Clinica Chimica Acta 460 (2016) 114–119

Contents lists available at ScienceDirect

Clinica Chimica Acta

journal homepage: www.elsevier.com/locate/clinchim

Method-to-method variability in urine albumin measurements

Anna E. Merrill [a], Jenna Khan [a], Jane A. Dickerson [b], Daniel T. Holmes [c,d], Neil S. Harris [e], Dina N. Greene [a,*]

[a] Department of Laboratory Medicine, University of Washington, Seattle, WA, United States
[b] Seattle Children's Hospital, Seattle, WA, United States
[c] St. Paul's Hospital, Vancouver, BC, Canada
[d] Department of Pathology and Laboratory Medicine, University of British Columbia, Vancouver, BC, Canada
[e] Department of Pathology, Immunology and Laboratory Medicine, University of Florida, Gainesville, FL, United States

ARTICLE INFO

Article history:
Received 13 April 2016
Received in revised form 10 June 2016
Accepted 13 June 2016
Available online 15 June 2016

Keywords:
Urine albumin
Microalbumin
AU680
Method validation
Hook effect
Standardization

ABSTRACT

Background: Urine albumin (uALB) is a useful marker in diagnosis and treatment of renal microvascular disease. Beckman Coulter recently re-formulated their uALB reagent for the AU series of instruments to increase the analytical measurement range (AMR).
Methods: Precision, linearity, reportable range, and analytical sensitivity for the reformulated reagent were determined using the AU680. In addition, the re-formulated AU reagent was compared to the previous generation AU reagent and to the Siemens Vista using residual urine specimens. The hook effect was evaluated on five instruments by spiking serum into albumin-free urine to generate a range of albumin concentrations.
Results: Precision and linearity within the AMR were confirmed, along with accuracy of dilutions to extend the reportable range. For patient sample correlation, the re-formulated reagent demonstrated a positive 11% bias relative to the original AU reagent and a negative 11% bias relative to the Vista. Concentrations of uALB > 3000 mg/dL produced falsely low results for both AU reagents. The DCA Vantage assay "hooked" at even lower uALB concentrations.
Conclusions: The re-formulated AU uALB reagent met the manufacturer claimed performance characteristics. The AU and DCA Vantage were the only instruments of those tested affected by the hook effect in the concentration range evaluated. uALB assays are clearly not standardized, yet clinical guidelines dictate result interpretation. The method-to-method biases we observed here have the potential to lead to clinically significant post-analytical errors in uALB interpretation.

1. Introduction

Persistent urinary albumin (uALB) excretion is an indicator of early diabetic nephropathy [1]. Furthermore, increased uALB concentrations have been associated with cardiovascular disease in both diabetic and non-diabetic patients [2,3]. Clinical practice guidelines published by the Kidney Disease: Improving Global Outcomes (KDIGO) Chronic Kidney Disease (CKD) Work Group and the American Diabetes Association (ADA) recommend evaluating the urine albumin-to-creatinine ratio (uACR, mg albumin/g creatinine) in a random urine sample to categorize the extent of kidney damage [1,4]. More specifically, albumin excretion rates can be classified as normal/mildly increased albuminuria (<30 mg/g), moderately increased albuminuria (30–300 mg/g), and severely increased albuminuria (>300 mg/g), and are utilized to facilitate CKD prognosis and treatment [4].

uALB is commonly quantified in the clinical laboratory using immunoturbidimetry or immunonephelometry. Beckman Coulter recently re-formulated their immunoturbidimetric uALB reagent for the AU series of instruments to increase the analytical measurement range (AMR) upper end from 30 to 45 mg/dL. The manufacturer also claimed increased confidence in measuring uALB concentrations between 600 and 2000 mg/dL. At these concentrations, "hook" effects can occur from saturation of the polyclonal antibodies due to the presence of excess antigen. This prevents formation of higher-order immune complexes, thereby generating less signal via light scattering than would be expected based on the true antigen concentration. The hook effect was first documented for uALB immunoturbidimetric measurements in 1990, where measured concentrations were artificially lowered when uALB was >50 mg/dL [5]. Hook effects for uALB remain a potential problem. In 2012, Pullan and Hitch introduced an automatic laboratory

* Corresponding author at: University of Washington, Department of Laboratory Medicine, 1959 NE Pacific St., NW120, Box 357110, Seattle, WA 98195, United States.
E-mail address: dngreene@uw.edu (D.N. Greene).

http://dx.doi.org/10.1016/j.cca.2016.06.015
0009-8981/

Table 1
Analytical specifications for 6 commercial urine albumin assays.

Assay	Assay type	Polyclonal anti-albumin antibody type	AMR (mg/dL)	Dilutions (system diluent)	Concentrations susceptible to hook effect (manufacturer claim)
Beckman AU680 original (OSR6167)	Immuno-turbidimetry	Goat	0.5–30	10×, 51× (water)	>600 mg/dL
Beckman AU680 re-formulated (B38858)	Immuno-turbidimetry	Goat	0.7–45	10×, 51× (water)	>2000 mg/dL
Siemens Vista	Immuno-nephelometry	Rabbit	0.5–34	20×, 40× (N Diluent)	>1269.3 mg/dL
Siemens BNII	Immuno-nephelometry	Rabbit	1.05–33.75	4×–1600× (N Diluent)	N/A
Roche Cobas	Immuno-turbidimetry	Sheep	1.2–40	11×, 50× (saline)	>4000 mg/dL
Siemens DCA Vantage	Immuno-turbidimetry	Goat	0.5–30	N/A	>500 mg/dL

computer flagging algorithm to detect uALB specimens potentially affected by antigen excess on the AU2700; briefly, urine samples meeting uACR and total protein criteria were subjected to manual dilutions prior to reporting uALB concentrations [6]. The propensity for the re-formulated AU uALB assay to display the hook effect has not been systematically evaluated and, to the best of our knowledge, no study has compared the hook effect across multiple modern uALB assays.

2. Materials and methods

2.1. Study samples

Residual, de-identified urine and serum samples from the University of Washington were used for all studies. This study was performed as part of ongoing quality assurance/quality improvement studies at the University of Washington, Department of Laboratory Medicine and therefore was not considered human subjects research.

2.2. Instrumentation/assay specifications

uALB quantification was performed on the Beckman Coulter AU680, Siemens Vista, Siemens BNII, Roche Cobas, and Siemens DCA Vantage at the University of Washington (Seattle, WA), Northwest Hospital (Seattle, WA), St. Paul's Hospital (Vancouver, B.C.), University of Florida (Gainesville, FL), and Seattle Children's Hospital (Seattle, WA), respectively. These immunoturbidimetric and immunonephelometric uALB assays utilize polyclonal anti-albumin antibodies to generate light scatter. Assay-specific analytical parameters are summarized in Table 1.

2.3. Precision

Precision experiments were performed using two concentrations of commercial quality control material (BioRad Liquichek Urine Chemistry). Intra-day precision included 20 consecutive measurements of each concentration. Inter-day precision was evaluated by analyzing each concentration once per day for 20 days.

2.4. Linearity/reportable range

Linearity within the manufacturer's claimed analytical measurement range (0.7–45 mg/dL) was confirmed using a high patient urine sample (49.4 mg/dL). This sample was diluted with an undetectable (<0.7 mg/dL) urine sample to generate multiple relative concentrations: 100%, 75%, 50%, 25%, and 10% samples. Each specimen was analyzed in triplicate and the measured concentration compared to the expected concentration based on the 100% sample. To extend the reportable range beyond the upper limit of the AMR, 10× and 51× dilutions were evaluated using recovery studies. For the 10× dilution, two urine specimens (46.93 and 38.87 mg/dL) were measured neat and after an on-board 10× dilution with water (expected 10× concentrations of 4.69 and 3.89 mg/dL). This process was repeated in triplicate for each sample and the recoveries of the diluted specimens relative to the neat specimens were calculated. For the 51× dilution, two urine specimens (375.30 and 385.10 mg/dL) were measured after an on-board 10× dilution with water (expected 10× concentrations of 37.53 and 38.51 mg/dL) and after a manual 51× dilution with saline (expected 51× concentrations of 7.36 and 7.55 mg/dL). This process was repeated in duplicate for each sample and the recoveries of the 51×-diluted specimens relative to the 10×-diluted specimens were calculated.

2.5. Analytical sensitivity

The limit of blank (LoB) was calculated using the equation $LoB = mean_{blank} + 3 \times SD_{blank}$. The mean and standard deviation (SD) of the blank were determined using 20 consecutive measurements of water.

2.6. Method comparison

Inter-assay variability was evaluated using 78 residual, de-identified urine specimens. These samples were compared between the original and re-formulated reagents on the Beckman Coulter AU680. Where sample volume allowed (n = 74), specimens were shipped refrigerated to Northwest Hospital (Seattle, WA) for analysis using the Siemens Vista.

2.7. Hook effect

The hook effect was evaluated across 5 platforms and 6 uALB reagents. A series of samples was prepared by spiking serum into a residual urine sample with undetectable albumin (n = 12; final albumin concentration = 0, 14, 28, 55, 110, 220, 440, 880, 1760, 2640, 3520, or 4400 mg/dL). Samples were aliquoted and frozen at −20 °C until analysis (within 7 days of freezing). Samples were shipped frozen to collaborating institutions under their respective Material Transfer Agreements (MTAs) with the University of Washington. Samples were

Table 2
Intra- and inter-day precision of the re-formulated urine albumin reagent. According to the manufacturer, imprecision should be <5% (intra-day) or 10% (inter-day). All precision studies were conducted using the same QC material. When comparing level 1 and level 2 inter-assay QC means, the re-formulated reagent demonstrated a bias of +1.21 mg/dL (+64%) and +2.39 mg/dL (+37%), respectively, relative to the original reagent.

	AU re-formulated reagent				AU original reagent	
	Intra-day precision (n = 20)		Inter-day precision (n = 20)		Inter-day precision (n = 20)	
	L1 (mg/dL)	L2 (mg/dL)	L1 (mg/dL)	L2 (mg/dL)	L1 (mg/dL)	L2 (mg/dL)
Mean	2.82	8.37	3.11	8.86	1.90	6.47
SD	0.03	0.11	0.24	0.39	0.08	0.19
CV	1.0%	1.3%	7.8%	4.4%	4.2%	3.0%