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# Effectiveness of URiSCAN 2 ACR strip test for albuminuria detection in screening of kidney disease

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**KEYWORDS**

Albumin/creatinine ratio (ACR);
Urine strip;
Screening test.

**ABSTRACT**

Albuminuria is a key marker for detection of kidney disease and an increased risk factor for cardiovascular diseases. Measurement of albumin/creatinine ratio (ACR) in urine is the best screening method to detect albuminuria. Nowadays, new version of URiSCAN 2 ACR strip test is developed to measure of albumin/creatinine ratio in urine and to report ACR value as semi-quantitative and quantitative results. Thus, we aimed to validate the effectiveness of the URiSCAN 2 ACR strip test by comparing with a quantitative automated analyzer, and to demonstrate the effectiveness of the strip test for screening chronic kidney disease in the community population. Measurements of ACR levels in 484 spot urine specimens of participants in CKDNET project were performed using the URiSCAN 2 Optima urine chemistry test system and using the SYNCHRON Lx20 PRO automated chemistry analyzer. The sensitivity, specificity, accuracy, positive predictive value and negative predictive value of URiSCAN 2 ACR strip to detect ACR were 93.2%, 62.9%, 67.6%, 31.2% and 98.1%, respectively. URiSCAN 2 ACR strip showed 63.0% concordance rate with quantitative automated assay, 68.8% false-positive and only 1.9% false-negative results. In addition, the best cut-off of ACR value for detection of chronic kidney disease using the urine strip test was in the range from 29.50 to 33.00 mg/g with area under the curve of 0.7413, while the area under the curve of quantitative automated analyzer was 0.7515. Sensitivity, specificity and accuracy of the strip test for detection of chronic kidney disease were 70.5%, 61.8% and 63.8%, respectively. URiSCAN 2 ACR strip provided a high sensitivity, high negative predictive value and few false negative results for detection of albuminuria. Thus, this ACR strip test might be served as an optimal method to rule-out microalbuminuria. However, diagnostic performance of URiSCAN 2 ACR strip test at cut-off of ACR $\geq$30 mg/g is fair for screening CKD in community population.

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## Introduction

Albuminuria is a marker of both active renal inflammation and renal injury. It is a strong risk factor associated with kidney disease, cardiovascular disease and a predictor of diabetic complications. Detection of albuminuria is a tool for early detection and monitoring kidney disease. Albuminuria usually appears before the decline of glomerular filtration rate (GFR). Thus, the screening test of albuminuria is a major role to prevent and to decline a development of chronic kidney disease (CKD)[1,2]. In healthy person, albumin excretion rate (AER) is less than 30 mg/24 hours or albumin/creatinine ratio (ACR) is less than 30 mg/g. Abnormal detection can be categorized when 30 to 300 mg/day of AER or 30-300 mg/g of ACR is called microalbuminuria, while >300 mg/day of AER and >300 mg/g of ACR is called macroalbuminuria[3].

Quantification of albumin in 24-hour urine collection (24-hr UAC) is the standard method to indicate albuminuria but it is an inconvenient, costly and has difficulty in sample collection causing poor patient compliance. There are several methods to detect the amount of protein in urine but the ACR is the best screening test for detection of albumin in spot urine. Previous studies showed that the results of spot ACR had high sensitivity (84.9%) and high specificity (95.8%) when compared with 24-hr UAC. In addition, they also showed positive correlation ($R^2$ = 0.905: coefficient of determination) and not significantly statistic difference (*p*-value = 0.724) for evaluation of microalbuminuria between the results from 24-hr UAC method and ACR method[4,5]. ACR is a convenient and alternatively rapid method with currently advocated by the major guidelines. The Kidney Disease Improving Global Outcomes (KDIGO) 2012 clinical practice guidelines determines that ACR is important for the diagnosis, prognosis and classification of CKD[3].

General technique to detect abnormal levels of proteinuria is urine dipstick. The usefulness of urine dipstick is not only rapid test but also a screening test in the community. This test is user-friendly, acceptably reliable and simple without additional equipment[6]. The importance of early detection of microalbuminuria is a marker of kidney damage such as glomerular disease. Additionally, early screening for microalbuminuria in diabetic patients with an initial stage of renal involvement has an advantage to prevent the progression of kidney complications.

URiSCAN 2 ACR strip test is a semi-quantitative urine dipstick as a screening tool for albumin and creatinine in urine. The URiSCAN Optima urine analyzer determines the ACR value with milligram of albumin per gram or mmol of creatinine. New version of the URiSCAN 2 ACR strip test reports both quantitative and semi-quantitative values of ACR. Thus, the objective of this study was to validate the effectiveness of the URiSCAN 2 ACR strip test by comparing with a quantitative automated analyzer, and to demonstrate the effectiveness of the strip test for screening chronic kidney disease in the community population.

## Materials and methods

### *Participants and samples collection*

This study was a cross-sectional community-based study investigating CKD events between June 2017 and August 2018 in Tambon Don Chang, Amphoe Meuang, and Tambon Khok Samran, Amphoe Ban Haet, Khon Kaen Province, Thailand. The study was a part of Chronic Kidney Disease Prevention in the Northeast of Thailand (CKDNET) project. The participants (more than 18 years old) were enrolled into this study. This study protocol (HE601035) was approved by the Ethics Committee of Khon Kaen University

The CKD subjects in this study presented with persistent kidney damage and/or loss of kidney function for more than 3 months according to the KDIGO 2012 guideline. Table 1 shows the baseline characteristics of all participants. The total of 484 random spot urine samples including 51 diabetes, 69 hypertension, 65 diabetes and hypertension and 299 non-diabetics and non-hypertension were collected. All samples were refrigerated in 2-4ºC during immediately transferred to the laboratory within 2 hours. The evaluation processes for all samples were performed at Community Laboratory, Faculty of Associated Medical Sciences, Khon Kaen University.

**Table 1** Characteristic of the 484 participants in this study

| Parameters | Total (n = 484) |
|---|---|
| Age: mean ± SD | 56.0 ± 12.5 |
| CKD: n(%) | 112 (23.1%) |
| Non-CKD: n(%) | 372 (76.9%) |
| Diabetes Mellitus: n(%) | 51 (10.5%) |
| Hypertension: n(%) | 69 (14.3%) |
| Diabetes and hypertension: n(%) | 65 (13.4%) |
| Non-diabetes and non-hypertension: n(%) | 299 (61.8%) |

***Laboratory quantitative ACR method***

SYNCHRON Lx20 PRO chemical analyzer (Beckman Coulter, Inc, USA) measured albumin level by turbid metric method (antigen-antibody complexes) and creatinine level by Jaffe rate method. The analytical range of albumin and creatinine in urine samples were 0.2-30.0 and 10-400 mg/dL, respectively. The quantitative ACR ratio (mg/g) was calculated by the results of albumin (mg/dL) and quantitative creatinine (g/L).

***ACR urine strip test***

URiSCAN 2 ACR strip test detected urinary albumin by dye binding method and detected creatinine by metal complex methods. URiSCAN Optima urine analyzer (YD Diagnostics Co., Ltd., Korea) read the color change on strips. The measurement of albumin concentration was categorized into four scales as negative, 1+, 2+, 3+ (0, 30, 80, and 150 mg/L) and five-scales of creatinine concentration as +/-, 1+, 2+, 3+, 4+ (10, 50, 100, 200, and 300 mg/dL). The results of ACR was also calculated and reported as a quantitative ACR were semi-quantitative. The quantitative results were a continuous value while semi-quantitative results were <30 mg/g, 30-300 mg/g and >300 mg/g referred to normoalbuminuria, microalbuminuria and macroalbuminuria, respectively.

***Statistical analysis***

Categories of ACR results in this study were positive and negative. The term "positive" was defined as samples with ACR results of ≥30 mg/g, whereas the definition of "negative" was samples with ACR results of <30 mg/g. The effectiveness of URiSCAN 2 ACR strip test for ACR detection was evaluated by sensitivity, specificity, accuracy, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (+LR) and negative likelihood ratio (-LR) compared with laboratory quantitative reference method. The diagnostic performance of strip test in CKD were analyzed by using receiver operating characteristic (ROC) curve, sensitivity, specificity, accuracy, positive predictive value, negative predictive value, positive and negative likelihood ratio. Statistical analyses were conducted by the SPSS for Windows version 19.0 (IBM Corp, Armonk, NY, USA).

## Results

***Comparison of the quantitative and semi-quantitative assay***

Reference quantitative ACR assay of 484 random urine samples for normoalbuminuria (<30 mg/g), microalbuminuria (30-300 mg/g) and macroalbuminuria (>300 mg/g) were 84.7%, 12.6% and 2.7%, respectively while the semi-quantitative ACR detected by strip test were 54.3%, 37.6% and 8.1% (Table 2).

**Table 2** Comparison of the ACR results between the quantitative assay and the semi-quantitative assay

| URiSCAN 2 ACR strip (mg/g) | Quantitative ACR (mg/g) | | | |
|---|---|---|---|---|
| | <30 | 30-300 | >300 | Total |
| <30 | 258 (53.3%) | 5 (1.0%) | 0 (0%) | 263 (54.3%) |
| 30-300 | 146 (30.2%) | 35 (7.2%) | 1 (0.2%) | 182 (37.6%) |
| >300 | 6 (1.2%) | 21 (4.3%) | 12 (2.5%) | 39 (8.1%) |
| Total | 410 (84.7%) | 61 (12.6%) | 13 (2.7%) | 484 (100%) |

The overall concordance rate of the ACR results between quantitative assay and semi-quantitative assay was 63.0%. The performance of URiSCAN 2 ACR strip test was 93.2% of sensitivity, 62.9% of specificity and 67.6% of accuracy. False positive and false negative rates of URiSCAN 2 ACR strip test for ACR detection were 68.8% and 1.9%, respectively. The value of their 95% confidence intervals for each parameter was also acceptable and presented (Table 3).

**Table 3** The performance of the URiSCAN 2 ACR strip test for detection of ACR

| | URiSCAN 2 ACR Strip |
|---|---|
| Sensitivity (%) | 93.2 (84.9-97.8) |
| Specificity (%) | 62.9 (58.1-67.6) |
| Accuracy (%) | 67.6 (63.2-71.7) |
| Concordance rate (%) | 63.0 (58.5-67.3) |
| False positive rate (%) | 68.8 (62.2-74.7) |
| False negative rate (%) | 1.9 (0.7-4.6) |
| Positive predictive value (%) | 31.2 (28.3-34.3) |
| Negative predictive value (%) | 98.1 (95.7-99.2) |
| Positive likelihood ratio | 2.5 (2.2-2.9) |
| Negative likelihood ratio | 0.1 (0.05-0.25) |

**Note:** Values shown are percentage (95% confidence intervals).

### *Cut-off of ACR for indicating CKD*

KDIGO 2012 guideline recommended the cut-off of ACR ≥30 mg/g for diagnosis and follow up of the complications of CKD. Based on the quantitative ACR reported by URiSCAN 2 ACR strip test, the efficiency for indicating CKD were analyzed using ROC analysis. The best cut-off of ACR value was 29.50 to 33.00 mg/g with AUC of 0.7413 (Figure 1). Sensitivity, specificity and accuracy of URiSCAN 2 ACR strip test for CKD detection were 70.5%, 61.8% and 63.8%, respectively. The diagnostic performance of URiSCAN 2 ACR strip test to indicate CKD was shown in table 4.

**Table 4** The diagnostic performance of the URiSCAN 2 ACR strip test for CKD detection

| | URiSCAN 2 ACR Strip |
|---|---|
| Sensitivity (%) | 70.5 (61.2-78.8) |
| Specificity (%) | 61.8 (56.7-66.8) |
| Accuracy (%) | 63.8 (59.4-68.1) |
| False positive rate (%) | 64.3 (57.5-70.5) |
| False negative rate (%) | 12.5 (8.9-17.3) |
| Positive predictive value (%) | 35.8 (31.8-39.9) |
| Negative predictive value (%) | 87.5 (83.8-90.4) |
| Positive likelihood ratio | 1.9 (1.6-2.2) |
| Negative likelihood ratio | 0.5 (0.4-0.6) |

**Note:** Values shown are percentage (95% confidence intervals).

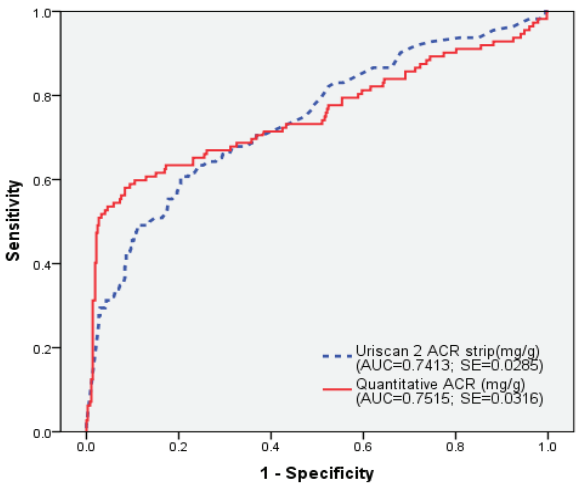


**Figure 1** Receiver operating characteristic curves (ROC) for presenting the effectiveness of URiSCAN 2 ACR strip test to indicate CKD in the community population.

**Abbreviations:** AUC, area under the curve; SE, standard error.

## Discussion

Detection of microalbuminuria is the earliest test for screening kidney disease and associated with the progression of CKD. It has a higher sensitivity and specificity than the total protein test[7,8]. KDIGOO 2012 guideline recommend that clinical laboratories should report ACR or protein/creatinine ratio (PCR) in spot urine samples rather than report the concentrations of albumin alone[3].

This study demonstrated the performance of the URiSCAN 2 ACR strip test to detect ACR. Our results showed a good sensitivity (93.2%), adequate specificity (62.9%) and concordance rate (63.0%) between the URiSCAN 2 ACR strip and the quantitative assay, which was comparable to those reported in the previous studies[9,10]. Lim et al[9] demonstrated sensitivity, specificity and concordance rate of the URiSCAN 2 ACR strip were 87.7%, 72.2% and 75.6%, respectively. Cho et al[10] evaluated the performance of URiSCAN Super cassette ACR strips and found that the, the sensitivity, specificity and concordance rate of the ACR strip test were 86.3%, 93.8% and 81.9%, respectively.

Lower specificity and concordance rate between URiSCAN 2 ACR strip and the quantitative assay in this study is due to the different assay of the reference method and the variation for disease heterogeneities among these populations in the study area. The high sensitivity of URiSCAN 2 ACR strip and the disagreement between this strip test and quantitative method for ACR detection were caused by high false positive results of the ACR results (68.8%) in this study. The false positive cases of higher albumin levels with lower creatinine levels might result in false positive ACR results. The previous study has shown the same pattern with these results[9]. However, false positive results can occur because of highly concentrated urine, especially diabetic patients. Urine of diabetic patients have more glucose than the level that the renal tubule can absorb. It is caused by highly concentrated urine in diabetic patients[11]. The prevalence of diabetic patients was 10.5%, diabetic patients with hypertension was 13.4% whereas 35.3% of all diabetic cases had positive result with glucose dipstick strip test. Moreover, 1.2% of false negative cases showed low specific gravity and low creatinine levels in urine (data not shown), suggesting that the urine was diluted. False negative results have been reported with diluted urine in the cases where predominant protein is not the albumin[12,13].

Furthermore, microalbuminuria is a marker of the inflammatory process. It is often present in patients with hypertension as well. This study showed a high incidence of albuminuria (ACR $\geq$30 mg/g) found in hypertensive patients with or without diabetes compared with non-diabetic and non-hypertensive patients (55.4% vs 27.0%) by using the quantitative assay (data not shown). The other study reported the same results as our study. In a study of 140 hypertensive patients with age of 50.1±11.6 years, the average ACR results was significantly higher in hypertensive than normotensive patients (2.17±2.67 mg/mmol and 1.72±2.97 mg/mmol, respectively, $P$=0.012)[14]. The increased ACR in hypertensive patients was due to two main mechanism, an increased glomerular hydrostatic pressure and an increased permeability in the glomerular basement membrane. Increased blood pressure, increased peripheral resistance and augmented volume load from increased flow pulsation could change the renal hemodynamics and damage the renal microvascular. Hence, hyperfiltration of albumin exceeds the absorption ability of the proximal tubules. In addition, the destruction of the lysosomal degradation pathway and the leakage of albuminuria is caused by the elevated angiotensin II and transforming growth factor-β1 in patients with hypertension[15,16].

Moreover, the data demonstrated that the URiSCAN 2 Optima urine chemistry test system for ACR detection is reliable to rule out increased excretion of urinary albumin with high NPV (98.1%) and low negative likelihood ratios (negative LR 0.1) at ACR value of >30 mg/g in spot urine. The procedures guide for clinical evaluation showed the reliably accepted cutoff value for rule-out microalbuminuria with a negative likelihood ratio of less than 0.1[17], whereas the performance of the URiSCAN 2 ACR strip test in our study for

rule-in albuminuria is poor (positive LR 2.5). The positive likelihood ratios must exceed 3.0 and the considered reliable value for a test's ability to rule-in a condition was a value of greater than 10[15]. Thus, the URiSCAN test system can reliably rule out microalbuminuria. In another ACR strip performance test study, Guy et al[18] evaluated the ability to rule in or rule out albuminuria by the CLINITEK microalbumin strip test (Siemens Healthcare Diagnostics Inc., Deerfield, US)[18]. Their data showed NPV above 90% and negative likelihood ratios less than 0.05. It was shown to be a reliable test for ruling out increased urinary albumin excretion at the same cutoff as in our study (AER>30 mg/24-hour).

In addition, this study accessed the effectiveness of urine ARC in distinguishing between CKD and non-CKD using the ROC curve. The best cutoff of ACR using URiSCAN 2 ACR strip for detection of CKD was in the range from 29.50 to 33.00 mg/g. The strip test had the same fair prediction (AUC 0.7413) as the quantitative automated assay (AUC 0.7515). Moreover, the URiSCAN 2 ACR strip test showed that PPV, NPV, positive LR and negative LR for diagnostic CKD were 35.8% 87.5%, 1.9 and 0.5%, respectively (Table 4). These results indicated that the effectiveness of the URiSCAN 2 ACR strip test as a screening test for ruling in CKD disease is poor; it is optimal for ruling out CKD disease. However, the previous studies have shown that the same cutoff of ACR (<3 mg/mmol or <30 mg/g) is used to determine albuminuria as with our study[19-21]. They showed the similarity of the high sensitivity and high NPV of the strip test for measuring the ACR. These results make this ACR strip test particularly useful for screening CKD in the general population, especially in the diabetic group.

There are several limitations for interpreting the results from this study. First, we have studied a few numbers of CKD samples when compared with non-CKD samples (approximately 1:4 of CKD vs. non-CKD) in this cohort. Imbalanced sample size may reduce the performance of the ACR strip test and increase the margin of error in this study. Secondly, the urine samples were collected from the wide-ranging parameter to enroll the population in the community into the study. The subjects were various groups instead of specific patients, which had a limitation in prediction of the test performance in specific diseases patients. Lastly, the measurement of ACR was only available from a single measurement. This will affect the imprecision of diagnostic performance of strip test in indicating CKD. KDIGO 2012 guideline recommends that the presence of kidney damage can be assumed by albuminuria. Findings evidence of albuminuria at least 2 occasions for more than 90 days should be classified as having CKD[3].

## Conclusion

URiSCAN 2 ACR strip provides the high sensitivity and high negative predictive value but few false negative results to detect albuminuria. Therefore, this ACR strip test might be served as an optimal method for excluding microalbuminuria. However, diagnostic performance of URiSCAN 2 ACR strip test at cut-off of ACR $\geq$30 mg/g is fair for screening CKD in community population.

### Take home messages

ACR strip test is the efficient method to rule-out microalbuminuria in random spot urine samples of the general population. It is still necessary to verify the effectiveness of the strip test for detection of microalbuminuria in chronic kidney disease patients in the community.

## Conflicts of interest

The authors declare no conflict of interest.

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