinion with Siemens DCA2000 and Roche Modular using 95 samples resulted in a linear correlation coefficient (r) of 0.99 for albumin (both methods) and 0.99 and 1.00, respectively, for creatinine. Total imprecision is 5.5% or lower for albumin, 3.8% or lower for creatinine, and 6.0% or lower for ACR.

Conclusions: The Afinion ACR assay provides a reliable, precise, and convenient point-of-care method for simultaneous determination of albumin, creatinine, and ACR in 5 minutes.

Key Words: ACR, Afinion, POC test, albumin, creatinine

(Point of Care 2009;8: 16–20)

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finion albumin-creatinine ratio (ACR) is an in vitro diagnostic test for quantitative determination of albumin, creatinine, and ACR in human urine. Measurement of urinary albumin aids in the early diagnosis of nephropathy.1,2 Sustained elevation of urinary albumin concentrations is known as microalbuminuria. The American Diabetes Association and the National Kidney Foundation define the medical decision points for ACR values as follows: normal, less than 30 mg/g; microalbuminuria, 30 to 300 mg/g; and clinical albuminuria, greater than 300 mg/g.2–4

Microalbuminuria is associated with several late complications of diabetes, such as retinopathy and neuropathy, as well as essential hypertension, preeclampsia, cardiovascular diseases, inflammatory conditions, and mortality. Today, ACR is a predictive marker of great importance in the early detection of kidney disease and the identification of patients at risk for complications from diabetes or hypertension.2–7

In this study, we report the development and performance of an ACR in vitro diagnostic test for quantitative determination of albumin, creatinine, and ACR in human urine using the Afinion AS100 Analyzer. The Afinion AS100 Analyzer is a compact, benchtop, multiassay analyzer for point-of-care (POC) testing, combining advanced immunoassay and optomechanical technology, with an integrated camera, computer, and LCD display. A simple sampling procedure and no requirement for manual calibration or chemistry handling make the Afinion ACR assay system well suited to POC testing. The reportable range of the Afinion ACR assay is 1.0 to 1250 mg/g for ACR. To ensure that correct albumin, creatinine, and ACR results are reported, the Afinion AS100 Analyzer performs optical, electronic, and mechanical controls of the capillary, the test cartridge, and all individual processing steps during the course of each analysis. When an error is detected by the built-in fail-safe mechanisms, the Analyzer terminates the test and displays an information code.

MATERIALS AND METHODS

Afinion ACR Test System

We have developed the Afinion ACR in vitro diagnostic test for quantitative determination of albumin, creatinine, and ACR in human urine on the Afinion AS100 Analyzer (Axis-Shield PoC, Oslo, Norway) (Fig. 1). The main components of the ACR test cartridge are the sampling device and the reaction container.

Urine is sampled (3.5 μL) using a sampling device integrated into the test cartridge. The test cartridge has a handle, a barcode label with lot-specific information, and an area for sample identification. The Afinion ACR test cartridge contains all the reagents necessary for determining albumin and creatinine in urine. The reagents and materials are prepared according to the manufacturing procedure at Axis-Shield PoC. The Afinion ACR test cartridges are packed separately in foil pouches with a desiccant bag.

Albumin is quantified using an immunometric membrane flow-through assay, using specific monoclonal antibodies immobilized to a nitrocellulose membrane and conjugated to colloidal gold particles. The gold-antibody conjugate binds to sample albumin bound to the membrane, making quantification possible by color measurements using the built-in digital camera.
Creatinine is quantified using a modified peroxidase-coupled kinetic enzymatic procedure, using 4-aminoantipyrine and 2,4,6-triiodo-3-hydroxybenzoic acid. Kinetic transmission measurements using LEDs, with dominant wavelength at 530 nm, are used to estimate the reaction end point. A blank sample measurement corrects for endogenous creatine.

The sample concentrations of albumin, creatinine, and the calculated ACR are displayed on the Afinion AS100 Analyzer screen. The Afinion ACR reportable ranges are the following: 5.0 to 200.0 mg/L for albumin, 1.5 to 30.0 mM for creatinine (EU units), and 0.1 to 140 mg/mmol for ACR (European Union). The corresponding United States units for creatinine and ACR are 16.4 to 339.9 mg/dL and 1.0 to 1225.0 mg/g, respectively.

Assay Calibration, Measuring Principles—Creatinine

A total of 3 standard curves are used for the quantification of creatinine in the sample, that is, (1) a creatinine standard curve, (2) a creatine standard curve, and finally (3) a standard curve correcting interference from creatine on the creatinine measurement. To minimize the assay time, the instrument software uses a specially designed algorithm to process the information from both fixed time and kinetic transmission readings, in combination with calibration curves 2 and 3, to predict the end point of the enzymatic creatinine reaction. This predicted end point value is then used as input to calibration curve 1 to calculate the creatinine concentration in the sample.

Both the creatinine and the creatine standard curve were established using creatine and creatinine standards traceable to SRM914a (SRM, Standard Reference Material, National Institute of Standards and Technology, Washington,Md). The creatinine standard curves are all established in millimolar (EU units). In the Afinion AS100 Analyzer, the creatinine result can be presented as US units (milligrams per deciliter) by use of the appropriate conversion factor (11.312 g/dmol).

Assay Calibration, Measuring Principles—Albumin

Albumin is quantified using a solid phase immunometric assay. The sample is aspirated through the antialbumin antibody-coated membrane, concentrating and immobilizing the albumin in the sample. A gold-antibody conjugate binds to the immobilized albumin, resulting in a red-stained membrane. Excess gold-antibody conjugate is removed in a washing step. The color is quantified using reflectance photometry using LEDs with dominant wavelength around 530 nm as illumination source. An albumin standard curve is used to calculate the albumin concentration in units of milligrams per liter in the urine sample. The quantification of albumin is traceable to the reference preparation ERM-DA470 (ERM; European Reference Material, Geel, Belgium).

Calculation of ACR

Based on the measurement of albumin and creatinine, ACR is calculated as follows:

$$\text{ACR (mg/mmol)} = \frac{\text{albumin (mg/L)}}{\text{creatinine (mM)}}$$

In the Afinion AS100 Analyzer, the ACR result can be converted to US units (milligrams per gram) by multiplication of ACR (milligrams per millimol) by the appropriate factor inserted (8.84017 mmol/g).

Samples

Urine samples were received from Aker Hospital, Først AS Med-Lab, Lovisenberg Hospital, and in-house (all in Oslo, Norway). Urine samples were stored at 2°C to 8°C for a maximum of 5 days or frozen until use.

Linearity

The linearity of Afinion ACR was studied by analyzing urine samples containing different proportions of albumin and creatinine. One sample with high albumin (201.8 mg/L) and creatinine levels (343.7 mg/dL) and one with low albumin (4.0 mg/L) and creatinine levels (11.3 mg/dL) were obtained, and 8 additional samples were prepared by mixing the 2 samples in different ratios. This resulted in a series of samples with a concentration range of 4.0 to 201.8 mg/L for albumin and 11.3 to 343.7 mg/dL for creatinine. The samples were measured in replicates of 4, and the mean values were used in the calculation. Linear regression was performed for both the albumin and creatinine sets of data.

Sample Dilution

For analytical recovery studies, saline was added to an albumin standard (206.9 mg/L) and a creatinine standard (416.2 mg/dL). The standards were analyzed undiluted (S1)
and diluted with saline 1+1 (S2), 1+2 (S3), and 1+3 (S4). The samples were measured in replicates of 4 using Afinion ACR. Recovery (in percentage) was calculated based on mean measured value/theoretical value.

**Imprecision**

Urine samples containing low, medium, and high levels of albumin, creatinine, and ACR were chosen for the precision study, divided into small portions (250 μL), and stored frozen until use. The samples were thawed at ambient temperature and mixed well before use. For assessing within-run, between-day, and total imprecision, samples were analyzed for 20 operating days with 2 replicates run twice each day, each separated by at least 2 hours. The study was performed according to National Committee for Clinical Laboratory Standards guideline EP5-A2. The between-instrument imprecision study was performed with 5 Afinion AS100 Analyzers in 1 operating day, with 10 replicates of each of the 3 urine samples (containing low, medium, and high levels of albumin, creatinine, and ACR) being processed by each analyzer.

**Method Comparison**

The Afinion ACR assay (Axis-Shield PoC) presented here was compared with 1 laboratory method (Modular; Roche Diagnostics GmbH, Penzberg, Germany) and 1 POC method (DCA 2000; Siemens, Kernenforsville, NC). Fresh urine samples (95 in total) were analyzed as singletons. The reagent kits used on Modular were Kreatinin plus and Albu-XS, both from Roche Diagnostics GmbH (Mannheim, Germany). The reagent used on DCA 2000 Analyzer was DCA 2000 Microalbumin/Creatinine kit from Siemens. The methods were compared by linear regression analysis and Bland-Altman analysis.

**Interfering Substances**

All chemicals used were of analytical grade. Glucose, creatine, urea, bilirubin, ascorbic acid, DL-beta-hydroxybutyric acid, acetalaminophen glucuronide, metformin, glyburide, acetone, and ibuprofen were all obtained from Sigma-Aldrich (Steinheim, Germany). Dimethyl sulfoxide and salicylic acid were from Merck (Darmstadt, Germany). Myoglobin and β2-microglobulin were from ProSpec-Tany TechnoGene Ltd (Rehovot, Israel). Immunoglobulin G (IgG) was from Biospecific/Fortron (Emeryville, Calif). Acetaminophen, salicylic acid, and acetylsalicylic acid were from Apotekerproduksjon, Norsk Medicinaldepot AS (Oslo, Norway). Acetocetate was from Fluka (Steinheim, Germany).

The effects of these compounds were analyzed using 2 urine samples, 1 with low albumin (<20 mg/L) and high creatinine (>170 mg/dL) concentrations and 1 with high albumin (>40 mg/L) and low creatinine (<110 mg/dL) concentrations, respectively. The samples were stored frozen until use. For each of the 2 urine samples used, a spiked and a nonspiked version of the sample were made by adding 5% to 10% vol/vol of a stock solution of the compound to be tested to the former and the corresponding volume of solvent only (without the compound present) to the latter. The samples were mixed and analyzed on the same day. The samples were run in replicates of 4. Substances that yielded a mean result deviating by more than ±15% from the corresponding nonspiked sample were considered to be interfering compounds.

The Afinion ACR assay corrects for endogenous creatine levels of up to 5 mM. Hence, analysis of urine samples with 5 mM or greater creatine level should be stopped by the fail-safe detection system, and in these cases, the test should be aborted. To verify this, urine samples containing 27 and 144 mg/dL creatinine levels were spiked with creatine (4.0, 5.0, 5.5, and 7.0 mM) and analyzed in replicates of 4.

In addition, urine samples were spiked with whole blood. A stock solution was prepared by adding 50 μL whole blood to 950 μL urine (5% vol/vol). This stock solution was diluted further with urine. The dilution series of urine spiked with whole blood were measured using the Afinion ACR assay and 2 commercially available urine test strip methods: Combir 3 Test E (Roche Diagnostics GmbH) and Multistix 5 (Bayer Diagnostics Manufacturing, Bridgend, United Kingdom).

**RESULTS**

**Linearity**

Linear regression statistics demonstrated that the methods are linear over the whole concentration range tested (ie, 4.0–201.8 mg/L for albumin and 11.3–343.7 mg/dL for creatinine). The linear regression yielded the following results for albumin, Y (predicted) = 0.98 + 4.2 mg/L (r = 1.00, S_x = 3.07), and for creatinine, Y (predicted) = 1.01 − 0.3 mg/dL (r = 1.00, S_x = 3.53).

**Sample Dilution**

The analysis of the nondiluted (S1) and the diluted standards (S2, S3, and S4) resulted in a recovery for albumin and creatinine of 97% to 107% and 104% to 107%, respectively.

**Imprecision**

The results of the within-run, between-day, and total imprecision are shown in Table 1.

The between-instrument imprecision study, measuring 3 different urine samples, resulted in a coefficient of variation (CV) ranging from 2.0% to 3.5% for albumin (mean values, 12.5, 30.4, and 101.9 mg/L), 1.4% to 3.6% for creatinine (mean values, 54.8, 157.8, and 280.6 mg/dL), and 2.4% to 3.3% for ACR (mean values, 4.4, 19.3, and 186.3 mg/g).

**Method Comparison**

The 95 urine samples analyzed had values distributed over the whole range of 5 to 200 mg/L albumin and 16.4 to 339.9 mg/dL creatinine. The albumin and creatinine results obtained with the Afinion ACR assay were compared with Modular and DCA 2000 using linear regression and Bland-Altman analysis, as shown in Figures 2 and 3. The difference versus average plot of albumin resulted in a bias of −0.16 and 0.86 mg/L.

<table>
<thead>
<tr>
<th>Sample Dilution</th>
<th>Within-Run, Between-Day, and Total Imprecision of Albumin, Creatinine, and ACR Values After 20 Days’ Testing, Twice a Day, of 3 Urine Samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean Albumin, mg/L</td>
<td>Within-Run Precision CV, %</td>
</tr>
<tr>
<td>S1</td>
<td>174.9</td>
</tr>
<tr>
<td>S2</td>
<td>55.3</td>
</tr>
<tr>
<td>S3</td>
<td>12.6</td>
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<tr>
<td>Mean creatinine mg/L</td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>51.4</td>
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<tr>
<td>S2</td>
<td>162.3</td>
</tr>
<tr>
<td>S3</td>
<td>348.1</td>
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<tr>
<td>Mean ACR (mg/g)</td>
<td></td>
</tr>
<tr>
<td>S1</td>
<td>340.6</td>
</tr>
<tr>
<td>S2</td>
<td>34.1</td>
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<tr>
<td>S3</td>
<td>3.6</td>
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</tbody>
</table>
with Afinion in comparison with Modular and DCA 2000, respectively. Correspondingly for creatinine, the difference versus average plot yielded 5.8 and 4.9 mg/dL with Afinion in comparison with Modular and DCA 2000. The linear regression analysis yielded the following results when Afinion ACR was compared with the Modular method: albumin \( Y \) (predicted) = 0.95 + 3.2 mg/L \( (r^2 = 0.99, S_{y,x} = 6.1) \) and creatinine \( Y \) (predicted) = 0.95 - 0.6 mg/dL \( (r^2 = 0.99, S_{y,x} = 6.4) \). All the samples were within the measuring range for creatinine, but 1 sample was greater than 200 mg/L for albumin with the Modular method. Hence, 94 samples were used in the linear regression analysis for albumin. The linear regression analysis yielded the following results when the Afinion ACR assay was compared with the DCA 2000 method: albumin \( Y \) (predicted) = 0.95 + 1.6 mg/L \( (r^2 = 0.99, S_{y,x} = 4.9) \) and creatinine \( Y \) (predicted) = 0.97 - 0.7 mg/dL \( (r^2 = 0.99, S_{y,x} = 9.2) \). All the samples were within the measurement range for creatinine, but 13 samples were greater than 200 mg/L for albumin with the DCA 2000 method. Hence, 82 samples were used in linear regression analysis for albumin in the comparison of the Afinion ACR with DCA 2000.

**Interfering Substances**

The endogenous interfering substances tested showed no interference in the Afinion ACR assay up to concentrations of: 20 mg/L myoglobin, 20 mg/L β2-microglobulin, 20 mg/L IgG, 0.06 mM bilirubin, 250 mM glucose, 500 mM urea, 13.8 mM acetone, 46.8 mM DL-β-hydroxybutyric acid, 7.8 mM acetacetate, and 16.7 mM ascorbic acid.

Urine samples with creatine of 5.0 mM or greater were correctly aborted by the fail-safe system in the analyzer (code 107). In urine samples with up to 4.0 mM creatine, no interference was observed.

No interference in the Afinion ACR assay was observed up to the concentrations of drugs or its metabolites tested in urine: 1.3 mM acenomphen, 30 mM acenominph phen glucuronide, 30 μM glyburide, 10 mM ibuprofen, 24.2 mM metformin (biguanide), 11 mM acetylsalicylic acid, and 14.5 mM salicylic acid.

**DISCUSSION**

The ACR assay presented simultaneously performs the analysis of samples for both albumin and creatinine. The creatinine reaction is performed within 4 minutes, using the advanced end point prediction algorithm, whereas the albumin assay, started just after the creatinine reaction, takes approximately 3 minutes. Both assays run in parallel and result in a total assay time of 5 minutes 35 seconds.

The results from the linearity study showed that both the albumin and creatinine assays were linear over the whole dynamic range. Additionally, it was demonstrated that urine samples containing high concentrations of albumin and/or creatinine can be properly recovered when diluted up to 4 times with saline. The Afinion ACR assay presented showed good correlation with the POC method DCA2000 microalbumin/creatinine assay and the automated laboratory Modular albumin and creatinine methods. As shown by the imprecision results, this ACR assay provides a precise POC method.

The total imprecision according to National Committee for Clinical Laboratory Standards protocol after 20 days’ testing, twice a day, of 3 urine samples yielded a total CV of 5.5% or lesser for albumin, CV of 3.8% or lesser for creatinine, and CV of 6.0% or lesser for ACR. The between-instrument imprecision performed with 5 analyzers resulted in CV of 3.2% or lesser for albumin, CV of 3.6% or lesser for creatinine, and CV of 3.3% or lesser for ACR.
No interference was observed for elevated concentrations of bilirubin, glucose, urea, human myoglobin, human β2-microglobulin, IgG, acetooacetate, acetone, ascorbic acid, and β-hydroxybutyric acid. To avoid interference with ascorbic acid in the creatinine part of the assay, ascorbate oxidase was added to the reagent. The effect of this is demonstrated by the results showing no interference of ascorbic acid, even with concentrations up to 16.7 mmol (3000 mg/L). Bilirubin interference was seen in several creatinine assays. Hexacyanoferrate is known to reduce the interference from bilirubin with hydrogen peroxide, and potassium hexacyanoferrate was therefore included in the reagent in the Afinion ACR assay. No bilirubin interference was seen when up to 60 μM was tested in urine. Compounds may potentially interfere with the peroxidase-coupled enzymatic reaction when determining creatinine. Some compounds may act as substrates, consume products, or alter the activity of the enzymes in the reagents.

With the exception of salicylic acid, the main metabolite of acetylsalicylic acid, no interference in the Afinion ACR assay was observed from exogenous interfering substances such as acetaminophen, acetaminophen glucuronide, glyburide, ibuprofen, and metformin (biguanide). The negative interference observed with high salicylic acid concentrations with the creatinine part of the assay is presumably related to this compound’s oxidation by and consumption of hydrogen peroxide.

In this Afinion ACR assay, kinetic transmission measurements are used to predict the end point for the creatinine reaction and to correct for sample creatine concentrations of up to 5 mM. The results showed that urine samples with 5.0 mM or greater were correctly aborted by the specific fail-safe creatinine high test (code 107) and that interference was not observed in urine samples with 4.0 mM creatine level in the Afinion ACR assay. This level is above the normal levels of creatine that are usually reported in serum, 0 to 0.6 mM creatine.

The concentration of albumin in blood plasma is approximately 35 to 50 g/L. As with all methods analyzing albumin in urine, the source of the protein measured cannot be identified. Consequently, blood in the urine sample will also give elevated levels of albumin. The Afinion AS100 Analyzer will process urine samples if the contamination of whole blood is limited. Interference with ACR in this assay was observed for urine samples with 25 erythrocytes/μL or greater, which was detected by commercially available urine test strips. Most commercially available urine test strips have an upper detection limit of 200 or 250 erythrocytes/μL. Test results that are classified as microalbuminuria or clinical albuminuria should be tested for blood in urine to avoid false-positive test results. Based on these results, it is recommended that samples with ACR result of greater than 30 mg/g and albumin result of greater than 20 mg/L should be tested for blood in urine with a commercially available urine test strip method. If the urine test strip result is 25 erythrocytes/μL or greater, the ACR and albumin results may be falsely elevated because of interference from blood in the urine sample. Directions for testing of blood in urine could have been related to the albumin result only because this is the analyte being affected. However, because ACR is equally important, we recommend testing for blood in urine when both the albumin and the ACR results are classified as microalbuminuria or clinical albuminuria.

CONCLUSIONS

The Afinion ACR assay for the Afinion AS100 Analyzer contains all the reagents necessary for determining the albumin and creatinine concentrations in urine samples. The new Afinion ACR test provides a reliable, precise, and convenient POC method for the simultaneous determination of albumin, creatinine, and ACR in only 5½ minutes. The user requirements for simplicity, robustness, safety, and convenience are met by the simple sampling procedure, the automated analysis of the Afinion ACR test cartridge, the touch screen, and the fail-safe detection system. The Afinion AS100 Analyzer itself is a compact, rapid, and fully automated desktop analyzer, easily operated from the touch screen. The analyzer measures both reflection and transmission with the integrated camera.

ACKNOWLEDGMENTS

The authors thank Inger-Lise Lauvstad, Kari Hansen, and Kerstin Bernström for excellent technical assistance.

REFERENCES