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RESEARCH ARTICLE

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Development and validation of point-of-care testing of albuminuria for early screening of chronic kidney disease

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Abstract

Introduction: Chronic kidney disease (CKD) is a significant global health issue. As the prevalence of renal replacement therapy (RRT) in Thailand is increasing, early detection and management of CKD is the most important step to prevent CKD progression and the need for RRT. Current diagnostic tests for CKD are non-specific and expensive. We aimed to develop and validate antibody-based-albumin point-of-care testing (POCT) to detect patients with impaired kidney function at early stage.

Methods: The prototype strip test was developed under the concept of competitive lateral flow immunochromatography assay, or strip test. Monoclonal antibodies (MAbs) to human serum albumin (HSA) were harvested from the hybridomas of spleen cells from immunized mice and mouse myeloma cells. Presence of MAbs was detected by enzyme-linked immunosorbent assay (ELISA). Spot urine was obtained from patients with kidney disease, type I, or type II Diabetes Mellitus upon their visit at King Chulalongkorn Memorial Hospital during 2018–2019. All samples were analyzed

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for urine albumin with our POCT (CU microalbumin) and the other two commercial POCTs (Microalbu PHAN and MICRAL). The results were validated against standard method for urine microalbumin measurement. A urine microalbumin concentration of less than 20 ug/ml was defined as normal. The sensitivity, specificity, and predictive values were calculated in comparison with the standard laboratory method.

Result: A total of 100 adult patients were included. CU microalbumin had a sensitivity of 86%, a specificity of 94%, and a positive predictive value of 96%. Our POCT showed good correlation with the laboratory results.

Conclusion: CU microalbumin correlated well with the standard method for quantitative measurement of urine albumin. Therefore, it has the potential for early screening of CKD, especially in primary health care facilities in resource limited settings.

KEYWORDS
chronic kidney disease, microalbuminuria, monoclonal antibody to albumin, point-of-care testing, urine strip test

1 | INTRODUCTION

The prevalence of chronic kidney disease (CKD) is increasing worldwide.[1,2] Accordingly, the number of patients with end-stage renal disease (ESRD) who need renal replacement therapy (RRT) has risen. In the United States, chronic kidney disease is one of the 10 leading causes of premature mortality.[2]

According to the population-based Thai Screening and Early Evaluation of Kidney Disease (Thai-SEEK) study,[3] the prevalence of CKD at any stage was about 17.5%. This number was much higher than the prevalence mentioned in other previous publications. However, less than 2% of the Thai general population in this study were aware that they had CKD.[3] The unawareness and limited accessibility to laboratory services, especially in remote areas, might cause underestimation of the prevalence. In addition, progressive increase in prevalence of patients on RRT was noted.[4] Impaired kidney function adversely affects not only health outcomes or quality of life but is also a burden on public health expenditure. The cost of RRT is 10 times more expensive than the cost of CKD care or other metabolic diseases. Allocation of health budget in Thailand has to be diverted to the treatment of ESRD patients rather than to prevent kidney failure and its complications.[5] Therefore, early detection of impaired kidney function is a prerequisite and urgently need for prevention of CKD progression in resource limited countries, especially in Thailand.

Microalbuminuria is an earlier biomarker for kidney damage and also a prognostic factor for CKD outcome.[6] Current technologies such as fluorescence immunoassays, high-performance liquid chromatography, immunonephelometry and radioimmunoassay have been developed.[7] However, most of these technologies require well-equipped labs with sophisticated equipment, well-trained personnel to operate the machine and is time consuming. Thus, there is an urgent need for point-of-care test (POCT) platforms that can quantitatively/semi-quantitatively detect microalbuminuria in relevant settings.

Currently, only few low-cost POCTs are available in Thailand. The commercial tools such as Microalbu PHAN® and Micral-Test® strips, have not been widely used and the cost is too expensive for developing countries to implement to the primary health care setting. Moreover, measuring principle of these tests is based on a chromogenic color indicator which can be easily misinterpreted.

In this study, we developed and validated point-of-care testing (POCT) for urine albumin based on immunoassay method (antibody-based-albumin POCT).

2 | MATERIALS AND METHODS

2.1 | Development of the antibody-albumin POCT

2.1.1 | Ethics statement

The protocol of this study was approved by The Institutional Review Board committee, Faculty of Medicine, Chulalongkorn University, Bangkok Thailand (COA No. 971/2017 and IRB No. 543/60). Written informed consent was obtained from each participant prior to enrollment.

2.1.2 | Preparation of monoclonal antibody

Female BALB/c mice were immunized thrice at 2-week interval with 50 μg human serum albumin (HSA) mixed with Freund's incomplete adjuvant (FIA) in proportion of 1:1 (v/v). One week after the last immunization, spleen cells were obtained and fused with SP2/0-Ag14 myeloma cells from The Institute of Biotechnology and Genetic Engineering Chulalongkorn University, following conventional methods for the generation of monoclonal antibodies (MAbs).[8,9] The levels of antibodies were measured by the enzyme-linked immunosorbent assay (ELISA; Table 1).

TABLE 1 Concentration of monoclonal antibody solutions (10 clones)

Number	Clone number	Code	Concentration of monoclonal antibody solutions (ug/ml)
1	1	4/B11/G3/B7/G12	54
2	2	4/B11/G3/F3/H2	52
3	4[a]	11/E12/D10/A3/G3	56
4	5	11/E12/D10/G6/C12	70
5	6	6/D1/D1/E3/G10	12
6	7	6/D1/G1/E7/G5	34
7	8	6/D2/D6/C11/A2	48
8	9	6/D2/D6/G11/A8	56
9	10	4/B11/G3/H11/D11	20
10	11	4/B11/G3/E12/E5	10

[a]No clone number 3.

2.1.3 | Specific indirect elisa assay

To detect Mabs, a 96-well plate was coated with 1 ng/well of human albumin and incubated at 4°C overnight. The wells were washed three times with washing buffer (phosphate buffer solution containing 0.1% Tween-20, PBST [Thermo Fisher scientific, USA]). Then, PBST 100 µl and MAb supernatants (1:500) 100 µl were added into each well and incubated at 37°C for 1 h. The wells were again washed three times with the washing buffer before adding secondary antibody (Sigma-Aldrich, Missouri, USA) (1:3000). The plate was then incubated at 37°C for 1 h and washed three times with the washing buffer. The substrate *o*-phenylenediamine in the presence of peroxide, OPD-H_2O_2 (Sigma-Aldrich, Missouri, USA) of 100 µl/well was added. The plate was further incubated at room temperature for 20 min in the dark. To stop the enzyme-substrate reaction, 50 µl of 2 M sulfuric acid was added. The Multiskan^{TM} GO micro plate reader (Thermo Fisher Scientific, Massachusetts, USA) was used to measure the absorbance at 492 nm.

2.1.4 | Isotyping of Mab

Mouse monoclonal antibody isotyping test kit (Bio-RAD, USA) was applied for determining the class and subclass of MAb. Each development tube was filled with 150 µl of 1 µg/ml Mab, incubated at room temperature for 30 s, and briefly vortexed. One isotyping strip, with the solid red end at the bottom, was placed into each development tube. A presence of blue bands in the two sections of the strip within 10 min would be corresponding to the isotype of MAb and its light chain.

2.1.5 | Affinity and kinetic measurement

The affinity and kinetic of Mab binding to HSA was determined by Biacore^{TM} T200 (GE Healthcare life sciences, Illinois, USA), a Surface Plasmon Resonance instrument. HBS-EP^{+} (GE Healthcare life sciences, Illinois, USA) was used as a system running buffer. Goat anti-mouse IgG1 (Sigma-Aldrich, Missouri, USA) was immobilized on the surface of the Protein G sensor chip (GE Healthcare life sciences, Illinois, USA). All ten clones of MAbs (ligand) were diluted to the concentration of 3 µg/ml with running buffer. A concentration of 20 nM and 320 nM of HSA (analyte) was injected with a flow rate of 30 µl/min for 30 s. The protein G sensor chip surface was regenerated with 10 mM Glycine HCl pH 1.5 (GE Healthcare life sciences, USA). The results were analyzed with BIAcore T200 evaluation software version 3.1 (GE Healthcare life sciences, USA).

2.1.6 | Protein purification

Purification of albumin in the urine was performed by affinity chromatography on Hi Trap^{TM} Blue HP column (GE Healthcare life sciences, Illinois, USA). 50 mM KH_2PO_4, pH 7.0, was used as a system binding buffer. All samples were applied at a flow rate of 1 ml/min. After washing the samples with PBST, the system was eluted manually with 50 mM KH_2PO_4, 1.5 M KCl, pH 7.0 at a flow rate of 1 ml/min. The urine samples were analyzed for proteins by using a UV spectrophotometer (measurement of the absorbance 280 and 214 nm).

2.1.7 | Preparation of antibody-conjugated gold nanoparticles

To detect microalbumin in urine, colloidal gold 40 nm was conjugated with Mab. The mixture was used as the detecting agent. First, a colloidal gold solution at pH 8.0 was stabilized with 200 mM potassium carbonate. Then, MAb (3 µg/ml) was added to the colloidal gold solution (pH 8.0). After gently stirring the mixture for 10 min at room temperature, 1% (w/v) Casein was added and the mixture was incubated at 2–8°C overnight. The conjugated mixture was centrifuged at 12,000 rpm for 30 min. The pellet was resuspended in a conjugate storage buffer. The gold particles were then mixed with a 10% (w/v) sucrose solution and 5% trehalose. The mixture was subsequently sprayed on the conjugated pad at the distribution of 10 µl/cm.

2.1.8 | Strip test interpretation

The prototype strip test was developed under the concept of competitive binding of albumin at the test line (T-line) and albumin in the urine to the anti-albumin Mab—gold conjugate. The specimen at the control line (C-line) was goat anti-mouse antibodies. If there was albumin in the sample, the gold—anti-albumin Mab conjugate would bind to the albumin in the sample instead of albumin at the T-line. As a result, this antibody-based POCT would be interpreted positive when the signal (red band) shown only at the C-line. A negative result for microalbumin would be when 2 red bands were shown at T-line and C-line. The red band must always appear on the C-line for reliable interpretation.

2.2 | Validation of the antibody-based-albumin POCT

2.2.1 | Urine samples collection

Twenty milliliters (20 ml) of the morning midstream or spot urine were collected from 100 samples such as 54 samples of CKD who were diagnosed as kidney injury following Kidney Disease: Improving Global Outcomes (KDIGO) guideline 2012[6] aged in range from 21–88 years, 8 samples of AKI patients age ranged from 29–83 years, 10 samples of type I or II Diabetes Mellitus (DM) for at least 5 years age ranged from 22–78 years, and 28 healthy for control group age ranged from 24–86 years, upon their visit at King Chulalongkorn Memorial Hospital during September 2018 to November 2019. All patients were adults who were at least 18 years of age. After mixing each urine sample by inversion, 100 µl of the sample was pipetted to the sample pad of the Microalbumin Test Kits. The results were read 15 min thereafter.

CU microalbumin, the other two commercial POCTs (MicroalbuPHAN® and Micral-Test®), and the standard method for urine albumin measurement were concurrently measured.

Turbidimetric immunoassay is the gold standard for the detection of microalbuminuria. This technique is a type of immunoassay based on the measurement of antigen-antibody formation, which is directly related to the concentration of antigen in the mixture. All strip tests were repeated thrice for more reliability. The standard urine microalbumin assay was regarded as a gold standard. A urine microalbumin of more than 20 ug/ml indicated an abnormality and the presence

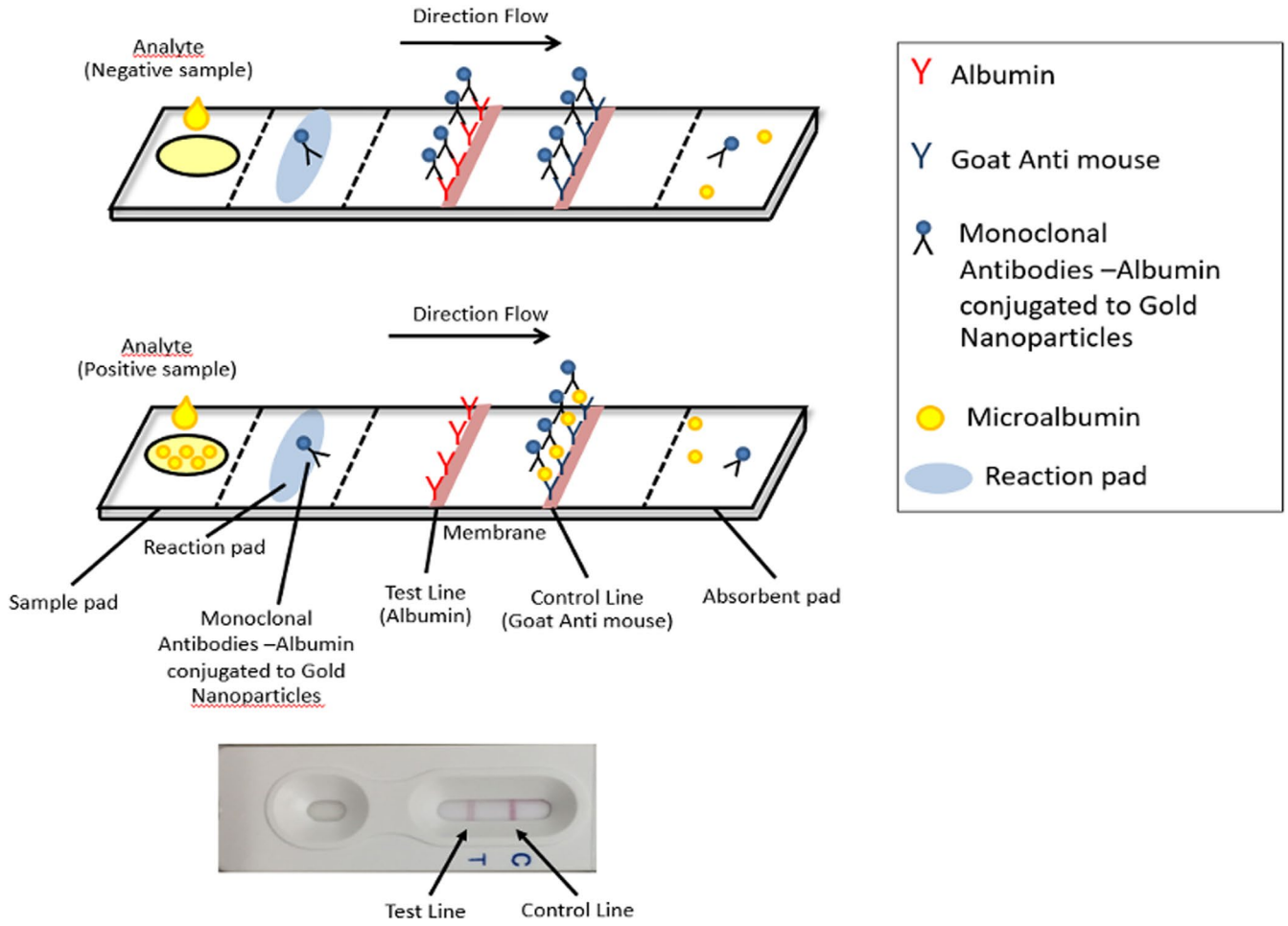


FIGURE 1 Diagram of a test strip showing a top-view of its components and principle of a competitive strip test assay with microalbuminuria

of microalbuminuria.[10] Figure 1 shows a diagram of a test strip with a top-view of its components and demonstrates the principle of a competitive strip test assay to detect microalbuminuria.

2.3 | Statistical analysis

Sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and diagnostic accuracy were calculated and corrected with 95% confidence interval of the diagnostic methods using Diagnostic test MedCalc Software.

3 | RESULTS

The concentration of MAbs in each clone are demonstrated in Table 1. All harvested MAbs consisted of immunoglobulin G1 (IgG1) subclass with *kappa* light chain. The kinetic studies of the reaction between MAbs and HSA are demonstrated in Table 2. The MAbs from the clone number 4,5,8,9 showed a lower dissociation constant (K_D) value than the others, reflecting higher affinity of the antibodies. The clone number 4 was applied for our antibody-based POCT development, as it showed the best sensitivity and specificity to the presence of microalbuminuria by ELISA.

A total of 100 urine samples were included in the analysis. Participant characteristics are shown in Table S1. The median level of microalbuminuria was 45.5 [12.48, 407.8] µg/ml by laboratory-measured urine microalbumin concentration. The results of microalbuminuria interpreted by the other three readers are shown in Table 3. Figure 2 shows interpretation of the results detected by the CU microalbumin test.

When compared to the gold standard, the CU microalbumin test had a sensitivity of 86%, specificity of 94%, positive predictive value (PPV) of 96%, negative predictive value (NPV) 74.6% and an accuracy of 87%. The MicroalbuPHAN® had a sensitivity of 95%, specificity of 64%, a PPV of 84%, NPV 86% and an accuracy of 84%. The Micral-Test® had a sensitivity of 98%, specificity of 61%, PPV of 83%, NPV 95% and an accuracy of 86% (Figure 3). When compared with the gold standard, the CU microalbumin test showed a Kappa test value of 0.915 with $p < 0.001$.

4 | DISCUSSION

The early stages of kidney impairment may be clinically silent, but progresses rapidly and leads to morbidity and mortality in the later stages. Detection of early CKD will allow for management to delay CKD progression and prevent the need of renal replacement therapy. Therefore, a combination of good sensitivity and high specificity is essential for screening to rule out CKD. When comparing to the other two commercial kits, the CU microalbumin has lower sensitivity, however, it reached higher specificity, PPV, and accuracy.

The level of microalbuminuria in this study varied from micro- to macroalbuminuria, which proves the efficacy of this test for screening in both general and high-risk populations. Thus, it is considered as a promising tool for front-line CKD screening before referral to nephrologists, especially in remote areas where the laboratory services are difficult to access.

The developed strip test, CU microalbumin, is a competitive strip test based on antigen-antibody complex formation labelled gold nanoparticles. It is simple, easy to use and inexpensive. When compared to current commercial tests, MicroalbuPHAN® strip and Micral-Test® strip, both tests are dipstick tests based on color change of acidic-basidic indicator. The urine test results are manually interpreted by comparing the color with the standard color chart where different color perception may interfere with the results of both tests. Therefore, it is essential to properly train personnel to accurately identify and interpret urine dipsticks.

Recently, immunosensor platforms have been developed to quantify microalbuminuria for quantitative measurement of microalbumin in urine. Shaikh et al, used monodisperse polystyrene(PS) nanoparticles coated with Ag nanoshells conjugated to HSA antibodies. Then, these nanoprobes would be trapped on the electrode surface using dieletrophoresis.[11] The second study by Omidfar et al, constructed an antibody labeled with AuNP (Ab-AuNP) and polyvinyl alcohol(PVA) modified screen-printed carbon electrode(SPCE) for quantitative

TABLE 2 Kinetics of the reaction between McAbs and HSA

Clone number	Code	Ka (1/MS)	Kd (1/s)	KD (M)	χ^2	*U*-value
1	4/B11/G3/B7/G12	7.48×10^4	0.0091	122.00 nM	0.12	1
2	4/B11/G3/F3/H2	7.51×10^4	0.0089	118.00 nM	0.19	1
4	11/E12/D10/A3/G3	1.04×10^5	0.0024	22.90 nM	1.05	1
5	11/E12/D10/G6/C12	8.28×10^4	0.0025	30.00 nM	0.63	1
6	6/D1/D1/E3/G10	1.32×10^4	0.0014	121.00 nM	0.01	2
8[a]	6/D2/D6/C11/A2	1.10×10^5	0.0011	10.43 nM	2.57	2
9	6/D2/D6/G11/A8	1.09×10^5	0.0011	10.41 nM	1.85	2
10	4/B11/G3/H11/D11	7.28×10^4	0.0090	123.00 nM	0.22	1
11	4/B11/G3/E12/E5	5.25×10^n	0.0090	172.00 nM	0.15	1

[a]Clone number 7 did not bind to albumin.

TABLE 3 Data interpretation of urine microalbumin performed by each POCT

	Microalbumin	Results								
		CU microalbumin			Microalbu Phan			MICRAL		
Samples	ug/ml	reader1	reader2	reader3	reader1	reader2	reader3	reader1	reader2	reader3
1	16.2	TN	TN	TN	TN	TN	FP	TN	TN	TN
2	79.3	TP	TP	TP	TP	TP	TP	TP	TP	TP
3	315.4	TP	TP	TP	TP	TP	TP	TP	TP	TP
4	360.2	TP	TP	TP	TP	TP	TP	TP	TP	TP
5	13.8	FP	FP	FP	FP	TN	FP	TN	TN	FP
6	919.5	TP	TP	TP	TP	TP	TP	TP	TP	TP
7	168.6	TP	TP	TP	TP	TP	TP	TP	TP	TP
8	1.8	TN	TN	TN	TN	TN	TN	TN	TN	TN
9	718.9	TP	TP	TP	TP	TP	TP	TP	TP	TP
10	55.1	TP	TP	TP	TP	TP	TP	TP	TP	TP
11	2194.8	TP	TP	TP	TP	TP	TP	TP	TP	TP
12	65.5	TP	TP	TP	TP	TP	TP	TP	TP	TP
13	483.2	TP	TP	TP	TP	TP	TP	TP	TP	TP
14	92.8	TP	TP	TP	TP	TP	TP	TP	TP	TP
15	22.5	TP	TP	TP	FN	FN	FN	TP	TP	TP
16	<5	TN	TN	TN	TN	TN	FP	TN	TN	TN
17	<5	TN	TN	TN	TN	TN	TN	TN	TN	TN
18	25.7	TP	TP	TP	TP	FN	TP	TP	TP	TP
19	826.2	TP	TP	TP	TP	TP	TP	TP	TP	TP
20	33.8	TP	TP	TP	TP	TP	TP	TP	TP	TP
21	33	FN	FN	FN	TP	TP	TP	TP	TP	TP
22	12.3	TN	TN	TN	TN	TN	TN	TN	FP	FP
23	241.8	TP	TP	TP	TP	TP	TP	TP	TP	TP
24	<5	TN	TN	TN	TN	TN	FP	FP	FP	FP
25	764.1	TP	TP	TP	TP	TP	TP	TP	TP	TP
26	10.1	TN	TN	TN	TN	TN	FP	FP	FP	FP
27	24.4	FN	FN	FN	TP	TP	TP	TP	TP	TP
28	5016.7	TP	TP	TP	TP	TP	TP	TP	TP	TP
29	37.1	TP	TP	TP	FN	TP	FN	TP	TP	TP
30	961.8	TP	TP	TP	TP	TP	TP	TP	TP	TP
31	1589.7	TP	TP	TP	TP	TP	TP	TP	TP	TP
32	62.7	TP	TP	TP	TP	TP	TP	TP	TP	TP
33	726.2	TP	TP	TP	TP	TP	TP	TP	TP	TP
34	42.1	TP	TP	TP	TP	TP	TP	TP	TP	TP
35	<5	TN	TN	TN	TN	TN	TN	TN	TN	TN
36	<0.5	TN	TN	TN	TN	TN	TN	TN	TN	TN
37	88	TP	TP	TP	TP	TP	TP	TP	TP	TP
38	190	TP	TP	TP	TP	TP	TP	TP	TP	TP
39	11	TN	TN	TN	FP	TN	FP	TN	TN	TN
40	<5	TN	TN	TN	TN	TN	TN	TN	TN	FP
41	696	TP	TP	TP	TP	TP	TP	TP	TP	TP
42	19	FP	FP	FP	TN	TN	TN	TN	FP	FP
43	65	TP	FN	TP	TP	TP	TP	TP	TP	TP

(Continues)

TABLE 3 (Continued)

	Microalbumin	Results								
		CU microalbumin			Microalbu Phan			MICRAL		
Samples	ug/ml	reader1	reader2	reader3	reader1	reader2	reader3	reader1	reader2	reader3
44	1108	TP	TP	TP	TP	TP	TP	TP	TP	TP
45	11	TN	TN	TN	FP	FP	FP	TN	FP	FP
46	13	TN	TN	TN	FP	FP	FP	TN	FP	FP
47	6170	TP	TP	TP	TP	TP	TP	TP	TP	TP
48	24	FN	FN	FN	TP	TP	TP	TP	TP	TP
49	9	TN	TN	TN	TN	TN	TN	TN	TN	FP
50	22	FN	FN	FN	TP	TP	TP	TP	TP	TP
51	10	TN	TN	TN	TN	TN	TN	TN	FP	FP
52	750	TP	TP	TP	TP	TP	TP	TP	TP	TP
53	455	TP	TP	TP	TP	TP	TP	TP	TP	TP
54	415	TP	TP	TP	TP	TP	TP	TP	TP	TP
55	687	TP	TP	TP	TP	TP	TP	TP	TP	TP
56	47	TP	FN	TP	TP	TP	TP	TP	TP	TP
57	14	TN	TN	TN	TN	TN	TN	TN	TN	TN
58	754	TP	TP	TP	TP	TP	TP	TP	TP	TP
59	9	TN	TN	TN	TN	TN	TN	FP	FP	FP
60	1990	TP	TP	TP	TP	TP	TP	TP	TP	TP
61	436	TP	TP	TP	TP	TP	TP	TP	TP	TP
62	16	TN	TN	TN	FP	FP	FP	TN	TN	TN
63	22	FN	FN	FN	TP	TP	TP	TP	TP	TP
64	33	FN	FN	FN	FN	FN	FN	FN	FN	FN
65	<5	TN	TN	TN	TN	TN	TN	TN	TN	FP
66	<5	TN	TN	TN	TN	TN	TN	TN	TN	TN
67	10	TN	TN	TN	TN	FP	FP	FP	FP	FP
68	14	TN	TN	TN	FP	FP	FP	FP	FP	FP
69	210	TP	TP	TP	TP	TP	TP	TP	TP	TP
70	190	TP	TP	TP	TP	TP	TP	TP	TP	TP
71	7	TN	TN	TN	TN	TN	TN	TN	TN	TN
72	80	TP	TP	TP	TP	TP	TP	TP	TP	TP
73	131	TP	TP	TP	TP	TP	TP	TP	TP	TP
74	18	TN	TN	TN	FP	FP	FP	FP	FP	FP
75	114	TP	TP	TP	TP	TP	TP	TP	TP	TP
76	<5	TN	TN	TN	TN	TN	TN	TN	TN	TN
77	<5	TN	TN	TN	TN	TN	TN	TN	TN	TN
78	220	TP	TP	TP	TP	TP	TP	TP	TP	TP
79	11	TN	TN	TN	FP	TN	FP	FP	FP	FP
80	10	TN	TN	TN	FP	TN	FP	FP	FP	FP
81	829	TP	TP	TP	TP	TP	TP	TP	TP	TP
82	793	TP	TP	TP	TP	TP	TP	TP	TP	TP
83	199	TP	TP	TP	TP	TP	TP	TP	TP	TP
84	40	FN	FN	FN	TP	TP	TP	TP	TP	TP
85	44	TP	FN	FN	TP	TP	TP	TP	TP	TP
86	7	TN	TN	TN	FP	TN	FP	TN	TN	FP

(Continues)

TABLE 3 (Continued)

	Microalbumin	Results								
		CU microalbumin			Microalbu Phan			MICRAL		
Samples	ug/ml	reader1	reader2	reader3	reader1	reader2	reader3	reader1	reader2	reader3
87	57	TP	TP	TP	TP	TP	TP	TP	TP	TP
88	110	TP	TP	TP	TP	TP	TP	TP	TP	TP
89	44	FN	FN	FN	TP	TP	TP	TP	TP	TP
90	2580	TP	TP	TP	TP	TP	TP	TP	TP	TP
91	386	TP	TP	TP	TP	TP	TP	TP	TP	TP
92	24	FN	FN	FN	FN	TP	TP	TP	TP	TP
93	1228	TP	TP	TP	TP	TP	TP	TP	TP	TP
94	17	TN	TN	TN	FP	FP	FP	TN	TN	TN
95	107	TP	TP	TP	TP	TP	TP	TP	TP	TP
96	8	TN	TN	TN	FP	FP	FP	TN	TN	FP
97	<5	TN	TN	TN	TN	TN	TN	TN	TN	TN
98	42	TP	FN	TP	TP	TP	TP	TP	TP	TP
99	64	TP	FN	TP	TP	TP	TP	TP	TP	TP
100	2804	TP	TP	TP	TP	TP	TP	TP	TP	TP

Abbreviations: FN, false negative; FP, false positive; TN, true negative; TP, true positive.

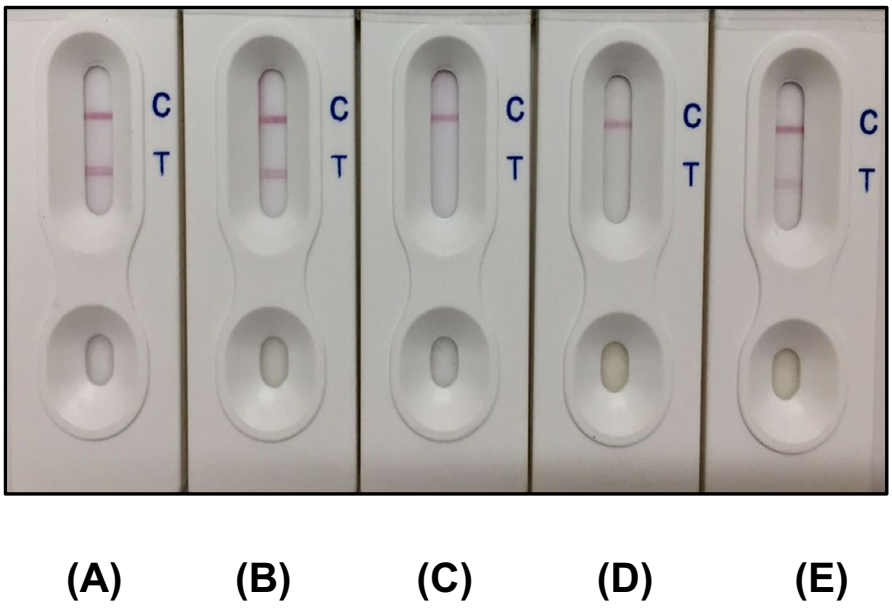
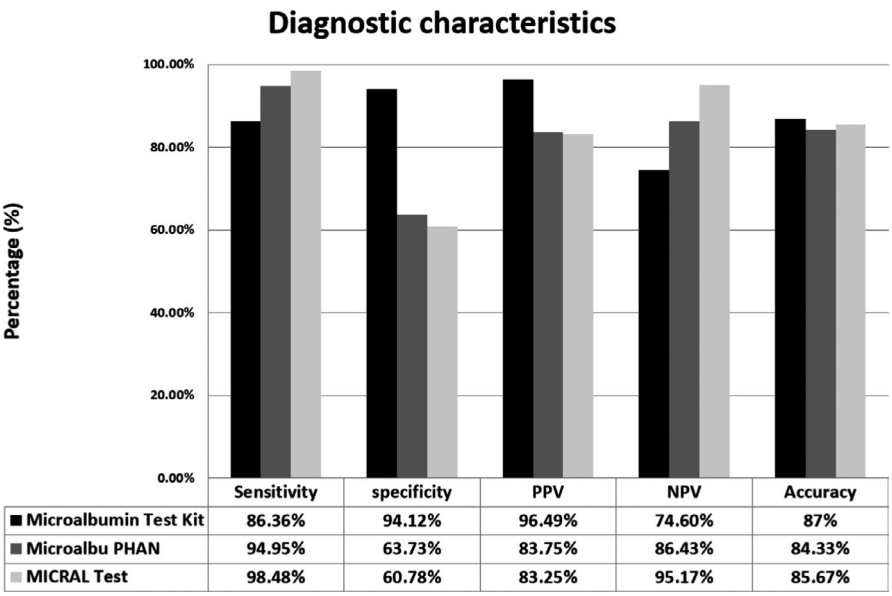


FIGURE 2 Test strips tested with different samples: (A) Phosphate buffer saline (negative control), (B) Urine without albumin (negative control), (C) Human serum albumin (positive control), (D) CKD with urine sample (positive), and (E) Healthy urine sample (negative). C, control line; T, test line

measurement of microalbuminuria. The current signal was recorded by voltammetric modes.[12] The last study by Tsai et al developed an immunosensor based on an carboxyl-enriched porous screen-printed carbon electrode (COOH-P-SPCE). The sensor could detect HSA in urine in a wide linear range from 10 to 300 ug/ml. Unfortunately, these immunosensors are not commercially available.[13]

Several limitations should be noted. First, the CU microalbumin was designed to detect microalbuminuria qualitatively for cost reduction in mass screening. To assess the degree of kidney damage, further quantitative investigations are still required. Second, our test is very specific to albumin detection as we developed the test in the concept of antibody-based POCT. Such kidney diseases with non-albumin proteinuria cannot be ruled out by our test. Third, CU microalbumin has lower sensitivity than the other two commercial tests used in this study. Thus, we plan to improve sensitivity of our test by developing a sandwich immunochromatographic assay in collaboration with the National Innovation Agency (NIA) and Siam Cement Group (SCG).

This is the first time in which Thailand can produce monoclonal antibodies with potential use at the commercial level. Therefore, this POCT can decrease the cost of importing commercial tests from overseas. In the future, we plan to integrate this POCT into



	Sensitivity	specificity	PPV	NPV	Accuracy
Microalbumin Test Kit	86.36%	94.12%	96.49%	74.60%	87%
Microalbu PHAN	94.95%	63.73%	83.75%	86.43%	84.33%
MICRAL Test	98.48%	60.78%	83.25%	95.17%	85.67%

FIGURE 3 Histogram showing the diagnostic characteristics of each Point-of-Care diagnostic device

our primary health care facilities and set up a remote telemedicine screening system for CKD. Health education and other useful recommendations are also given individually according to the patient's underlying diseases in an online format. In addition, we also plan to use our test in research areas as one of the outcomes of implementation of integrated CKD care to delay progression of CKD in patients with metabolic diseases.

5 | CONCLUSION

CU microalbumin correlated well with the standard method for quantitative measurement of urine albumin. Therefore, it has the potential for point-of-care testing in early screening of CKD.

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CONFLICT OF INTEREST

None.

AUTHORS' CONTRIBUTION

Conceptualization: NS, TP, KK; Methodology: NS, TP, KK, PK; Formal analysis and investigation: NS, TP, KK, PK, NV Writing – NW, PK, NS, original draft preparation: NS, TP, KK, PK, NV; Writing – review and editing: NV, PK, TP, KK, CT, NL, SP, SK, KK, KT, SE, NS; Funding acquisition: NS; Supervision: NS, TP, KK.

CONSENT TO PARTICIPATE

All subjects were informed about the risk and benefit of the study. Informed consent was signed before enrolling to the study.

CONSENT FOR PUBLICATION

All authors whose names appear on the submission (1) made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; (2) drafted the work or revised it critically for important intellectual content; (3) approved the version to be published; and (4) agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

CODE AVAILABILITY

Custom code.

DATA AVAILABILITY STATEMENT

The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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