Evaluation of point-of-care tests for detecting microalbuminuria in diabetic patients

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Abstract

Background: Microalbuminuria, the presence of low levels of albumin in the urine, indicates renal damage and is recognised as a risk factor for the progression of renal and cardiovascular disease. Several international scientific bodies recommend microalbuminuria screening. Point-of-care testing (POCT) of microalbuminuria allows immediate identification of risk, and monitoring of treatment effects. In this study, two POCT instruments were evaluated as microalbuminuria screening methods.

Method: Spot urine specimens from diabetic patients were analysed with the quantitative HemoCue® urine albumin analyser (n = 245), and the semiquantitative Clinitek® microalbumin urine dipstick (n = 204). These results were compared to the respective data for laboratory-determined albumin (nephelometry), creatinine (modified Jaffe) and albumin-to-creatinine ratio (ACR).

Results: Linear regression analysis demonstrated a good correlation for the HemoCue® urine albumin with the laboratory-determined albumin concentration (y = 0.8557x + 0.2487y, r = 0.97). The sensitivities for the HemoCue® and Clinitek® POCT systems were 79.6% and 83.8%, and the specificities 97.1% and 93.8% respectively. Positive and negative predictive values for the HemoCue® were 95.6% and 85.8%, and were 88.6% and 91.0% the Clinitek®. The repeatability of both instruments was excellent. Both instruments are easy to use, and more cost-effective than the laboratory methods for albumin and ACR.

Conclusion: Both the HemoCue® and the Clinitek® microalbumin POCT systems for albuminuria are easy to use and inexpensive, and are adequately accurate as a screening method. Although the HemoCue® POCT system measures only urine albumin concentration, its sensitivity and specificity compared well with that of the Clinitek® POCT system, which determines the ACR.

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Introduction

The prevention of chronic disorders is one of the most important cornerstones of primary health care. Screening for microalbuminuria is valuable in primary health care, as microalbuminuria is the earliest manifestation of nephropathy, and an independent marker of increased cardiovascular morbidity and mortality in diabetic,[1] as well as non-diabetic, patients.[2] The presence of microalbuminuria serves as a signal for vascular disease screening, as well as aggressive intervention to reduce all cardiovascular risk factors. Early detection of microalbuminuria is crucial, as optimal treatment of these patients can slow the development of and progression to persistent albuminuria (overt nephropathy), and reduce cardiovascular risks.[1]

Microalbuminuria is a persistent, increased urinary excretion of albumin above the reference range for healthy non-diabetic subjects, but at a concentration that is not generally detectable by crude clinical tests, such as dipsticks designed to measure total urine protein. The reference point for albuminuria remains the accurate collection of a timed or 24-hour urine specimen, but this is cumbersome for the patient, and a difficult procedure to control effectively. This is because the urine is frequently over- or undercollected, with subsequent over- or underestimation of albumin. Since the creatinine excretion in urine is fairly constant throughout a 24-hour period, measurements of the albumin-to-creatinine ratio (ACR) allow correction of variations in urinary concentration, avoiding the confounding effect of variations in urine volume on the urine albumin concentration (Table I shows the diagnostic thresholds).

Table I: Diagnostic thresholds for urine albumin excretion[1,3]

| [a]Category | 24-hour urine albumin (mg/24 hours) | Overnight/timed urine albumin (µg/minute) | Spot urine | |
			Urine albumin (mg/l)	[b]ACR (mg/mmol)
Normal	< 30	< 20	< 20	< 3.4
Microalbuminuria	30-299	20-199	20-199	3.4-33.9
Macroalbuminuria	> 300	> 200	> 200	> 34.0

a = two of three urine specimens collected within a three-to-six-month period should be abnormal before diagnosing microalbuminuria, b = albumin-to-creatinine ratio

The assessment of cardiovascular risk factors and renal damage is a common task in general practice. In a clinic setting, screening needs to be simple and cost-effective with acceptable accuracy. There is an increasing demand for urine albumin tests that are suitable for point-of-care testing (POCT). The advantage of POCT for detecting microalbuminuria is rapid results, and thus immediate motivation for lifestyle changes by the patient, and the possibility of early intervention, with the goal of delaying the onset of overt nephropathy and reducing the cardiovascular risks. In this study, we evaluate two POCT instruments that are currently available for albuminuria detection, the HemoCue® urine albumin analyser, and Clinitek® microalbumin reagent strips,

Method

Urine specimens

Spot urine specimens of diabetic patients received at the National Health Laboratory Services (NHLS), Universitas Hospital, Bloemfontein, for the routine analysis of albuminuria were used. The specimens were obtained between July 2007-August 2008. The number of specimens analysed by the POCT instruments were subject to the availability of reagents for the respective POCT instruments at that time. The study was approved by the ethics committee of the Faculty of Health Sciences of the University of the Free State.

Sample processing

All received specimens were kept at 4˚C until analysis. Analysis was carried out within 48 hours of the specimens being received. Turbid specimens were centrifuged, and all specimens were allowed to warm to room temperature before analysis. The urine specimens were first screened for proteins with the Bayer Diagnostics (Siemens) Multistix standard urine protein reagent strip. All specimens screened for proteinuria with results 3+ (corresponding to urine proteins > 3.0 g/l), or greater, on the dipstick were excluded from the study.

Laboratory urine albumin and creatinine method

- Urine creatinine concentration (mmol/l) was measured on the Beckman-Coulter LX20® (modified Jaffe method).

- Urine albumin concentration (mg/l) was measured on the Dade Behring BN Prospec® Nephelometer (immunonephelometry).

- ACR was calculated and expressed as mg albumin/ mmol creatinine.

POCT urine albumin and creatinine method

All urine specimens were analysed according to the manufacturer's instructions with the HemoCue® 201 urine albumin (HemoCue®, Ängelholm, Sweden) POCT system and Clinitek® microalbumin urine reagent strips, read by the Clinitek® status urine chemistry reflectance photometer (Siemens® Medical Solutions Diagnostics, formerly Bayer).

The HemoCue® 201 urine albumin analyser is a POCT system that measures urine albumin quantitatively, using specially designed micro-cuvettes that contain an immunochemical antigen-antibody reaction using antihuman antibodies specific to human albumin. This reaction forms agglutination complexes that create turbidity, which is read by the HemoCue® albumin 201 photometer. The turbidity is proportional to the concentration of albumin in urine. The sample volume is 18 µl, and results are displayed within 90 seconds. The HemoCue® albumin 201 measurement range is 5-150 mg/l. To obtain results above 150 mg/l, specimens can be diluted with isotonic sodium chloride, and the results multiplied manually by the dilution factor.[4]

The Clinitek® microalbumin urine reagent strips contain two reagent areas to test for albumin and creatinine in urine respectively. The semiquantitative results are read by a Clinitek® status reflectance photometer and the results are expressed within 60 seconds as an ACR in mg/mmol. The ACR is determined by the instrument via photometry, and it is not merely a calculation of the semiquantitative values assigned to albumin and creatinine. The results are expressed as "normal" (ACR < 3.4 mg/mmol), "abnormal" (ACR 3.4-33.9 mg/mmol) and "high abnormal" (ACR > 33.9 mg/mmol).[5]

Quality control

The NHLS Department of Chemical Pathology belongs to an external quality assurance scheme (EQAS) for both the urine creatinine and albumin analytes, and runs a daily internal quality-control programme. Performances for the period of the study were within acceptable limits. The reproducibility of the HemoCue® POCT system was assessed by analysing two levels of control material with each batch of specimens. The overall coefficient of variation (CV) was calculated. The repeatability of the Clinitek® microalbumin POCT system was evaluated by analysing urine specimens with different concentrations of albumin and creatinine repeatedly, and comparing the semiquantitative results. The linearity, detection limits and reportable range of both POCT instruments for urine albumin were confirmed by making serial dilutions of 20% human albumin stock solution with 0.9% sodium chloride. The concentrations of the dilutions were confirmed with the laboratory nephelometric method.

Data analysis

All the data were captured on Excel spreadsheets. A urine albumin concentration of less than 20 mg/l, or less than 3.4 mg/mmol creatinine, was regarded as normal, with microalbuminuria defined as a concentration of 20-199 mg/l or 3.4-33.9 mg/mmol creatinine. The two POCT results were compared with the laboratory-measured albumin and creatinine concentrations, and correlations with quantitative values were carried out by means of linear regression and Bland-Altman plots. The sensitivity, specificity, predictive values and likelihood ratios for each POCT system were calculated in comparison to the laboratory method.

Results

HemoCue® POCT system

Two hundred and forty-five urine specimens were tested on the HemoCue® urine albumin analyser (measurement range: 5-150 mg/l):

- Forty-three specimens (43) tested < 5 mg/l.
- One hundred and ninety-one specimens (191) tested 5-150 mg/l.
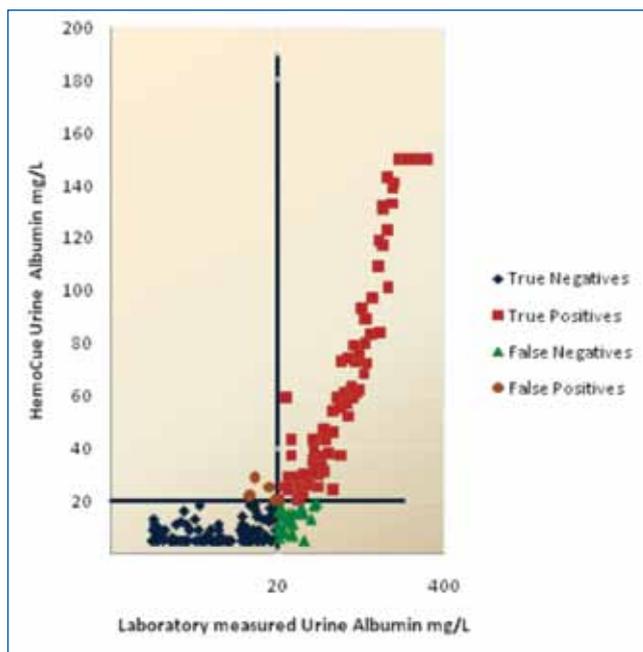- Eleven specimens (11) tested > 150 mg/l.

Figure 1: HemoCue® urine albumin vs. laboratory urine albumin in mg/l

Table II and Figure 1 illustrate the results of the HemoCue® urine albumin compared to the laboratory-determined albumin concentration at a cut-off of 20 mg/l for microalbuminuria. The sensitivity of the HemoCue® test was 79.6%, and specificity was 97.1%. There were 4.4% false positives, and 14.2% false negatives. However, it is noteworthy to mention that most of the specimens that tested falsely negative with the HemoCue® method had a laboratory albumin concentration just above the cut-off point of 20 mg/l.

There was adequate correlation between the HemoCue® and the laboratory quantitation of urine albumin (see Figure 2), with a correlation coefficient of 0.97, and no significant constant bias (intercept) of 0.25 (95% confidence interval 1.4-1.9). The laboratory method gave slightly higher values at high urine albumin concentrations (see Figure 3) with a proportional bias (slope) of 0.86 (95% confidence interval 0.82-0.89).
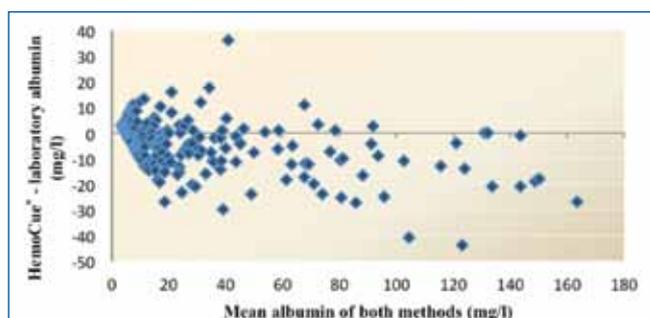
Two levels of control material were analysed with each batch of specimens on the HemoCue® POCT system as part of internal quality control. The overall CV was 13.1% at 27 mg/l (n = 16) and 6.4% at 86 mg/l (n = 17).

Table II: Distribution of urine albumin analysed by the HemoCue® POCT method and the laboratory methods, with a cut-off point of 20 mg/l for microalbuminuria
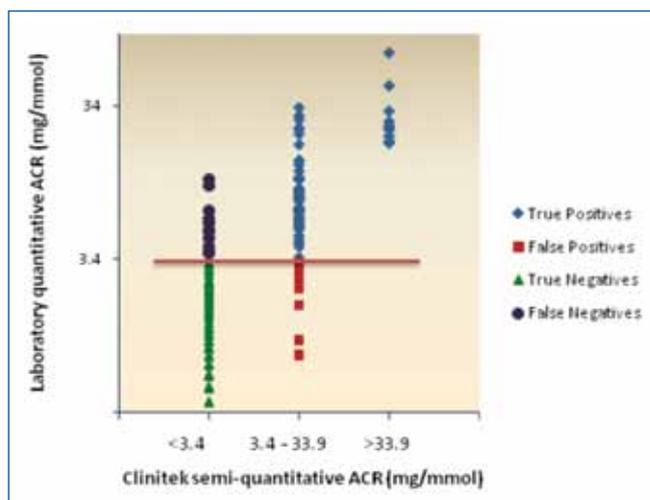
	Laboratory albumin < 20 mg/l microalbuminuria negative	Laboratory albumin ≥ 20 mg/l microalbuminuria positive	Total
HemoCue® albumin < 20 mg/l	True negatives 133 (85.8%)	False negatives 22 (14.2%)	155
HemoCue® albumin ≥ 20 mg/l	False positives 4 (4.4%)	True positives 86 (95.6%)	90
Total	137	108	245
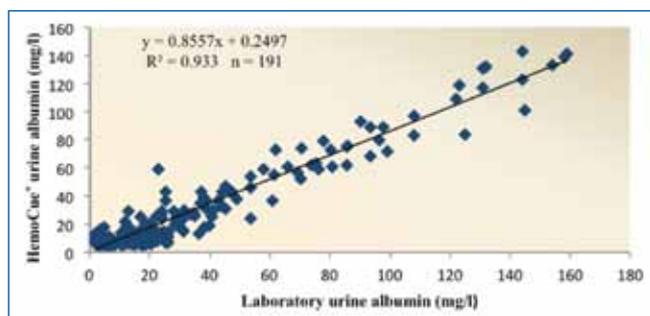
Figure 2: Correlation between urine albumin concentrations measured by the laboratory (BN ProSpec® Nephelometer) and the HemoCue® POCT methods

Figure 3: Bland-Altman bias plot showing correlation between urine albumin analysed with the HemoCue® and that analysed using the laboratory method

Figure 4: Laboratory quantitative albumin-to-creatinine ratio (ACR) versus Clinitek® semiquantitative ACR (mg/mmol)

Clinitek® microalbumin POCT system

Two hundred and four urine specimens were analysed by the Clinitek® microalbumin urine dipstick POCT system:

- One hundred and thirty-four specimens (134) tested normal (ACR < 3.4 mg/mmol).
- Sixty specimens (60) tested abnormal (ACR 3.4-33.9 mg/mmol).
- Ten specimens (10) tested high abnormal (ACR > 33.9 mg/mmol).

Figure 4 and Table III illustrate the results of the Clinitek® microalbumin ACR compared with the quantitated laboratory ACR at a cut-off of 3.4 mg/mmol for microalbuminuria. The sensitivity and specificity for the Clinitek® microalbumin POCT system were respectively 83.8% and 93.8% with false positive and false negatives of 11.4% and 9.0% respectively.

The repeatability of the Clinitek® microalbumin dipstick was evaluated using patient urine specimens with different concentrations of albumin and creatinine, as well as positive and negative ACRs. The Clinitek® microalbumin dipstick gave excellent repeatability (n = 10) on different levels of the semiquantitative albumin and creatinine concentrations, as well as ACRs.

Discussion

There is increasing evidence that suggests that the detection and treatment of microalbuminuria in diabetic patients is important in identifying patients at increased risk of both micro- and macrovascular disease, and to prevent or slow the progression of vascular damage.[1,2] POCT of microalbuminuria allows immediate identification of the risk and monitoring of the treatment effects. In this study, the goal was to evaluate two new office-based methods to assess urinary albumin excretion.

The HemoCue® albumin 201 is a POCT method for the quantitative determination of albumin in urine (measuring range 5-50 mg/l), using an immunochemical method.

Table III: Distribution of urine albumin measured semiquantitatively by the Clinitek® microalbumin POCT system compared with the quantitative laboratory method, using a albumin-to-creatinine ratio cut-off of 3.4 mg/mmol

	Laboratory [b]ACR < 3.4 mg/mmol microalbuminuria negative	Laboratory ACR ≥ 3.4 mg/mmol microalbuminuria positive	Total	Laboratory ACR mean (range) in mg/mmol
[a]Clinite® ACR "normal"	True negatives 122 (91.0%)	False negatives 12 (9.0%)	134	1.62 (0.25-11.39)
Clinitek® ACR "abnormal"	False positives 8 (11.4%)	True positives 62 (88.6%)	70	9.47 (0.78-32.81)
Total	130	74	204	

a = Clinitek® albumin-to-creatinine ratios are semiquantitatively expressed as "normal", "abnormal" or "high abnormal" correlating with the respective albumin-to-creatinine ratios of < 3.4, 3.4-33.9 and > 33.9 mg/mmol, b = albumin-to-creatinine ratio

The linear regression analysis demonstrated a good correlation in the range of 5-150 mg/l for the HemoCue® system (y = 0.8557x + 0.2497y, r = 0.97), especially considering that presently there is no international calibrator for urine albumin. There was also a good correlation between the HemoCue® and laboratory albumin concentration (see Figure 2), with the laboratory albumin results being slightly higher than those of the HemoCue® (see Figure 3). The diagnostic accuracy of the two POCT systems as compared to our laboratory methods is illustrated in Table IV.

Table IV: The diagnostic accuracy of the two POCT systems for microalbuminuria vs. laboratory methods

	HemoCue® urine albumin	Clinitek® urine albumin
Sensitivity% (95% CI)	79.6 (70.6)	83.8 (73.0-91.0)
Specificity% (95% CI)	97.1 (92.2-99.1)	93.8 (87.8-97.1)
[a]PPV% (95% CI)	95.6 (88.4-98.6)	88.6 (78.2-94.6)
[b]NPV% (95% CI)	85.8 (79.1-90.7)	91.0 (84.6-95.1)

a = positive predictive value; b = negative predictive value

The HemoCue® urine albumin POCT system is considered simple, very easy to use, and delivers rapid results (within two minutes). The cost per test compares favourably with the cost of a laboratory-based albumin determination. Both spot and timed urine specimens can be used with this system. This analyser measures urine albumin quantitatively, which allows for clinical decision-making and follow-up of patients. To date, it is the only POCT system for urine albumin on the market that delivers quantitative results. Haemoglobin, myoglobin, and most other drugs do not interfere with the interpretation of this test.[4] Although the instrument delivers quantitative results, it does not correct the albumin concentration per creatinine, and false high or low values may be obtained for severely concentrated or diluted spot urine specimens. Another shortcoming of the HemoCue® POCT system is that its measurement range is only 5-150 mg/l. This means that for specimens with an albumin concentration above 150 mg/l, a dilution should be made with normal saline, which is time-consuming and may prove to be difficult for an inexperienced health professional in an office or clinic setting. The HemoCue® urine albumin analyser can only be used to analyse urine albumin concentrations, and is a rather costly instrument. Other factors that need to be taken into consideration are that the HemoCue® reagent needs to be refrigerated, and that visually turbid specimens should only be analysed after centrifugation, which may prove to be difficult in a clinic setting.

Clinitek® microalbumin dipsticks test for albumin and creatinine semiquantitatively by measuring colour changes on the strips using the Clinitek® status reflectance photometer, which calculates an ACR to allow for varying urine concentrations. The semiquantitative Clinitek® microalbumin urine dipstick albumin and creatinine results correlate well with the quantitative results of the laboratory method. This urine dipstick is easy to use, delivering rapid results. The Clinitek® status photometer is a multi-purpose analyser, and can also read Multistix® standard urine dipsticks, as well as βhCG cassettes. This is a cost-effective test, compared to the laboratory-determined ACR. Although the results expressed by this system are semiquantitative, the albumin is corrected for creatinine, eliminating the effect of variations in the urine concentration. The reagent strips for this test have a long shelf life, and need to be stored in a cool, dry place only. Refrigeration is not necessary. The dipsticks are easy to use and the instructions on the Clinitek® status analyser are clear and easy to follow, but the system is operator dependent. It is important to read the creatinine pad on the dipstick exactly 60 seconds after immersing the dipstick into the sample. The creatinine pad may continue to change colour after 60 seconds, and improper timing can cause the creatinine, and the resulting ACR, to be incorrect. There might be substances in the urine that interfere with the interpretation of the results. The presence of visible haemoglobin, or myoglobin, may cause falsely elevated results, for both the albumin and creatinine. According to the manufacturer, contamination of the urine with soaps, detergents, antiseptics, or preservatives other than boric acid may also affect the results.[5] Substances that cause abnormal urine colour (such as drugs containing azo dyes, nitrofurantoin and riboflavin) may mask or enhance colour development on the pad, causing false positive or negative results. The results are only semiquantitative, and are not ideal for follow-up of patients.

In general, the drawback of POCT systems is that they are not sensitive or accurate enough to be used for diagnostic purposes. Sacks et al[6] propose that screening tests for microalbuminuria should be positive in > 95% of patients with microalbuminuria, and that the positive tests should be confirmed by analysis in an accredited laboratory. Sacks et al[6] also recommend that the analytical CV of methods used to measure microalbuminuria should be < 15%. In this study, the HemoCue® urine albumin POCT system fulfils these proposed specifications, but it is important to take into account that the HemoCue® POCT instrument measures the albumin concentration only, and does not correct for creatinine in a random spot urine specimen.

The results of this study correlate with those of previously published studies that show a good correlation between the HemoCue® and a nephelometrically determined albumin concentration.[7] Sarafidis et al[8] found a sensitivity of 92%, a specificity of 98%, a positive predictive value (PPV) of 92%, and a negative predictive value (NPV) of 98%, compared with a immunoturbidimetric method in patients with various risk factors for chronic kidney disease and cardiovascular disease. In this study, the sensitivity of the HemoCue® was slightly lower (78.8%), but with similar specificity and predictive values.

In this study, the performance of the Clinitek® microalbumin test was similar to, or better than, that reported in previously published studies. Le Floch et al[9] found a sensitivity of 79%, a specificity of 81%, a PPV of 46%, and an NPV of 95%, in diabetic patients. Wilde et al[10] found sensitivities for pregnant patients ranging from 19-59%, and specificities from 45.4-84.2%, with sensitivity of 52.4% and a specificity of 97.6% in the community cohort. The differences in the various studies are probably as result of the diverse patient populations involved, as well as the inclusion or exclusion of macroalbuminuric specimens. The results in this study on diabetic patients confirm the findings reported by other investigators, namely that the Clinitek® microalbumin test is valuable, particularly in confirming the absence of microalbuminuria in urine specimens (the NPV in this study was 91.0%).

A possible limitation of this study is that it was conducted in a laboratory environment, and all the analyses were carried out by one person. In a clinical setup, different people are likely to use the POCT systems, and a difference in the results might occur if the specimens are not all handled in the same way, or not used according to manufacturers' recommendations. Our urine specimens were centrifuged before analysis, which will not be possible in the clinic setting, and this may influence results.

In conclusion, both the HemoCue® and the Clinitek® microalbumin POCT systems are easy to use, inexpensive, and reasonably accurate as screening methods. Although the HemoCue® test measures albumin concentration only, its sensitivity and specificity compared well with the Clinitek® POCT system, which determines the ACR.

Acknowledgements

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