RESEARCH REPORT

Master of medicine in Chemical Pathology

University of Witwatersrand Faculty of Health Sciences

Title

The use of point of care testing for the diagnosis of chronic kidney disease

Submitted by:

Dr Sean Currin (MBChB)

Student number: 350639

Supervisors:

Prof. Jaya George (MBBCh, DTM&H, MSc, FCPath, PHD)

Professor and Head

Department of Chemical Pathology

NHLS and University of Witwatersrand

Dr June Fabian (MBBCh, FCP (SA), Cert Neph(SA))

Medical Research Council/Wits University Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences, University of Witwatersrand

Wits Donald Gordon Medical Centre, School of Clinical Medicine, Faculty of Health Sciences, University of Witwatersrand

Table of contents

1.

Published paper 1: Evaluating chronic kidney disease in rural South Africa: comparing estimated glomerular filtration rate using point-of-care creatinine to iohexol measured GFR

Sean Currin*, Mwawi Gondwe, Nokthula Mayindi, Shingirai Chipungu, Bongekile Khoza, Lungile Khambule, Tracy Snyman, Stephen Tollman, June Fabian and Jaya George, on behalf of the ARK Consortium

Evaluating chronic kidney disease in rural South Africa: comparing estimated glomerular filtration rate using point-of-care creatinine to iohexol measured GFR

https://doi.org/10.1515/cclm-2020-1882
Received December 29, 2020; accepted March 3, 2021;
published online March 12, 2021

Abstract

Objectives: The prevalence of chronic kidney disease is rising rapidly in low- and middle-income countries. Serum creatinine and estimation of glomerular filtration rate (GFR) are critical diagnostic tools, yet access to centralised laboratory services remains limited in primary care resource-limited settings. The aim of this study was to evaluate point-of-care (POC) technologies for serum creatinine measurement and to compare their performance to a gold standard measurement using iohexol measured GFR (mGFR).

Methods: POC creatinine was measured using iSTAT® and StatSensor® devices in capillary and venous whole blood, and laboratory creatinine was measured using the compensated kinetic Jaffe method in 670 participants from a rural area in South Africa. GFR estimating equations Chronic Kidney Disease Epidemiology Collaboration and Modification of Diet in Renal Disease (CKD-EPI and MDRD) for POC and laboratory creatinine were compared to iohexol mGFR.

Results: Calculated GFR for laboratory and POC creatinine measurements overestimated GFR (positive bias of 1.9–34.1 mL/min/1.73 m^2). However, all POC devices had less positive bias than the laboratory Jaffe method (1.9–14.7 vs. 34.1 for MDRD, and 8.4–19.9 vs. 28.6 for CKD-EPI). Accuracy within 30% of mGFR ranged from 0.56 to 0.72 for POC devices and from 0.36 to 0.43 for the laboratory Jaffe method. POC devices showed wider imprecision with coefficients of variation ranging from 4.6 to 10.2% compared to 3.5% for the laboratory Jaffe method.

Conclusions: POC estimated GFR (eGFR) showed improved performance over laboratory Jaffe eGFR, however POC devices suffered from imprecision and large bias. The laboratory Jaffe method performed poorly, highlighting the need for laboratories to move to enzymatic methods to measure creatinine.

Keywords: chronic kidney disease; creatinine; eGFR; iohexol mGFR; low- and middle-income countries (LMICs); point-of-care.

***Corresponding author:** Dr. **Sean Currin**, Department of Chemical Pathology, Faculty of Health Sciences, University of Witwatersrand, 7 York Road, Parktown, Johannesburg, 2193, South Africa; and Department of Chemical Pathology, National Health Laboratory Service, Johannesburg, South Africa, Phone: +2711 489 8499, E-mail: sean.currin@wits.ac.za. https://orcid.org/0000-0003-2487-6763

Mwawi Gondwe, Nokthula Mayindi, Shingirai Chipungu and Bongekile Khoza, Medical Research Council/Wits University Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

Lungile Khambule, Department of Chemical Pathology, University of Witwatersrand, Johannesburg, South Africa

Tracy Snyman and Jaya George, Department of Chemical Pathology, University of Witwatersrand, Johannesburg, South Africa; and Department of Chemical Pathology, National Health Laboratory Service, Johannesburg, South Africa

Stephen Tollman, Medical Research Council/Wits University Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa; and International Network for the Demographic Evaluation of Populations and their Health (INDEPTH) Network, Accra, Ghana

June Fabian, Medical Research Council/Wits University Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa; and Wits Donald Gordon Medical Centre, School of Clinical Medicine, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

Introduction

Chronic kidney disease (CKD) has a prevalence of 10.7% in sub-Saharan Africa, and is associated with significant

morbidity and mortality [1]. Low- and middle-income countries (LMIC) suffer a disproportionate mortality associated with CKD when compared to high-income countries [2]. Therefore, screening strategies that aim to identify patients with early stages of CKD in primary care settings and ideally, prevent progression of CKD, are of profound importance.

Glomerular filtration rate (GFR) is considered the best overall index of kidney function and is an important criterion in the diagnosis and staging of CKD [3]. GFR cannot be quantitated directly. However, it can be assessed by the measurement of exogenous (inulin, Cr-EDTA, iohexol, iothalamate) or endogenous markers [4]. Exogenous markers are impractical for routine clinical use due to their limited access, high cost and duration of time required for testing. Estimated GFR (eGFR) equations based on endogenous markers and additional factors, such as age and sex, are recommended for the routine assessment of kidney function [3]. The creatinine based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) and Modification of Diet in Renal Disease (MDRD) equations are the most commonly used [5]. The 2009 CKD-EPI creatinine equation is recommended for use in adults [3].

In resource-limited settings a lack of access to central laboratory services hinders the ability to implement effective CKD screening programs. Even when central laboratory services are available, logistical factors in primary health care clinics can impact the accuracy of creatinine measurements, such as sub-optimal storage of collected samples and delays in transport to the central laboratory [6]. Point-of-care (POC) testing offers an important solution to this problem by offering access to actionable results in real-time for clinical decision making [7]. While the 2012 KDIGO guidelines recognise that limited laboratory access is a problem, they fall short of recommending POC testing in such settings [8].

The aim of our study was to evaluate the performance of POC creatinine by comparing eGFR equations to iohexol measured GFR (mGFR), and to compare this performance with that of laboratory eGFR. Additionally, we looked at the performance of the MDRD and CKD-EPI equations for these different creatinine measurements.

Materials and methods

Our study formed part of the African Research on Kidney (ARK) study, which aimed to determine the population prevalence of CKD and associated risk factors in three sub-Saharan African countries, Malawi, South Africa, and Uganda. Detailed methods for the ARK study have been published [9]. The South African component was conducted in the Agincourt Health and Demographic Surveillance Site in Bushbuckridge, a rural sub-district of the Mpumalanga province in South Africa.

Participants from the community were invited to participate in this second phase of the study, to measure GFR using intravenous iohexol. The initial study comprised a population-based sample of 2021 adults aged 20–80 years to determine CKD prevalence and investigate associated risk factors. For the iohexol study, we stratified a subsample of the main study by sex and eGFR to ensure adequate sampling across all stages of CKD.

Measured GFR was determined by plasma excretion of iohexol. A 5 mL bolus of intravenous iohexol (Omnipaque™, GE Healthcare, Illinois) was administered in the antecubital fossa of one arm, followed by repeat plasma sampling from the antecubital fossa of the contralateral arm after 5, 120, 180 and 240 min. Prior to iohexol administration a baseline venous blood sample was obtained, which was used for real-time testing with the iSTAT® (Abbott Laboratories, Illinois) and StatSensor® (Nova Biomedical Corporation, Massachusetts) POC devices. At the same time, a capillary blood sample taken from a fingerprick was also analysed on the StatSensor® POC device. There were no adverse events related to the intravenous administration of iohexol.

On the day of iohexol testing, for each participant, samples for creatinine and iohexol were refrigerated (2–8 °C) until all participants for the day had been processed (usually by 3 pm), after which all samples were centrifuged at 2,000 *g* for 10 min. Samples were stored and transported at −80 °C to a central laboratory for iohexol and laboratory creatinine measurements.

Plasma concentrations of iohexol were determined using ultra-performance liquid chromatography with tandem mass spectrometry using a published method [10] in an ISO 15189 accredited laboratory which participates in the EQUALIS external quality assurance programme for iohexol (Uppsala, Sweden). The calibration curve and internal standard were determined with certified reference materials for iohexol (CRM: USP H0J211) and ioversol (CRM: USP 34510F), respectively. Internal quality control was prepared using the iohexol certified reference material and EQUALIS samples were included in every run as an additional quality check. The slope-intercept method using the 120, 180 and 240 time points (during the second exponential phase of iohexol elimination) was used to calculate mGFR by plotting iohexol concentration against time [11]. mGFR results were then corrected for body surface area [12].

Laboratory creatinine was determined in a central laboratory using an isotope dilution mass spectrometry (IDMS) traceable compensated kinetic Jaffe method (Cobas c500®, Roche Diagnostics, Mannheim). POC creatinine was measured at the time of collection in venous whole blood on the iSTAT® and in venous whole blood and capillary blood on the StatSensor®, both POC devices make use of an enzymatic amperometric method with an enzyme cascade reaction. Laboratory creatinine was monitored with internal and external quality control schemes, while POC creatinine was monitored with internal quality control. Inter-day coefficients of variation (CV)s were determined by assessing the variation in quality control materials over the period of the study.

Statistical analysis was performed using Tibco Statistica® (version 13.5.0.17, United States) and MedCalc® (version 19.2.1, Belgium). POC creatinine was compared to laboratory creatinine using Passing–Bablok regression and Bland–Altman agreement. eGFR was calculated according to the IDMS-traceable MDRD equation and the 2009 CKD-EPI equation. All subsequent eGFR results are presented

without the African-American coefficient applied, based on prior studies [13, 14] demonstrating that inclusion of the coefficient overestimates GFR in sub-Saharan Africans. eGFR was compared to mGFR with Passing–Bablok regression, Spearman rank correlation coefficient (r) and Lin's concordance correlation coefficient were determined. Bias was defined as the median of individual differences between eGFR and mGFR, while imprecision was defined as the interquartile range (IQR) of the differences between eGFR and mGFR. Outliers were excluded if their residuals lay outside of 2.5 standard deviations on initial Passing–Bablok regression [15, 16]. Accuracy within 30% of mGFR (P30) was calculated. The ability of the eGFR equations to correctly classify mGFR: <60, 60–89, and ≥90 mL/min/1.73 m^2 (corresponding to KDIGO GFR categories for CKD of G3–5, G2 and G1, respectively) was calculated. Differences between the laboratory and POC devices were tested using the exact McNemar test. Results were considered statistically significant if $p<0.05$.

Repeat analysis with laboratory Jaffe creatinine adjusted by a factor of +9.0 µmol/L was performed (Supplementary Table S1). This adjustment was based on an inter-site calibration study performed within the larger ARK study comparing laboratory Jaffe creatinine methodology performed in South Africa and Malawi with an enzymatic laboratory creatinine method in Uganda.

Results

Study population

Figure 1 depicts the flow of participants through the study. All participants were Black African adults with a broad spectrum of kidney function. eGFR equations for laboratory and POC creatinine overestimated GFR when compared to mGFR, except in the ≥90 mL/min/1.73 m^2 group where a mixed picture was seen (Table 1). Mean POC eGFRs showed less overestimation compared to mGFR than the mean laboratory eGFRs.

POC vs. laboratory creatinine

All three POC creatinine devices showed positive bias compared to laboratory creatinine with Bland–Altman agreements of 17.4 ± 15.3, 9.4 ± 6.3, and 15.1 ± 13.0 µmol/L for StatSensor® capillary, iSTAT®, and StatSensor® venous respectively. Passing–Bablok regression analysis yielded proportional errors (slope) of: 1.45 (95% CI: 1.33–1.57), 1.16 (95% CI: 1.13–1.20), and 1.17 (95% CI: 1.03–1.33); constant errors (*y*-intercept) of: −7.68 (95% CI: −14.43 to −1.33), 0.16 (95% CI: −2.20 to 2.13), and 6.50 (95% CI: −2.33 to 14.58); and Spearman rank correlation coefficients (r) of: 0.57 (95% CI: 0.52–0.62) ($p<0.0001$), 0.92 (95% CI: 0.91–0.93) ($p<0.0001$), and 0.65 (95% CI: 0.57–0.72) ($p<0.0001$) for StatSensor® capillary, iSTAT®, and StatSensor® venous respectively (Figure 2).

eGFR (using POC creatinine and laboratory creatinine) vs. mGFR

Passing–Bablok regression demonstrated that all eGFR equations using POC and laboratory creatinine measurements suffered from proportional and constant error when compared to mGFR. The largest constant error was seen in laboratory creatinine eGFR using the CKD-EPI equation whilst the smallest constant error was seen in StatSensor® capillary creatinine eGFR using the MDRD equation (*y*-intercept: 62.76 vs. 3.91). StatSensor® capillary creatinine eGFR using the MDRD equation showed the least proportional error, while laboratory creatinine eGFR using the CKD-EPI equation showed the most proportional error (slope: 1.01 vs. 0.59). MDRD eGFR showed less proportional

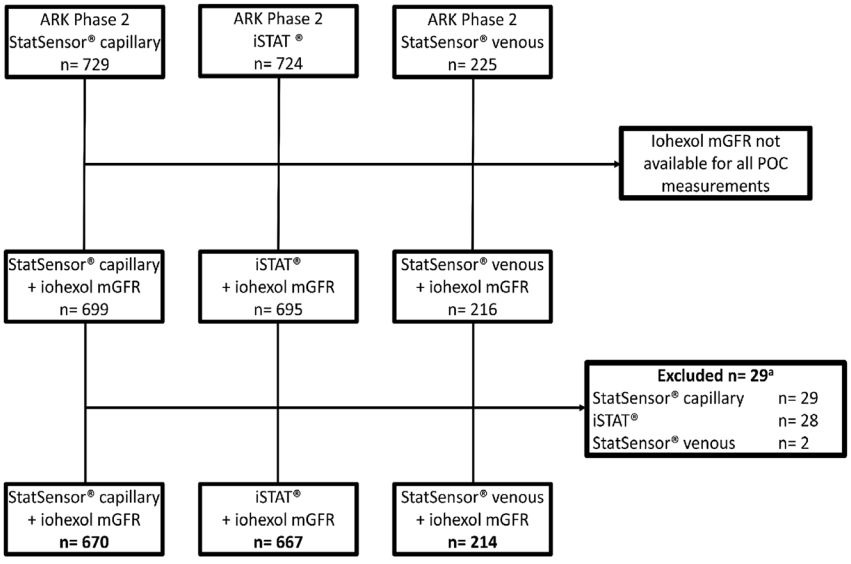

Figure 1: Participant flow through study. [a]Outliers excluded on initial Passing–Bablok comparison between mGFR and POC eGFR, excluded based on residuals >2.5 SD.

Table 1: Characteristics according to mGFR.

Characteristic	mGFR (categorised by GFR CKD stage)[a]			
	All	<60 (G3–5)	60–89 (G2)	≥90 (G1)
n, %	674	134 (20)	286 (42)	254 (38)
Age, years	43 (14)	50 (14)	46 (14)	37 (11)
BMI, kg/m^2	28.6 (6.4)	28.9 (5.9)	29.7 (6.6)	27.2 (6.2)
Body surface area, m^2	1.91 (0.23)	1.90 (0.22)	1.94 (0.24)	1.87 (0.22)
Male, n, %	211 (31)	38 (28)	81 (28)	92 (36)
mGFR[a]	81 (24)	47 (10)	76 (8)	105 (13)
Laboratory eGFR (MDRD)[a]	116 (27)	98 (26)	112 (22)	129 (25)
Laboratory eGFR (CKD-EPI)[a]	111 (17)	98 (21)	109 (14)	120 (11)
StatSensor capillary eGFR (MDRD)[a]	87 (26)	72 (19)	85 (24)	97 (28)
StatSensor capillary eGFR (CKD-EPI)[a]	93 (21)	79 (20)	91 (20)	102 (19)
iSTAT eGFR (MDRD)[a]	97 (23)	83 (22)	94 (20)	108 (22)
iSTAT eGFR (CKD-EPI)[a]	102 (19)	89 (22)	100 (17)	112 (13)
StatSensor venous eGFR (MDRD)[a]	85 (21)[b]	71 (27)[c,f]	84 (19)[d]	93 (21)[e]
StatSensor venous eGFR (CKD-EPI)[a]	92 (19)[b]	77 (32)[c,f]	90 (16)[d]	101 (17)[e]

All data mean (SD) unless otherwise indicated. [a]In mL/min/1.73 m^2; [b]n=214; [c]n=31; [d]n=97; [e]n=86; [f]median (interquartile range). All eGFR equations exclude the African-American coefficient.

and constant error than the CKD-EPI eGFR for all POC and laboratory creatinine measurements. All POC creatinine eGFR measurements, except for iSTAT® MDRD, showed less proportional and constant error than the respective laboratory creatinine eGFR equations. Additionally, Lin's concordance correlation coefficient showed improved accuracy for the POC eGFR measurements over the respective laboratory eGFR equations (Table 2, Figures 3 and 4).

Bias and precision

All calculated eGFR equations by POC creatinine or laboratory creatinine measurements showed positive bias compared to mGFR when looking at the all mGFR values category. Less bias was observed for the MDRD equation compared to the CKD-EPI equation for all POC creatinine measurements, except in the mGFR >90 mL/min/1.73 m^2 group where a mixed picture was seen. The observed biases for POC MDRD eGFR ranged from 1.9 ± 33.5 to 14.7 ± 32.0 mL/min/1.73 m^2, and for POC CKD-EPI eGFR from 8.4 ± 31.0 to 19.9 ± 28.5 mL/min/1.73 m^2 when looking at the all mGFR values category. For each calculated eGFR equation, the largest bias was observed with laboratory creatinine with all POC devices yielding less bias than the laboratory (Table 3).

Inter-day CVs varied for the different creatinine measuring devices, the iSTAT® had a CV of 5.9% at a level of 88 µmol/L, the two StatSensor devices had CVs of 7.4 and 10.2% at 84 µmol/L and CVs of 4.6 and 5.5% at 530 µmol/L, while the laboratory Jaffe assay had a CV of 3.5% at both levels of 89.3 and 342 µmol/L. When looking at IQRs, the MDRD equation showed less imprecision at a mGFR <60 mL/min/1.73 m^2 for all POC measurements, at mGFR ≥60 mL/min/1.73 m^2 the CKD-EPI equation showed the least imprecision. However, for laboratory eGFR the CKD-EPI equation showed less imprecision across all mGFR categories. When comparing POC eGFR imprecision to that of laboratory eGFR a mixed picture was seen, all methods suffered from imprecision; when the CKD-EPI equation was utilized the laboratory showed the least imprecision, however when the MDRD equation was utilized POC (generally the iSTAT device) showed the least imprecision. Capillary sampling with the StatSensor device showed the highest levels of imprecision except in the mGFR <60 mL/min/1.73 m^2 category (Table 3).

Accuracy

Overall, the P30 for the POC and laboratory creatinine eGFR equations ranged from 36 to 72%. All P30s showed improved accuracy with increasing mGFR. Below 90 mL/min/1.73 m^2 the MDRD eGFR showed greater accuracy (higher P30s) than the CKD-EPI eGFR in all POC and laboratory creatinine measurements, above 90 mL/min/1.73 m^2 the CKD-EPI eGFR showed greater accuracy. In the different mGFR groups, POC P30s continued to show

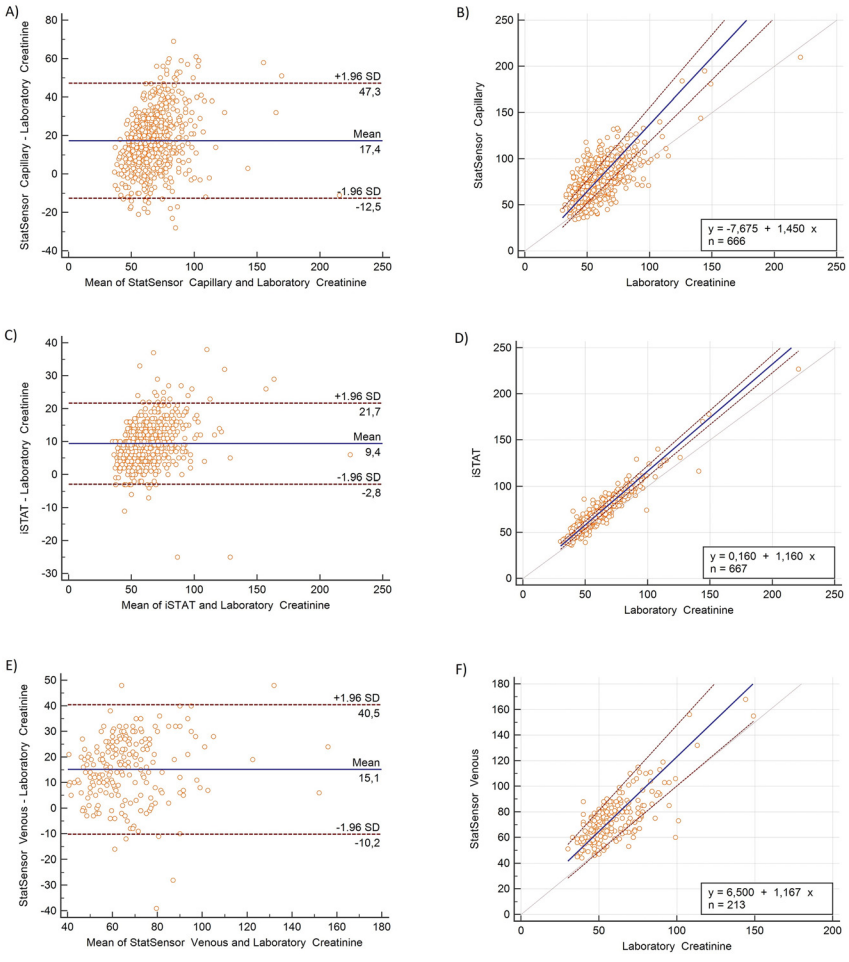

Figure 2: POC vs. laboratory creatinine. Bland–Altman agreement charts of: (A) the StatSensor® capillary creatinine (µmol/L), (C) iSTAT® creatinine (µmol/L), and (E) StatSensor® venous creatinine (µmol/L) compared to laboratory creatinine (µmol/L). Passing–Bablok regression comparing: (B) StatSensor® capillary creatinine, (D) iSTAT® creatinine, and (F) StatSensor® venous creatinine to laboratory creatinine.

Table 2: Analytical comparison of POC and laboratory eGFR equations compared to mGFR.

	Passing–Bablok regression				Lin's concordance correlation coefficient[d]
	Slope/proportional error[a]	*y*-intercept/constant error[b]	r[c]	p-value	
Laboratory eGFR (MDRD)	1.16 (1.05–1.27)	20.47 (10.94–29.23)	0.44 (0.38–0.50)	<0.0001	0.25 (0.21–0.29)
Laboratory eGFR (CKD-EPI)	0.59 (0.53–0.65)	62.76 (57.60–67.88)	0.50 (0.45–0.56)	<0.0001	0.24 (0.21–0.28)
StatSensor capillary eGFR (MDRD)	1.01 (0.89–1.14)	3.91 (–6.10 to 12.04)	0.34 (0.27–0.41)	<0.0001	0.35 (0.28–0.41)
StatSensor capillary eGFR (CKD-EPI)	0.88 (0.79–0.98)	22.38 (13.77–30.18)	0.40 (0.33–0.46)	<0.0001	0.37 (0.31–0.42)
iSTAT eGFR (MDRD)	0.92 (0.84–1.01)	21.35 (13.35–28.75)	0.45 (0.39–0.51)	<0.0001	0.40 (0.35–0.45)
iSTAT eGFR (CKD-EPI)	0.70 (0.64–0.77)	45.18 (38.90–50.84)	0.49 (0.43–0.54)	<0.0001	0.34 (0.29–0.38)
StatSensor venous eGFR (MDRD)	0.90 (0.74–1.08)	10.24 (–4.41 to 22.10)	0.37 (0.25–0.48)	<0.0001	0.42 (0.31–0.53)
StatSensor venous eGFR (CKD-EPI)	0.84 (0.70–1.00)	22.10 (8.33–33.73)	0.45 (0.33–0.55)	<0.0001	0.47 (0.37–0.56)

[a]Slope (95% confidence interval); [b]*y*-intercept (95% confidence interval); [c]Spearman rank correlation coefficient (95% confidence interval); [d]Lin's concordance correlation coefficient (95% confidence interval). All eGFR equations exclude the African-American coefficient.

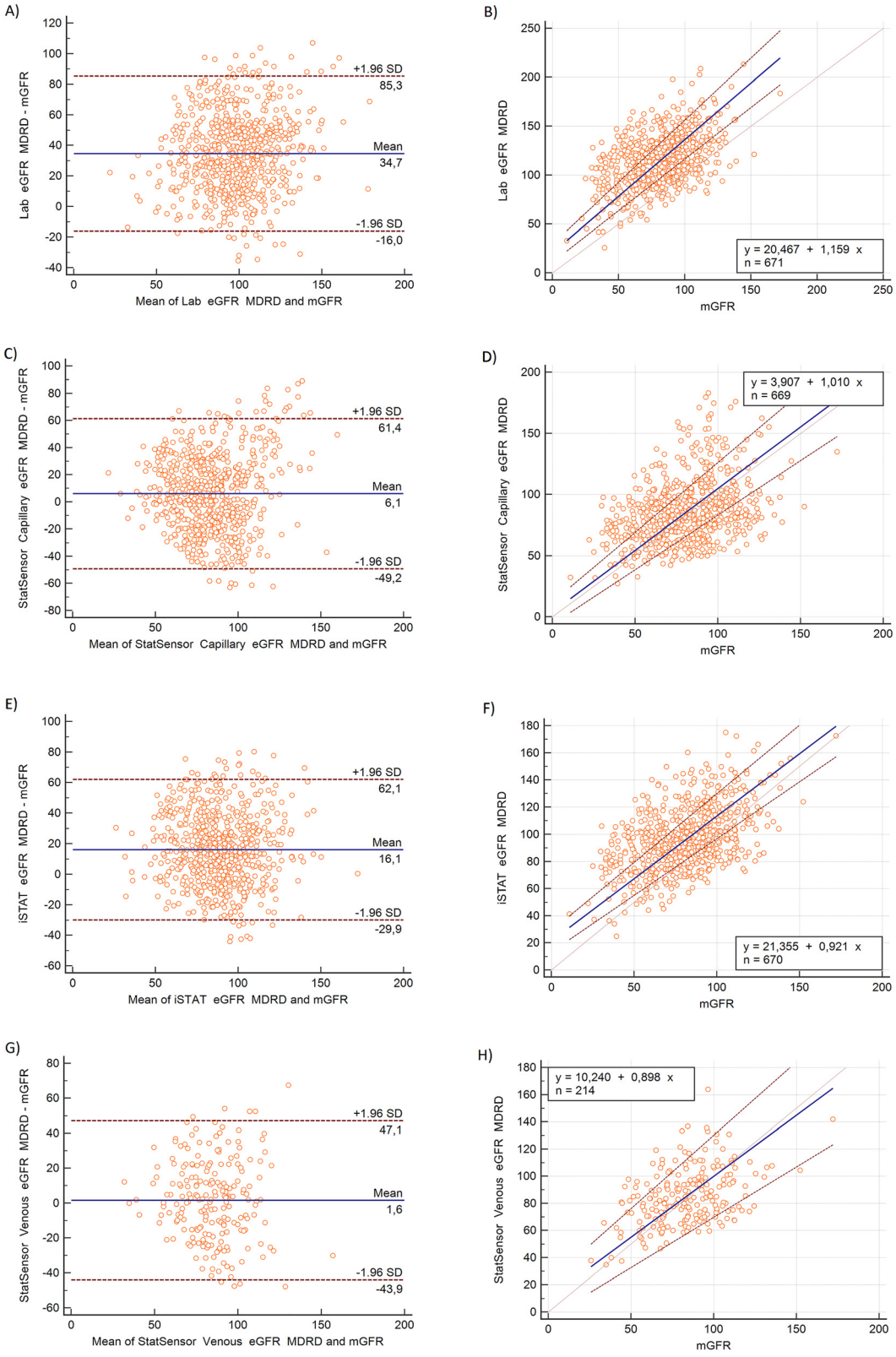

Figure 3: MDRD eGFR (without the African-American coefficient) vs. iohexol mGFR. Bland–Altman agreement charts of: (A) Laboratory MDRD eGFR, (C) StatSensor® capillary MDRD eGFR, (E) iSTAT® MDRD eGFR, and (G) StatSensor® venous MDRD eGFR compared to iohexol mGFR. Passing–Bablok regression comparing: (B) Laboratory MDRD eGFR, (D) StatSensor® capillary MDRD eGFR, (F) iSTAT® MDRD eGFR, and (H) StatSensor® venous MDRD eGFR compared to iohexol mGFR.

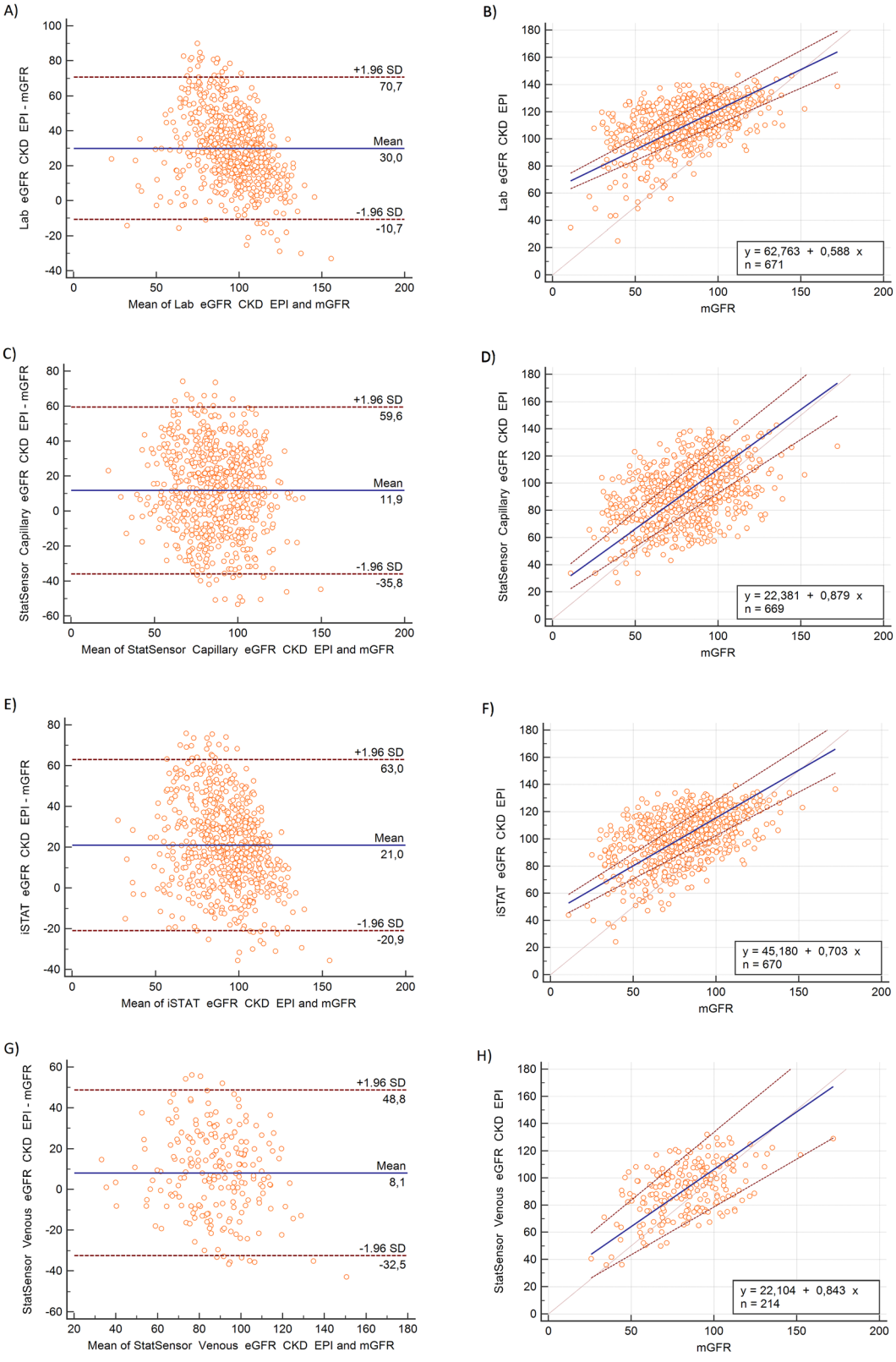

Figure 4: CKD-EPI eGFR (without the African-American coefficient) vs. iohexol mGFR. Bland–Altman agreement charts of: (A) Laboratory CKD-EPI eGFR, (C) StatSensor® capillary CKD-EPI eGFR, (E) iSTAT® CKD-EPI eGFR, and (G) StatSensor® venous CKD-EPI eGFR compared to iohexol mGFR. Passing–Bablok regression comparing: (B) Laboratory CKD-EPI eGFR, (D) StatSensor® capillary CKD-EPI eGFR, (F) iSTAT® CKD-EPI eGFR, and (H) StatSensor® venous CKD-EPI eGFR compared to iohexol mGFR.

Table 3: Accuracy within 30% of mGFR (P30), bias and imprecision according to CKD stage.

	Bias[a]				Imprecision[b]				P30[c]			
	All	mGFR <60[d] (G3–5)	mGFR 60–89[d] (G2)	mGFR ≥90[d] (G1)	All	mGFR <60[d] (G3–5)	mGFR 60–89[d] (G2)	mGFR ≥90[d] (G1)	All	mGFR <60[d] (G3–5)	mGFR 60–89[d] (G2)	mGFR ≥90[d] (G1)
Laboratory eGFR (MDRD)	34.1 (31.1–36.3)	52.7 (45.8–59.7)	35.7 (30.7–38.6)	22.6 (18.3–27.7)	35.4 (30.4–40.7)	38.1 (28.7–51.1)	29.2 (21.9–37.0)	33.3 (26.0–41.9)	36 (32–40)	10 (5–16)	27 (22–33)	60 (53–66)
Laboratory eGFR (CKD-EPI)	28.6 (27.0–30.6)	54.5 (51.2–58.8)	33.8 (31.7–35.3)	16.1 (14.2–17.3)	26.8 (23.5–30.7)	27.3 (18.1–38.6)	19.4 (14.5–23.3)	17.9 (13.7–23.5)	43 (40–47)	7 (3–12)	25 (20–31)	83 (78–88)
StatSensor capillary eGFR (MDRD)	4.6 (1.3–7.7)	28.0 (23.2–31.7)	7.3 (3.7–10.4)	−12.4 (−16.9 to −9.2)	39.8 (34.2–46.4)	26.5 (17.5–35.6)	30.4 (21.6–38.6)	38.8 (25.6–47.6)	61 (57–65)	29 (22–38)	68 (62–73)	70 (64–76)
StatSensor capillary eGFR (CKD-EPI)	11.4 (10.0–14.3)	35.3 (31.2–38.4)	16.5 (12.8–19.5)	−1.3 (−5.4 to 2.4)	35.0 (29.8–41.8)	29.3 (17.8–40.2)	29.4 (22.0–36.9)	28.7 (22.8–37.1)	62 (58–65)	21 (14–29)	60 (54–66)	85 (80–89)
iSTAT eGFR (MDRD)	14.7 (12.1–16.5)	39.1 (33.0–42.0)	16.0 (13.8–18.6)	3.1 (−1.1 to 5.4)	32.0 (26.4–37.7)	27.8 (21.4–40.1)	23.0 (18.3–31.1)	29.5 (21.7–36.5)	62 (58–66)	18 (12–26)	61 (55–66)	87 (82–90)
iSTAT eGFR (CKD-EPI)	19.9 (17.7–21.4)	47.6 (42.2–51.0)	25.8 (21.3–27.8)	8.5 (5.3–10.5)	28.5 (23.9–33.9)	28.9 (22.7–38.6)	21.4 (16.8–27.3)	20.1 (14.5–26.4)	56 (52–60)	14 (8–21)	44 (38–50)	92 (88–95)
StatSensor venous eGFR (MDRD)	1.9 (−2.1 to 4.6)	20.8 (11.0–31.6)	6.4 (2.2–11.0)	−14.0 (−22.8 to −8.6)	33.5 (22.5–43.3)	28.6 (10.0–42.5)	27.9 (13.4–37.8)	30.4 (18.1–41.0)	71 (64–77)	42 (25–61)	74 (64–83)	78 (68–86)
StatSensor venous eGFR (CKD-EPI)	8.4 (5.1–12.4)	24.5 (13.2–36.3)	14.1 (10.9–18.0)	−3.4 (−9.1 to 2.5)	31.0 (21.0–37.8)	33.0 (11.8–50.2)	22.8 (17.0–35.1)	28.0 (16.3–40.4)	72 (65–78)	35 (19–55)	66 (56–75)	92 (84–97)

[a]Median bias of individual differences between eGFR and mGFR (in mL/min/1.73 m^2 with 95% confidence intervals); [b]interquartile range (IQR) of the differences between eGFR and mGFR (in mL/min/1.73 m^2 with 95% confidence intervals); [c]% (95% confidence intervals); [d]in mL/min/1.73 m^2. All eGFR equations exclude the African-American coefficient.

improved accuracy compared to laboratory P30s (Table 3). When serum creatinine was adjusted by a factor of +9.0 µmol/L improved P30s ranging from 62 to 71% were seen. Additional analysis using the revised Lund–Malmö equation yielded P30s ranging from 58 to 80% with improvement noted for POC devices and laboratory results (Supplementary Table S1).

The percentage of total samples correctly classified into the eGFR groups of <60, 60–89 and >90 mL/min/1.73 m^2 ranged from 41.7% (95% CI: 38.0–45.6) to 52.8% (95% CI: 45.9–59.7). The POC creatinine eGFR equations correctly classified more samples into the correct eGFR groups than the respective laboratory creatinine equations, although the laboratory equations showed improved performance in the mGFR >90 mL/min/1.73 m^2 group. Below 90 mL/min/1.73 m^2 the MDRD equation correctly classified more samples than the CKD-EPI equation, above 90 mL/min/1.73 m^2 the CKD-EPI equation correctly classified more samples (Table 4).

Discussion

In this study we used iohexol mGFR to evaluate the clinical utility of the iSTAT® and StatSensor® POC devices that measure creatinine and subsequently estimate kidney function using eGFR. Additionally, we evaluated the performance of the MDRD and CKD-EPI equations and compared the performance of the POC devices to that of laboratory Jaffe creatinine. Despite falling well short of the 90% of eGFRs within 30% of mGFR goal [17], POC eGFR showed improved accuracy compared to laboratory Jaffe eGFR when screening for CKD in a resource-limited setting. All current eGFR estimating equations performed poorly in our study sample.

A number of studies [18–21] have shown the utility of the iSTAT® and StatSensor® POC devices for the diagnosis of acute kidney injury. Our study showed more bias compared to laboratory creatinine than these studies possibly due to differences in laboratory creatinine methods. Studies with less bias [18–21] used the enzymatic method while studies which used the IDMS-traceable Jaffe method, including our own, showed more bias [22]. We found the iSTAT® device showed less imprecision than the StatSensor® device, consistent with previous studies [18, 19].

Comparison of POC eGFR to iohexol mGFR showed greater bias and inaccuracy than a previous study [23] from Belgium, which found a bias of 0.1 ± 17.6 mL/min/1.73 m^2 with a P30 of 81% for StatSensor® capillary eGFR (CKD-EPI). Our P30 values for POC devices were low, and varied across the range of GFR, with the worst performance at low GFR. Bukabau et al. [13] reported a similarly low P30 of 31.3% in an African population with mGFR <60 mL/min/1.73 m^2 for the MDRD and CKD-EPI equations, with a laboratory enzymatic creatinine method.

It has been shown that serum creatinine suffers from significant positive bias when there is a delay in sample separation of 24 h, this is true for Jaffe but not enzymatic methods [6, 24]. In our study POC creatinine measurements were performed in real-time, while laboratory creatinine measurements were performed on samples with a maximum delayed separation of between 8 and 9 h utilising an IDMS-traceable Jaffe method. This delay is not expected to have significantly influenced results, although if

Table 4: Percentage of samples correctly classified into mGFR groups <60, 60–89 and ≥90.

	mGFR <60[c] (G3–5)	mGFR 60–89[c] (G2)	mGFR ≥90[c] (G1)	Total correctly classified[d]
Laboratory eGFR (MDRD)	8.2 (4.2–14.2)	14.7 (10.8–19.4)	95.6 (92.3–97.8)	43.8 (40.0–47.7)
Laboratory eGFR (CKD-EPI)	7.5 (3.6–13.3)	7.0 (4.3–10.6)	99.2 (97.2–99.9)	41.7 (38.0–45.6)
StatSensor capillary eGFR (MDRD)	24.6 (17.6–32.8)	48.4 (42.5–54.4)	52.0 (45.6–58.3)	45.0 (41.2–48.9)
p[a]	<0.0001	<0.0001	<0.0001	0.5451
StatSensor capillary eGFR (CKD-EPI)	19.4 (13.1–27.1)	38.2 (32.6–44.2)	73.2 (67.3–78.6)	47.5 (43.7–51.4)
p[b]	0.0004	<0.0001	<0.0001	0.0028
iSTAT eGFR (MDRD)	15.0 (9.4–22.2)	39.6 (33.9–45.6)	77.4 (71.7–82.4)	49.0 (45.1–52.8)
p[a]	0.0117	<0.0001	<0.0001	0.0048
iSTAT eGFR (CKD-EPI)	12.0 (7.0–18.8)	23.9 (19.0–29.2)	93.7 (89.9–96.3)	47.8 (43.9–51.6)
p[b]	0.0703	<0.0001	0.0002	<0.0001
StatSensor venous eGFR (MDRD)	35.5 (19.2–54.6)	57.7 (47.3–67.7)	53.5 (42.4–64.3)	52.8 (45.9–59.7)
p[a]	0.1250	<0.0001	<0.0001	0.4215
StatSensor venous eGFR (CKD-EPI)	29.0 (14.2–48.0)	45.4 (35.2–55.8)	69.8 (58.9–79.2)	52.8 (45.9–59.7)
p[b]	0.3750	<0.0001	<0.0001	0.1249

All data percentage (95% confidence interval). [a]vs. laboratory eGFR (MDRD) using exact NcNemar test; [b]vs. laboratory eGFR (CKD-EPI) using exact NcNemar test; [c]in mL/min/1.73 m^2; [d]total samples correctly classified into CKD stages: G3–5, G2 and G1. All eGFR equations exclude the African-American coefficient.

present a positive bias on laboratory creatinine with lowered eGFR would be expected (the opposite pattern from what was observed in our laboratory samples). Importantly in LMIC, these methodological and pre-analytical factors are common in clinical practice. For example, in South Africa, samples from rural areas are transported to central laboratories and only processed 12–72 h post collection [6] and the Jaffe method is used in all but a few large urban centres due to cost considerations [25] – despite an initiative for all laboratories to use the enzymatic method [26]. The IDMS-traceable Jaffe method has been shown not to reach the desirable specifications of the Laboratory Working Group of the National Kidney Disease Education Program [27] – nevertheless, it is commonly used with 93% of studies from sub-Saharan Africa which reported creatinine methodology making use of the Jaffe method [28].

In LMIC the potential public health benefits associated with POC screening for early detection and monitoring of CKD is an exciting possibility. Despite the bias and imprecision seen in POC eGFRs, these devices showed improved performance compared to laboratory Jaffe eGFR. The performance of POC devices to detect eGFR in the range 60–89 mL/min/1.73 m^2 is of particular interest. With limited access to renal replacement therapy for severe kidney disease, it is advantageous to detect individuals with early disease who may benefit from renal protective measures. There was improved accuracy in this area compared to laboratory Jaffe measurements, as well as compared to measurements below 60 mL/min/1.73 m^2. Nevertheless, the large bias, imprecision and misclassifications mean that results remain inaccurate with current eGFR estimating equations.

Enzymatic methods are more specific for creatinine than Jaffe methods which are vulnerable to interference from numerous substances including proteins, glucose and ketone bodies [29]. Various assay techniques such as kinetic reactions and compensated assays are used to improve specificity. The laboratory assay used in our study was an IDMS-traceable compensated kinetic Jaffe assay. Compensated assays assume a fixed concentration of non-specific interferences which are subtracted from the total creatinine value, bias will be introduced if this assumption is incorrect [30, 31]. The positive bias for creatinine which the enzymatic POC devices demonstrated compared to the laboratory Jaffe assay may reflect overcompensation of the Jaffe assay in our population. When laboratory creatinine results were adjusted by a factor of +9.0 μmol/L resulting eGFR equations showed similar P30s to that obtained for POC (Supplementary Table S1). Ideally all laboratories should move to enzymatic methods for the measurement of creatinine, while compensated Jaffe equations should be used with caution especially if this compensation has not been validated in a particular population. The poor performance of the laboratory Jaffe assay in our study may reflect overcompensation of the Jaffe method in our population.

Our study focussed primarily on accuracy; however, imprecision makes an equally important contribution to analytical variation. Jaffe methods are known with higher imprecision than enzymatic methods [27 ,32, 33]. The inter-day CVs of the POC creatinine devices were greater than that seen with the laboratory Jaffe assay. This high imprecision with POC assays, despite the use of enzymatic methodology, is an important distinguishing factor between laboratory and POC testing [34]. High levels of imprecision may result in total error that hinders the interpretation of creatinine and subsequent eGFR [32].

The ability of the CKD-EPI equation to quantify GFR above 60 mL/min/1.73 m^2 offers significant advantages over the MDRD equation and avoids classifying patients based on a GFR cut-off which dichotomises all measurements [5, 35]. In keeping, we found the MDRD equation to suffer from greater imprecision above a mGFR of 60 mL/min/1.73 m^2. Despite these apparent short-comings, we were surprised by the improved accuracy of the MDRD equation with higher P30s and sensitivities up to a mGFR of 90 mL/min/1.73 m^2 compared to the CKD-EPI equation.

In keeping with other studies from sub-Saharan Africa [13, 14] we found a positive bias when using the African-American coefficient for the MDRD and CKD-EPI equations, and both led to substantial overestimation of GFR (Supplementary Figures S1 and S2). The revised Lund–Malmö equation outperformed the MDRD and CKD-EPI equations (Supplementary Table S1), suggesting that a new equation developed from an African population group may further improve performance. Despite the improved performance seen with the revised Lund–Malmö equation it remains rarely used in sub-Saharan Africa.

POC devices have the potential to provide immediately actionable results and mitigate pre-analytical errors, such as delay in separation commonly seen in LMIC. The iSTAT® utilises venous whole blood while the StatSensor® can be used with capillary and venous whole blood. Capillary sampling offers added benefit by eliminating the need for phlebotomy skills, and is preferred by patients [36]. Capillary sampling showed improved performance over corresponding laboratory creatinine, however it showed the highest levels of imprecision. Thus, venous sampling should remain the preferred option, with capillary sampling considered as an alternative only if venous sampling is unavailable and if the device has been validated for this purpose.

POC testing has limitations such as: refrigerated supply storage (both devices), susceptibility to lot-to-lot variations and a lack of monitoring by laboratory professionals (especially external quality control). Cost considerations need to be taken into account especially in resource-limited settings. POC testing has a significantly higher cost compared to laboratory measurements, especially when additional components such as the purchase of quality control materials and the need for refrigeration are considered. However, cost savings may be achieved if POC testing leads to improved detection, and reduced numbers of patients progressing to advanced CKD. Both devices, and others, have previously been reviewed regarding suitability in low-income countries, including estimated cost per test [37].

Our study had a number of strengths: (i) the large sample size drawn from a well characterised population-based study of rural South Africans; (ii) the study procedures were standardised and conducted in a well-controlled research environment; (iii) two different POC devices using venous whole blood and capillary blood were evaluated; (iv) participants had a wide range of iohexol mGFRs including 134 participants with a mGFR less than 60 mL/min/1.73 m^2. Our study also had limitations: (i) the poor performance of laboratory creatinine likely reflects the Jaffe methodology used despite enzymatic methods being the preferred choice [27, 33, 38–40], however without a parallel analysis using a laboratory enzymatic method this assumption remains untested; (ii) although plasma clearance of iohexol is a well described method for measuring GFR, the MDRD and CKD-EPI equations were developed using urinary clearance of iothalamate and as such, methodologic bias may be introduced by using iohexol mGFR [4]; (iii) laboratory creatinine was processed after delayed sample separation (although less than the 24 h level at which changes become significant [6, 24]); (iv) POC testing was carried out by trained staff under controlled conditions, which may not be reproducible in a busy clinic environment; (v) roughly one-third of participants had venous measurements assessed with the StatSensor® due to cost considerations.

In conclusion, our study demonstrates poor performance of the compensated kinetic Jaffe method to measure creatinine in an under-resourced primary care setting and highlights the recommendation for laboratories to move to enzymatic methods. In these situations POC devices, despite their poor performance compared to iohexol mGFR, show improved accuracy in predicting GFR compared to Jaffe methodology. The inaccuracy and wide imprecision with current eGFR estimating equations mean that POC devices should be used with caution.

Acknowledgments: We thank Abbott Laboratories for the supply of the iSTAT® device, control materials and cartridges. We thank Nova Biomedical Corporation for the supply of the StatSensor® devices, control materials and biosensor strips.

Research funding: We thank our funders: (i) the South African Medical Research Council, with funds from the South African National Department of Health, MRC UK (via the Newton Fund) and GSK R&D; (ii) Faculty Research Committee Individual Research Grant, University of Witwatersrand; (iii) The International Society of Nephrology Clinical Research Program.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' Institutional Review Board (Human Research Ethics Committee of the University of the Witwatersrand) or equivalent committee. (M160937 and M190434).

References

1. George JA, Brandenburg J-T, Fabian J, Crowther NJ, Agongo G, Alberts M, et al. Kidney damage and associated risk factors in rural and urban sub-Saharan Africa (AWI-Gen): a cross-sectional population study. Lancet Glob Health 2019;7:e1632–43.
2. Stanifer JW, Muiru A, Jafar TH, Patel UD. Chronic kidney disease in low- and middle-income countries. Nephrol Dial Transplant 2016; 31:868–74.
3. KDIGO Working Group. Kidney disease: improving global outcomes (KDIGO). KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl 2013;3:1–150.
4. Levey AS, Inker LA. GFR as the "gold standard": estimated, measured, and true. Am J Kidney Dis 2016;67:9–12.
5. Miller WG, Jones GRD. Estimated glomerular filtration rate; laboratory implementation and current global status. Adv Chron Kidney Dis 2018;25:7–13.
6. Chikomba CE, Padoa CJ, Tanyanyiwa D. Evaluation of the impact of delayed centrifugation on the diagnostic performance of serum creatinine as a baseline measure of renal function before antiretroviral treatment. South Afr J HIV Med 2020;21:a1056.
7. Kazmierczak SC. Point-of-care testing quality: some positives but also some negatives. Clin Chem 2011;57:1219–20.
8. Levin A, Stevens PE. Summary of KDIGO 2012 CKD Guideline: behind the scenes, need for guidance, and a framework for moving forward. Kidney Int 2014;85:49–61.

9. Kalyesubula R, Fabian J, Nakanga W, Newton R, Ssebunnya B, Prynn J, et al. How to estimate glomerular filtration rate in sub-Saharan Africa: design and methods of the African research into kidney diseases (ARK) study. BMC Nephrol 2020;21:1–12.
10. Annesley TM, Clayton LT. Ultraperformance liquid chromatography-tandem mass spectrometry assay for iohexol in human serum. Clin Chem 2009;55:1196–202.
11. Fleming JS, Zivanovic MA, Blake GM, Burniston M, Cosgriff PS. Guidelines for the measurement of glomerular filtration rate using plasma sampling. Nucl Med Commun 2004;25:759–69.
12. Haycock GB, Schwartz GJ, Wisotsky DH. Geometric method for measuring body surface area: a height–weight formula validated in infants, children, and adults. J Pediatr 1978;93:62–6.
13. Bukabau JB, Yayo E, Gnionsahé A, Monnet D, Pottel H, Cavalier E, et al. Performance of creatinine- or cystatin C-based equations to estimate glomerular filtration rate in sub-Saharan African populations. Kidney Int 2019;95:1181–9.
14. Van Deventer HE, George JA, Paiker JE, Becker PJ, Katz IJ. Estimating glomerular filtration rate in black South Africans by use of the modification of diet in renal disease and Cockcroft–Gault equations. Clin Chem 2008;54:1197–202.
15. Parrinello CM, Grams ME, Couper D, Ballantyne CM, Hoogeveen RC, Eckfeldt JH, et al. Recalibration of blood analytes over 25 years in the atherosclerosis risk in communities study: impact of recalibration on chronic kidney disease prevalence and incidence. Clin Chem 2015;61:938–47.
16. Ricos C, Ramon F, Salas A, Buno A, Calafell R, Morancho J, et al. Minimum analytical quality specifications of inter-laboratory comparisons: agreement among Spanish EQAP organizers. Clin Chem Lab Med 2012;50:455–61.
17. National Kidney Foundation. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 2002;39:S1-266.
18. Snaith B, Harris MA, Shinkins B, Jordaan M, Messenger M, Lewington A. Point-of-care creatinine testing for kidney function measurement prior to contrast-enhanced diagnostic imaging: evaluation of the performance of three systems for clinical utility. Clin Chem Lab Med 2018;56:1269–76.
19. van der Heijden C, Roosens L, Cluckers H, Van Craenenbroeck AH, Peeters B. Analytical and clinical performance of three hand-held point-of-care creatinine analyzers for renal function measurements prior to contrast-enhanced imaging. Clin Chim Acta 2019:13–9. https://doi.org/10.1016/j.cca.2019.06.025.
20. Kosack CS, De Kieviet W, Bayrak K, Milovic A, Page AL. Evaluation of the Nova StatSensor® Xpress™ creatinine point-of-care handheld analyzer. PloS One 2015;10:1–11.
21. Snaith B, Harris MA, Shinkins B, Messenger M, Lewington A, Jordaan M, et al. Point of care creatinine testing in diagnostic imaging: a feasibility study within the outpatient computed tomography setting. Eur J Radiol 2019:82–7. https://doi.org/10.1016/j.ejrad.2019.01.007.
22. Griffin BR, Butler-Dawson J, Dally M, Krisher L, Cruz A, Weitzenkamp D, et al. Unadjusted point of care creatinine results overestimate acute kidney injury incidence during field testing in Guatemala. PloS One 2018;13:1–12.
23. Stojkovic V, Delanaye P, Collard G, Ferrante N, Le Goff C, Lutteri L, et al. Estimated glomerular filtration rate using a point of care measure of creatinine in patients with iohexol determinate GFR. Clin Chim Acta 2019;499:123–7.
24. Ford L, Berg J. Delay in separating blood samples affects creatinine measurement using the Roche kinetic Jaffe method. Ann Clin Biochem 2008;45:83–7.
25. Delanghe JR, Speeckaert MM. Creatinine determination according to Jaffe – what does it stand for? NDT Plus 2011;4:83–6.
26. National Institute for Health and Care Excellence. Chronic kidney disease in adults: assessment and management. NICE Guideline CG182; 2014.
27. Boutten A, Bargnoux AS, Carlier MC, Delanaye P, Rozet E, Delatour V, et al. Enzymatic but not compensated Jaffe methods reach the desirable specifications of NKDEP at normal levels of creatinine. Results of the French multicentric evaluation. Clin Chim Acta 2013;18:132–5.
28. Fabian J, George JA, Etheredge HR, van Deventer M, Kalyesubula R, Wade AN, et al. Methods and reporting of kidney function: a systematic review of studies from sub-Saharan Africa. Clin Kidney J 2019;12:778–87.
29. Panteghini M. Enzymatic assays for creatinine: time for action. Clin Chem Lab Med 2008;46:567–72.
30. Parry DM Use of single-value protein compensation of the Jaffe creatinine assay contributes to clinically significant inaccuracy in results. Clin Chem 2008;54:215–6.
31. Cobbaert CM, Baadenhuijsen H, Weykamp CW. Prime time for enzymatic creatinine methods in pediatrics. Clin Chem 2009;55: 549–58.
32. Delanaye P, Cavalier E, Pottel H. Serum creatinine: not so simple! Nephron 2017;136:302–8.
33. Hoste L, Deiteren K, Pottel H, Callewaert N, Martens F. Routine serum creatinine measurements: how well do we perform? BMC Nephrol 2015;16:1–9.
34. van Lint CL, van der Boog PJM, Romijn FPHTM, Schenk PW, van Dijk S, Rövekamp TJM, et al. Application of a point of care creatinine device for trend monitoring in kidney transplant patients: fit for purpose? Clin Chem Lab Med 2015; 53:1547–56.
35. Dalton RN. Serum creatinine and glomerular filtration rate: perception and reality. Clin Chem 2010;56:687–9.
36. Woods K, Douketis JD, Schnurr T, Kinnon K, Powers P, Crowther MA. Patient preferences for capillary vs. venous INR determination in an anticoagulation clinic: a randomized controlled trial. Thromb Res 2004;114:161–5.
37. Raimann JG, Riella MC, Levin NW. International Society of Nephrology's 0by25 initiative (zero preventable deaths from acute kidney injury by 2025): focus on diagnosis of acute kidney injury in low-income countries. Clin Kidney J 2018;11:12–9.
38. Piéroni L, Delanaye P, Boutten A, Bargnoux AS, Rozet E, Delatour V, et al. A multicentric evaluation of IDMS-traceable creatinine enzymatic assays. Clin Chim Acta 2011;412:2070–5.
39. Drion I, Cobbaert C, Groenier KH, Weykamp C, Bilo HJ, Wetzels JF, et al. Clinical evaluation of analytical variations in serum creatinine measurements: why laboratories should abandon Jaffe techniques. BMC Nephrol 2012;8:133.
40. Miller WG, Bachmann LM, Delanghe JR, Inker LA, Jones GRD, Vassalotti JA. Optimal use of biomarkers for chronic kidney disease. Clin Chem 2019;65:949–55.

Supplementary Material: The online version of this article offers supplementary material (https://doi.org/10.1515/cclm-2020-1882).

2.

Published paper 1: Supplementary material

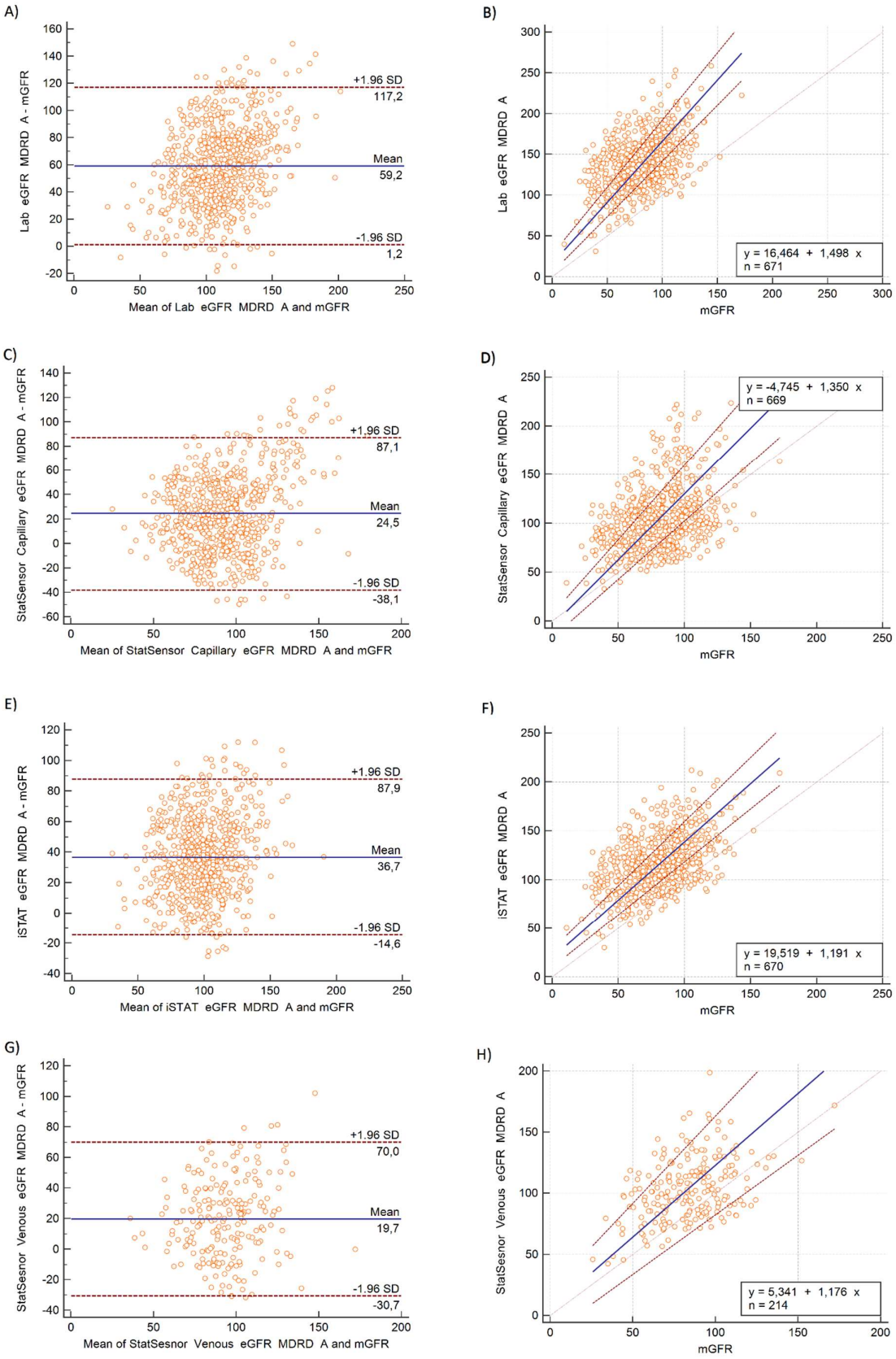

Figure S1: MDRD eGFR (with African-American coefficient) vs iohexol mGFR

Bland-Altman agreement charts of: A) Laboratory MDRD eGFR, C) StatSensor® capillary MDRD eGFR, E) iSTAT® MDRD eGFR, and G) StatSensor® venous MDRD eGFR compared to iohexol mGFR

Passing-Bablok regression comparing: B) Laboratory MDRD eGFR, D) StatSensor® capillary MDRD eGFR, F) iSTAT® MDRD eGFR, and H) StatSensor® venous MDRD eGFR compared to iohexol mGFR

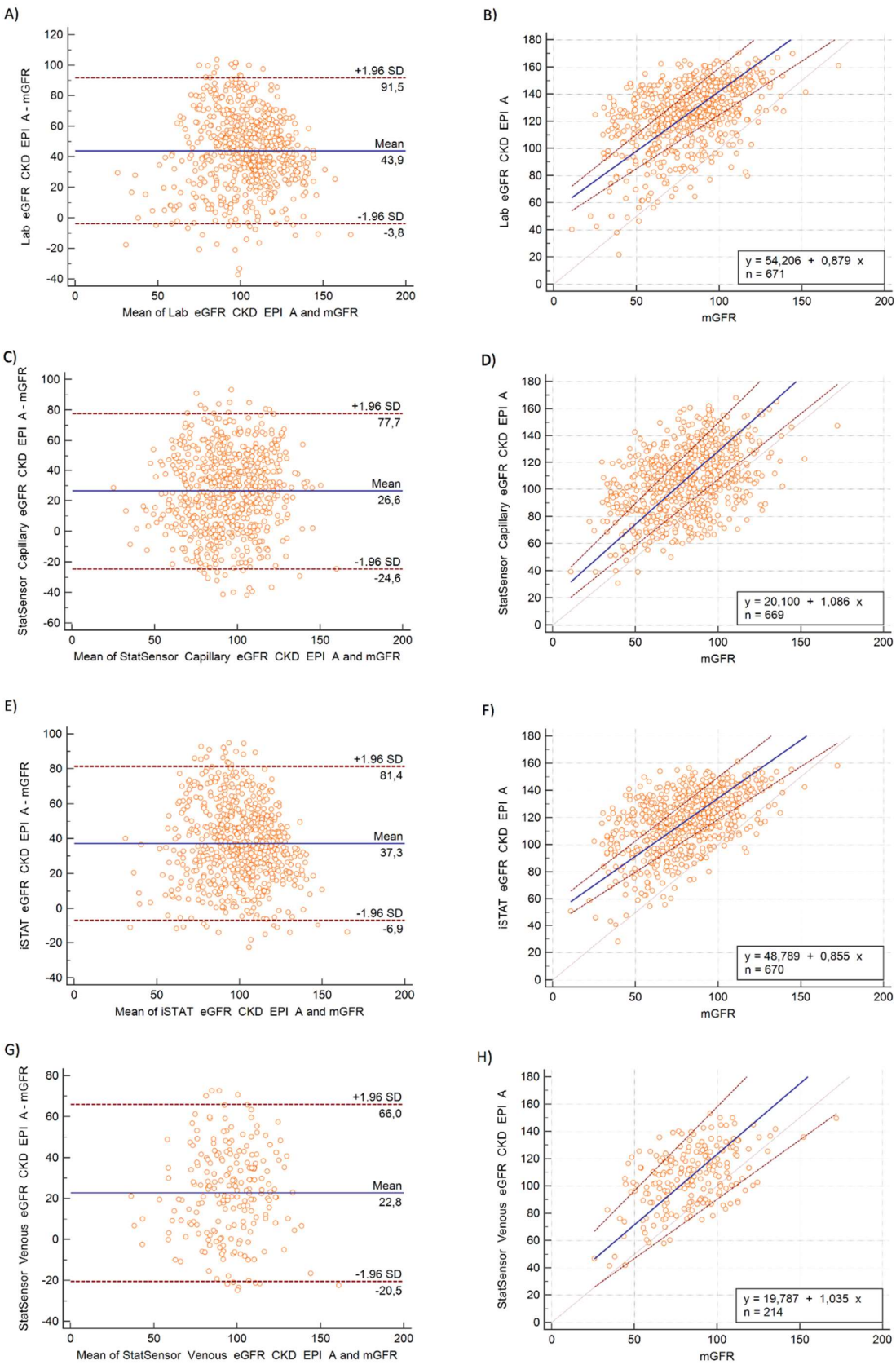

Figure S2: CKD-EPI eGFR (with African-American coefficient) vs iohexol mGFR

Bland-Altman agreement charts of: A) Laboratory CKD-EPI eGFR, C) StatSensor® capillary CKD-EPI eGFR, E) iSTAT® CKD-EPI eGFR, and G) StatSensor® venous CKD-EPI eGFR compared to iohexol mGFR

Passing-Bablok regression comparing: B) Laboratory CKD-EPI eGFR, D) StatSensor® capillary CKD-EPI eGFR, F) iSTAT® CKD-EPI eGFR, and H) StatSensor® venous CKD-EPI eGFR compared to iohexol mGFR

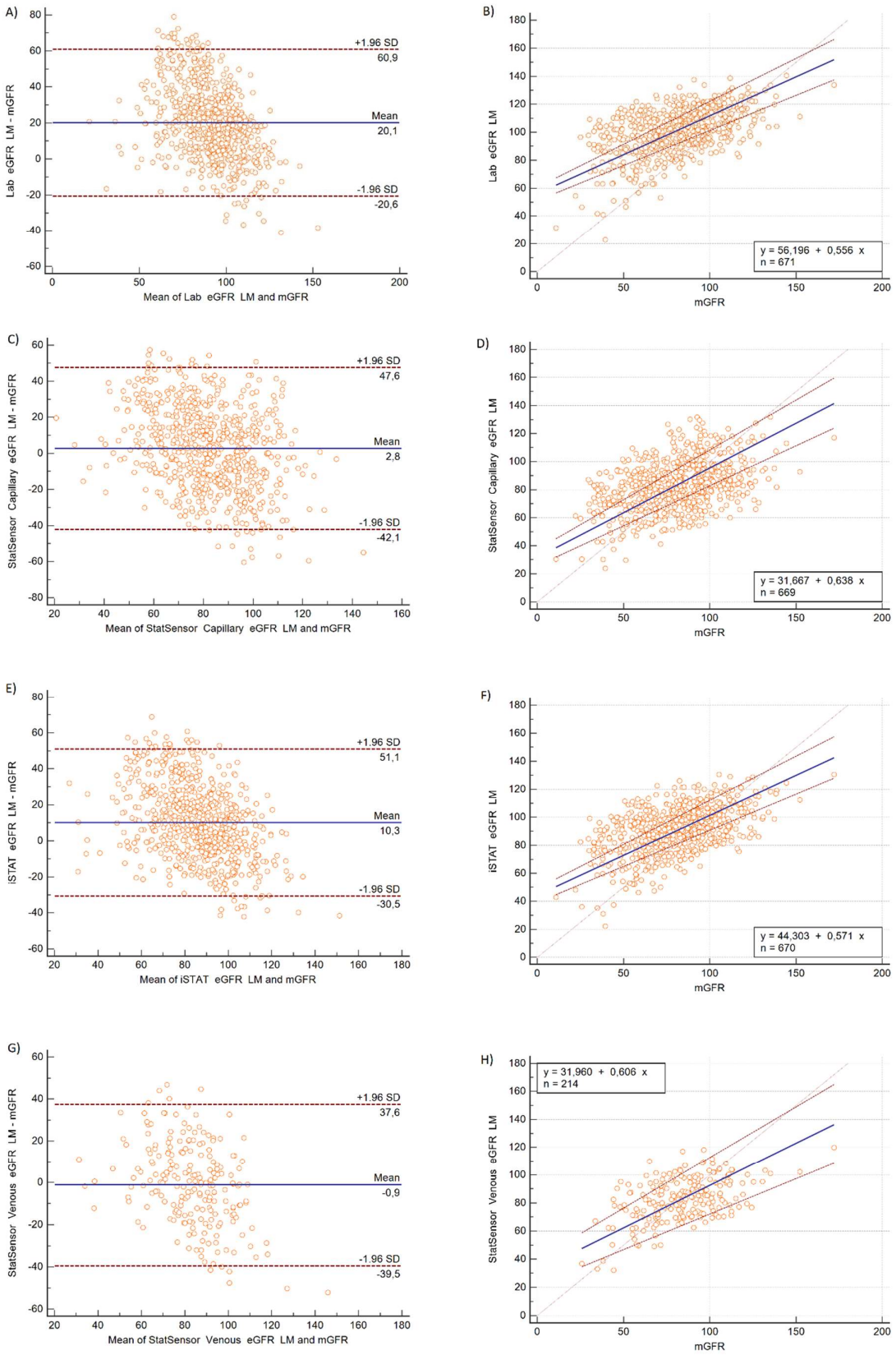

Figure S3: Revised Lund-Malmö eGFR vs iohexol mGFR

Bland-Altman agreement charts of: A) Laboratory rLM eGFR, C) StatSensor® capillary rLM eGFR, E) iSTAT® rLM eGFR, and G) StatSensor® venous rLM eGFR compared to iohexol mGFR

Passing-Bablok regression comparing: B) Laboratory rLM eGFR, D) StatSensor® capillary rLM eGFR, F) iSTAT® rLM eGFR, and H) StatSensor® venous rLM eGFR compared to iohexol mGFR

Table S1: Accuracy within 30% of mGFR (P30), including revised Lund-Malmö equation and adjusted laboratory creatinine

	P30			
	All	mGFR <60[a] (G3-5)	mGFR 60-89[a] (G2)	mGFR ≥90[a] (G1)
Laboratory eGFR (MDRD)	36 (32 – 40)	10 (5 – 16)	27 (22 – 33)	60 (53 – 66)
Laboratory eGFR (CKD-EPI)	43 (40 – 47)	7 (3 – 12)	25 (20 – 31)	83 (78 – 88)
Laboratory eGFR (Revised Lund-Malmö)	58 (54 – 61)	10 (6 -17)	45 (39 – 51)	98 (95 – 99)
StatSensor Capillary eGFR (MDRD)	61 (57 – 65)	29 (22 – 38)	68 (62 – 73)	70 (64 – 76)
StatSensor Capillary eGFR (CKD-EPI)	62 (58 – 65)	21 (14 – 29)	60 (54 – 66)	85 (80 – 89)
StatSensor Capillary eGFR (Revised Lund-Malmö)	69 (66 – 73)	27 (20 – 35)	78 (72 – 82)	83 (78 – 87)
iSTAT eGFR (MDRD)	62 (58 – 66)	18 (12 – 26)	61 (55 – 66)	87 (82 – 90)
iSTAT eGFR (CKD-EPI)	56 (52 – 60)	14 (8 – 21)	44 (38 – 50)	92 (88 – 95)
iSTAT eGFR (Revised Lund-Malmö)	71 (68 – 75)	17 (11 – 25)	74 (68 – 79)	97 (94 – 99)
StatSensor Venous eGFR (MDRD)	71 (64 – 77)	42 (25 – 61)	74 (64 – 83)	78 (68 – 86)
StatSensor Venous eGFR (CKD-EPI)	72 (65 – 78)	35 (19 – 55)	66 (56 – 75)	92 (84 – 97)
StatSensor Venous eGFR (Revised Lund-Malmö)	80 (74 – 85)	45 (27 – 64)	87 (78 – 93)	85 (76 – 92)
Laboratory adjusted[b] eGFR (MDRD)	62 (58 – 66)	15 (9 – 22)	60 (54 – 65)	90 (86 – 94)
Laboratory adjusted[b] eGFR (CKD-EPI)	55 (51 – 59)	12 (7 – 19)	41 (35 – 47)	94 (90 – 97)
Laboratory adjusted[b] eGFR (Revised Lund-Malmö)	71 (68 – 75)	14 (9 – 21)	76 (70 – 81)	97 (94 – 99)

[a] in mL/min/1.73m^2

[b] adjusted by a factor of +9.0µmol/L based on inter-site calibration study comparing laboratory Jaffe and enzymatic methods

All data: % (95% confidence intervals)

Note: all eGFR equations exclude the African-American coefficient

Table S2: Samples correctly classified according to KDIGO GFR staging

eGFR[a]	Laboratory eGFR (MDRD)	Laboratory eGFR (CKD-EPI)	Laboratory eGFR (LM)	StatSensor Capillary eGFR (MDRD)	StatSensor Capillary eGFR (CKD-EPI)	StatSensor Capillary eGFR (LM)	iSTAT eGFR (MDRD)	iSTAT eGFR (CKD-EPI)	iSTAT eGFR (LM)	StatSensor Venous eGFR (MDRD)	StatSensor Venous eGFR (CKD-EPI)	StatSensor Venous eGFR (LM)
mGFR >90												
>90	**241 (96)**	**250 (99)**	**202 (80)**	**130 (52)**	**183 (73)**	**99 (40)**	**195 (77)**	**236 (94)**	**149 (59)**	**46 (53)**	**60 (70)**	**32 (37)**
60 - 89	11 (4)	2 (1)	50 (20)	108 (43)	65 (26)	139 (56)	57 (23)	16 (6)	102 (40)	38 (44)	26 (30)	48 (56)
30 - 59	0 (0)	0 (0)	0 (0)	12 (5)	2 (1)	12 (5)	0 (0)	0 (0)	1 (0)	2 (2)	0 (0)	6 (7)
15 - 29	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<15	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
mGFR 60 - 89												
>90	241 (85)	264 (93)	189 (66)	106 (37)	155 (54)	68 (24)	165 (58)	212 (74)	114 (40)	34 (35)	51 (53)	22 (23)
60 - 89	**42 (15)**	**20 (7)**	**92 (32)**	**138 (48)**	**109 (38)**	**175 (61)**	**113 (40)**	**68 (24)**	**149 (52)**	**56 (58)**	**44 (45)**	**69 (71)**
30 - 59	2 (1)	1 (0)	4 (1)	41 (14)	21 (7)	42 (15)	7 (2)	5 (2)	22 (8)	7 (7)	2 (2)	6 (6)
15 - 29	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<15	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
mGFR 30 - 59												
>90	83 (64)	96 (74)	60 (47)	25 (19)	39 (30)	16 (12)	49 (38)	70 (55)	40 (31)	5 (17)	7 (23)	3 (10)
60 - 89	38 (29)	26 (20)	55 (43)	74 (57)	66 (51)	75 (58)	62 (48)	45 (35)	66 (52)	15 (50)	15 (50)	16 (53)
30 - 59	**7 (5)**	**6 (5)**	**12 (9)**	**29 (22)**	**23 (18)**	**34 (26)**	**16 (13)**	**12 (9)**	**19 (15)**	**10 (33)**	**8 (27)**	**9 (30)**
15 - 29	1 (1)	1 (1)	2 (2)	1 (1)	1 (1)	4 (3)	1 (1)	1 (1)	3 (2)	0 (0)	0 (0)	2 (7)
<15	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
mGFR 15 - 29												
>90	2 (50)	2 (50)	1 (25)	1 (25)	1 (25)	0 (0)	0 (0)	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)
60 - 89	0 (0)	0 (0)	1 (25)	1 (25)	2 (50)	1 (25)	2 (50)	1 (25)	2 (50)	0 (0)	0 (0)	0 (0)
30 - 59	2 (50)	2 (50)	2 (50)	2 (50)	1 (25)	2 (50)	2 (50)	2 (50)	1 (25)	1 (100)	1 (100)	0 (0)
15 - 29	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**1 (25)**	**0 (0)**	**0 (0)**	**1 (25)**	**0 (0)**	**0 (0)**	**1 (100)**
<15	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
mGFR <15												
>90	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

60 - 89	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
30 - 59	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)
15 - 29	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
<15	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**

All data n (%)
CKD-EPI and MDRD equations presented without the African-American coefficient
Correctly classified samples highlighted in **bold**
[a]eGFR in mL/min/1.73m^2

3.

Published paper 2: Diagnostic accuracy of semiquantitative point of care urine albumin to creatinine ratio and urine dipstick analysis in a primary care resource limited setting in South Africa

Currin et al. BMC Nephrology (2021) 22:103
https://doi.org/10.1186/s12882-021-02290-5

BMC Nephrology

RESEARCH ARTICLE Open Access

Diagnostic accuracy of semiquantitative point of care urine albumin to creatinine ratio and urine dipstick analysis in a primary care resource limited setting in South Africa

Sean D. Currin[1,2*], Mwawi S. Gondwe[3], Nokthula B. Mayindi[3], Shingirai Chipungu[3], Bongekile L. Khoza[3], Stephen Tollman[3,4], June Fabian[3,5†], Jaya A. George[1,2†] and On behalf of the ARK Consortium

Abstract

Background: The prevalence of chronic kidney disease (CKD) is predicted to rise over the next few decades. In resource-limited settings access to central laboratory services is limited. Point-of-care (POC) urine dipstick testing offers the potential to detect markers of kidney damage (albuminuria) as well as markers of other disease processes. We evaluated the diagnostic accuracy of the semi-quantitative albumin-creatinine ratio (ACR) Sysmex UC-1000 POC urine dipstick system as well as the extent of other abnormal dipstick findings in urine.

Methods: 700 participants from a rural area in South Africa were screened for albuminuria. A spot urine sample was used to measure POC and central laboratory ACR. We determined the sensitivity, specificity, positive predictive value and negative predictive value of the POC ACR, and recorded dipstick parameters.

Results: The prevalence of albuminuria was 11.6% (95%CI; 9.3–14.2). Those with albuminuria had higher mean diastolic (82 vs 79 mmHg, $p = 0.019$) and systolic (133 vs 128 mmHg, $p = 0.002$) blood pressures and a higher proportion of diabetes mellitus (17.6 vs 4.9%, $p < 0.001$). The sensitivity of the POC ACR system was 0.79, specificity 0.84, positive predictive value 0.39 and negative predictive value 0.97. The sensitivity improved to 0.80, 0.85, 0.85 and 0.89 in those with elevated blood pressure, diabetes mellitus, HIV positive status, and those 65 years and older, respectively. Abnormalities other than albuminuria were detected in 240 (34.3%) of the samples; 88 (12.6%) were positive for haematuria, 113 (16.1%) for leucocytes, 66 (9.4%) for nitrites and 27 (3.9%) for glycosuria.

(Continued on next page)

* Correspondence: sean.currin@wits.ac.za
June Fabian and Jaya A George acted jointly in the last authorship role.
[1]Department of Chemical Pathology, Faculty of Health Sciences, University of Witwatersrand, 7 York Road, Parktown, Johannesburg 2193, South Africa
[2]National Health Laboratory Service, Johannesburg, South Africa
Full list of author information is available at the end of the article

(Continued from previous page)
Conclusion: Our study shows that POC ACR has good negative predictive value and could be used to rule out albuminuria when screening for CKD. Additionally, a high proportion of participants had other urine abnormalities detected with dipsticks which may reflect kidney disease or co-morbid untreated genitourinary pathology such as urinary tract infections or endemic schistosomiasis with important implications for CKD.

Keywords: Point of care, Urine albumin creatinine ratio, Dipstick, Chronic kidney disease

Background

Sub-Saharan Africa (SSA) has a high burden of infectious diseases (ID), mostly from HIV and tuberculosis, and an emerging burden of non-communicable diseases (NCD) [1, 2]. Together, ID and NCD are risk factors for chronic kidney disease (CKD), which has a prevalence of 10.7% in SSA [3]. The prevalence of CKD is predicted to rise disproportionately in low- and middle-income countries (LMIC) [4]. Therefore, early detection of CKD and appropriate management of risk factors for progression is an essential public health priority.

Persistent albuminuria is one criterion for the diagnosis of CKD, and an independent risk factor for adverse kidney and cardiovascular outcomes [5–9]. Albuminuria, measured on more than one occasion, as a spot urine albumin to creatinine ratio (ACR), is now included as one of the diagnostic criteria for CKD [10]. Screening for albuminuria is recommended in certain high-risk groups such as those with diabetes mellitus, however results should be confirmed by a central laboratory [11, 12].

The lack of access to central laboratory services in LMICs is a major hurdle obstructing widespread implementation of CKD screening programs. While limited laboratory access is acknowledged in the 2012 KDIGO guidelines, there are no recommendations for using point of care (POC) devices in resource-limited settings (RLS) [10]. POC testing provides actionable results in real-time for clinical decision-making [13]. In South Africa (SA), the efficacy of POC testing is well established for diagnosing and managing patients with HIV and tuberculosis [14]. Using the existing framework established for POC testing with ID, we propose that POC testing for screening and early detection of CKD is feasible in our setting. Previous studies [15–22] have shown good negative predictive values with fair sensitivity for semi-quantitative ACR POC testing, which suggests utility as a screening test for CKD. Additionally, the ability of urine dipsticks to detect other disease markers besides albuminuria offers added benefit.

The aim of our study was to evaluate the diagnostic accuracy of a POC semi-quantitative ACR system in a RLS in rural SA, and evaluate additional urinary abnormalities discovered on dipstick analysis.

Methods

Study design

Our sub-study comprised part of the prospective African Research on Kidney (ARK) study, the methods of which have been published [23]. To summarise, the ARK study was conducted in the Agincourt Health and Demographic Surveillance Site, Mpumalanga province, SA. There were two phases of the ARK study; the first phase (November 2017 – October 2018) screened a population-based sample of 2020 rural Africans for kidney disease and associated risk factors, those between the ages of 20 and 80 were considered eligible; the second phase (November 2018 – July 2019) comprised a subsample of the first phase, stratified by CKD stage. (Fig. 1).

In phase one of the ARK study, a CKD-risk questionnaire was administered, blood pressure (BP) was measured (as per the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure guidelines [24]), and voluntary counselling and HIV testing was offered to all participants according to the SA Department of Health Guidelines [25]. In the field, POC tests were performed including urine dipstick analysis using Roche

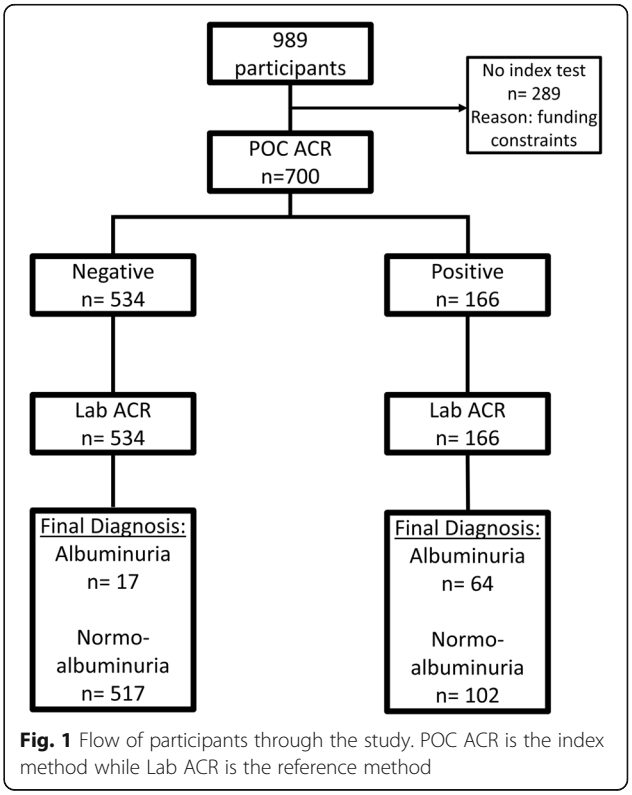

Fig. 1 Flow of participants through the study. POC ACR is the index method while Lab ACR is the reference method

(Mannheim, Germany) Combur 10® test strips on fresh spot urine samples.

The second phase of the ARK study recruited 989 participants and included the same BP and HIV testing protocols as the first phase. Spot urine samples were refrigerated overnight (2 - 8 °C) and processed the following morning for storage at -80 °C. Urine samples were transported at -80 °C from the study site to a central laboratory where they were processed over a nine-day period. Each day, 81 samples were separated into two aliquots, one aliquot was tested using central laboratory ACR, the other aliquot was tested using a POC semi-quantitative dipstick method. Central laboratory ACR results were not known while POC ACR was performed and vice versa. 700 urine samples out of the 989 where selected based on the order in which they were sent to the central laboratory, all 700 samples were processed. Funding constraints limited the sample size to 700 out of 989.

POC ACR and dipstick analysis

Urine dipstick analysis was performed using Sysmex Corporation (Kobe, Japan) 12S® urine dipsticks together with the Sysmex UC-1000® semi-automated urine chemistry analyser. The 12S dipsticks measure the following parameters: urobilinogen, red blood cells, haemoglobin, protein, glucose, ketones, bilirubin, nitrites, specific gravity, leukocytes, pH, creatinine, and albumin with colorimetric dipstick assays. The Sysmex UC-1000® system optically reads reaction results from 12S dipsticks using multi-wavelength reflectance photometry thereby providing semi-quantitative results. The semi-automated reading of strips eliminates inter-individual variation in interpreting dipstick results. The albumin concentration is measured as 10, 30, 80, or 150 mg/L using the protein error of pH indicator method, while the creatinine concentration is measured as 10, 50, 100, 200, or 300 mg/dL using the Benedict-Behre method; thereafter the ACR is calculated as dilute (albumin 10 mg/L and creatinine 10 mg/dL), normal (< 30 mg/g), 1+ (30–300 mg/g), or 2+ (> 300 mg/g). Internal quality control was performed each day before any samples were run.

Measurement of laboratory ACR

Laboratory albumin and creatinine measurements were performed on the Cobas c502 module® (Roche Diagnostics, Mannheim), with urine albumin (mg/L) determined using an immunoturbidimetric assay while urine creatinine (mg/dL) was determined using the kinetic Jaffe method. This was considered an appropriate reference method due to widespread usage in central laboratories in South Africa. During the nine days of measurements a single lot was used for both urine albumin and creatinine assays, internal quality control was performed twice daily as per standard practices within the central laboratory, together with an external quality assurance program.

Statistical analysis

Laboratory measurement of ACR was used as the reference method to which POC ACR was compared. Laboratory ACR was considered positive if ≥30 mg/g and negative if < 30 mg/g. POC ACR was considered positive if falling in the "1+" or "2+" (≥30 mg/g) groups whilst negative if falling in the normal group (< 30 mg/g). Samples in which the laboratory albumin was < 3 mg/L (limit of quantification) were considered as negative, as were POC ACR samples which were dilute (albumin < 10 mg/L and creatinine < 10 mg/dL). All 700 samples were included for analysis.

Statistical analysis was performed using Tibco Statistica 13® and MedCalc 19.1.3®. Continuous data were compared using the students T-test and Mann-Whitney test when appropriate, while categorical data were compared using χ^2 test. The prevalence of albuminuria, sensitivity, specificity, positive (PPV) and negative predictive values (NPV), and likelihood ratios of POC testing were calculated using cross-tabulation. 95% confidence intervals were calculated according to the exact Clopper-Pearson method for sensitivity and specificity, according to the Miettinen-Nurminen method for likelihood ratios and according to the Mercaldo-Wald method for predictive values. A p-value < 0.05 was considered statistically significant.

Results

Study population

We analysed 700 urine samples; participant demographics and clinical characteristics are listed in Table 1. Mean systolic and diastolic BP were significantly higher amongst those with laboratory albuminuria, as was a history of diabetes mellitus; however, a history of hypertension was not. The prevalence of albuminuria was 11.6% (95%CI; 9.3–14.2), based on the central laboratory ACR measurements (≥30 mg/g).

Clinical performance of the POC ACR

The central laboratory method identified 81 participants as having albuminuria (≥ 30 mg/g) whilst the POC method, performed on the same day, identified 166 participants with albuminuria. No adverse events were reported during testing or collection. In total the POC reader misclassified 119 samples (17%) with 102 false positives, and 17 false negatives. Sensitivity of the POC ACR to detect albuminuria (ACR ≥ 30 mg/g) was 0.790 (95%CI; 0.689–0.865), and specificity was 0.835 (95%CI; 0.804–0.862). A positive likelihood ratio of 4.80 (95%CI; 3.86–5.89) and negative likelihood ratio of 0.25 (95%CI;

Table 1 Demographic and clinical features

	Laboratory ACR (mg/g)			p[c]
	< 30	≥30	> 300	
Number of patients	619	81	9	
Age (years)	45 (25)[a]	43 (23)[a]	55 (25)[a]	0.680[d]
BMI (kg/m^2)	28.1 (6.2)	27.3 (6.5)	27.0 (7.0)[a]	0.239[e]
Systolic BP (mmHg)	128 (14.9)	133 (18.1)	144 (24.0)[a]	0.002[e]
Diastolic BP (mmHg)	79 (8.6)	82 (9.9)	83 (7.0)[a]	0.019[e]
Male (%)[b]	211 (34.1)	31 (38.3)	4 (44.4)	0.457[f]
Hypertension (%)[b]	154 (26.0)	25 (33.8)	3 (37.5)	0.153[f]
Diabetes Mellitus (%)[b]	29 (4.9)	13 (17.6)	2 (25.0)	< 0.001[f]
Smoker (%)[b]	85 (14.3)	11 (14.9)	0 (0)	0.902[f]
HIV reactive (%)[b]	119 (20.1)	20 (27.0)	1 (12.5)	0.246[f]

Data presented as mean (SD) or n (%) unless otherwise indicated
[a]Data presented as median (IQR)
[b]Missing values for all patients = 33
[c]Comparison between laboratory negative (< 30 mg/g) and laboratory positive (≥30 mg/g) groups
[d]Mann-Whitney test
[e]Students T-test
[f] χ^2 test
Hypertension, diabetes mellitus, smoker status obtained from medical questionnaire
HIV status obtained from medical questionnaire. If previously tested, participants were asked their status; those who did not know their status, or previously tested negative, were offered voluntary counselling and testing during the home screening (see methods)
Measured blood pressure (BP): see methods

0.16–0.37) was obtained for POC ACR. Predictive values of 0.968 (95%CI; 0.952–0.979) for negative results and 0.386 (95%CI; 0.337–0.436) for positive results were obtained. Sensitivity and specificity to predict ACR > 300 mg/g was 1.00 (95%CI;0.664–1.00) and 0.987 (95%CI; 0.975–0.994) respectively; with predictive values of 1.00 (95%CI; 0.995–1.00) for negative results and 0.50 (95%CI;0.343–0.657) for positive results.

The sensitivity and specificity of the POC ACR system varied among different groups of participants. There was reduced sensitivity (76%) in those with a history of hypertension (medical questionnaire), however those with raised blood pressure [26] showed an improved sensitivity and specificity of 80 and 86% respectively. The same is true of those with a history of diabetes mellitus, with an improved sensitivity of 85%, however, specificity was lower (66%) in this group. Smokers, those with HIV, and those 65 years or older showed improved sensitivity. PPV remained low in all groups ranging from 0.26 to 0.52, while NPV was high ranging from 0.90 to 0.99. (Table 2).

False negative values ranged from 30.9–57.5 mg/g, and those for false positives ranged from 1.8–29.2 mg/g. All nine samples with laboratory ACR > 300 mg/g were correctly identified as "2+" (> 300 mg/g) according to POC ACR, a further nine samples were classified as > 300 mg/g while laboratory ACR classified them as albuminuria (> 30 mg/g) but less than 300 mg/g. POC ACR correctly classified 572 (82%, 95%CI;79–85) of the samples into the correct KDIGO albuminuria stages of A1 (< 30 mg/g), A2(30 – 300 mg/g) and A3 (> 300 mg/g). Accuracy was 97% (95%CI;95–98), 31% (95%CI;24–39), and 50% (95%CI;26–74) in groups A1, A2 and A3 according to POC ACR. (Fig. 2).

Additional urine dipstick abnormalities

240 (34.3%) of the 700 samples had abnormal findings other than ACR, protein to creatinine ratio, pH, or specific gravity. 65% (53/81) of laboratory confirmed albuminuria samples had additional abnormalities on urine dipstick. While 30% (187/619) of the samples without laboratory confirmed albuminuria demonstrated additional urinary dipstick abnormalities. Using the dipstick method 88 (12.6%) participants had haematuria including 50 in the laboratory negative group, and 113 (16.1%) had leukocyturia.

Urine dipstick findings were compared between those with laboratory positive and negative ACR. Haematuria and glycosuria were significantly higher in the laboratory positive compared to the laboratory negative group (46.9% vs 8.1% and 11.1% vs 2.9% respectively). All abnormalities were seen with a higher proportion in those with laboratory positive ACR except for leucocytes, although not statistically significant. (Table 3).

Effect of freezing on semi-quantitative POC dipstick parameters

Freezing had a minimal effect on dipstick parameters with a small increase in false negatives and no increase in false positives being seen for haematuria and leukocyturia. (See Supplementary for detailed results and methodology).

Discussion

Our study represents the largest evaluation of a POC ACR compared to laboratory ACR to date. Similar to previous studies [15–22] we found that the POC analyser has good rule out utility for albuminuria, as indicated by the NPV. Our study differs from previous studies in that we simultaneously evaluated additional urine dipstick parameters aside from albuminuria. Similar studies [15–21] have looked at semi-quantitative POC ACR from different manufacturers, however this is the first published study on the Sysmex UC-1000® instrument. The fully automated Sysmex UC-3500® has previously been validated showing excellent precision and good analytical accuracy [22].

When using ACR to screen for albuminuria, the recommended sample is an early morning urine sample. Previous studies have used random urine [15, 18, 20] or early morning samples [16, 17, 19, 21] with sensitivity of

Table 2 Classification of albuminuria as positive or negative by POC and laboratory ACR

All patients (700)			**Sensitivity**	**0,79**
	Positive POC ($n = 166$)	Negative POC ($n = 534$)	Specificity	0,84
Positive lab ACR ($n = 81$)	64	17	PPV	0,39
Negative lab ACR ($n = 619$)	102	517	NPV	0,97
Hypertension (179)			Sensitivity	0,76
	Positive POC ($n = 47$)	Negative POC ($n = 132$)	Specificity	0,82
Positive lab ACR ($n = 25$)	19	6	PPV	0,40
Negative lab ACR ($n = 154$)	28	126	NPV	0,95
DM (42)			Sensitivity	0,85
	Positive POC ($n = 21$)	Negative POC (n = 21)	Specificity	0,66
Positive lab ACR (n = 13)	11	2	PPV	0,52
Negative lab ACR ($n = 29$)	10	19	NPV	0,90
Smoker (96)			Sensitivity	0,82
	Positive POC ($n = 26$)	Negative POC ($n = 70$)	Specificity	0,80
Positive lab ACR ($n = 11$)	9	2	PPV	0,35
Negative lab ACR ($n = 85$)	17	68	NPV	0,97
HIV (139)			Sensitivity	0,85
	Positive POC ($n = 41$)	Negative POC ($n = 98$)	Specificity	0,80
Positive lab ACR ($n = 20$)	17	3	PPV	0,41
Negative lab ACR ($n = 119$)	24	95	NPV	0,97
BP: Diastolic ≥ 80 OR Systolic ≥ 130 (389)			Sensitivity	0,80
	Positive POC ($n = 93$)	Negative POC ($n = 296$)	Specificity	0,86
Positive lab ACR ($n = 56$)	45	11	PPV	0,48
Negative lab ACR ($n = 333$)	48	285	NPV	0,96
Age ≥ 65 (105)			Sensitivity	0,89
	Positive POC ($n = 31$)	Negative POC ($n = 74$)	Specificity	0,76
Positive lab ACR (n = 9)	8	1	PPV	0,26
Negative lab ACR ($n = 96$)	23	73	NPV	0,99

Hypertension, diabetes mellitus (DM), smoker status obtained from medical questionnaire
HIV status obtained from medical questionnaire. If previously tested, participants were asked their status; those who did not know their status, or previously tested negative, were offered voluntary counselling and testing during the home screening (see methods)
Measured blood pressure (BP): see methods
PPV: positive predictive value
NPV: negative predictive value

random urine samples being 0.83 [15], 0.79 [18], and 0.63 [20] while the sensitivity of early morning samples was 0.90 [16], 0.90 [17], 0.75 [19], 0.92 [21] and 0.80 [21], suggesting a possible advantage of early morning sampling [27].

Our findings show the POC device would be useful for excluding albuminuria, but any positive result would need to be confirmed with a laboratory ACR. This in keeping with other studies where NPV ranged from 0.89–0.99 [15, 16, 18, 19, 21, 22] with one study reporting a NPV of 0.71 [17]; while positive predictive value was low for all studies ranging from 0.46–0.82 [15–19, 21, 22]. Similarly, only one study [16] reported a positive likelihood ratio above 10 indicating utility in ruling in disease.

The improved sensitivity seen in participant groups with known risk factors for CKD could help guide screening strategies. The discordance in sensitivities between a history of hypertension (medical questionnaire) and raised BP during examination could point to the effect of antihypertensives, particularly angiotensin-converting enzyme inhibitors, on albuminuria [28]. Screening strategies targeted at those with a history of diabetes mellitus, the elderly, those who present with elevated blood pressure, and those with HIV are justified, and in keeping with management guidelines [29, 30]. Our findings corroborate those of previous studies: in the study by Graziani et al. [16] the sensitivity and NPV of POC ACR improved marginally from 0.90 to 0.91 and 0.90 to 0.98 respectively in the general population versus

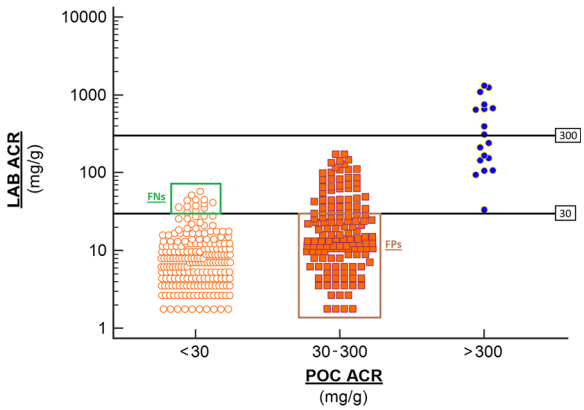

Fig. 2 Laboratory ACR (reference) versus POC ACR (index). The horizontal lines represent 30 mg/g and 300 mg/g. 30 mg/g represents the threshold for albuminuria while 300 mg/g is the threshold for 2+ albuminuria (as defined by the POC system). FNs represent false negative results by the POC method while FPs represent false positive results by the POC method

a cohort with a history of type 2 diabetes mellitus. McTaggart et al. [15] found improved sensitivity and NPV: 0.83 to 0.91 and 0.95 to 0.98 respectively in the general population compared to participants with hypertension. The study by Nah et al. [21] was conducted in prediabetic and diabetic populations with sensitivity of 0.92 and 0.80, and NPV of 0.99 and 0.91 respectively. Our sensitivity of 0.79 and NPV of 0.97 are in keeping with random urine samples in the general population. Although the loss of specificity (increased false positives) seen in those with diabetes mellitus in our study suggests the need for confirmatory laboratory testing, the increased sensitivity in this group of participants is valuable in detecting diabetic nephropathy. Although increased sensitivity was seen in subgroup analysis, the low PPV and high NPV remained in all sub-populations suggesting that laboratory confirmation of positive results is required while negative results exclude disease.

When using POC ACR, 82% of samples were correctly classified into the correct KDIGO albuminuria stages, however, the poor performance in those with albuminuria according to POC ACR suggests category shifting in this group. Despite POC ACR having 100% sensitivity in those with significant albuminuria (> 300 mg/g or stage A3), 50% of positive samples were false positive results. The high number of false positives and relatively few false negatives suggests that POC ACR tends to shift patients into higher concentrations of albuminuria, thus higher ACR categories. As such, negative results carry more significance, further emphasized by the 97% accuracy seen in those with POC ACR < 30 mg/g.

Surprisingly, our study showed a high prevalence of abnormal urine findings besides ACR- highlighting the extent of abnormalities which can be picked up with dipstick analysis in our population. Similar findings were demonstrated during phase one of the ARK study (haematuria: 32.6% and leukocyturia: 30.3%), while the effects of freezing at -80 °C were shown to have minimal effects (Supplementary). Our aims were not to validate these findings but rather to expose the extent of abnormalities seen. The epidemiology of CKD in LMICs is complex and often comprises the influence of multiple factors including NCDs, IDs, host and environmental factors [31]. Our findings suggest that strategies to decrease the impact of CKD should involve screening, diagnosing and managing CKD, but also screening for, and managing other risk factors [32].

We found a high prevalence of haematuria on dipstick analysis. Potential explanations for isolated haematuria could be endemic urogenital schistosmiasis, a known cause of CKD in LMICs [31], or glomerular pathology that could include infectious (or other) glomerulonephritides, urogenital tuberculosis, and interstitial

Table 3 Other dipstick findings besides ACR in phase 1 and phase 2

	Blood[e]	Urobilinogen	Bilirubin	Glucose	Leucocytes	Nitrites
Phase 2 (*n* = 700) (Sysmex 12S® strips)[a]						
Lab ACR ≥30 (n and %)	38 (46.9)	1 (1.2)	2 (2.5)	9 (11.1)	12 (14.8)	12 (14.8)
Lab ACR < 30 (n and %)	50 (8.1)	2 (0.3)	4 (0.6)	18 (2.9)	101 (16.3)	54 (8.7)
TOTAL (%)	88 (12.6)	3 (0.4)	6 (0.9)	27 (3.9)	113 (16.1)	66 (9.4)
p[d]	< 0.001	0.238	0.094	< 0.001	0.730	0.078
Phase 1 (*n* = 1993)[c] (Roche Combur 10® strips)[b]						
TOTAL (%)	649 (32.6)	36 (1.8)	46 (2.3)	64 (3.2)	603 (30.3)	49 (2.5)

All data n (%)
[a]Performed on urine after a freeze thaw cycle, semi-quantitative analysis performed with Sysmex UC-1000® device (see methods)
[b]Performed on fresh urine during phase one, semi-quantitative analysis performed with visual inspection (see methods)
[c]Missing data for 27 (2 refused, 25 missing data)
[d] χ^2 test Lab ACR ≥30 vs Lab ACR < 30 (all phase 2)
[e]Haemoglobin or erythrocytes detected by dipstick method

nephritis. The high level of haematuria observed in the population is concerning: haematuria is associated with an increased risk of progression to ESRD [33], and plays a pathological role in renal tubular damage [34]. These dipstick findings need to be validated in our population, however previous studies have recommended dipstick analysis when screening for haematuria due to high sensitivity and reduced costs [35–38].

All the dipstick parameters except for leucocytes occurred with higher frequency in those with laboratory albuminuria; of these haematuria and glycosuria had statistical significance. This is in keeping with the role haematuria plays in various renal pathologies as well as the risk for kidney disease which is inferred from diabetes mellitus (a possible cause for glycosuria amongst others). Although not statistically significant the relatively higher prevalence of leucocytes in urine of those with laboratory negative findings was an unexpected finding. Previous studies [39–42] including a study performed on South African participants with CKD [43] showed that the presence of JC viruria was protective of developing CKD, with 43-fold higher odds of developing kidney disease in those without JC viruria. Whether these protective virurias stimulate leucocyte shedding in the urine of individuals is yet to be studied but could be a possible explanation for the raised leucocytes seen in laboratory negative individuals.

The limitations of dipstick screening such as false positives, interferences from various drugs, a lack a specificity and the need for confirmatory laboratory or microscopy testing are well known [44]. In RLS where access to central laboratory services is often limited the goals and uses of POC testing are very different to resource replete settings. Better patient outcomes with POC testing can be achieved even with decreased performance compared to laboratory-based testing if POC testing ensures improved linkage to care and retention in treatment [45]. The ability of dipstick testing to detect the potential presence of multiple risk factors for CKD and other co-morbid conditions such as diabetes mellitus, schistosomiasis, urinary tract infections, urological abnormalities, liver disease, and numerous others; together with conventional (albuminuria) and non-conventional (haematuria) markers of CKD in a single test is beneficial. Semi-quantitative (and semi-automated) results are preferable over subjective visual dipstick inspection, such semi-quantitative results aid primary care nurses who are often responsible for following screening guidelines in RLS.

Our study had several limitations: the POC testing was performed in a laboratory environment on the same day as laboratory measurements and not at the patient side, in a less controlled environment it is possible the POC instrument will not perform to the same standards. The urine samples were kept in a fridge overnight and then frozen the next day at -80 °C before being shipped frozen to the central laboratory. Previous studies have shown good stability of urine albumin and ACR with long term storage at -70 °C [46, 47], urine albumin and ACR is stable for up to 7 days when stored at 4 °C [48, 49]. To our knowledge no studies exist on the stability of urine dipstick analysis in previously frozen samples. Dipstick analysis on samples post refrigeration up to 48 h has shown: glucose to be stable up to 48 h at 4 °C; nitrites stable up to 24 h at 4 °C; leucocytes, haemoglobin, red blood cells, bilirubin show increased false negative results at 4 °C from between 4 and 8 h [50, 51]. It is possible the POC device would perform better on fresh urine as this is the recommended sample from the manufacturer (due to bilirubin and urobilinogen deterioration), however frozen and refrigerated samples can be used if returned to room temperature [52]. Despite the widespread use of immunoassays and the kinetic Jaffe method to measure laboratory ACR, these methods remain far from ideal gold standards with which to compare POC ACR. There is currently no reference method or material for urine albumin; as such methods are not standardised despite this being a goal of the National Kidney Disease Education Program [53, 54]. Comparison of results between different methods have generally not found large biases [53, 55]. Although isotope dilution mass spectrometry-traceability has been established for the Jaffe method and a primary reference material exists the lack of matrix-specific secondary reference materials for urine creatinine mean that calibration is not ideal [53]. Consequently, the use of laboratory ACR as the gold standard in our study was a pragmatic rather than ideal choice. Our study also has several advantages, the large sample size from a rural population in South Africa represents an important demographic group where POC testing may be most beneficial. While random urine sampling is not the recommended sample type (versus concentrated early morning sampling) it represents the situation that will be encountered across rural clinics in South Africa. We were able to process all 700 samples, without having to exclude outliers- and so better represent the clinical situation which would be encountered by health care workers.

Conclusions

We have demonstrated the good rule out utility of a POC ACR dipstick device, which could aid in excluding at risk patients who do not require confirmatory central laboratory testing in RLS. We have also shown the large number of other abnormal findings seen with urine dipstick testing in our population- which could have implications for establishing screening guidelines for multiple diseases or risks for CKD with a single test. Our study population and findings are relevant in rural Africa with limited access to central laboratory services.

Abbreviations
CKD: Chronic kidney disease; POC: Point-of-care; ACR: Albumin-creatinine ratio; PPV: Positive predictive value; NPV: Negative predictive value; SSA: Sub-Saharan Africa; ID: Infectious diseases; NCD: Non-communicable diseases; LMIC: Low- and middle-income countries; RLS: Resource-limited settings; SA: South Africa; ARK: African Research on Kidney; BP: Blood pressure

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12882-021-02290-5.

Additional file 1. Table S1: Classification of haematuria and leukocyturia pre and post freezing compared to laboratory.

Additional file 2. Phase 1: FW questionnaire CKD risk – family.

Additional file 3. Phase 1: FW questionnaire CKD Risk - participant a.

Additional file 4. Phase 1: FW questionnaire CKD Risk - participant b.

Acknowledgments
We thank Sysmex Corporation for supplying the UC-1000 system, 12S dipsticks and quality control material for measurement of urine dipstick and albumin to creatinine ratio.

Authors' contributions
SC wrote the manuscript and performed analysis and interpretation of data, while participating in elements of conception and design. The manuscript forms part of the requirements for the fulfilment of a MMed degree for SC. JG and JF acted as supervisors for the project- providing conception and design as well as guidance with data interpretation. Both supervisors actively revised the manuscript with important contributions and gave their final approval for the submitted manuscript. ST participated in conceptualising the study and editing the final draft of the paper. MG acted as the project manager, participating in project implementation and oversight, data collection, data checking; and reviewed the final draft of the paper. NM acted as the assistant project manager, participating in oversight of study procedures, data collection, data checking; and reviewed the final draft of the paper. SC acted as the laboratory manager, participating in oversight of laboratory procedures, quality control, specimen collection and processing, storage and shipping; and reviewed the final draft of the paper. BK acted as a medical laboratory technician, participating in quality control procedures, specimen collection and processing, storage and shipping; and reviewed the final draft of the paper. All authors read and approved the final manuscript.

Funding
Research was jointly supported by (i) the South African Medical Research Council, with funds from the South African National Department of Health, MRC UK (via the Newton Fund) and GSK R&D; (ii) Faculty Research Committee Individual Research Grant, University of Witwatersrand; (iii) The International Society of Nephrology Clinical Research Program.
Funders played no role in the design of the study; collection, analysis or interpretation of data or in writing the manuscript.

Availability of data and materials
The data underlying this article will be shared on reasonable request to the corresponding author.

Declarations

Ethics and approval and consent to participate
Study approval was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (M160937 and M190434) and trained fieldworkers obtained written, informed consent from participants in their first language.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Chemical Pathology, Faculty of Health Sciences, University of Witwatersrand, 7 York Road, Parktown, Johannesburg 2193, South Africa. [2]National Health Laboratory Service, Johannesburg, South Africa. [3]Faculty of Health Sciences, Medical Research Council/Wits University Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, University of Witwatersrand, Johannesburg, South Africa. [4]International Network for the Demographic Evaluation of Populations and their Health (INDEPTH) Network, Accra, Ghana. [5]Wits Donald Gordon Medical Centre, Faculty of Health Sciences, School of Clinical Medicine, University of Witwatersrand, Johannesburg, South Africa.

Received: 28 August 2020 Accepted: 2 March 2021
Published online: 20 March 2021

References

1. Naghavi M, Abajobir AA, Abbafati C, et al. Global, regional, and national age-sex specifc mortality for 264 causes of death, 1980-2016: a systematic analysis for the global burden of disease study 2016. Lancet. 2017; 390(10100):1151–210. https://doi.org/10.1016/S0140-6736(17)32152-9.
2. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006;3(11):e442. https://doi.org/10.1371/journal.pmed.0030442.
3. George JA, Brandenburg J-T, Fabian J, et al. Kidney damage and associated risk factors in rural and urban sub-Saharan Africa (AWI-gen): a cross-sectional population study. Lancet Glob Heal. 2019;7(12):e1632–43. https://doi.org/10.1016/S2214-109X(19)30443-7.
4. Liyanage T, Ninomiya T, Jha V, et al. Worldwide access to treatment for end-stage kidney disease: a systematic review. Lancet. 2015;385(9981):1975–82. https://doi.org/10.1016/S0140-6736(14)61601-9.
5. Klausen K, Borch-Johnsen K, Feldt-Rasmussen B, et al. Very low levels of microalbuminuria are associated with increased risk of coronary heart disease and death independently of renal function, hypertension, and diabetes. Circulation. 2004;110(1):32–5. https://doi.org/10.1161/01.CIR.0000133312.96477.48.
6. Ärnlöv J, Evans JC, Meigs JB, et al. Low-grade albuminuria and incidence of cardiovascular disease events in nonhypertensive and nondiabetic individuals: the Framingham heart study. Circulation. 2005;112(7):969–75. https://doi.org/10.1161/CIRCULATIONAHA.105.538132.
7. Verhave JC, Gansevoort RT, Hillege HL, Bakker SJL, De Zeeuw D, de Jong PE. An elevated urinary albumin excretion predicts de novo development of renal function impairment in the general population. Kidney Int Suppl. 2004;92:S18–21. https://doi.org/10.1111/j.1523-1755.2004.09205.x.
8. Gerstein HC, Mann JFE, Yi Q, et al. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. J Am Med Assoc. 2001;286(4):421–6. https://doi.org/10.1001/jama.286.4.421.
9. Park HY, Schumock GT, Pickard AS, Akhras K. A structured review of the relationship between microalbuminuria and cardiovascular events in patients with diabetes mellitus and hypertension. Pharmacotherapy. 2003; 23(12):1611–6. https://doi.org/10.1592/phco.23.15.1611.31962.
10. KDIGO Working Group. Kidney Disease: Improving Global Outcomes (KDIGO). KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl. 2013;3(1):1–150.
11. Ninomiya T, Perkovic V, De Galan BE, et al. Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes. J Am Soc Nephrol. 2009;20(8):1813–21. https://doi.org/10.1681/ASN.2008121270.
12. Sacks DB, Arnold M, Bakris GL, et al. Executive summary: guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. Clin Chem. 2011;57(6):793–8. https://doi.org/10.1373/clinchem.2011.163634.
13. Kazmierczak SC. Point-of-care testing quality: some positives but also some negatives. Clin Chem. 2011;57(9):1219–20. https://doi.org/10.1373/clinchem.2011.171538.
14. Cox HS, Mbhele S, Mohess N, et al. Impact of Xpert MTB/RIF for TB diagnosis in a primary care clinic with High TB and HIV prevalence in South Africa: a pragmatic randomised trial. PLoS Med. 2014;11(11):e1001760. https://doi.org/10.1371/journal.pmed.1001760.
15. McTaggart MP, Price CP, Pinnock RG, Stevens PE, Newall RG, Lamb EJ. The diagnostic accuracy of a urine albumin-creatinine ratio point-of-care test for detection of albuminuria in primary care. Am J Kidney Dis. 2012;60(5):787–94. https://doi.org/10.1053/j.ajkd.2012.05.009.

16. Graziani MS, Gambaro G, Mantovani L, et al. Diagnostic accuracy of a reagent strip for assessing urinary albumin excretion in the general population. Nephrol Dial Transplant. 2009;24(5):1490–4. https://doi.org/10.1093/ndt/gfn639.
17. Guy M, Newall R, Borzomato J, Kalra PA, Price C. Diagnostic accuracy of the urinary albumin: creatinine ratio determined by the CLINITEK microalbumin and DCA 2000+ for the rule-out of albuminuria in chronic kidney disease. Clin Chim Acta. 2009;399(1–2):54–8. https://doi.org/10.1016/j.cca.2008.09.006.
18. Le Floch JP, Marre M, Rodier M, Passa PH. Interest of clinitek® microalbumin in screening for microalbuminuria: results of a multicentre study in 302 diabetic patients. Diabetes Metab. 2001;27(1):36–9.
19. Croal BL, Mutch WJ, Clark BM, et al. The clinical application of a urine albumin: Creatinine ratio point-of-care device. Clin Chim Acta. 2001;307(1–2):15–21. https://doi.org/10.1016/S0009-8981(01)00450-8.
20. Meinhardt U, Ammann RA, Flück C, Diem P, Mullis PE. Microalbuminuria in diabetes mellitus - efficacy of a new screening method in comparison with timed overnight urine collection. J Diabetes Complicat. 2003;17(5):254–7. https://doi.org/10.1016/S1056-8727(02)00180-0.
21. Nah EH, Cho S, Kim S, Cho HI. Comparison of urine albumin-to-creatinine ratio (ACR) between ACR strip test and quantitative test in prediabetes and diabetes. Ann Lab Med. 2017;37(1):28–33. https://doi.org/10.3343/alm.2017.37.1.28.
22. Oyaert M, Delanghe JR. Semiquantitative, fully automated urine test strip analysis. J Clin Lab Anal. 2019;33(5):1–7. https://doi.org/10.1002/jcla.22870.
23. Kalyesubula R, Fabian J, Nakanga W, et al. How to estimate glomerular filtration rate in sub-Saharan Africa: design and methods of the African research into kidney diseases (ARK) study. BMC Nephrol. 2020;21(1):1–12. https://doi.org/10.1186/s12882-020-1688-0.
24. Chobanian AV, Bakris GL, Black HR, et al. The seventh report of the joint National Committee on prevention, detection, evaluation, and treatment of High blood pressure: the JNC 7 report. J Am Med Assoc. 2003;289(19):2560–72. https://doi.org/10.1001/jama.289.19.2560.
25. National Department of Health. National Consolidated Guidelines for the Prevention of Mother-To-Child Transmission of HIV (PMTCT) and the Management of HIV in Children, Adolescents and Adults. Dep Heal Repub South Africa. 2015:1–136.
26. Whelton PK, Carey RM, Aronow WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA guideline for the prevention, detection, evaluation, and Management of High Blood Pressure in adults a report of the American College of Cardiology / American Heart Association task force on clinical P. Hypertension. 2017;71(6):e13–e115. https://doi.org/10.1161/HYP.0000000000000065/-/DC1.The.
27. Witte EC, Heerspink HJL, De Zeeuw D, Bakker SJL, De Jong PE, Gansevoort R. First morning voids are more reliable than spot urine samples to assess microalbuminuria. J Am Soc Nephrol. 2009;20(2):436–43. https://doi.org/10.1681/ASN.2008030292.
28. Zhang WR, Craven TE, Malhotra R, et al. Kidney damage biomarkers and incident chronic kidney disease during blood pressure reduction: a case-control study. Ann Intern Med. 2018;169(9):610–8. https://doi.org/10.7326/M18-1037.
29. KDOQI. Clinical practice guidelines and clinical practice recommendations for diabetes and chronic kidney disease. Am J Kidney Dis. 2007;47(5 Suppl 3):S11–145. https://doi.org/10.1053/j.ajkd.2006.12.005.
30. Diana NE, Naicker S. Update on current management of chronic kidney disease in patients with HIV infection. Int J Nephrol Renovasc Dis. 2016;9:223–34. https://doi.org/10.2147/IJNRD.S93887.
31. Stanifer JW, Muiru A, Jafar TH, Patel UD. Chronic kidney disease in low- and middle-income countries. Nephrol Dial Transplant. 2016;31(6):868–74. https://doi.org/10.1093/ndt/gfv466.
32. Jha V, Arici M, Collins AJ, et al. Understanding kidney care needs and implementation strategies in low- and middle-income countries: conclusions from a "kidney disease: improving global outcomes" (KDIGO) controversies conference. Kidney Int. 2016;90(6):1164–74. https://doi.org/10.1016/j.kint.2016.09.009.
33. Vivante A, Afek A, Frenkel-Nir Y, et al. Persistent asymptomatic isolated microscopic hematuria in Israeli adolescents and young adults and risk for end-stage renal disease. J Am Med Assoc. 2011;306(7):729–36. https://doi.org/10.1001/jama.2011.1141.
34. Moreno JA, Sevillano Á, Gutiérrez E, et al. Glomerular hematuria: Cause or consequence of renal inflammation? Int J Mol Sci. 2019;20(9). https://doi.org/10.3390/ijms20092205.
35. Bataille A, Wetzstein M, Hertig A, Vimont S, Rondeau E, Galichon P. Evidence of dipstick superiority over urine microscopy analysis for detection of hematuria. BMC Res Notes. 2016;9(1):435. https://doi.org/10.1186/s13104-016-2240-y.
36. Anigilaje EA, Adedoyin OT. Correlation between dipstick urinalysis and urine sediment microscopy in detecting haematuria among children with sickle cell anaemia in steady state in Ilorin, Nigeria. Pan Afr Med J. 2013;15:135. https://doi.org/10.11604/pamj.2013.15.135.1854.
37. Arm JP, Peile EB, Rainford DJ, Strike PW, Tettmar RE. Significance of dipstick haematuria. 1. Correlation with microscopy of the urine. Br J Urol. 1986;58(2):211–7. https://doi.org/10.1111/j.1464-410x.1986.tb09029.x.
38. Arm JP, Peile EB, Rainford DJ. Significance of dipstick haematuria. 2. Correlation with pathology. Br J Urol. 1986;58(2):218–23. https://doi.org/10.1111/j.1464-410x.1986.tb09030.x.
39. Divers J, Núñez M, High KP, et al. JC polyoma virus interacts with APOL1 in African Americans with nondiabetic nephropathy. Kidney Int. 2013;84(6):1207–13. https://doi.org/10.1038/ki.2013.173.
40. Freedman BI, Kistler AL, Skewes-Cox P, et al. JC polyoma viruria associates with protection from chronic kidney disease independently from apolipoprotein L1 genotype in African Americans. Nephrol Dial Transplant. 2018;33(11):1960–7. https://doi.org/10.1093/ndt/gfx368.
41. Pires EP, Bernardino-Vallinoto CV, Alves DM, et al. Prevalence of infection by JC and BK polyomaviruses in kidney transplant recipients and patients with chronic renal disease. Transpl Infect Dis. 2011;13(6):633–7. https://doi.org/10.1111/j.1399-3062.2011.00614.x.
42. Kruzel-Davila E, Divers J, Russell GB, et al. JC viruria is associated with reduced risk of diabetic kidney disease. J Clin Endocrinol Metab. 2019;104(6):2286–94. https://doi.org/10.1210/jc.2018-02482.
43. Nqebelele NU, Dickens C, Dix-Peek T, Duarte R, Naicker S. JC virus and APOL1 risk alleles in Black south Africans with hypertension-attributed CKD. Kidney Int Rep. 2019;4(7):939–45. https://doi.org/10.1016/j.ekir.2019.05.006.
44. Simerville JA, Maxted WC, Pahira JJ. Urinalysis: a comprehensive review. Am Fam Physician. 2005;71(6):1153–62.
45. Drain PK, Hyle EP, Noubary F, et al. Diagnostic point-of-care tests in resource-limited settings. Lancet Infect Dis. 2014;14(3):239–49. https://doi.org/10.1016/S1473-3099(13)70250-0.
46. Chapman DP, Gooding KM, McDonald TJ, Shore AC. Stability of urinary albumin and creatinine after 12 months storage at −20 °C and −80 °C. Pract Lab Med. 2019;15(March):e00120. https://doi.org/10.1016/j.plabm.2019.e00120.
47. Kania K, Byrnes EA, Beilby JP, Webb SAR, Strong KJ. Urinary proteases degrade albumin: implications for measurement of albuminuria in stored samples. Ann Clin Biochem. 2010;47(Pt 2):151–7. https://doi.org/10.1258/acb.2009.009247.
48. Herrington W, Illingworth N, Staplin N, et al. Effect of processing delay and storage conditions on urine albumin-to-creatinine ratio. Clin J Am Soc Nephrol. 2016;11(10):1794–801. https://doi.org/10.2215/CJN.13341215.
49. Osberg I, Chase HP, Garg SK, et al. Effects of storage time and temperature on measurement of small concentrations of albumin in urine. Clin Chem. 1990;36(8 Pt 1):1428–30. https://doi.org/10.1093/clinchem/36.8.1428.
50. Froom P, Bieganiec B, Ehrenrich Z, Barak M. Stability of common analytes in urine refrigerated for 24 h before automated analysis by test strips. Clin Chem. 2000;46(9):1384–6.
51. Ekşioğlu MK, Madenci ÖÇ, Yücel N, et al. The effectiveness of BD vacutainer® plus urinalysis preservative tubes in preservation of urine for chemical strip analysis and particle counting. Biochem Medica. 2016;26(2):224–32. https://doi.org/10.11613/BM.2016.024.
52. Sysmex Corporation. MEDITAPE UC-12S (Package Insert).; 2017.
53. Miller WG, Bruns DE, Hortin GL, et al. Current issues in measurement and reporting of urinary albumin excretion. Clin Chem. 2009;55(1):24–38. https://doi.org/10.1373/clinchem.2008.106567.
54. Speeckaert MM, Speeckaert R, Van De Voorde L, Delanghe JR. Immunochemically unreactive albumin in urine: fiction or reality? Crit Rev Clin Lab Sci. 2011;48(2):87–96. https://doi.org/10.3109/10408363.2011.591366.
55. Seegmiller JC, Sviridov D, Larson TS, Borland TM, Hortin GL, Lieske JC. Comparison of urinary albumin quantification by immunoturbidimetry, competitive immunoassay, and protein-cleavage liquid chromatography-tandem mass spectrometry. Clin Chem. 2009;55(11):1991–4. https://doi.org/10.1373/clinchem.2009.129833.

Publisher's Note

4.

Published paper 2: Supplementary material

Supplementary Material

Freezing and dipstick parameters

Methodology

The effects of sample freezing on dipstick analysis was investigated by comparing 45 samples for haematuria and leukocyturia according to the semi-quantitative POC device before and after storage at -80^{0}C for seven days, in both cases this was compared to laboratory results (Beckman Coulter Iris-IQ200®) before freezing. Samples were classified as positive or negative. POC methods were considered positive if trace or above (1+, 2+, or 3+) was detected by semi-quantitative results. Laboratory considered positive for haematuria if erythrocytes >10 cells/uL and positive for leukocyturia if leukocytes > 25 cells/uL (based on cut-offs used on the POC device).

Results

Sensitivity and specificity were compared for haematuria and leukocyturia on fresh and frozen samples compared to laboratory measurements. Sensitivity for haematuria and leukocyturia decreased slightly post freezing: 78% vs 74%, and 88% vs 75% respectively. Specificity was unchanged post freezing for haematuria (68%) and slightly improved: 52% vs 67% for leukocyturia.

Table S1: Classification of haematuria and leukocyturia pre and post freezing compared to laboratory

Haematuria (fresh urine)				
	Positive POC (n=25)	Negative POC (n=20)		
Positive lab (n=23)	18	5	Sensitivity	0,78
Negative lab (n=22)	7	15	Specificity	0,68
Haematuria (post freezing)				
	Positive POC (n=24)	Negative POC (n=21)		
Positive lab ACR (n=23)	17	6	Sensitivity	0,74
Negative lab ACR (n=22)	7	15	Specificity	0,68
Leukocyturia (fresh urine)				
	Positive POC (n=31)	Negative POC (n=14)		
Positive lab (n=24)	21	3	Sensitivity	0,88
Negative lab (n=21)	10	11	Specificity	0,52
Leukocyturia (post freezing)				
	Positive POC (n=25)	Negative POC (n=20)		
Positive lab (n=24)	18	6	Sensitivity	0,75
Negative lab (n=21)	7	14	Specificity	0,67

Haematuria positive if red blood cells or haemoglobin detected in urine according to POC dipstick results

5.

Protocol

UNIVERSITY OF WITWATERSRAND

FACULTY OF HEALTH SCIENCES

SCHOOL OF MEDICINE

Research Protocol

The use of point of care testing for the diagnosis of chronic kidney disease

In Partial fulfillment of the requirements for the degree Master of Medicine: Chemical Pathology

Submitted by:

Dr Sean Currin (MBChB)

Student number: 350639

Supervisors:

Prof. Jaya George (MBBCh, DTM&H, MSc, FCPath, PHD)

Professor and Head

Department of Chemical Pathology

NHLS and University of Witwatersrand

Dr June Fabian (MBBCh, FCP (SA), Cert Neph(SA))

Wits Donald Gordon Medical Center

Department of Internal Medicine

University of Witwatersrand

Introduction

Sub-Saharan Africa (SSA), with a population of over 960 million people, suffers from high rates of infectious diseases, malnutrition, and chronic non-communicable diseases (NCDs) - many of which are risk factors for chronic kidney disease (CKD). Projections for developing regions including SSA, expect a dramatic increase in diabetes, hypertension, and obesity (1, 2) all of which may lead to an increased prevalence of CKD. A study of 570 participants from rural Kwa-Zulu Natal found that: 33% were HIV-positive with high rates of NCD risk factors; 71% were overweight (body mass index (BMI) 25 - 29.9 kg/m^2) or obese (BMI $\geq$ 30 kg/m^2); 4% had hyperglycaemia (random plasma glucose >11.0 mmol/l); 33% had hypertension (blood pressure >140/90 mmHg); and 20% had hyperlipidaemia (total cholesterol >5.2 mmol/l) (3). These are all risk factors for CKD.

The extent of CKD is largely unknown across SSA whilst the complications of CKD place a considerable burden on already stretched health care resources. The high cost of renal replacement therapy renders this condition fatal amongst the poor. A recent systematic review estimates the prevalence of CKD to be 13.9% in SSA(4). A single population based study in South Africa (SA) found that the prevalence of CKD was between 14.8 – 23.9% (5).

CKD is defined as "the presence of reduced kidney function (decreased glomerular filtration rate (GFR)) and/or kidney damage (e.g. increased urinary albumin excretion) for a minimum period of 3 months (6). Measurement of both GFR and a marker of kidney damage for example: albumin to creatinine ratio (ACR) is therefore necessary in the diagnosis of CKD. ACR is the most commonly used marker of kidney damage, it has been shown to be more sensitive than protein to creatinine ratio at detecting early kidney disease and is recommended in classifying the various stages of CKD (6, 7). A GFR <60 ml/min/1.73 m^2 implies the loss of approximately 50%

of kidney function and its significance lies in the association with an increased risk of kidney failure and death; whilst albuminuria confers an independent risk of kidney and cardiovascular disease (7).

GFR is considered the best overall index of kidney function and can be determined by the measurement of exogenous (Cr-EDTA, Iohexol, Iothalamate) or endogenous serum markers. Exogenous markers are impractical for routine clinical use due to their limited access, high cost and duration of time required for the test. As a result, various prediction equations have been developed that estimate GFR (eGFR) using endogenous markers (namely serum creatinine, but also cystatin C): e.g. Cockcroft and Gault (C+G), the Modification of Diet in Renal Disease (4-v MDRD) and Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations (8, 9). These equations are based on the measurement of serum creatinine which is influenced by muscle mass, sex, and diet. The equations adjust for muscle mass by the incorporation of a factor for sex and ethnicity. However, work from our center has shown that they perform better without this ethnicity adjustment and remain inaccurate (10, 11).

In many resource limited settings (RLS) diagnostic services are centralized and inaccessible to the majority of the population. To address this, the World Health Organization (WHO) has mandated new clinical diagnostic tools that can function in settings with limited access to a central laboratory. Point-of-care (POC) testing is testing conducted at or near the site of patient care. It can provide results to a clinician without having to wait days or even hours for sample transport and laboratory processing(12). Many POC tests have been designed for use in developed countries, and their application may not be readily transferrable to RLS. For example, in resource-replete emergency departments or intensive care units, the goal of POC testing is to obtain immediate test results to help guide an emergent intervention. In RLS, POC testing is

more applicable in outpatient clinics or mobile testing units; where the aim is to provide access to care through early diagnosis. In these settings, POC testing expedites diagnostic testing without requiring the services of a remote clinical laboratory. This accelerates treatment initiation, increases access to next steps in care, and improves health outcomes. Patients with NCDs will access healthcare facilities for prolonged periods- often lifelong, POC benefits these patients not only in aiding diagnosis but also in ensuring more efficient follow-up with actionable results available. POC testing also benefits healthcare providers by enabling them to make decisions, initiate treatment, and make appropriate referrals in a single visit, rather than on multiple visits; thus reducing workload. The development of POC tests for use in RLS has primarily focused on infectious diseases that require prompt diagnosis and treatment, such as HIV, tuberculosis, and malaria. For example, rapid HIV antibody tests are accurate and reliable, and increase the proportion of people who receive their test results with early initiation of treatment (13). Diagnosis of NCDs face similar barriers to infectious diseases, namely restricted access to central laboratories, lack of awareness about the need for testing in persons with minimal or no symptoms of disease, stigma, the high cost to patients of accessing clinic-based testing (including travel and time away from work), and inadequate or non-existent primary care services(14).

POC devices for the measurement of creatinine (with eGFR) and ACR are currently used in hospitals and clinics; their potential benefit in RLS is of interest in the diagnosis of CKD. Published studies of POC creatinine results compared to laboratory measured creatinine vary depending on population, manufacturer and sample type: e.g. whole blood vs capillary (15). In a study by Shephard et al. the Nova StatSensor was compared to IDMS traceable enzymatic laboratory creatinine; using a cut-off of 130µmol/L sensitivity of 86.8% and specificity of 100%

was reported, bias was however large at -47µmol/L in this population (16). This large bias was likely due to the poor concordance seen between devices and laboratory measured creatinine when creatinine is markedly raised (>600 µmol/L) as seen in other studies (15, 17). At these high creatinine values the clinical outcome or classification of patients into CKD staging may not be affected by the relative inaccuracy of POC devices at such levels. In a similar study by Haneder et al. of 401 patients in a radiology department the overall bias was 0 µmol/L (with 95% limits of agreement between -35 and 35) (18). To our knowledge no studies exist comparing POC eGFR to mGFR. A systematic review and meta-analysis in 2014 which reviewed 16 studies comparing Clinitek and DCA POC ACR systems (both Siemens HealthCare Diagnostics) and Aution POC ACR system (Arkray) to clinical laboratory ACR measurements showed combined sensitivity of 76% and combined specificity of 93% (19). This is in keeping with the conclusions drawn from the McTaggart et al study that the Clinitek ACR POC system demonstrates good rule-out value with relatively poor rule-in value (positive predictive value) (20). To date no studies have been published looking at the Sysmex UC-1000 semi-quantitative ACR POC system. Better patient outcomes with POC testing can be achieved even with decreased performance compared to laboratory-based testing if POC testing ensures improved linkage to care and retention in treatment (21).

Our study is nested within the larger study "Prevalence, Characterization and Response to Chronic Kidney Disease in an urban and rural setting in South Africa" based in Agincourt, Mpumalanga which aims to (i)determine the prevalence of CKD in a rural South African cohort (ii) establish an accurate method for estimating kidney function at community level in the South African population and regionally, in conjunction with collaborating sister studies and (iii) investigate the risk factors, including novel gene loci, for incident and progressive CKD in urban

and rural South African cohorts. Our aim is to evaluate POC testing versus laboratory testing for the diagnosis of CKD using creatinine measurement for the estimation of GFR and urine ACR for the measurement of urinary albumin excretion.

Study Objectives

- To compare POC eGFR by MDRD and CKD-EPI equations with mGFR by iohexol
- To compare POC serum creatinine to laboratory serum creatinine
- To compare POC ACR to laboratory ACR

Methods

Study design: prospective analytical study.

Study setting: the study will be conducted at the Wits Agincourt site and at the Contract Laboratory Services (CLS).

Study population: participants in the Agincourt cohort known with CKD (determined in previous studies) will be included in the study.

Sample size: 1000 serum samples will be analyzed from participants who will be separated into four groups based on eGFR: >90, 60 - 90; 45 – 59; and < 45. 650 urine samples will be obtained from these participants as evenly distributed from the four groups as is possible. Method comparison studies require a minimum of 40 samples which cover the measuring range of the test, classically 40 – 100 samples are used (22, 23). Previous studies comparing POC urine ACR to laboratory urine ACR with similar statistical tests applied have used sample sizes from 501 – 642 (20, 24, 25).

Participants will come in to the Agincourt site to have their GFRs measured using exogenous iohexol following standard practices. Preceding iohexol infusion venous blood will be collected by the attending nursing sisters in gel serum separator tubes for 1000 participants, a drop of venous blood from this sample will be analyzed on a POC creatinine device (Abbott Technologies iSTAT), additionally a fingerprick capillary sample will be collected and analyzed on the Nova Biomedical StatSensor POC creatinine device, 400 samples using a drop of venous blood will be analyzed on the Nova StatSensor. The Nova StatSensor and Abbot iSTAT both use the enzymatic method with amperometric detection of the generated H_2O_2 to determine creatinine. Commercially available quality control samples will be analysed with each run. At the same time a urine sample will be collected for ACR measurement from 650 participants. All samples (urine and venous blood) will be centrifuged, the serum and urine samples will then be stored at -80^{0}C and shipped to Johannesburg monthly. The point of care creatinine results will be recorded by the sisters and stored in the participant's files. (See attached standard operating procedures as appendices). Quality will be assessed and maintained by the regular performance and recording of quality control samples which will be monitored. The Abbott iSTAT was selected as it is widely used in clinics and hospitals throughout Gauteng, the Nova StatSensor was selected due to its rapid action (within 30 seconds) and provision of an eGFR result, the Sysmex UC-1000 was selected as no published studies have been done on this platform to date.

Serum creatinine will be run at CLS laboratory on a Cobas 501 (Roche Diagnostics, Mannheim) by the kinetic Jaffe method which is traceable to isotope-dilution mass spectrometry reference method. The laboratory creatinine results will be compared to the POC creatinine results. The eGFR by POC (using the MDRD equation as well as CKD-EPI equations) will be compared to the measured GFR obtained by iohexol method.

The frozen urine samples will be transported to NHLS Charlotte Maxeke Johannesburg Academic Hospital Laboratory. Samples will be thawed, mixed and separated; half of the sample will be run on the laboratory Roche Cobas 8000 analyzer to determine laboratory albumin to creatinine ratio (ACR), the other half of the sample will be analyzed with the Sysmex Corporation POC ACR platform. The Sysmex corporation system is a semi-automated semi-quantitative urine chemistry analyzer (UC-1000) which optically reads inserted test strips colour changes. The laboratory ACR will then be compared to the POC ACR. Commercially available quality control samples will be analyzed with each run to ensure quality.

Data Analysis

POC eGFR to mGFR

We will calculate the eGFR with the POC creatinine results using the following equations:
MDRD equation with and without the African American correction factor:

- eGFR (mL/min/1.73m^2 = 175 x [S-Cr (µmol/L)]$^{-1.154}$ x age (years)$^{-0.203}$ x (0.742 if female) x (1.212 if African American)

2009 CKD-EPI creatinine eGFR equation with and without the African American correction factor:

- eGFR (mL/min/1.73m^2) = 141 x min(S_{Cr}/κ, 1)$^{\alpha}$ x max(S_{Cr}/κ, 1)$^{-1.209}$ x 0.993^{Age} x (1.018 if female) x (1.159 if African American)

  Note: Scr (µmo/L), κ = 61.9 (females) or 79.6 (males), α = -0.329 (females) or -0.411 (males), min = indicates the minimum of S_{Cr}/κ or 1, max = indicates the maximum of S_{Cr}/κ or 1, age = years

Data will be expressed as mean ± SD if parametric and median (interquartile range, IQR) if non-parametric. Method comparison will be performed with Bland Altman bias plots and the significance of the bias compared to mGFR will be tested with Wilcoxon match pairs signed ranks test. Deming regression will be used to calculate the correlation coefficients and coefficient of determination will be calculated. Calculation of mean percentage bias, standard deviation of the difference and 95% limits of agreement will be performed as well as accuracy within 10% (P10) and accuracy within 30% (P30) of mGFR. Receiver operating characteristics (ROC) curve analysis will be assessed for the sensitivity and specificity to correctly predict eGFR<90 and < 60 ml/min/1.73m^2 and misclassification of patients around these levels with eGFR identified. All data will be assessed for normality using Shapiro-Wilk test. A p value of <0.05 is considered significant. If appropriate a correction factor will be determined for POC eGFR to better reflect mGFR.

POC creatinine to laboratory creatinine (serum)

Method comparison for creatinine performance on POC instruments versus laboratory creatinine will be conducted using CLSI EP9 protocol (26), bias will be analysed using Bland Altman plots, Deming (weighted) regression will be used to calculate the correlation coefficient and co-efficient of determination will be calculated.

POC ACR (Urine) to laboratory ACR

Urine analysis for albumin and creatinine will be performed on a Cobas 8000 (Roche Diagnostics) from which ACR will be calculated as the laboratory gold standard or reference method to which the POC methods will be compared. The POC device classifies ACR (mg/mmol) into 1 of 3 groups: normal (<3.4), microalbuminuria (3.4 – 33.9), and

macroalbuminuria (>33.9). Any urine sample that is too dilute to obtain an ACR by POC or laboratory ACR will be excluded from the study.

Laboratory ACR ≥ 3.4mg/mmol will be considered as positive, whilst values <3.4mg/mmol will be considered as negative. For POC testing: samples in the groups of 3.4 – 33.9mg/mmol or >33.9mg/mmol will be considered positive, whilst samples falling in the <3.4mg/mmol group will be considered negative by point of care method. The prevalence of albuminuria, sensitivity, specificity, positive and negative predictive values, and likelihood ratios of the point of care system will be calculated using standard approaches. ROC curve analysis will be performed.

Research outputs

1) The research report will go towards the requirement of obtaining a Master of Medicine: Chemical Pathology degree for Dr SD Currin
2) The findings of the research will guide the use of POC devices for the diagnosis of patients with CKD, especially in RLS
3) The publication of findings in a peer reviewed journal

Ethical consideration

Ethics has been approved by the Human Research Ethics Committee (Medical) of the University of Witwatersrand for the parent study to which this research is attached, clearance certificate number M160937. Individual ethical clearance has been applied for through the same committee.

Timing

	Feb	Mar	Apr	May	Jun	Aug	Oct	Dec

	‘19	‘19	‘19	‘19	and Jul ‘19	and Sep ‘19	and Nov ‘19	’19
Preparation								
Literature review								
Prepare protocol								
Submit to MMed committee for assessment								
Submit application for ethical clearance								
Study phase								
Sample and data collection								
Sample analysis								
Data analysis								
Writing of report								

Funding

Kits will be provided for the study from the different point of care manufacturers, including quality control materials and strips.

Costs:

1) Laboratory analysis:

- Urine albumin R73.38/ sample

- Urine creatinine R30.35/ sample
- TOTAL for ACR: R103.73/ sample
- Therefore total cost for 650 ACR samples= R67 424.50

2) Publication cost: R15 000

3) Travel costs (present at local and international conferences) R25 000

TOTAL: R107 424.50

An application for funding has been applied for through the Medical Faculty Research Endowment Fund. Additional funding will also be applied for through the NHLS Research Trust Development Grant.

Problems

All urine samples are coming from participants with some degree of CKD, as such the distribution of ACR results cannot be guaranteed. There may be fewer than desired ACR in each range.

The POC creatinine results are being compared to laboratory creatinine (Jaffe method) results. Although the Jaffe method is traceable to isotope dilution mass spectrometry reference method, the preferred method for the laboratory creatinine results is the enzymatic method.

No international reference method currently exists for urine albumin, although various groups are working on the harmonization of this analyte. As such bias in the laboratory urine albumin results cannot be excluded.

Resources

1. Kelly T, Yang W, Chen CS, Reynolds K, He J. Global burden of obesity in 2005 and projections to 2030. Int J Obes (Lond). 2008;32:1431-7.

2. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006;3:e442.

3. van Heerden A, Barnabas RV, Norris SA, Micklesfield LK, van Rooyen H, Celum C. High prevalence of HIV and non-communicable disease (NCD) risk factors in rural KwaZulu-Natal, South Africa. J Int AIDS Soc. 2017;20:e25012.

4. Stanifer JW, Jing B, Tolan S, Helmke N, Mukerjee R, Naicker S. The epidemiology of chronic kidney disease in sub-Saharan Africa: a systematic review and meta-analysis. Lancet Glob Health. 2014;2:e174.

5. Matsha TE, Yako YY, Rensburg MA, Hassan MS, Kengne AP, Erasmus RT. Chronic kidney diseases in mixed ancestry south African populations: prevalence, determinants and concordance between kidney function estimators. BMC Nephrol. 2013;14:75.

6. K/DOQI clinical practice guidelines for chronic kidney disease:: evaluation, classification, and stratification. Am J Kidney Dis. 2002;39:S1.

7. Inker LA, Astor BC, Fox CH, Isakova T, Lash JP, Peralta CA, et al. KDOQI US Commentary on the 2012 KDIGO Clinical Practice Guideline for the Evaluation and Management of CKD. Am J Kidney Dis. 2014;63:713-35.

8. Levey AS, Bosch JP, Lewis JB. A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Int Med. 1999;130:461-70.

9. Levey AS, Stevens LA, Schmid CH, Zhang YL, Castro AF, Feldman HI. A new equation to estimate glomerular filtration rate. Ann Intern Med. 2009;150:604-12.

10. van Deventer HE, George JA, Paiker JE, Becker PJ, Katz IJ. Estimating glomerular filtration rate in black South Africans by use of the modification of diet in renal disease and Cockcroft-Gault equations. Clin Chem. 2008;54:1197-202.

11. van Deventer HE, Paiker JE, Katz IJ, George JA. A comparison of cystatin C- and creatinine-based prediction equations for the estimation of glomerular filtration rate in black South Africans. Nephrol Dial Transplant. 2011;26:1553-8.

12. Urdea M, Penny LA, Olmsted SS, Giovanni MY, Kaspar P, Shepherd A, et al. Requirements for high impact diagnostics in the developing world. Nature. 2006;444 S1:73-9.

13. Malonza IM, Richardson BA, Kreiss JK, Bwayo JJ, Stewart GC. The effect of rapid HIV-1 testing on uptake of perinatal HIV-1 interventions: a randomized clinical trial. AIDS. 2003;17:113-8.

14. Petti CA, Polage CR, Quinn TC, Ronald AR, Sande MA. Laboratory medicine in Africa: a barrier to effective health care. Clin Infect Dis. 2006;42:377-82.

15. Martinez Lomakin F, Tobar C. Accuracy of point-of-care serum creatinine devices for detecting patients at risk of contrast-induced nephropathy: a critical overview. Crit Rev Clin Lab Sci. 2014;51:332-43.

16. Shephard M, Peake M, Corso O, Shephard A, Mazzachi B, Spaeth B, et al. Assessment of the Nova StatSensor whole blood point-of-care creatinine analyzer for the measurement of kidney function in screening for chronic kidney disease. Clin Chem Lab Med. 2010;48:1113-9.

17. Kosack CS, de Kieviet W, Bayrak K, Milovic A, Page AL. Evaluation of the Nova StatSensor(R) Xpress(TM) Creatinine point-of-care handheld analyzer. PLoS One. 2015;10:e0122433.

18. Haneder S, Gutfleisch A, Meier C, Brade J, Hannak D, Schoenberg SO, et al. Evaluation of a handheld creatinine measurement device for real-time determination of serum creatinine in radiology departments. World J Radiol. 2012;4:328-34.

19. McTaggart MP, Newall RG, Hirst JA, Bankhead CR, Lamb EJ, Roberts NW, et al. Diagnostic accuracy of point-of-care tests for detecting albuminuria: a systematic review and meta-analysis. Ann Intern Med. 2014;160:550-7.

20. McTaggart MP, Price CP, Pinnock RG, Stevens PE, Newall RG, Lamb EJ. The diagnostic accuracy of a urine albumin-creatinine ratio point-of-care test for detection of albuminuria in primary care. Am J Kidney Dis. 2012;60:787-94.

21. Drain PK, Hyle EP, Noubary F, Freedberg KA, Wilson D, Bishai WR, et al. Diagnostic point-of-care tests in resource-limited settings. Lancet Infect Dis. 2014;14:239-49.

22. JO Westgard. Method Validation: The Comparison of Methods Experiment. 3rd edition ed: Westgard QC, Inc; 2008.

23. Linnet K. Necessary sample size for method comparison studies based on regression analysis. Clin Chem. 1999;45:882-94.

24. Kumar V, Hebbar S, Bhat A, Panwar S, Vaishnav M, Muniraj K, et al. Application of a Nanotechnology-Based, Point-of-Care Diagnostic Device in Diabetic Kidney Disease. Kidney Int Rep. 2018;3:1110-8.

25. Davidson EM, Croal BL. Introduction of an Albumin-to-Creatinine Ratio Point-of-Care Device: Analytic, Clinical, and Cost-effectiveness Aspects. Point Care. 2003;2:89-95.

26. PA Wayne. Selection Criteria for Point of Care testing devices; Approved guideline. Clinical Laboratory and Standards Institute document POCT 09-A. 2010.

Appendix

SOP: LABORATORY, NURSES AND FIELDWORKERS

POINT OF CARE: QC PROCEDURE FOR THE NOVA STAT SENSOR FOR CREATININE MEASUREMENT

SITE	AGINCOURT – SOUTH AFRICA		
TITLE	Point of Care: QC for the NOVA STAT SENSOR for creatinine measurement		
VERSION	1.0		
DIVISION	Phase 2: ARK Laboratory Staff		
PURPOSE	To ensure that procedures for the QC for the NOVA STAT SENSOR are followed in Phase 2 of the ARK Study		
CONTENTS	1.0 PURPOSE and SCOPE 2.0 RESPONSIBILITIES AND SAFETY 3.0 EQUIPMENT 4.0 PROCEDURE		
WRITTEN BY	June Fabian		
APPROVAL	NAME	SIGNATURE	DATE
	June Fabian		
	Mwawi Gondwe		
	Busisiwe Mayindi		
	Shingirai Chipungu		

1.0 PURPOSE AND SCOPE

To describe the use of the NOVA STAT SENSOR portable handheld analyzer used to analyze capillary and venous blood for creatinine and eGFR at the point of care.

The NOVA STAT SENSOR analyzer contains a microprocessor that performs all calculations for reporting results. Results are displayed numerically with the appropriate units.

The aim of the procedure is to ensure that appropriate validation of the creatinine measurement using the NOVA STAT SENSOR analyser allows for comparison to:

- Serum creatinine as measured by CLS
- Measured GFR, as measured by the NHLS research laboratory and the

- Depending on the results above, this would allow for correlation and development of an appropriate correction factor for eGFR with the NOVA STAT SENSOR point of care device.

2.0 RESPONSIBILITIES AND SAFETY

It is the responsibility of the Agincourt Research Laboratory Manager to ensure that equipment is appropriately cleaned, maintained in good working order, and available for research personnel as requested.

It is the responsibility of the principal investigator and managerial staff to ensure that all research and technical staff are adequately trained to use the NOVA STAT SENSOR system.

All sterile procedures, as per standard laboratory practice and according to standard universal precautions for nurses must be followed during this procedure.

3.0 EQUIPMENT

Ensure all the required materials are available

- Non-sterile gloves
- NOVA STAT SENSOR test strip
- NOVA STAT SENSOR machine (must be charged)
- NOVA STAT SENSOR QC vials – level 1 and 3
- Recording sheets

4.0. PROCEDURE

The QC procedure will be performed each morning prior to commencement of testing of ARK participants: QC level 1 (low levels of creatinine) and level 3 (high levels of creatinine). The QC level 1 and 3 testing will be done every alternate day, i.e. Level 1 on day 1, level 3 on day 2, except for when a new container of test strips is opened, in which case, both level 1 and 3 will be conducted on the same day – which is the day of issue of the new container to the research team.

About the machine and the test strips

- The NOVA STAT SENSOR machine will be stored in the Agincourt Research Laboratory (ARL)
- Each morning, before commencement of the study procedures, the nurses and fieldworkers will fetch the NOVA STAT SENSOR machine from the ARL and the machine will be returned at the end of each day.
- The nurses and fieldworkers will check that NOVA STAT SENSOR has been charged overnight and is in good working order. If not, this will be reported to the ARL manager, and the Project Site Manager (PSM)

- Only fresh blood samples can be used with the NOVA STAT SENSOR.
- The fieldworkers will perform a capillary blood specimen at the time of the arrival of all participants – before iohexol is administered
- The nurses will use a drop of venous blood, taken as part of the baseline bloods before iohexol is administered
- The specimen should be collected carefully, handled properly to ensure accurate results and tested immediately for the most accurate results.
- Test strips are stored in the fridge between 2-8 degrees Celsius in the ARL fridge. They must not be frozen.
- The test strips will be stored at room temperature by the nurses and the fieldworkers after they have been issued by the ARL.
- Once opened, the test strips can be stored at room temperature for 90 days. The test strip container must be closed as soon as the test strip has been removed for testing.

About the QC testing material

- The manufacturer has supplied level 1 and level 3 creatinine vials for testing
- These vials need to be stored between 2 – 8 degrees Celsius – even after they have been opened
- When performing the QC procedure, remove from the fridge, perform the test, and place back into fridge as soon as the procedure is completed

QC Procedure for Level 1 and Level 3

- Switch the machine on
- At the bottom of the screen, touch the "login"
- The red bar at the top will ask you to "Enter operator ID"
- To enter your operator ID, touch the screen, entering your 3 digit operator ID and then press the small green "accept" sign at the bottom of the screen
- The red bar at the top will ask say "Patient Test"
- On this screen, at the bottom, you will see "QC" - touch the "QC" box to select the QC test, then press the small green "accept" sign at the bottom of the screen
- The red bar at the top will ask you to enter "Strip Lot"
- At the bottom left of the screen, touch the "erase" sign (this will remove the test strip number from the least test that was performed)
- Then, at the bottom left of the screen, touch the "scan" sign and line up the horizontal red line of the scanner with the middle of the barcode to scan it
- Once the number is scanned, it will appear at the top of the screen – check that the number on the screen corresponds with the lot number next to the barcode on the test strip container, and then press the small green "accept" sign at the bottom of the screen

- The red bar at the top will ask you to "Enter QC lot"
- Then, take the level 1 QC vial, touch the "scan" sign and line up the horizontal red line of the scanner with the middle of the barcode on the level 1 QC vial to scan it
- Once the number is scanned, it will appear at the top of the screen – check that the number on the screen corresponds with the lot number next to the barcode on the test strip container, and then press the small green "accept" sign at the bottom of the screen
- Then insert the creatinine test strip, with the white part at the top – and apply one drop of the level 1 QC liquid
- **The test strip must fill completely. If the test strip does not fill completely, do not touch the test strip to add more liquid, discard the test strip and repeat the test with a new strip.**
- In 30 seconds the results will display on the screen as Creat, with a value in umol / L
- Please record this on the QC logging sheet
- If out of range, please inform the Agincourt Laboratory Manager, who will then inform the PI of the study.
- The machine will now be ready for the next test
- Repeat all the above procedures using the level 3 QC vial for the second component of the QC

REFERENCE RANGES FOR CREATININE QC FOR THE NOVOSTAT SENSOR

- ***Level 1: low creatinine (umol/L): 44 – 84 – 124 range***
  Using this QC material, this is interpreted as: lowest acceptable level is 44umol/L; highest acceptable level is 124umol/L and mean is 84umol/L. If the level recorded by the device is out of range is lower than 44 or higher than 124, then this must be reported to the laboratory manager and the PI of the study

- ***Level 3: high creatinine (umol/L): 398 – 530 – 663 range***
  Using this QC material, this is interpreted as: lowest acceptable level is 398umol/L; highest acceptable level is 663umol/L and mean is 530umol/L. If the level recorded by the device is out of range i.e. lower than 398 or higher than 663, then this must be reported to the laboratory manager and the PI of the study.

Full Name	Operator ID for the NOVA STAT SENSOR machine
Shingirai Chipungu	001
Bongekile Khoza	002
Conrad Mogane	003
Phumzile Dlamini	004
Florah Sihlangu	005
Nontsikeleko Patients Mahime	006
Fortunate Ngobeni	007
Busisiwe Mayindi	008
Mwawi Gondwe	009

June Fabian	010
Nyiko or Bianca	011

SIGNATURES

This signature page indicates that you have read and understood this SOP.

Full Name	Role in the study	Signature	Date

SOP: LABORATORY, NURSES AND FIELDWORKERS

POINT OF CARE: QC PROCEDURE FOR THE i-STAT SENSOR FOR CREATININE MEASUREMENT

SITE	AGINCOURT – SOUTH AFRICA		
TITLE	Point of Care: QC for the –STAT blood analyser for creatinine measurement		
VERSION	1.0		
DIVISION	Phase 2: ARK Laboratory Staff		
PURPOSE	To ensure that procedures for the QC for the i-STAT blood analyser are followed in Phase 2 of the ARK Study		
CONTENTS	5.0 PURPOSE and SCOPE 6.0 RESPONSIBILITIES AND SAFETY 7.0 EQUIPMENT 8.0 PROCEDURE		
WRITTEN BY	June Fabian		
APPROVAL	NAME	SIGNATURE	DATE
	June Fabian		
	Mwawi Gondwe		
	Busisiwe Mayindi		
	Shingirai Chipungu		

3.0 PURPOSE AND SCOPE

To describe the use of the i-STAT blood analyser portable handheld analyzer used to analyze capillary and venous blood for creatinine and eGFR at the point of care.

The i-STAT blood analyser contains a microprocessor that performs all calculations for reporting results. Results are displayed numerically with the appropriate units.

The aim of the procedure is to ensure that appropriate validation of the creatinine measurement using the i-STAT blood analyser allows for comparison to:

- Serum creatinine as measured by CLS
- Measured GFR, as measured by the NHLS research laboratory and the

- Depending on the results above, this would allow for correlation and development of an appropriate correction factor for eGFR with the i-STAT blood analyser point of care device.

4.0 RESPONSIBILITIES AND SAFETY

It is the responsibility of the Agincourt Research Laboratory Manager to ensure that equipment is appropriately cleaned, maintained in good working order, and available for research personnel as requested.

It is the responsibility of the principal investigator and managerial staff to ensure that all research and technical staff are adequately trained to use the i-STAT blood analyser.

All sterile procedures, as per standard laboratory practice and according to standard universal precautions for nurses must be followed during this procedure.

3.0 EQUIPMENT

Ensure all the required materials are available

- Non-sterile gloves
- i-STAT blood analyser cartridge
- i-STAT blood analyser machine (must be charged)
- i-STAT blood analyser QC vials – level 2
- Recording sheets

4.0. PROCEDURE

The QC procedure will be performed once weekly: QC level 2 creatinine.

About the machine and the test strips

- The i-STAT blood analyser will be stored in the Agincourt Research Laboratory (ARL)
- Each morning, before commencement of the study procedures, the nurses and fieldworkers will fetch the i-STAT blood analyser from the ARL and the machine will be returned at the end of each day.
- The nurses and fieldworkers will check that i-STAT blood analyser has been charged overnight and is in good working order. If not, this will be reported to the ARL manager, and the Project Site Manager (PSM)
- Only fresh blood samples can be used with the i-STAT blood analyser.
- The fieldworkers will perform a capillary blood specimen at the time of the arrival of all participants – before iohexol is administered

- The nurses will use a drop of venous blood, taken as part of the baseline bloods before iohexol is administered
- The specimen should be collected carefully, handled properly to ensure accurate results and tested immediately for the most accurate results.
- Test strips are stored in the fridge between 2-8 degrees Celsius in the ARL fridge. They must not be frozen.
- The test strips will be stored at room temperature by the nurses and the fieldworkers after they have been issued by the ARL.
- Once opened, the test strips can be stored at room temperature for 90 days. The test strip container must be closed as soon as the test strip has been removed for testing.

About the QC testing material

- The manufacturer has supplied level 2 creatinine vials for testing
- These vials need to be stored between 2 – 8 degrees Celsius – even after they have been opened
- When performing the QC procedure, remove from the fridge, perform the test, and place back into fridge as soon as the procedure is completed
- For the QC vial that has been opened, cover with tape when returning to the fridge until the contents of the vial have been used

QC Procedure for Level 2

- Switch on the i-STAT blood analyser (bottom right button)
- Press "MENU" – bottom left button
- Press option "3" for Quality tests
- Press option "1" for Control tests
- Enter your operator ID
- Confirm your operator ID by entering a second time
- Scan the level 2 control lot number
- Scan the test cartridge lot number
- Remove the test cartridge from the packaging
- Place the test cartridge on a flat surface
- Use a pipette tipped syringe to add 95ul to the test cartridge sample well
- Insert the test cartridge into the device
- Record the creatinine (umol/L) on the i-STAT QC logging sheet
- NB: Do not attempt to remove the cartridge while it is running the test

REFERENCE RANGES FOR CREATININE QC FOR THE i-STAT SENSOR

- ***Level 2: for LOT 311103***

These have been received from the manufacturer (see separate document)

Creatinine (umol/L): mean = 88; minimum = 53; maximum 124

Full Name	Operator ID for the i-STAT blood analyser
Shingirai Chipungu	001
Bongekile Khoza	002
Conrad Mogane	003
Phumzile Dlamini	004
Florah Sihlangu	005
Nontsikeleko Patients Mahime	006
Fortunate Ngobeni	007
Busisiwe Mayindi	008
Mwawi Gondwe	009
June Fabian	010
Nyiko or Bianca	011

SIGNATURES

This signature page indicates that you have read and understood this SOP.

Full Name	Role in the study	Signature	Date

6.

Appendix A: Ethics Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Dr Sean Currin

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M190434

NAME: (Principal Investigator)	Dr Sean Currin
DEPARTMENT:	School of Pathology, Chemical Pathology Agincourt Study Site, Mpumalanga
PROJECT TITLE:	The use of point of care testing for the diagnosis of chronic kidney disease
DATE CONSIDERED:	2019/04/26
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Jaya George and June Fabian
APPROVED BY:	Dr. CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	30/05/2019

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **April** and will therefore be due in the month of **April** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date 31/05/2019

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

7.

Appendix B: Turnitin Reports

a0044271:CCLM4.docx

by Sean Currin

Submission date: 10-Mar-2021 07:05AM (UTC-0800)
Submission ID: 1529326875
File name: 9e5c_Assignments_dd95b938-05d7-42e6-948b-32a0a36a046f_CCLM4.docx (100.56K)
Word count: 6604
Character count: 37223

Evaluating chronic kidney disease in rural South Africa: comparing estimated glomerular filtration rate using point of care creatinine to iohexol measured GFR

Sean Currin[1,2], Mwawi Gondwe[3], Nokthula Mayindi[3], Shingirai Chipungu[3], Bongekile Khoza[3], Lungile Khambule[1], Tracy Snyman[1,2], Stephen Tollman[3,4], June Fabian[3,5], Jaya George[1,2], on behalf of the ARK Consortium

[1] Department of Chemical Pathology, University of Witwatersrand, Johannesburg, South Africa

[2] Department of Chemical Pathology, National Health Laboratory Service, South Africa

[3] Medical Research Council/Wits University Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

[4] International Network for the Demographic Evaluation of Populations and their Health (INDEPTH) Network, Accra, Ghana

[5] Wits Donald Gordon Medical Centre, School of Clinical Medicine, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

Correspondence:

Dr Sean Currin

Chemical Pathology Registrar

University of Witwatersrand, Faculty of Health Sciences, Department of Chemical Pathology

7 York Road, Parktown, Johannesburg, 2193, South Africa

Telephone: +2711 489 8499

Email: sean.currin@wits.ac.za

Word count: 3500

Number of tables and figures: 8

Supplementary included: 5 figures and tables

Short title: POC eGFR vs mGFR

Abstract

Introduction: The prevalence of chronic kidney disease is rising rapidly in low- and middle-income countries. Serum creatinine and estimation of glomerular filtration rate (GFR) are critical diagnostic tools, yet access to centralised laboratory services remains limited in primary care resource-limited settings. The aim of this study was to evaluate point-of-care (POC) technologies for serum creatinine measurement and to compare their performance to a gold standard measurement using iohexol mGFR.

Methods: POC creatinine was measured using iSTAT® and StatSensor® devices in capillary and venous whole blood, and laboratory creatinine was measured using the compensated kinetic Jaffe method in 670 participants from a rural area in South Africa. GFR estimating equations (CKD-EPI and MDRD) for POC and laboratory creatinine were compared to iohexol mGFR.

Results: Calculated GFR for laboratory and POC creatinine measurements overestimated GFR (positive bias of 1.9 - 34.1ml/min/1.73m^2). However, all POC devices had less positive bias than the laboratory Jaffe method (1.9 - 14.7 vs 34.1 for MDRD, and 8.4 – 19.9 vs 28.6 for CKD-EPI). Accuracy within 30% of mGFR ranged from 0.56 – 0.72 for POC devices and from 0.36 – 0.43 for the laboratory Jaffe method. POC devices showed wider imprecision with coefficients of variation ranging from 4.6 - 10.2% compared to 3.5% for the laboratory Jaffe method.

Conclusion: POC eGFR showed improved performance over laboratory Jaffe eGFR, however POC devices suffered from imprecision and large bias. The laboratory Jaffe method performed poorly, highlighting the need for laboratories to move to enzymatic methods to measure creatinine.

Keywords: Point-of-care, creatinine, chronic kidney disease, eGFR, iohexol mGFR, LMICs

Introduction

Chronic kidney disease (CKD) has a prevalence of 10.7% in sub-Saharan Africa, and is associated with significant morbidity and mortality [1]. Low- and middle-income countries (LMIC) suffer a disproportionate mortality associated with CKD when compared to high-income countries [2]. Therefore, screening strategies that aim to identify patients with early stages of CKD in primary care settings and ideally, prevent progression of CKD, are of profound importance.

Glomerular filtration rate (GFR) is considered the best overall index of kidney function and is an important criterion in the diagnosis and staging of CKD [3]. GFR cannot be quantitated directly. However, it can be assessed by the measurement of exogenous (Inulin, Cr-EDTA, Iohexol, Iothalamate) or endogenous markers [4]. Exogenous markers are impractical for routine clinical use due to their limited access, high cost and duration of time required for testing. Estimated GFR (eGFR) equations based on endogenous markers and additional factors, such as age and sex, are recommended for the routine assessment of kidney function [3]. The creatinine based Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) and Modification of Diet in Renal Disease (MDRD) equations are the most commonly used [5]. The 2009 CKD-EPI creatinine equation is recommended for use in adults [3].

In resource limited settings a lack of access to central laboratory services hinders the ability to implement effective CKD screening programs. Even when central laboratory services are available, logistical factors in primary health care clinics can impact the accuracy of creatinine measurements, such as sub-optimal storage of collected samples and delays in transport to the central laboratory [6]. Point-of-care (POC) testing offers an important solution to this problem by offering access to actionable results in real-time for clinical decision making [7]. While the 2012 KDIGO guidelines recognise that limited laboratory access is a problem, they fall short of recommending POC testing in such settings [8].

The aim of our study was to evaluate the performance of POC creatinine by comparing eGFR equations to iohexol measured GFR (mGFR), and to compare this performance with that of laboratory eGFR. Additionally, we looked at the performance of the MDRD and CKD-EPI equations for these different creatinine measurements.

Methods

Our study formed part of the African Research on Kidney (ARK) study, which aimed to determine the population prevalence of CKD and associated risk factors in three sub-Saharan African countries, Malawi, South Africa, and Uganda. Detailed methods for the ARK study have been published [9]. The South African component was conducted in the Agincourt Health and Demographic Surveillance Site in Bushbuckridge, a rural sub-district of the Mpumalanga province in South Africa.

Participants from the community were invited to participate in this second phase of the study, to measure GFR using intravenous iohexol. The initial study comprised a population-based sample of 2021 adults aged 20-80 years to determine CKD prevalence and investigate associated risk factors. For the iohexol study, we stratified a subsample of the main study by sex and eGFR to ensure adequate sampling across all stages of CKD.

Measured GFR was determined by plasma excretion of iohexol. A five-millilitre bolus of intravenous iohexol (Omnipaque™, GE Healthcare, Illinois) was administered in the antecubital fossa of one arm, followed by repeat plasma sampling from the antecubital fossa of the contralateral arm after 5, 120, 180 and 240 minutes. Prior to iohexol administration a baseline venous blood sample was obtained, which was used for real-time testing with the iSTAT® (Abbott Laboratories, Illinois) and StatSensor® (Nova Biomedical Corporation, Massachusetts) POC devices. At the same time, a capillary blood sample taken from a fingerprick was also analysed on the StatSensor® POC device. There were no adverse events related to the intravenous administration of iohexol.

On the day of iohexol testing, for each participant, samples for creatinine and iohexol were refrigerated (2 – 8^{0}C) until all participants for the day had been processed (usually by 15h00), after which all samples were centrifuged at 2000G for 10 minutes. Samples were stored and transported at -80^{0}C to a central laboratory for iohexol and laboratory creatinine measurements.

Plasma concentrations of iohexol were determined using ultraperformance liquid chromatography with tandem mass spectrometry using a published method [10] in an ISO 15189 accredited laboratory which participates in the EQUALIS external quality assurance programme for iohexol (Uppsala, Sweden). The calibration curve and internal standard were determined with certified reference materials for iohexol (CRM: USP H0J211) and ioversol (CRM: USP 34510F), respectively. Internal quality control was prepared using the iohexol certified reference material and EQUALIS samples were included in every run as an additional quality check. The slope-intercept method using the 120, 180 and 240 time points (during the second exponential phase of iohexol elimination) was used to calculate mGFR by plotting iohexol concentration against time [11]. mGFR results were then corrected for body surface area [12].

Laboratory creatinine was determined in a central laboratory using an isotope dilution mass spectrometry (IDMS) traceable compensated kinetic Jaffe method (Cobas c500®, Roche Diagnostics, Mannheim). POC creatinine was measured at the time of collection in venous whole blood on the iSTAT® and in venous whole blood and capillary blood on the StatSensor®, both POC devices make use of an enzymatic amperometric method with an enzyme cascade reaction. Laboratory creatinine was monitored with internal and external quality control schemes, while POC creatinine was monitored with internal quality control. Inter-day coefficients of variation (CV)s were determined by assessing the variation in quality control materials over the period of the study.

Statistical analysis was performed using Tibco Statistica® (version 13.5.0.17, United States) and MedCalc® (version 19.2.1, Belgium). POC creatinine was compared to laboratory creatinine using Passing-Bablok regression and Bland-Altman agreement. Estimated glomerular filtration rate (eGFR)

was calculated according to the IDMS-traceable MDRD equation and the 2009 CKD-EPI equation. All subsequent eGFR results are presented without the African-American coefficient applied, based on prior studies [13,14] demonstrating that inclusion of the coefficient overestimates GFR in sub-Saharan Africans. eGFR was compared to mGFR with Passing-Bablok regression, Spearman rank correlation coefficient (r) and Lin's concordance correlation coefficient were determined. Bias was defined as the median of individual differences between eGFR and mGFR, while imprecision was defined as the interquartile range (IQR) of the differences between eGFR and mGFR. Outliers were excluded if their residuals lay outside of 2.5 standard deviations on initial Passing-Bablok regression [15,16]. Accuracy within 30% of mGFR (P30) was calculated. The ability of the eGFR equations to correctly classify mGFR: <60, 60 – 89, and ≥90mL/min/1.73m^2 (corresponding to KDIGO GFR categories for CKD of G3-5, G2 and G1, respectively) was calculated. Differences between the laboratory and POC devices were tested using the exact McNemar test. Results were considered statistically significant if $p < 0.05$.

Repeat analysis with laboratory Jaffe creatinine adjusted by a factor of +9.0µmol/L was performed (Supplementary Table S1). This adjustment was based on an inter-site calibration study performed within the larger ARK study comparing laboratory Jaffe creatinine methodology performed in South Africa and Malawi with an enzymatic laboratory creatinine method in Uganda.

Results

Study population

Figure 1 depicts the flow of participants through the study. All participants were Black African adults with a broad spectrum of kidney function. eGFR equations for laboratory and POC creatinine overestimated GFR when compared to mGFR, except in the ≥90mL/min/1.73m^2 group where a

mixed picture was seen (Table 1). Mean POC eGFRs showed less overestimation compared to mGFR than the mean laboratory eGFRs.

POC vs laboratory creatinine

All three POC creatinine devices showed positive bias compared to laboratory creatinine with Bland-Altman agreements of 17.4 ± 15.3, 9.4 ± 6.3, and 15.1 ± 13.0 µmol/L for StatSensor® capillary, iSTAT®, and StatSensor® venous respectively. Passing-Bablok regression analysis yielded proportional errors (slope) of: 1.45 (95%CI: 1.33 to 1.57), 1.16 (95%CI: 1.13 to 1.20), and 1.17 (95%CI: 1.03 to 1.33); constant errors (y-intercept) of: -7.68 (95%CI: -14.43 to -1.33), 0.16 (95%CI: -2.20 to 2.13), and 6.50 (95%CI: -2.33 to 14.58); and Spearman rank correlation coefficients (r) of: 0.57 (95%CI: 0.52 to 0.62) (p<0.0001), 0.92 (95%CI: 0.91 to 0.93) (p<0.0001), and 0.65 (95%CI: 0.57 to 0.72) (p<0.0001) for StatSensor® capillary, iSTAT®, and StatSensor® venous respectively. (Figure 2)

eGFR (using POC creatinine and laboratory creatinine) vs mGFR

Passing-Bablok regression demonstrated that all eGFR equations using POC and laboratory creatinine measurements suffered from proportional and constant error when compared to mGFR. The largest constant error was seen in laboratory creatinine eGFR using the CKD-EPI equation whilst the smallest constant error was seen in StatSensor® capillary creatinine eGFR using the MDRD equation (y-intercept: 62.76 vs 3.91). StatSensor® capillary creatinine eGFR using the MDRD equation showed the least proportional error, while laboratory creatinine eGFR using the CKD-EPI equation showed the most proportional error (slope: 1.01 vs 0.59). MDRD eGFR showed less proportional and constant error than the CKD-EPI eGFR for all POC and laboratory creatinine measurements. All POC creatinine eGFR measurements, except for iSTAT® MDRD, showed less proportional and constant error than the respective laboratory creatinine eGFR equations. Additionally, Lin's concordance correlation coefficient showed improved accuracy for the POC eGFR measurements over the respective laboratory eGFR equations. (Table 2, Figure 3 and Figure 4)

Bias and precision

All calculated eGFR equations by POC creatinine or laboratory creatinine measurements showed positive bias compared to mGFR when looking at the all mGFR values category. Less bias was observed for the MDRD equation compared to the CKD-EPI equation for all POC creatinine measurements, except in the mGFR >90mL/min/1.73m^2 group where a mixed picture was seen. The observed biases for POC MDRD eGFR ranged from 1.9 ± 33.5 to 14.7 ± 32.0mL/min/1.73m^2, and for POC CKD-EPI eGFR from 8.4 ± 31.0 to 19.9 ± 28.5mL/min/1.73m^2 when looking at the all mGFR values category. For each calculated eGFR equation, the largest bias was observed with laboratory creatinine with all POC devices yielding less bias than the laboratory. (Table 3)

Inter-day CVs varied for the different creatinine measuring devices, the iSTAT® had a CV of 5.9% at a level of 88µmol/L, the two StatSensor devices had CVs of 7.4% and 10.2% at 84µmol/L and CVs of 4.6% and 5.5% at 530 µmol/L, while the laboratory Jaffe assay had a CV of 3.5% at both levels of 89.3µmol/L and 342µmol/L. When looking at IQRs, the MDRD equation showed less imprecision at a mGFR <60mL/min/1.73m^2 for all POC measurements, at mGFR ≥60mL/min/1.73m^2 the CKD-EPI equation showed the least imprecision. However, for laboratory eGFR the CKD-EPI equation showed less imprecision across all mGFR categories. When comparing POC eGFR imprecision to that of laboratory eGFR a mixed picture was seen, all methods suffered from imprecision; when the CKD-EPI equation was utilized the laboratory showed the least imprecision, however when the MDRD equation was utilized POC (generally the iSTAT device) showed the least imprecision. Capillary sampling with the StatSensor device showed the highest levels of imprecision except in the mGFR <60mL/min/1.73m^2 category. (Table 3)

Accuracy

Overall, the P30 for the POC and laboratory creatinine eGFR equations ranged from 36 – 72%. All P30s showed improved accuracy with increasing mGFR. Below 90mL/min/1.73m^2 the MDRD eGFR showed greater accuracy (higher P30s) than the CKD-EPI eGFR in all POC and laboratory creatinine

measurements, above 90mL/min/1.73m^2 the CKD-EPI eGFR showed greater accuracy. In the different mGFR groups, POC P30s continued to show improved accuracy compared to laboratory P30s (Table 3). When serum creatinine was adjusted by a factor of +9.0µmol/L improved P30s ranging from 62 – 71% were seen. Additional analysis using the revised Lund-Malmö equation yielded P30s ranging from 58 – 80% with improvement noted for POC devices and laboratory results. (Supplementary Table S1).

The percentage of total samples correctly classified into the eGFR groups of <60, 60-89 and >90mL/min/1.73m^2 ranged from 41.7% (95%CI: 38.0 – 45.6) to 52.8% (95%CI: 45.9 – 59.7). The POC creatinine eGFR equations correctly classified more samples into the correct eGFR groups than the respective laboratory creatinine equations, although the laboratory equations showed improved performance in the mGFR >90mL/min/1.73m^2 group. Below 90mL/min/1.73m^2 the MDRD equation correctly classified more samples than the CKD-EPI equation, above 90mL/min/1.73m^2 the CKD-EPI equation correctly classified more samples. (Table 4)

Discussion

In this study we used iohexol mGFR to evaluate the clinical utility of the iSTAT® and StatSensor® POC devices that measure creatinine and subsequently estimate kidney function using eGFR. Additionally, we evaluated the performance of the MDRD and CKD-EPI equations and compared the performance of the POC devices to that of laboratory Jaffe creatinine. Despite falling well short of the 90% of eGFRs within 30% of mGFR goal [17], POC eGFR showed improved accuracy compared to laboratory Jaffe eGFR when screening for CKD in a resource limited setting. All current eGFR estimating equations performed poorly in our study sample.

A number of studies [18–21] have shown the utility of the iSTAT® and StatSensor® POC devices for the diagnosis of acute kidney injury. Our study showed more bias compared to laboratory creatinine than these studies possibly due to differences in laboratory creatinine methods. Studies with less

bias [18–21] used the enzymatic method while studies which used the IDMS traceable Jaffe method, including our own, showed more bias [22]. We found the iSTAT® device showed less imprecision than the StatSensor® device, consistent with previous studies [18,19].

Comparison of POC eGFR to iohexol mGFR showed greater bias and inaccuracy than a previous study [23] from Belgium, which found a bias of 0.1 ± 17.6mL/min/1.73m^2 with a P30 of 81% for StatSensor® capillary eGFR (CKD-EPI). Our P30 values for POC devices were low, and varied across the range of GFR, with the worst performance at low GFR. Bukabau et al [13] reported a similarly low P30 of 31.3% in an African population with mGFR<60mL/min/1.73m^2 for the MDRD and CKD-EPI equations, with a laboratory enzymatic creatinine method.

It has been shown that serum creatinine suffers from significant positive bias when there is a delay in sample separation of 24 hours, this is true for Jaffe but not enzymatic methods [6,24]. In our study POC creatinine measurements were performed in real-time, while laboratory creatinine measurements were performed on samples with a maximum delayed separation of between 8 and 9 hours utilising an IDMS traceable Jaffe method. This delay is not expected to have significantly influenced results, although if present a positive bias on laboratory creatinine with lowered eGFR would be expected (the opposite pattern from what was observed in our laboratory samples). Importantly in LMIC, these methodological and pre-analytical factors are common in clinical practice. For example, in South Africa, samples from rural areas are transported to central laboratories and only processed 12 – 72 hours post collection [6] and the Jaffe method is used in all but a few large urban centres due to cost considerations [25] - despite an initiative for all laboratories to use the enzymatic method [26]. The IDMS traceable Jaffe method has been shown not to reach the desirable specifications of the Laboratory Working Group of the National Kidney Disease Education Program [27]- nevertheless, it is commonly used with 93% of studies from sub-Saharan Africa which reported creatinine methodology making use of the Jaffe method [28].

In LMIC the potential public health benefits associated with POC screening for early detection and monitoring of CKD is an exciting possibility. Despite the bias and imprecision seen in POC eGFRs, these devices showed improved performance compared to laboratory Jaffe eGFR. The performance of POC devices to detect eGFR in the range 60 – 89mL/min/1.73m^2 is of particular interest. With limited access to renal replacement therapy for severe kidney disease, it is advantageous to detect individuals with early disease who may benefit from renal protective measures. There was improved accuracy in this area compared to laboratory Jaffe measurements, as well as compared to measurements below 60mL/min/1.73m^2. Nevertheless, the large bias, imprecision and misclassifications mean that results remain inaccurate with current eGFR estimating equations.

Enzymatic methods are more specific for creatinine than Jaffe methods which are vulnerable to interference from numerous substances including proteins, glucose and ketone bodies [29]. Various assay techniques such as kinetic reactions and compensated assays are used to improve specificity. The laboratory assay used in our study was an IDMS traceable compensated kinetic Jaffe assay. Compensated assays assume a fixed concentration of non-specific interferences which are subtracted from the total creatinine value, bias will be introduced if this assumption is incorrect [30,31]. The positive bias for creatinine which the enzymatic POC devices demonstrated compared to the laboratory Jaffe assay may reflect overcompensation of the Jaffe assay in our population. When laboratory creatinine results were adjusted by a factor of +9.0µmol/L resulting eGFR equations showed similar P30s to that obtained for POC (Supplementary Table S1). Ideally all laboratories should move to enzymatic methods for the measurement of creatinine, while compensated Jaffe equations should be used with caution especially if this compensation has not been validated in a particular population. The poor performance of the laboratory Jaffe assay in our study may reflect overcompensation of the Jaffe method in our population.

Our study focussed primarily on accuracy; however, imprecision makes an equally important contribution to analytical variation. Jaffe methods are known with higher imprecision than

enzymatic methods [27,32,33]. The inter-day CVs of the POC creatinine devices were greater than that seen with the laboratory Jaffe assay. This high imprecision with POC assays, despite the use of enzymatic methodology, is an important distinguishing factor between laboratory and POC testing [34]. High levels of imprecision may result in total error that hinders the interpretation of creatinine and subsequent eGFR [32].

The ability of the CKD-EPI equation to quantify GFR above 60mL/min/1.73m^2 offers significant advantages over the MDRD equation and avoids classifying patients based on a GFR cut-off which dichotomises all measurements [5,35]. In keeping, we found the MDRD equation to suffer from greater imprecision above a mGFR of 60mL/min/1.73m^2. Despite these apparent short-comings, we were surprised by the improved accuracy of the MDRD equation with higher P30s and sensitivities up to a mGFR of 90mL/min/1.73m^2 compared to the CKD-EPI equation.

In keeping with other studies from sub-Saharan Africa [13,14] we found a positive bias when using the African-American coefficient for the MDRD and CKD-EPI equations, and both led to substantial overestimation of GFR (Supplementary Figures S1 and S2). The revised Lund-Malmö equation outperformed the MDRD and CKD-EPI equations (Supplementary Table S1), suggesting that a new equation developed from an African population group may further improve performance. Despite the improved performance seen with the revised Lund-Malmö equation it remains rarely used in sub-Saharan Africa.

POC devices have the potential to provide immediately actionable results and mitigate pre-analytical errors, such as delay in separation commonly seen in LMIC. The iSTAT® utilises venous whole blood while the StatSensor® can be used with capillary and venous whole blood. Capillary sampling offers added benefit by eliminating the need for phlebotomy skills, and is preferred by patients [36]. Capillary sampling showed improved performance over corresponding laboratory creatinine, however it showed the highest levels of imprecision. Thus, venous sampling should remain the

preferred option, with capillary sampling considered as an alternative only if venous sampling is unavailable and if the device has been validated for this purpose.

POC testing has limitations such as: refrigerated supply storage (both devices), susceptibility to lot-to-lot variations and a lack of monitoring by laboratory professionals (especially external quality control). Cost considerations need to be taken into account especially in resource-limited settings. POC testing has a significantly higher cost compared to laboratory measurements, especially when additional components such as the purchase of quality control materials and the need for refrigeration are considered. However, cost savings may be achieved if POC testing leads to improved detection, and reduced numbers of patients progressing to advanced CKD. Both devices, and others, have previously been reviewed regarding suitability in low-income countries, including estimated cost per test [37].

Our study had a number of strengths: i) the large sample size drawn from a well characterised population-based study of rural South Africans; ii) the study procedures were standardised and conducted in a well-controlled research environment; iii) two different POC devices using venous whole blood and capillary blood were evaluated; iv) participants had a wide range of iohexol mGFRs including 134 participants with a mGFR less than 60mL/min/1.73m^2. Our study also had limitations: i) the poor performance of laboratory creatinine likely reflects the Jaffe methodology used despite enzymatic methods being the preferred choice [27,33,38–40], however without a parallel analysis using a laboratory enzymatic method this assumption remains untested; ii) although plasma clearance of iohexol is a well described method for measuring GFR, the MDRD and CKD-EPI equations were developed using urinary clearance of iothalamate and as such, methodologic bias may be introduced by using iohexol mGFR [4]; iii) laboratory creatinine was processed after delayed sample separation (although less than the 24 hour level at which changes become significant [6,24]); iv) POC testing was carried out by trained staff under controlled conditions, which may not

be reproducible in a busy clinic environment; v) roughly one-third of participants had venous measurements assessed with the StatSensor® due to cost considerations.

In conclusion, our study demonstrates poor performance of the compensated kinetic Jaffe method to measure creatinine in an under-resourced primary care setting and highlights the recommendation for laboratories to move to enzymatic methods. In these situations POC devices, despite their poor performance compared to iohexol mGFR, show improved accuracy in predicting GFR compared to Jaffe methodology. The inaccuracy and wide imprecision with current eGFR estimating equations mean that POC devices should be used with caution.

List of abbreviations

CKD: chronic kidney disease

CKD-EPI: Chronic Kidney Disease Epidemiology Collaboration

CV: coefficient of variation

eGFR: estimated glomerular filtration rate

GFR: glomerular filtration rate

IDMS: isotope dilution mass spectrometry

ISO: International Organization for Standardization

IQR: interquartile range

KDIGO: Kidney Disease Improving Global Outcomes

LMIC: low- and middle-income countries

MDRD: Modification of Diet in Renal Disease

mGFR: measured glomerular filtration rate

POC: point-of-care

P30: accuracy within 30% of iohexol measured glomerular filtration rate

Acknowledgements: We thank Abbott Laboratories for the supply of the iSTAT® device, control materials and cartridges. We thank Nova Biomedical Corporation for the supply of the StatSensor® devices, control materials and biosensor strips.

Research funding: We thank our funders: (i) the South African Medical Research Council, with funds from the South African National Department of Health, MRC UK (via the Newton

Fund) and GSK R&D; (ii) Faculty Research Committee Individual Research Grant, University of Witwatersrand; (iii) The International Society of Nephrology Clinical Research Program.

Author contributions: All authors have accepted responsibility for the entire content of this manuscript and approved its submission.

Competing interests: Authors state no conflict of interest.

Informed consent: Informed consent was obtained from all individuals included in this study.

Ethical approval: Research involving human subjects complied with all relevant national regulations, institutional policies and is in accordance with the tenets of the Helsinki Declaration (as revised in 2013), and has been approved by the authors' Institutional Review Board (Human Research Ethics Committee of the University of the Witwatersrand) or equivalent committee. (M160937 and M190434).

Supplementary material

Figure S1: MDRD eGFR (with African-American coefficient) vs iohexol mGFR.

Figure S2: CKD-EPI eGFR (with African-American coefficient) vs iohexol mGFR.

Figure S3: Revised Lund-Malmö eGFR vs iohexol mGFR.

Table S1: Accuracy within 30% of mGFR (P30) according to CKD stage, including revised Lund-Malmö equation.

Table S2: Samples correctly classified according to KDIGO GFR staging.

References

1. George JA, Brandenburg J-T, Fabian J, Crowther NJ, Agongo G, Alberts M, et al. Kidney damage and associated risk factors in rural and urban sub-Saharan Africa (AWI-Gen): a cross-sectional population study. Lancet Glob Heal [Internet]. 2019;7:e1632–43.

2. Stanifer JW, Muiru A, Jafar TH, Patel UD. Chronic kidney disease in low- and middle-income countries. Nephrol Dial Transplant. 2016;31:868–74.

3. KDIGO Working Group. Kidney Disease: Improving Global Outcomes (KDIGO). KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. Kidney Int Suppl. 2013;3:1–150.

4. Levey AS, Inker LA. GFR as the "gold Standard": Estimated, Measured, and True. Am J Kidney Dis. 2016;67:9–12.

5. Miller WG, Jones GRD. Estimated Glomerular Filtration Rate; Laboratory Implementation and Current Global Status. Adv Chronic Kidney Dis [Internet]. 2018;25:7–13.

6. Chikomba CE, Padoa CJ, Tanyanyiwa D. Evaluation of the impact of delayed centrifugation on the diagnostic performance of serum creatinine as a baseline measure of renal function before antiretroviral treatment. South Afr J HIV Med. 2020;21:a1056.

7. Kazmierczak SC. Point-of-care testing quality: Some positives but also some negatives. Clin Chem. 2011;57:1219–20.

8. Levin A, Stevens PE. Summary of KDIGO 2012 CKD Guideline: Behind the scenes, need for guidance, and a framework for moving forward. Kidney Int [Internet]. 2014;85:49–61.

9. Kalyesubula R, Fabian J, Nakanga W, Newton R, Ssebunnya B, Prynn J, et al. How to estimate glomerular filtration rate in sub-Saharan Africa: Design and methods of the African Research into Kidney Diseases (ARK) study. BMC Nephrol. 2020;21:1–12.

10. Annesley TM, Clayton LT. Ultraperformance liquid chromatography-tandem mass spectrometry assay for iohexol in human serum. Clin Chem. 2009;55:1196–202.

11. Fleming JS, Zivanovic MA, Blake GM, Burniston M, Cosgriff PS. Guidelines for the measurement of glomerular filtration rate using plasma sampling. Nucl Med Commun. 2004;25:759–69.

12. Haycock GB, Schwartz GJ, Wisotsky DH. Geometric method for measuring body surface area:

A height-weight formula validated in infants, children, and adults. J Pediatr. 1978;93:62–6.

13. Bukabau JB, Yayo E, Gnionsahé A, Monnet D, Pottel H, Cavalier E, et al. Performance of creatinine- or cystatin C–based equations to estimate glomerular filtration rate in sub-Saharan African populations. Kidney Int. 2019;95:1181–9.

14. Van Deventer HE, George JA, Paiker JE, Becker PJ, Katz IJ. Estimating glomerular filtration rate in black South Africans by use of the modification of diet in renal disease and Cockcroft-Gault equations. Clin Chem. 2008;54:1197–202.

15. Parrinello CM, Grams ME, Couper D, Ballantyne CM, Hoogeveen RC, Eckfeldt JH, et al. Recalibration of blood analytes over 25 years in the Atherosclerosis Risk in Communities Study: Impact of recalibration on chronic kidney disease prevalence and incidence. Clin Chem. 2015;61:938–47.

16. Ricos C, Ramon F, Salas A, Buno A, Calafell R, Morancho J, et al. Minimum analytical quality specifications of inter-laboratory comparisons: Agreement among Spanish EQAP organizers. Clin Chem Lab Med. 2012;50:455–61.

17. K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis. 2002 Feb;39:S1-266.

18. Snaith B, Harris MA, Shinkins B, Jordaan M, Messenger M, Lewington A. Point-of-care creatinine testing for kidney function measurement prior to contrast-enhanced diagnostic imaging: Evaluation of the performance of three systems for clinical utility. Clin Chem Lab Med. 2018;56:1269–76.

19. van der Heijden C, Roosens L, Cluckers H, Van Craenenbroeck AH, Peeters B. Analytical and clinical performance of three hand-held point-of-care creatinine analyzers for renal function measurements prior to contrast-enhanced imaging. Clin Chim Acta [Internet]. 2019;Oct:13–9.

20. Kosack CS, De Kieviet W, Bayrak K, Milovic A, Page AL. Evaluation of the Nova StatSensor®

Xpress™ creatinine point-of-care handheld analyzer. PLoS One. 2015;10:1–11.

21. Snaith B, Harris MA, Shinkins B, Messenger M, Lewington A, Jordaan M, et al. Point of care creatinine testing in diagnostic imaging: A feasibility study within the outpatient computed tomography setting. Eur J Radiol [Internet]. 2019;Mar:82–7.

22. Griffin BR, Butler-Dawson J, Dally M, Krisher L, Cruz A, Weitzenkamp D, et al. Unadjusted point of care creatinine results overestimate acute kidney injury incidence during field testing in Guatemala. PLoS One. 2018;13:1–12.

23. Stojkovic V, Delanaye P, Collard G, Ferrante N, Le Goff C, Lutteri L, et al. Estimated glomerular filtration rate using a point of care measure of creatinine in patients with iohexol determinate GFR. Clin Chim Acta. 2019;Dec:123–7.

24. Ford L, Berg J. Delay in separating blood samples affects creatinine measurement using the Roche kinetic Jaffe method. Ann Clin Biochem. 2008 Jan;45:83–7.

25. Delanghe JR, Speeckaert MM. Creatinine determination according to Jaffe - What does it stand for? NDT Plus. 2011;4:83–6.

26. National Institute for Health and Care Excellence. Chronic kidney disease in adults: assessment and management. NICE Guideline CG182; 2014.

27. Boutten A, Bargnoux AS, Carlier MC, Delanaye P, Rozet E, Delatour V, et al. Enzymatic but not compensated Jaffe methods reach the desirable specifications of NKDEP at normal levels of creatinine. Results of the French multicentric evaluation. Clin Chim Acta. 2013;18:132–5.

28. Fabian J, George JA, Etheredge HR, van Deventer M, Kalyesubula R, Wade AN, et al. Methods and reporting of kidney function: a systematic review of studies from sub-Saharan Africa. Clin Kidney J. 2019 Dec;12:778–87.

29. Panteghini M. Enzymatic assays for creatinine: time for action. Clin Chem Lab Med.

2008;46:567–72.

30. Parry DM. Use of single-value protein compensation of the Jaffe creatinine assay contributes to clinically significant inaccuracy in results. Vol. 54, Clinical chemistry. England; 2008. p. 215–6.

31. Cobbaert CM, Baadenhuijsen H, Weykamp CW. Prime time for enzymatic creatinine methods in pediatrics. Clin Chem. 2009 Mar;55:549–58.

32. Delanaye P, Cavalier E, Pottel H. Serum Creatinine: Not So Simple! Nephron. 2017;136:302–8.

33. Hoste L, Deiteren K, Pottel H, Callewaert N, Martens F. Routine serum creatinine measurements: How well do we perform? BMC Nephrol. 2015;16:1–9.

34. van Lint CL, van der Boog PJM, Romijn FPHTM, Schenk PW, van Dijk S, Rövekamp TJM, et al. Application of a point of care creatinine device for trend monitoring in kidney transplant patients: fit for purpose? Clin Chem Lab Med. 2015 Sep;53:1547–56.

35. Dalton RN. Serum Creatinine and Glomerular Filtration Rate: Perception and Reality. Clin Chem [Internet]. 2010 May 1;56:687–9.

36. Woods K, Douketis JD, Schnurr T, Kinnon K, Powers P, Crowther MA. Patient preferences for capillary vs. venous INR determination in an anticoagulation clinic: a randomized controlled trial. Thromb Res. 2004;114:161–5.

37. Raimann JG, Riella MC, Levin NW. International Society of Nephrology's 0by25 initiative (zero preventable deaths from acute kidney injury by 2025): Focus on diagnosis of acute kidney injury in low-income countries. Clin Kidney J. 2018;11:12–9.

38. Piéroni L, Delanaye P, Boutten A, Bargnoux AS, Rozet E, Delatour V, et al. A multicentric evaluation of IDMS-traceable creatinine enzymatic assays. Clin Chim Acta. 2011;412:2070–5.

39. Drion I, Cobbaert C, Groenier KH, Weykamp C, Bilo HJ, Wetzels JF, et al. Clinical evaluation of

analytical variations in serum creatinine measurements: Why laboratories should abandon Jaffe techniques. BMC Nephrol. 2012;8:133.

40. Miller WG, Bachmann LM, Delanghe JR, Inker LA, Jones GRD, Vassalotti JA. Optimal Use of Biomarkers for Chronic Kidney Disease. Clin Chem [Internet]. 2019 Aug 1;65:949–55.

Table 1: Characteristics according to mGFR

Characteristic	mGFR (categorised by GFR CKD Stage)[a]			
	All	<60 (G3-5)	60-89 (G2)	≥90 (G1)
n (%)	674	134 (20)	286 (42)	254 (38)
Age (years)	43 (14)	50 (14)	46 (14)	37 (11)
BMI (kg/m^2)	28.6 (6.4)	28.9 (5.9)	29.7 (6.6)	27.2 (6.2)
Body surface area (m^2)	1.91 (0.23)	1.90 (0.22)	1.94 (0.24)	1.87 (0.22)
Male (n and %)	211 (31)	38 (28)	81 (28)	92 (36)
mGFR[a]	81 (24)	47 (10)	76 (8)	105 (13)
Laboratory eGFR (MDRD)[a]	116 (27)	98 (26)	112 (22)	129 (25)
Laboratory eGFR (CKD-EPI)[a]	111 (17)	98 (21)	109 (14)	120 (11)
StatSensor Capillary eGFR (MDRD)[a]	87 (26)	72 (19)	85 (24)	97 (28)
StatSensor Capillary eGFR (CKD-EPI)[a]	93 (21)	79 (20)	91 (20)	102 (19)
iSTAT eGFR (MDRD)[a]	97 (23)	83 (22)	94 (20)	108 (22)
iSTAT eGFR (CKD-EPI)[a]	102 (19)	89 (22)	100 (17)	112 (13)
StatSensor Venous eGFR (MDRD)[a]	85 (21)[b]	71 (27)[c,f]	84 (19)[d]	93 (21)[e]
StatSensor Venous eGFR (CKD-EPI)[a]	92 (19)[b]	77 (32)[c,f]	90 (16)[d]	101 (17)[e]

All data mean (SD) unless otherwise indicated

[a] in mL/min/1.73m^2

[b] n= 214

[c] n= 31

[d] n= 97

[e] n= 86

[f] median (interquartile range)

Note: all eGFR equations exclude the African-American coefficient

Table 2: Analytical comparison of POC and laboratory eGFR equations compared to mGFR

	Passing-Bablok regression				Lin's concordance correlation coefficient[d]
	Slope/proportional error[a]	Y intercept/constant error[b]	r[c]	p	
Laboratory eGFR (MDRD)	1.16 (1.05 - 1.27)	20.47 (10.94 - 29.23)	0.44 (0.38 - 0.50)	<0.0001	0.25 (0.21 – 0.29)
Laboratory eGFR (CKD-EPI)	0.59 (0.53 - 0.65)	62.76 (57.60 - 67.88)	0.50 (0.45 - 0.56)	<0.0001	0.24 (0.21 – 0.28)
StatSensor Capillary eGFR (MDRD)	1.01 (0.89 - 1.14)	3.91 (-6.10 - 12.04)	0.34 (0.27 - 0.41)	<0.0001	0.35 (0.28 – 0.41)
StatSensor Capillary eGFR (CKD-EPI)	0.88 (0.79 - 0.98)	22.38 (13.77 - 30.18)	0.40 (0.33 - 0.46)	<0.0001	0.37 (0.31 – 0.42)
iSTAT eGFR (MDRD)	0.92 (0.84 - 1.01)	21.35 (13.35 - 28.75)	0.45 (0.39 - 0.51)	<0.0001	0.40 (0.35 – 0.45)
iSTAT eGFR (CKD-EPI)	0.70 (0.64 - 0.77)	45.18 (38.90 - 50.84)	0.49 (0.43 - 0.54)	<0.0001	0.34 (0.29 – 0.38)
StatSensor Venous eGFR (MDRD)	0.90 (0.74 - 1.08)	10.24 (-4.41 - 22.10)	0.37 (0.25 - 0.48)	<0.0001	0.42 (0.31 – 0.53)
StatSensor Venous eGFR (CKD-EPI)	0.84 (0.70 - 1.00)	22.10 (8.33 - 33.73)	0.45 (0.33 - 0.55)	<0.0001	0.47 (0.37 – 0.56)

42

[a] slope (95% confidence interval)

[b] y-intercept (95% confidence interval)

[c] Spearman rank correlation coefficient (95% confidence interval)

[d] Lin's concordance correlation coefficient (95% confidence interval)

Note: all eGFR equations exclude the African-American coefficient

Table 3: Accuracy within 30% of mGFR (P30), bias and imprecision according to CKD stage

	Bias[a]				Imprecision[b]				P30[c]			
	All	mGFR <60[d] (G3-5)	mGFR 60-89[d] (G2)	mGFR ≥90[d] (G1)	All	mGFR <60[d] (G3-5)	mGFR 60-89[d] (G2)	mGFR ≥90[d] (G1)	All	mGFR <60[d] (G3-5)	mGFR 60-89[d] (G2)	mGFR ≥90[d] (G1)
Laboratory eGFR (MDRD)	34.1 (31.1 - 36.3)	52.7 (45.8 - 59.7)	35.7 (30.7 - 38.6)	22.6 (18.3 - 27.7)	35.4 (30.4 - 40.7)	38.1 (28.7 - 51.1)	29.2 (21.9 - 37.0)	33.3 (26.0 - 41.9)	36 (32 – 40)	10 (5 – 16)	27 (22 – 33)	60 (53 – 66)
Laboratory eGFR (CKD-EPI)	28.6 (27.0 - 30.6)	54.5 (51.2 - 58.8)	33.8 (31.7 - 35.3)	16.1 (14.2 - 17.3)	26.8 (23.5 - 30.7)	27.3 (18.1 - 38.6)	19.4 (14.5 - 23.3)	17.9 (13.7 - 23.5)	43 (40 – 47)	7 (3 – 12)	25 (20 – 31)	83 (78 – 88)
StatSensor Capillary eGFR (MDRD)	4.6 (1.3 - 7.7)	28.0 (23.2 - 31.7)	7.3 (3.7 - 10.4)	-12.4 (-16.9 - -9.2)	39.8 (34.2 - 46.4)	26.5 (17.5 - 35.6)	30.4 (21.6 - 38.6)	38.8 (25.6 - 47.6)	61 (57 – 65)	29 (22 – 38)	68 (62 – 73)	70 (64 – 76)
StatSensor Capillary eGFR (CKD-EPI)	11.4 (10.0 - 14.3)	35.3 (31.2 - 38.4)	16.5 (12.8 - 19.5)	-1.3 (-5.4 - 2.4)	35.0 (29.8 - 41.8)	29.3 (17.8 - 40.2)	29.4 (22.0 - 36.9)	28.7 (22.8 - 37.1)	62 (58 – 65)	21 (14 – 29)	60 (54 – 66)	85 (80 – 89)
iSTAT eGFR (MDRD)	14.7 (12.1 - 16.5)	39.1 (33.0 - 42.0)	16.0 (13.8 - 18.6)	3.1 (-1.1 - 5.4)	32.0 (26.4 - 37.7)	27.8 (21.4 - 40.1)	23.0 (18.3 - 31.1)	29.5 (21.7 - 36.5)	62 (58 – 66)	18 (12 – 26)	61 (55 – 66)	87 (82 – 90)
iSTAT eGFR (CKD-EPI)	19.9 (17.7 - 21.4)	47.6 (42.2 - 51.0)	25.8 (21.3 - 27.8)	8.5 (5.3 - 10.5)	28.5 (23.9 - 33.9)	28.9 (22.7 - 38.6)	21.4 (16.8 - 27.3)	20.1 (14.5 - 26.4)	56 (52 – 60)	14 (8 – 21)	44 (38 – 50)	92 (88 – 95)
StatSensor Venous eGFR (MDRD)	1.9 (-2.1 - 4.6)	20.8 (11.0 - 31.6)	6.4 (2.2 - 11.0)	-14.0 (-22.8 - -8.6)	33.5 (22.5 - 43.3)	28.6 (10.0 - 42.5)	27.9 (13.4 - 37.8)	30.4 (18.1 - 41.0)	71 (64 – 77)	42 (25 – 61)	74 (64 – 83)	78 (68 – 86)
StatSensor Venous eGFR (CKD-EPI)	8.4 (5.1 - 12.4)	24.5 (13.2 - 36.3)	14.1 (10.9 - 18.0)	-3.4 (-9.1 - 2.5)	31.0 (21.0 - 37.8)	33.0 (11.8 - 50.2)	22.8 (17.0 - 35.1)	28.0 (16.3 - 40.4)	72 (65 – 78)	35 (19 – 55)	66 (56 – 75)	92 (84 – 97)

[a] ian bias of individual differences between eGFR and mGFR (in mL/min/1.73m^2 with 95% confidence intervals)

[b] interquartile range (IQR) of the differences between eGFR and mGFR (in mL/min/1.73m^2 with 95% confidence intervals)

[c] % (95% confidence intervals)

[d] in mL/min/1.73m^2

Note: all eGFR equations exclude the African-American coefficient

Table 4: Percentage of samples correctly classified into mGFR groups <60, 60-89 and ≥90

	mGFR <60[c] (G3-5)	mGFR 60-89[c] (G2)	mGFR ≥90[c] (G1)	Total correctly classified[d]
Laboratory eGFR (MDRD)	8.2 (4.2 – 14.2)	14.7 (10.8 – 19.4)	95.6 (92.3 – 97.8)	43.8 (40.0 – 47.7)
Laboratory eGFR (CKD-EPI)	7.5 (3.6 – 13.3)	7.0 (4.3 – 10.6)	99.2 (97.2 – 99.9)	41.7 (38.0– 45.6)
StatSensor Capillary eGFR (MDRD)	24.6 (17.6 – 32.8)	48.4 (42.5 – 54.4)	52.0 (45.6 – 58.3)	45.0 (41.2 – 48.9)
p[a]	<0.0001	<0.0001	<0.0001	0.5451
StatSensor Capillary eGFR (CKD-EPI)	19.4 (13.1 – 27.1)	38.2 (32.6 – 44.2)	73.2 (67.3 – 78.6)	47.5 (43.7 – 51.4)
p[b]	0.0004	<0.0001	<0.0001	0.0028
iSTAT eGFR (MDRD)	15.0 (9.4 – 22.2)	39.6 (33.9 – 45.6)	77.4 (71.7 – 82.4)	49.0 (45.1 – 52.8)
p[a]	0.0117	<0.0001	<0.0001	0.0048
iSTAT eGFR (CKD-EPI)	12.0 (7.0 – 18.8)	23.9 (19.0 – 29.2)	93.7 (89.9 – 96.3)	47.8 (43.9 – 51.6)
p[b]	0.0703	<0.0001	0.0002	<0.0001
StatSensor Venous eGFR (MDRD)	35.5 (19.2 – 54.6)	57.7 (47.3 – 67.7)	53.5 (42.4 – 64.3)	52.8 (45.9 – 59.7)
p[a]	0.1250	<0.0001	<0.0001	0.4215
StatSensor Venous eGFR (CKD-EPI)	29.0 (14.2 – 48.0)	45.4 (35.2 – 55.8)	69.8 (58.9 – 79.2)	52.8 (45.9 – 59.7)
p[b]	0.3750	<0.0001	<0.0001	0.1249

All data percentage (95% confidence interval)

[a] versus laboratory eGFR (MDRD) using exact NcNemar test

[b] versus laboratory eGFR (CKD-EPI) using exact NcNemar test

[c] in mL/min/1.73m^2

[d] total samples correctly classified into CKD stages: G3-5, G2 and G1

Note: all eGFR equations exclude the African-American coefficient

Figure 1: Participant flow through study

[a] outliers excluded on initial Passing-Bablok comparison between mGFR and POC eGFR, excluded based on residuals > 2.5 SD

Figure 2: POC vs laboratory creatinine

Bland-Altman agreement charts of: (A) the StatSensor® capillary creatinine (µmol/L), (C) iSTAT® creatinine (µmol/L), and (E) StatSensor® venous creatinine (µmol/L) compared to laboratory creatinine (µmol/L)

Passing-Bablok regression comparing: (B) StatSensor® capillary creatinine, (D) iSTAT® creatinine, and (F) StatSensor® venous creatinine to laboratory creatinine

Figure 3: MDRD eGFR (without the African-American coefficient) vs iohexol mGFR

Bland-Altman agreement charts of: (A) Laboratory MDRD eGFR, (C) StatSensor® capillary MDRD eGFR, (E) iSTAT® MDRD eGFR, and (G) StatSensor® venous MDRD eGFR compared to iohexol mGFR

Passing-Bablok regression comparing: (B) Laboratory MDRD eGFR, (D) StatSensor® capillary MDRD eGFR, (F) iSTAT® MDRD eGFR, and (H) StatSensor® venous MDRD eGFR compared to iohexol mGFR

Figure 4: CKD-EPI eGFR (without the African-American coefficient) vs iohexol mGFR

Bland-Altman agreement charts of: (A) Laboratory CKD-EPI eGFR, (C) StatSensor® capillary CKD-EPI eGFR, (E) iSTAT® CKD-EPI eGFR, and (G) StatSensor® venous CKD-EPI eGFR compared to iohexol mGFR

Passing-Bablok regression comparing: (B) Laboratory CKD-EPI eGFR, (D) StatSensor® capillary CKD-EPI eGFR, (F) iSTAT® CKD-EPI eGFR, and (H) StatSensor® venous CKD-EPI eGFR compared to iohexol mGFR

a0044271:CCLM4.docx

ORIGINALITY REPORT

15%	12%	13%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	www.degruyter.com Internet Source	1%
2	www.biomedcentral.com Internet Source	1%
3	academic.oup.com Internet Source	1%
4	worldwidescience.org Internet Source	1%
5	Violeta Stojkovic, Pierre Delanaye, Gregory Collard, Nunzio Ferrante, Caroline Le Goff, Laurence Lutteri, Etienne Cavalier. "Estimated glomerular filtration rate using a point of care measure of creatinine in patients with iohexol determinate GFR", Clinica Chimica Acta, 2019 Publication	1%
6	bmcnephrol.biomedcentral.com Internet Source	<1%
7	caremore.com Internet Source	<1%

8	hdl.handle.net Internet Source	<1%
9	www.research.lancs.ac.uk Internet Source	<1%
10	eprints.gla.ac.uk Internet Source	<1%
11	eprints.whiterose.ac.uk Internet Source	<1%
12	bmcmedicine.biomedcentral.com Internet Source	<1%
13	www.sajs.co.za Internet Source	<1%
14	Q. Huang, Xuefeng Sun, Y. Chen, M. Zhang, L. Tang, S. Liu, R. Wei, S. Wang, J. Zhou, X. Cao, W. Zhang, G. Cai, X. Chen. "A study of the applicability of GFR evaluation equations for an elderly Chinese population", The journal of nutrition, health & aging, 2015 Publication	<1%
15	journals.lww.com Internet Source	<1%
16	Wyatt, Christina M., George J. Schwartz, Willis Owino Ong'or, Joseph Abuya, Alison G. Abraham, Charles Mboku, Loice B. M'mene, Winnie J. Koima, Mathew Hotta, Paula Maier,	<1%

Paul E. Klotman, and Kara Wools-Kaloustian. "Estimating Kidney Function in HIV-Infected Adults in Kenya: Comparison to a Direct Measure of Glomerular Filtration Rate by Iohexol Clearance", PLoS ONE, 2013.
Publication

---

17 Daniel Scarr, Petter Bjornstad, Leif E. Lovblom, Julie A. Lovshin et al. "Estimating GFR by Serum Creatinine, Cystatin C, and β2-Microglobulin in Older Adults: Results From the Canadian Study of Longevity in Type 1 Diabetes", Kidney International Reports, 2019 <1%
Publication

---

18 jamanetwork.com <1%
Internet Source

---

19 Céline L. van Lint, Paul J.M. van der Boog, Fred P.H.T.M. Romijn, Paul W. Schenk et al. "Application of a point of care creatinine device for trend monitoring in kidney transplant patients: fit for purpose?", Clinical Chemistry and Laboratory Medicine (CCLM), 2015 <1%
Publication

---

20 L. E. Scott, L. D. Noble, J. Moloi, L. Erasmus, W. D. F. Venter, W. Stevens. "Evaluation of the Abbott m2000 RealTime Human Immunodeficiency Virus Type 1 (HIV-1) Assay for HIV Load Monitoring in South Africa <1%

Compared to the Roche Cobas AmpliPrep-Cobas Amplicor, Roche Cobas AmpliPrep-Cobas TaqMan HIV-1, and BioMerieux NucliSENS EasyQ HIV-1 Assays", Journal of Clinical Microbiology, 2009
Publication

---

21 Mannix Imani Masimango, Ernest Kiswaya Sumaili, Pierre Wallemacq, Espoir Bwenge Malembaka et al. "Prevalence and risk factors of chronic kidney disease in South-Kivu, Democratic Republic of Congo: a large-scale population study.", Kidney International Reports, 2020 <1%
Publication

---

22 van Deventer, H. E., J. E. Paiker, I. J. Katz, and J. A. George. "A comparison of cystatin C- and creatinine-based prediction equations for the estimation of glomerular filtration rate in black South Africans", Nephrology Dialysis Transplantation, 2011. <1%
Publication

---

23 Christopher B. Pierce, Alvaro Muñoz, Derek K. Ng, Bradley A. Warady, Susan L. Furth, George J. Schwartz. "Age- and sex-dependent clinical equations to estimate glomerular filtration rates in children and young adults with chronic kidney disease", Kidney International, 2020 <1%
Publication

24	link.springer.com Internet Source	<1%
25	journals.sagepub.com Internet Source	<1%
26	www.jmir.org Internet Source	<1%
27	tel.archives-ouvertes.fr Internet Source	<1%
28	www.ajol.info Internet Source	<1%
29	www.ias2015.org Internet Source	<1%
30	Jonas Björk, Sten Erik Bäck, Natalie Ebert, Marie Evans et al. "GFR estimation based on standardized creatinine and cystatin C: a European multicenter analysis in older adults", Clinical Chemistry and Laboratory Medicine (CCLM), 2018 Publication	<1%
31	www.nature.com Internet Source	<1%
32	www.tandfonline.com Internet Source	<1%
33	www.wjgnet.com Internet Source	<1%

34	Cheng, Ya-Yun, Neng-Chyan Huang, Yu-Tzu Chang, Junne-Ming Sung, Kun-Hung Shen, Chang-Chih Tsai, and How-Ran Guo. "Associations between Arsenic in Drinking Water and the Progression of Chronic Kidney Disease: A Nationwide Study in Taiwan", Journal of Hazardous Materials, 2016. Publication	<1%
35	Hans Pottel, Frank Martens. "Are eGFR equations better than IDMS-traceable serum creatinine in classifying chronic kidney disease?", Scandinavian Journal of Clinical and Laboratory Investigation, 2009 Publication	<1%
36	Sten Westgard, Victoria Petrides, Sharon Schneider, Marvin Berman, Jörg Herzogenrath, Anthony Orzechowski. "Assessing precision, bias and sigma-metrics of 53 measurands of the Alinity ci system", Clinical Biochemistry, 2017 Publication	<1%
37	Submitted to University of Aberdeen Student Paper	<1%
38	fr.scribd.com Internet Source	<1%
39	pubmed.ncbi.nlm.nih.gov Internet Source	<1%

40	www.j3.jstage.jst.go.jp Internet Source	<1%
41	www.uptodate.com Internet Source	<1%
42	Christian Schimper, Rupert Keckeis, Constanta Ibanescu, Eduard Burtscher, Avinash P. Manian, Thomas Bechtold. "Influence of steam and dry heat pretreatment on fibre properties and cellulase degradation of cellulosic fibres", Biocatalysis and Biotransformation, 2009 Publication	<1%
43	F. Iliadis, T. Didangelos, A. Ntemka, A. Makedou, E. Moralidis, A. Gotzamani-Psarakou, T. Kouloukourgiotou, D. Grekas. "Glomerular filtration rate estimation in patients with type 2 diabetes: creatinine- or cystatin C-based equations?", Diabetologia, 2011 Publication	<1%
44	Namki Hong, Jaewon Oh, Yong-ho Lee, Jong Chan Youn et al. "Comparison of association of glomerular filtration rate with metabolic syndrome in a community-based population using the CKD-EPI and MDRD study equations", Clinica Chimica Acta, 2014 Publication	<1%
45	Obiols, Julien, Anne-Sophie Bargnoux, Nils	<1%

Kuster, Pierre Fesler, Laurence Piéroni, Stéphanie Badiou, Anne-Marie Dupuy, Jean Ribstein, and Jean-Paul Cristol. "Validation of a new standardized cystatin C turbidimetric assay: Evaluation of the three novel CKD-EPI equations in hypertensive patients", Clinical Biochemistry, 2013.
Publication

46	Researchonline.lshtm.ac.uk Internet Source	<1%
47	Youngsun Yeo, Duck-Jong Han, Dae Hyuk Moon, Jung Sik Park, Won Seok Yang, Jai Won Chang, Seung Woon Byun, Su-Kil Park. "Suitability of the IDMS-Traceable MDRD Equation Method to Estimate GFR in Early Postoperative Renal Transplant Recipients", Nephron Clinical Practice, 2010 Publication	<1%
48	bmcgeriatr.biomedcentral.com Internet Source	<1%
49	e-sciencecentral.org Internet Source	<1%
50	ir.ymlib.yonsei.ac.kr Internet Source	<1%
51	jlpm.amegroups.com Internet Source	<1%

52	journals.plos.org Internet Source	<1%
53	orbi.uliege.be Internet Source	<1%
54	www.basilita.com Internet Source	<1%
55	www.karger.com Internet Source	<1%
56	www.utupub.fi Internet Source	<1%
57	Łukasz Kuźma, Jolanta Małyszko, Hanna Bachórzewska- Gajewska, Sławomir Dobrzycki. "Exposure to Air Pollution and Renal Function - An Underestimated Threat?", Research Square, 2021 Publication	<1%
58	Kate Simeon, Monisha Sharma, Jienchi Dorward, Jessica Naidoo et al. "Comparative cost analysis of point-of-care versus laboratory-based testing to initiate and monitor HIV treatment in South Africa", PLOS ONE, 2019 Publication	<1%
59	Lu-Xi Zou, Ling Sun, Susanne B. Nicholas, Yan Lu, Satyesh Sinha K, Ruixue Hua. "Comparison of bias and accuracy using cystatin C and	<1%

creatinine in CKD-EPI equations for GFR estimation", European Journal of Internal Medicine, 2020
Publication

---

60 Marijana Vučić Lovrenčić, Vanja Radišić Biljak, Kristina Blaslov, Sandra Božičević, Lea Smirčić Duvnjak. "Impact of creatinine methodology on glomerular filtration rate estimation in diabetes", World Journal of Diabetes, 2017 <1%
Publication

---

61 bjgp.org <1%
Internet Source

---

62 AS Levey, LA Inker. "Assessment of Glomerular Filtration Rate in Health and Disease: A State of the Art Review", Clinical Pharmacology & Therapeutics, 2017 <1%
Publication

---

63 Hughes, J.T., K. O'Dea, K. Piera, F. Barzi, A. Cass, W.E. Hoy, R.J. MacIsaac, and L.J. Maple-Brown. "Associations of serum adiponectin with markers of cardio-metabolic disease risk in Indigenous Australian adults with good health, diabetes and chronic kidney disease", Obesity Research & Clinical Practice, 2015. <1%
Publication

---

64 Janet Seeley, Elizabeth Kabunga, Joseph Ssembatya, Laurie A. Tomlinson et al. <1%

"Understanding kidney disease in rural central Uganda – Findings from a qualitative study", Global Public Health, 2020
Publication

---

65 Justine B. Bukabau, Ernest K. Sumaili, Etienne Cavalier, Hans Pottel et al. "Performance of glomerular filtration rate estimation equations in Congolese healthy adults: The inopportunity of the ethnic correction", PLOS ONE, 2018 <1%
Publication

---

66 Kazunori Murata, Nikola A. Baumann, Amy K. Saenger, Timothy S. Larson, Andrew D. Rule, John C. Lieske. "Relative Performance of the MDRD and CKD-EPI Equations for Estimating Glomerular Filtration Rate among Patients with Varied Clinical Presentations", Clinical Journal of the American Society of Nephrology, 2011 <1%
Publication

---

67 www.asn-online.org <1%
Internet Source

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		

a0044271:CCLM4.docx

GRADEMARK REPORT

FINAL GRADE

/0

GENERAL COMMENTS

Instructor

PAGE 1

PAGE 2

PAGE 3

PAGE 4

PAGE 5

PAGE 6

PAGE 7

PAGE 8

PAGE 9

PAGE 10

PAGE 11

PAGE 12

PAGE 13

PAGE 14

PAGE 15

PAGE 16

PAGE 17

PAGE 18

PAGE 19

PAGE 20

a0044271:BMC.REV.no_mark-up.doc

by Sean Currin

Submission date: 10-Mar-2021 07:30AM (UTC-0800)
Submission ID: 1529347313
File name: ents_4195209b-4193-43f4-9a69-66c459127eb2_BMC.REV.no_mark-up.doc (340K)
Word count: 6668
Character count: 39768

Title

Diagnostic accuracy of semiquantitative point of care urine albumin to creatinine ratio and urine dipstick analysis in a primary care resource limited setting in South Africa

Sean D Currin

MBChB

Department of Chemical Pathology, University of Witwatersrand, Johannesburg, South Africa and National Health Laboratory Service

Mwawi S Gondwe

Masters in Population Studies

Medical Research Council/Wits University Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

Nokthula B Mayindi

BA (Health Science and Social Services)

Medical Research Council/Wits University Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

Shingirai Chipungu

Higher National Diploma in Applied Biological Technology

Medical Research Council/Wits University Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

Bongekile L Khoza

BSc. (Medical Sciences)

Medical Research Council/Wits University Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

Stephen Tollman

MBBCh, PHD

[1]Medical Research Council/Wits University Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

[2]International Network for the Demographic Evaluation of Populations and their Health (INDEPTH) Network, Accra, Ghana

June Fabian*

MBBCh, FCP, Cert Neph

[1]Medical Research Council/Wits University Rural Public Health and Health Transitions Research Unit (Agincourt), School of Public Health, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

[2]Wits Donald Gordon Medical Centre, School of Clinical Medicine, Faculty of Health Sciences, University of Witwatersrand, Johannesburg, South Africa

Jaya A George*

MBBCh, DTM&H, MSc, FCPath, PhD

Professor and Head

Department of Chemical Pathology, University of Witwatersrand, Johannesburg, South Africa and National Health Laboratory Service

* Both authors acted jointly in the last authorship role.

On behalf of the ARK Consortium

Correspondence:

Sean Currin

University of Witwatersrand, Faculty of Health Sciences, Department of Chemical Pathology

7 York Road, Parktown, Johannesburg, 2193, South Africa

Telephone: +2711 489 8499

Email: sean.currin@wits.ac.za

Abstract

Background: the prevalence of chronic kidney disease (CKD) is predicted to rise over the next few decades. In resource-limited settings access to central laboratory services is limited. Point-of-care (POC) urine dipstick testing offers the potential to detect markers of kidney damage (albuminuria) as well as markers of other disease processes. We evaluated the diagnostic accuracy of the semi-quantitative albumin-creatinine ratio (ACR) Sysmex UC-1000 POC urine dipstick system as well as the extent of other abnormal dipstick findings in urine.

Methods: 700 participants from a rural area in South Africa were screened for albuminuria. A spot urine sample was used to measure POC and central laboratory ACR. We determined the sensitivity, specificity, positive predictive value and negative predictive value of the POC ACR, and recorded dipstick parameters.

Results: the prevalence of albuminuria was 11.6% (95%CI; 9.3 – 14.2). Those with albuminuria had higher mean diastolic (82 vs 79mmHg, p=0.019) and systolic (133 vs 128mmHg, p=0.002) blood pressures and a higher proportion of diabetes mellitus (17.6 vs 4.9%, p<0.001). The sensitivity of the POC ACR system was 0.79, specificity 0.84, positive predictive value 0.39 and negative predictive value 0.97. The sensitivity improved to 0.80, 0.85, 0.85 and 0.89 in those with elevated blood pressure, diabetes mellitus, HIV positive status, and those 65 years and older, respectively. Abnormalities other than albuminuria were detected in 240 (34.3%) of the samples; 88 (12.6%) were positive for haematuria, 113 (16.1%) for leucocytes, 66 (9.4%) for nitrites and 27 (3.9%) for glycosuria.

Conclusion: our study shows that POC ACR has good negative predictive value and could be used to rule out albuminuria when screening for CKD. Additionally, a high proportion of participants had other urine abnormalities detected with dipsticks which may reflect kidney disease or co-morbid untreated genitourinary pathology such as urinary tract infections or endemic schistosomiasis with important implications for CKD.

Keywords: point of care, urine albumin creatinine ratio, dipstick, chronic kidney disease

Background

Sub-Saharan Africa (SSA) has a high burden of infectious diseases (ID), mostly from HIV and tuberculosis, and an emerging burden of non-communicable diseases (NCD).[1,2] Together, ID and NCD are risk factors for chronic kidney disease (CKD), which has a prevalence of 10.7% in SSA.[3] The prevalence of CKD is predicted to rise disproportionately in low- and middle-income countries (LMIC).[4] Therefore, early detection of CKD and appropriate management of risk factors for progression is an essential public health priority.

Persistent albuminuria is one criterion for the diagnosis of CKD, and an independent risk factor for adverse kidney and cardiovascular outcomes.[5–9] Albuminuria, measured on more than one occasion, as a spot urine albumin to creatinine ratio (ACR), is now included as one of the diagnostic criteria for CKD.[10] Screening for albuminuria is recommended in certain high-risk groups such as those with diabetes mellitus, however results should be confirmed by a central laboratory.[11,12]

The lack of access to central laboratory services in LMICs is a major hurdle obstructing widespread implementation of CKD screening programs. While limited laboratory access is acknowledged in the 2012 KDIGO guidelines, there are no recommendations for using point of care (POC) devices in resource-limited settings (RLS).[10] POC testing provides actionable results in real-time for clinical decision-making.[13] In South Africa (SA), the efficacy of POC testing is well established for diagnosing and managing patients with HIV and tuberculosis.[14] Using the existing framework established for POC testing with ID, we propose that POC testing for screening and early detection of CKD is feasible in our setting. Previous studies[15–22] have shown good negative predictive values with fair sensitivity for semi-quantitative ACR POC testing, which suggests utility as a screening test for CKD. Additionally, the ability of urine dipsticks to detect other disease markers besides albuminuria offers added benefit.

The aim of our study was to evaluate the diagnostic accuracy of a POC semi-quantitative ACR system in a RLS in rural SA, and evaluate additional urinary abnormalities discovered on dipstick analysis.

Methods

Study design

Our sub-study comprised part of the prospective African Research on Kidney (ARK) study, the methods of which have been published.[23] To summarise, the ARK study was conducted in the Agincourt Health and Demographic Surveillance Site, Mpumalanga province, SA. There were two phases of the ARK study; the first phase (November 2017 – October 2018) screened a population-based sample of 2020 rural Africans for kidney disease and associated risk factors, those between the ages of 20 and 80 were considered eligible; the second phase (November 2018 – July 2019) comprised a subsample of the first phase, stratified by CKD stage. (Figure 1)

Figure 1: Flow of participants through the study. POC ACR is the index method while Lab ACR is the reference method.

In phase one of the ARK study, a CKD-risk questionnaire was administered, blood pressure (BP) was measured (as per the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure guidelines[24]), and voluntary counselling and HIV testing was offered to all participants according to the SA Department of Health Guidelines.[25] In the field, POC tests were performed including urine dipstick analysis using Roche (Mannheim, Germany) Combur 10® test strips on fresh spot urine samples.

The second phase of the ARK study recruited 989 participants and included the same BP and HIV testing protocols as the first phase. Spot urine samples were refrigerated overnight (2 - 8^{0}C) and processed the following morning for storage at -80^{0}C. Urine samples were transported at -80^{0}C from the study site to a central laboratory where they were processed over a nine-day period. Each day, 81 samples were separated into two aliquots, one aliquot was tested using central laboratory ACR, the other aliquot was tested using a POC semi-quantitative dipstick method. Central laboratory ACR results were not known while POC ACR was performed and vice versa. 700 urine samples out of the

989 where selected based on the order in which they were sent to the central laboratory, all 700 samples were processed. Funding constraints limited the sample size to 700 out of 989.

POC ACR and dipstick analysis

Urine dipstick analysis was performed using Sysmex Corporation (Kobe, Japan) 12S® urine dipsticks together with the Sysmex UC-1000® semi-automated urine chemistry analyser. The 12S dipsticks measure the following parameters: urobilinogen, red blood cells, haemoglobin, protein, glucose, ketones, bilirubin, nitrites, specific gravity, leukocytes, pH, creatinine, and albumin with colorimetric dipstick assays. The Sysmex UC-1000® system optically reads reaction results from 12S dipsticks using multi-wavelength reflectance photometry thereby providing semi-quantitative results. The semi-automated reading of strips eliminates inter-individual variation in interpreting dipstick results. The albumin concentration is measured as 10, 30, 80, or 150 mg/L using the protein error of pH indicator method, while the creatinine concentration is measured as 10, 50, 100, 200, or 300 mg/dL using the Benedict-Behre method; thereafter the ACR is calculated as dilute (albumin 10mg/L and creatinine 10mg/dL), normal (<30 mg/g), 1+ (30 – 300 mg/g), or 2+ (>300 mg/g). Internal quality control was performed each day before any samples were run.

Measurement of laboratory ACR

Laboratory albumin and creatinine measurements were performed on the Cobas c502 module® (Roche Diagnostics, Mannheim), with urine albumin (mg/L) determined using an immunoturbidimetric assay while urine creatinine (mg/dL) was determined using the kinetic Jaffe method. This was considered an appropriate reference method due to widespread usage in central laboratories in South Africa. During the nine days of measurements a single lot was used for both urine albumin and creatinine assays, internal quality control was performed twice daily as per standard practices within the central laboratory, together with an external quality assurance program.

Statistical Analysis

Laboratory measurement of ACR was used as the reference method to which POC ACR was compared. Laboratory ACR was considered positive if ≥30 mg/g and negative if <30 mg/g. POC ACR was considered positive if falling in the "1+" or "2+" (≥30 mg/g) groups whilst negative if falling in the normal group (<30 mg/g). Samples in which the laboratory albumin was <3 mg/L (limit of quantification) were considered as negative, as were POC ACR samples which were dilute (albumin <10 mg/L and creatinine <10 mg/dL). All 700 samples were included for analysis.

Statistical analysis was performed using Tibco Statistica 13® and MedCalc 19.1.3®. Continuous data were compared using the students T-test and Mann-Whitney test when appropriate, while categorical data were compared using χ^2 test. The prevalence of albuminuria, sensitivity, specificity, positive (PPV) and negative predictive values (NPV), and likelihood ratios of POC testing were calculated using cross-tabulation. 95% confidence intervals were calculated according to the exact Clopper-Pearson method for sensitivity and specificity, according to the Miettinen-Nurminen method for likelihood ratios and according to the Mercaldo-Wald method for predictive values. A p-value <0.05 was considered statistically significant.

Results

Study population

We analysed 700 urine samples; participant demographics and clinical characteristics are listed in Table 1. Mean systolic and diastolic BP were significantly higher amongst those with laboratory albuminuria, as was a history of diabetes mellitus; however, a history of hypertension was not. The prevalence of albuminuria was 11.6% (95%CI; 9.3 – 14.2), based on the central laboratory ACR measurements (≥30 mg/g).

Table 1: Demographic and clinical features

	Laboratory ACR (mg/g)			p[c]
	<30	≥30	>300	
Number of patients	619	81	9	
Age (years)	45 (25)[a]	43 (23)[a]	55 (25)[a]	0.680[d]
BMI (kg/m^2)	28.1 (6.2)	27.3 (6.5)	27.0 (7.0)[a]	0.239[e]
Systolic BP (mmHg)	128 (14.9)	133 (18.1)	144 (24.0)[a]	0.002[e]
Diastolic BP (mmHg)	79 (8.6)	82 (9.9)	83 (7.0)[a]	0.019[e]
Male (%)[b]	211 (34.1)	31 (38.3)	4 (44.4)	0.457[f]
Hypertension (%)[b]	154 (26.0)	25 (33.8)	3 (37.5)	0.153[f]
Diabetes Mellitus (%)[b]	29 (4.9)	13 (17.6)	2 (25.0)	<0.001[f]
Smoker (%)[b]	85 (14.3)	11 (14.9)	0 (0)	0.902[f]
HIV reactive (%)[b]	119 (20.1)	20 (27.0)	1 (12.5)	0.246[f]

Data presented as mean (SD) or n (%)

[a]Data presented as median (IQR)

[b]Missing values for all patients = 33

[c]Comparison between laboratory negative (<30mg/g) and laboratory positive (≥30mg/g) groups

[d]Mann-Whitney test

[e]Students T-test

[f]χ^2 test

Hypertension, diabetes mellitus, smoker status obtained from medical questionnaire.

HIV status obtained from medical questionnaire. If previously tested, participants were asked their status; those who did not know their status, or previously tested negative, were offered voluntary counselling and testing during the home screening (see methods)

Measured blood pressure (BP): see methods

Clinical performance of the POC ACR

The central laboratory method identified 81 participants as having albuminuria (≥ 30 mg/g) whilst the POC method, performed on the same day, identified 166 participants with albuminuria. No adverse events were reported during testing or collection. In total the POC reader misclassified 119 samples (17%) with 102 false positives, and 17 false negatives. Sensitivity of the POC ACR to detect albuminuria (ACR ≥ 30mg/g) was 0.790 (95%CI; 0.689 – 0.865), and specificity was 0.835 (95%CI; 0.804 – 0.862). A positive likelihood ratio of 4.80 (95%CI; 3.86 – 5.89) and negative likelihood ratio of

0.25 (95%CI; 0.16 – 0.37) was obtained for POC ACR. Predictive values of 0.968 (95%CI; 0.952 – 0.979) for negative results and 0.386 (95%CI; 0.337 – 0.436) for positive results were obtained. Sensitivity and specificity to predict ACR >300mg/g was 1.00 (95%CI;0.664 – 1.00) and 0.987 (95%CI;0.975 – 0.994) respectively; with predictive values of 1.00 (95%CI; 0.995 – 1.00) for negative results and 0.50 (95%CI;0.343 – 0.657) for positive results.

The sensitivity and specificity of the POC ACR system varied among different groups of participants. There was reduced sensitivity (76%) in those with a history of hypertension (medical questionnaire), however those with raised blood pressure[26] showed an improved sensitivity and specificity of 80 and 86% respectively. The same is true of those with a history of diabetes mellitus, with an improved sensitivity of 85%, however, specificity was lower (66%) in this group. Smokers, those with HIV, and those 65 years or older showed improved sensitivity. PPV remained low in all groups ranging from 0.26 to 0.52, while NPV was high ranging from 0.90 to 0.99. (Table 2)

Table 2: Classification of albuminuria as positive or negative by POC and laboratory ACR

All patients (700)			Sensitivity	0,79
	Positive POC (n=166)	Negative POC (n=534)	Specificity	0,84
Positive lab ACR (n=81)	64	17	PPV	0,39
Negative lab ACR (n=619)	102	517	NPV	0,97
Hypertension (179)			Sensitivity	0,76
	Positive POC (n=47)	Negative POC (n=132)	Specificity	0,82
Positive lab ACR (n=25)	19	6	PPV	0,40
Negative lab ACR (n=154)	28	126	NPV	0,95
DM (42)			Sensitivity	0,85
	Positive POC (n=21)	Negative POC (n=21)	Specificity	0,66
Positive lab ACR (n=13)	11	2	PPV	0,52
Negative lab ACR (n=29)	10	19	NPV	0,90
Smoker (96)			Sensitivity	0,82
	Positive POC (n=26)	Negative POC (n=70)	Specificity	0,80
Positive lab ACR (n=11)	9	2	PPV	0,35
Negative lab ACR (n=85)	17	68	NPV	0,97
HIV (139)			Sensitivity	0,85
	Positive POC (n=41)	Negative POC (n=98)	Specificity	0,80
Positive lab ACR (n=20)	17	3	PPV	0,41
Negative lab ACR (n=119)	24	95	NPV	0,97

POC ACR and dipstick analysis in RLS

BP: Diastolic ≥ 80 OR Systolic ≥ 130 (389)			Sensitivity	0,80
	Positive POC (n=93)	Negative POC (n=296)	Specificity	0,86
Positive lab ACR (n=56)	45	11	PPV	0,48
Negative lab ACR (n=333)	48	285	NPV	0,96
Age ≥ 65 (105)			Sensitivity	0,89
	Positive POC (n=31)	Negative POC (n=74)	Specificity	0,76
Positive lab ACR (n=9)	8	1	PPV	0,26
Negative lab ACR (n=96)	23	73	NPV	0,99

Hypertension, diabetes mellitus (DM), smoker status obtained from medical questionnaire.
HIV status obtained from medical questionnaire. If previously tested, participants were asked their status; those who did not know their status, or previously tested negative, were offered voluntary counselling and testing during the home screening (see methods)
Measured blood pressure (BP): see methods
PPV: positive predictive value
NPV: negative predictive value

False negative values ranged from 30.9 - 57.5 mg/g, and those for false positives ranged from 1.8 – 29.2 mg/g. All nine samples with laboratory ACR > 300mg/g were correctly identified as "2+" (>300 mg/g) according to POC ACR, a further nine samples were classified as > 300mg/g while laboratory ACR classified them as albuminuria (>30 mg/g) but less than 300 mg/g. POC ACR correctly classified 572 (82%, 95%CI;79 – 85) of the samples into the correct KDIGO albuminuria stages of A1 (<30mg/g), A2(30 – 300mg/g) and A3 (>300mg/g). Accuracy was 97% (95%CI;95-98), 31% (95%CI;24-39), and 50% (95%CI;26 – 74) in groups A1, A2 and A3 according to POC ACR. (Figure 2)

Figure 2: Laboratory ACR (reference) versus POC ACR (index). The horizontal lines represent the laboratory cut-offs of 30 mg/g and 300 mg/g. 30 mg/g represents the threshold for albuminuria while 300 mg/g is the threshold for 2+ albuminuria (as defined by the POC system). FNs represent false negative results by the POC method while FPs represent false positive results by the POC method.

Additional Urine Dipstick Abnormalities

240 (34.3%) of the 700 samples had abnormal findings other than ACR, protein to creatinine ratio, pH, or specific gravity. 65% (53/81) of laboratory confirmed albuminuria samples had additional abnormalities on urine dipstick. While 30% (187/619) of the samples without laboratory confirmed albuminuria demonstrated additional urinary dipstick abnormalities. Using the dipstick method 88

(12.6%) participants had haematuria including 50 in the laboratory negative group, and 113 (16.1%) had leukocyturia.

Urine dipstick findings were compared between those with laboratory positive and negative ACR. Haematuria and glycosuria were significantly higher in the laboratory positive compared to the laboratory negative group (46.9% vs 8.1% and 11.1% vs 2.9% respectively). All abnormalities were seen with a higher proportion in those with laboratory positive ACR except for leucocytes, although not statistically significant. (Table 3)

Table 3: Other dipstick findings besides ACR in phase 1 and phase 2

	Blood[e]	Urobilinogen	Bilirubin	Glucose	Leucocytes	Nitrites
Phase 2 (n=700) (Sysmex 12S® strips)[a]						
Lab ACR ≥30 (n and %)	38 (46.9)	1 (1.2)	2 (2.5)	9 (11.1)	12 (14.8)	12 (14.8)
Lab ACR <30 (n and %)	50 (8.1)	2 (0.3)	4 (0.6)	18 (2.9)	101 (16.3)	54 (8.7)
TOTAL (%)	88 (12.6)	3 (0.4)	6 (0.9)	27 (3.9)	113 (16.1)	66 (9.4)
p[d]	<0.001	0.238	0.094	<0.001	0.730	0.078
Phase 1 (n=1993)[c] (Roche Combur 10® strips)[b]						
TOTAL (%)	649 (32.6)	36 (1.8)	46 (2.3)	64 (3.2)	603 (30.3)	49 (2.5)

All data n (%)

[a] Performed on urine after a freeze thaw cycle, semi-quantitative analysis performed with Sysmex UC-1000® device (see methods)

[b] Performed on fresh urine during phase one, semi-quantitative analysis performed with visual inspection (see methods)

[c] Missing data for 27 (2 refused, 25 missing data)

[d] χ^2 test Lab ACR ≥30 vs Lab ACR <30 (all phase 2)

[e] Haemoglobin or erythrocytes detected by dipstick method

Effect of freezing on semi-quantitative POC dipstick parameters

Freezing had a minimal effect on dipstick parameters with a small increase in false negatives and no increase in false positives being seen for haematuria and leukocyturia. (See Supplementary for detailed results and methodology)

Discussion

Our study represents the largest evaluation of a POC ACR compared to laboratory ACR to date. Similar to previous studies[15–22] we found that the POC analyser has good rule out utility for albuminuria, as indicated by the NPV. Our study differs from previous studies in that we simultaneously evaluated additional urine dipstick parameters aside from albuminuria. Similar studies[15–21] have looked at semi-quantitative POC ACR from different manufacturers, however this is the first published study on the Sysmex UC-1000® instrument. The fully automated Sysmex UC-3500 has previously been validated showing excellent precision and good analytical accuracy.[22]

When using ACR to screen for albuminuria, the recommended sample is an early morning urine sample. Previous studies have used random urine[15,18,20] or early morning samples[16,17,19,21] with sensitivity of random urine samples being 0.83,[15] 0.79,[18] and 0.63[20] while the sensitivity of early morning samples was 0.90,[16] 0.90,[17] 0.75,[19] 0.92[21] and 0.80,[21] suggesting a possible advantage of early morning sampling.[27]

Our findings show the POC device would be useful for excluding albuminuria, but any positive result would need to be confirmed with a laboratory ACR. This in keeping with other studies where NPV ranged from 0.89 – 0.99[15,16,18,19,21,22] with one study reporting a NPV of 0.71[17]; while positive predictive value was low for all studies ranging from 0.46 – 0.82.[15–19,21,22] Similarly, only one study[16] reported a positive likelihood ratio above 10 indicating utility in ruling in disease.

The improved sensitivity seen in participant groups with known risk factors for CKD could help guide screening strategies. The discordance in sensitivities between a history of hypertension (medical questionnaire) and raised BP during examination could point to the effect of antihypertensives, particularly angiotensin-converting enzyme inhibitors, on albuminuria.[28] Screening strategies targeted at those with a history of diabetes mellitus, the elderly, those who present with elevated blood pressure, and those with HIV are justified, and in keeping with management guidelines.[29,30] Our findings corroborate those of previous studies: in the study by Graziani et al.[16] the sensitivity

and NPV of POC ACR improved marginally from 0.90 to 0.91 and 0.90 to 0.98 respectively in the general population versus a cohort with a history of type 2 diabetes mellitus. McTaggart et al.[15] found improved sensitivity and NPV: 0.83 to 0.91 and 0.95 to 0.98 respectively in the general population compared to participants with hypertension. The study by Nah et al.[21] was conducted in prediabetic and diabetic populations with sensitivity of 0.92 and 0.80, and NPV of 0.99 and 0.91 respectively. Our sensitivity of 0.79 and NPV of 0.97 are in keeping with random urine samples in the general population. Although the loss of specificity (increased false positives) seen in those with diabetes mellitus in our study suggests the need for confirmatory laboratory testing, the increased sensitivity in this group of participants is valuable in detecting diabetic nephropathy. Although increased sensitivity was seen in subgroup analysis, the low PPV and high NPV remained in all sub-populations suggesting that laboratory confirmation of positive results is required while negative results exclude disease.

When using POC ACR, 82% of samples were correctly classified into the correct KDIGO albuminuria stages, however, the poor performance in those with albuminuria according to POC ACR suggests category shifting in this group. Despite POC ACR having 100% sensitivity in those with significant albuminuria (>300mg/g or stage A3), 50% of positive samples were false positive results. The high number of false positives and relatively few false negatives suggests that POC ACR tends to shift patients into higher concentrations of albuminuria, thus higher ACR categories. As such, negative results carry more significance, further emphasized by the 97% accuracy seen in those with POC ACR <30mg/g.

Surprisingly, our study showed a high prevalence of abnormal urine findings besides ACR-highlighting the extent of abnormalities which can be picked up with dipstick analysis in our population. Similar findings were demonstrated during phase one of the ARK study (haematuria: 32.6% and leukocyturia: 30.3%), while the effects of freezing at -80^{0}C were shown to have minimal effects (Supplementary). Our aims were not to validate these findings but rather to expose the

extent of abnormalities seen. The epidemiology of CKD in LMICs is complex and often comprises the influence of multiple factors including NCDs, IDs, host and environmental factors.[31] Our findings suggest that strategies to decrease the impact of CKD should involve screening, diagnosing and managing CKD, but also screening for, and managing other risk factors.[32]

We found a high prevalence of haematuria on dipstick analysis. Potential explanations for isolated haematuria could be endemic urogenital schistosmiasis, a known cause of CKD in LMICs[31], or glomerular pathology that could include infectious (or other) glomerulonephritides, urogenital tuberculosis, and interstitial nephritis. The high level of haematuria observed in the population is concerning: haematuria is associated with an increased risk of progression to ESRD,[33] and plays a pathological role in renal tubular damage.[34] These dipstick findings need to be validated in our population, however previous studies have recommended dipstick analysis when screening for haematuria due to high sensitivity and reduced costs.[35–38]

All the dipstick parameters except for leucocytes occurred with higher frequency in those with laboratory albuminuria; of these haematuria and glycosuria had statistical significance. This is in keeping with the role haematuria plays in various renal pathologies as well as the risk for kidney disease which is inferred from diabetes mellitus (a possible cause for glycosuria amongst others). Although not statistically significant the relatively higher prevalence of leucocytes in urine of those with laboratory negative findings was an unexpected finding. Previous studies[39–42] including a study performed on South African participants with CKD[43] showed that the presence of JC viruria was protective of developing CKD, with 43-fold higher odds of developing kidney disease in those without JC viruria. Whether these protective virurias stimulate leucocyte shedding in the urine of individuals is yet to be studied but could be a possible explanation for the raised leucocytes seen in laboratory negative individuals.

The limitations of dipstick screening such as false positives, interferences from various drugs, a lack a specificity and the need for confirmatory laboratory or microscopy testing are well known.[44] In RLS

where access to central laboratory services is often limited the goals and uses of POC testing are very different to resource replete settings. Better patient outcomes with POC testing can be achieved even with decreased performance compared to laboratory-based testing if POC testing ensures improved linkage to care and retention in treatment.[45] The ability of dipstick testing to detect the potential presence of multiple risk factors for CKD and other co-morbid conditions such as diabetes mellitus, schistosomiasis, urinary tract infections, urological abnormalities, liver disease, and numerous others; together with conventional (albuminuria) and non-conventional (haematuria) markers of CKD in a single test is beneficial. Semi-quantitative (and semi-automated) results are preferable over subjective visual dipstick inspection, such semi-quantitative results aid primary care nurses who are often responsible for following screening guidelines in RLS.

Our study had several limitations: the POC testing was performed in a laboratory environment on the same day as laboratory measurements and not at the patient side, in a less controlled environment it is possible the POC instrument will not perform to the same standards. The urine samples were kept in a fridge overnight and then frozen the next day at -80^{0}C before being shipped frozen to the central laboratory. Previous studies have shown good stability of urine albumin and ACR with long term storage at -70^{0}C,[46,47] urine albumin and ACR is stable for up to 7 days when stored at 4^{0}C.[48,49] To our knowledge no studies exist on the stability of urine dipstick analysis in previously frozen samples. Dipstick analysis on samples post refrigeration up to 48 hours has shown: glucose to be stable up to 48 hours at 4^{0}C; nitrites stable up to 24 hours at 4^{0}C; leucocytes, haemoglobin, red blood cells, bilirubin show increased false negative results at 4^{0}C from between 4 and 8 hours.[50,51] It is possible the POC device would perform better on fresh urine as this is the recommended sample from the manufacturer (due to bilirubin and urobilinogen deterioration), however frozen and refrigerated samples can be used if returned to room temperature.[52] Despite the widespread use of immunoassays and the kinetic Jaffe method to measure laboratory ACR, these methods remain far from ideal gold standards with which to compare POC ACR. There is currently no reference method or material for urine albumin; as such methods are not standardised despite this

being a goal of the National Kidney Disease Education Program.[53,54] Comparison of results between different methods have generally not found large biases.[53,55] Although isotope dilution mass spectrometry-traceability has been established for the Jaffe method and a primary reference material exists the lack of matrix-specific secondary reference materials for urine creatinine mean that calibration is not ideal.[53] Consequently, the use of laboratory ACR as the gold standard in our study was a pragmatic rather than ideal choice. Our study also has several advantages, the large sample size from a rural population in South Africa represents an important demographic group where POC testing may be most beneficial. While random urine sampling is not the recommended sample type (versus concentrated early morning sampling) it represents the situation that will be encountered across rural clinics in South Africa. We were able to process all 700 samples, without having to exclude outliers- and so better represent the clinical situation which would be encountered by health care workers.

Conclusions

In conclusion, we have demonstrated the good rule out utility of a POC ACR dipstick device, which could aid in excluding at risk patients who do not require confirmatory central laboratory testing in RLS. We have also shown the large number of other abnormal findings seen with urine dipstick testing in our population- which could have implications for establishing screening guidelines for multiple diseases or risks for CKD with a single test. Our study population and findings are relevant in rural Africa with limited access to central laboratory services.

Abbreviations

CKD: chronic kidney disease

POC: point-of-care

ACR: albumin-creatinine ratio

PPV: positive predictive value

NPV: negative predictive value

SSA: Sub-Saharan Africa

ID: infectious diseases

NCD: non-communicable diseases

LMIC: low- and middle-income countries

RLS: resource-limited settings

SA: South Africa

ARK: African Research on Kidney

BP: blood pressure

Declarations

Ethics and consent to participate

Study approval was obtained from the Human Research Ethics Committee (Medical) of the University of the Witwatersrand (M160937 and M190434) and trained fieldworkers obtained written, informed consent from participants in their first language.

Consent for publication

Not applicable.

Data availability

The data underlying this article will be shared on reasonable request to the corresponding author.

Competing interests

The authors declare that they have no competing interests.

Funding

Research was jointly supported by (i) the South African Medical Research Council, with funds from the South African National Department of Health, MRC UK (via the Newton Fund) and GSK R&D; (ii) Faculty Research Committee Individual Research Grant, University of Witwatersrand; (iii) The International Society of Nephrology Clinical Research Program.

Funders played no role in the design of the study; collection, analysis or interpretation of data or in writing the manuscript.

Author contributions

SC wrote the manuscript and performed analysis and interpretation of data, while participating in elements of conception and design. The manuscript forms part of the requirements for the fulfilment of a MMed degree for SC. JG and JF acted as supervisors for the project- providing conception and design as well as guidance with data interpretation. Both supervisors actively revised the manuscript with important contributions and gave their final approval for the submitted manuscript. ST participated in conceptualising the study and editing the final draft of the paper. MG acted as the project manager, participating in project implementation and oversight, data collection, data checking; and reviewed the final draft of the paper. NM acted as the assistant project manager, participating in oversight of study procedures, data collection, data checking; and reviewed the final draft of the paper. SC acted as the laboratory manager, participating in oversight of laboratory procedures, quality control, specimen collection and processing, storage and shipping; and reviewed the final draft of the paper. BK acted as a medical laboratory technician, participating in quality control procedures, specimen collection and processing, storage and shipping; and reviewed the final draft of the paper.

Acknowledgments

We thank Sysmex Corporation for supplying the UC-1000 system, 12S dipsticks and quality control material for measurement of urine dipstick and albumin to creatinine ratio.

References

1. Naghavi M, Abajobir AA, Abbafati C, et al. Global, regional, and national age-sex specifc mortality for 264 causes of death, 1980-2016: A systematic analysis for the Global Burden of Disease Study 2016. *Lancet*. 2017;390(10100):1151-1210. doi:10.1016/S0140-6736(17)32152-9

2. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. *PLoS Med*. 2006;3(11):e442. doi:10.1371/journal.pmed.0030442

3. George JA, Brandenburg J-T, Fabian J, et al. Kidney damage and associated risk factors in rural and urban sub-Saharan Africa (AWI-Gen): a cross-sectional population study. *Lancet Glob Heal*. 2019;7(12):e1632-e1643. doi:10.1016/S2214-109X(19)30443-7

4. Liyanage T, Ninomiya T, Jha V, et al. Worldwide access to treatment for end-stage kidney disease: A systematic review. *Lancet*. 2015;385(9981):1975-1982. doi:10.1016/S0140-6736(14)61601-9

5. Klausen K, Borch-Johnsen K, Feldt-Rasmussen B, et al. Very low levels of microalbuminuria are associated with increased risk of coronary heart disease and death independently of renal function, hypertension, and diabetes. *Circulation*. 2004;110(1):32-35. doi:10.1161/01.CIR.0000133312.96477.48

6. Ärnlöv J, Evans JC, Meigs JB, et al. Low-grade albuminuria and incidence of cardiovascular disease events in nonhypertensive and nondiabetic individuals: The framingham heart study. *Circulation*. 2005;112(7):969-975. doi:10.1161/CIRCULATIONAHA.105.538132

7. Verhave JC, Gansevoort RT, Hillege HL, Bakker SJL, De Zeeuw D, de Jong PE. An elevated urinary albumin excretion predicts de novo development of renal function impairment in the

general population. *Kidney Int Suppl*. 2004;(92):S18-21. doi:10.1111/j.1523-1755.2004.09205.x

8. Gerstein HC, Mann JFE, Yi Q, et al. Albuminuria and risk of cardiovascular events, death, and heart failure in diabetic and nondiabetic individuals. *J Am Med Assoc*. 2001;286(4):421-426. doi:10.1001/jama.286.4.421

9. Park HY, Schumock GT, Pickard AS, Akhras K. A Structured Review of the Relationship between Microalbuminuria and Cardiovascular Events in Patients with Diabetes Mellitus and Hypertension. *Pharmacotherapy*. 2003;23(12):1611-1616. doi:10.1592/phco.23.15.1611.31962

10. KDIGO Working Group. Kidney Disease: Improving Global Outcomes (KDIGO). KDIGO 2012 clinical practice guideline for the evaluation and management of chronic kidney disease. *Kidney Int Suppl*. 2013;3(1):1-150.

11. Ninomiya T, Perkovic V, De Galan BE, et al. Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes. *J Am Soc Nephrol*. 2009;20(8):1813-1821. doi:10.1681/ASN.2008121270

12. Sacks DB, Arnold M, Bakris GL, et al. Executive summary: Guidelines and recommendations for laboratory analysis in the diagnosis and management of diabetes mellitus. *Clin Chem*. 2011;57(6):793-798. doi:10.1373/clinchem.2011.163634

13. Kazmierczak SC. Point-of-care testing quality: Some positives but also some negatives. *Clin Chem*. 2011;57(9):1219-1220. doi:10.1373/clinchem.2011.171538

14. Cox HS, Mbhele S, Mohess N, et al. Impact of Xpert MTB/RIF for TB Diagnosis in a Primary Care Clinic with High TB and HIV Prevalence in South Africa: A Pragmatic Randomised Trial. *PLoS Med*. 2014;11(11):e1001760. doi:10.1371/journal.pmed.1001760

15. McTaggart MP, Price CP, Pinnock RG, Stevens PE, Newall RG, Lamb EJ. The diagnostic

accuracy of a urine albumin-creatinine ratio point-of-care test for detection of albuminuria in primary care. *Am J Kidney Dis*. 2012;60(5):787-794. doi:10.1053/j.ajkd.2012.05.009

16. Graziani MS, Gambaro G, Mantovani L, et al. Diagnostic accuracy of a reagent strip for assessing urinary albumin excretion in the general population. *Nephrol Dial Transplant*. 2009;24(5):1490-1494. doi:10.1093/ndt/gfn639

17. Guy M, Newall R, Borzomato J, Kalra PA, Price C. Diagnostic accuracy of the urinary albumin: creatinine ratio determined by the CLINITEK Microalbumin and DCA 2000+ for the rule-out of albuminuria in chronic kidney disease. *Clin Chim Acta*. 2009;399(1-2):54-58. doi:10.1016/j.cca.2008.09.006

18. Le Floch JP, Marre M, Rodier M, Passa PH. Interest of clinitek® microalbumin in screening for microalbuminuria: Results of a multicentre study in 302 diabetic patients. *Diabetes Metab*. 2001;27(1):36-39.

19. Croal BL, Mutch WJ, Clark BM, et al. The clinical application of a urine albumin: Creatinine ratio point-of-care device. *Clin Chim Acta*. 2001;307(1-2):15-21. doi:10.1016/S0009-8981(01)00450-8

20. Meinhardt U, Ammann RA, Flück C, Diem P, Mullis PE. Microalbuminuria in diabetes mellitus - Efficacy of a new screening method in comparison with timed overnight urine collection. *J Diabetes Complications*. 2003;17(5):254-257. doi:10.1016/S1056-8727(02)00180-0

21. Nah EH, Cho S, Kim S, Cho HI. Comparison of urine albumin-to-creatinine ratio (ACR) between acr strip test and quantitative test in prediabetes and diabetes. *Ann Lab Med*. 2017;37(1):28-33. doi:10.3343/alm.2017.37.1.28

22. Oyaert M, Delanghe JR. Semiquantitative, fully automated urine test strip analysis. *J Clin Lab Anal*. 2019;33(5):1-7. doi:10.1002/jcla.22870

23. Kalyesubula R, Fabian J, Nakanga W, et al. How to estimate glomerular filtration rate in sub-

Saharan Africa: Design and methods of the African Research into Kidney Diseases (ARK) study. *BMC Nephrol*. 2020;21(1):1-12. doi:10.1186/s12882-020-1688-0

24. Chobanian A V., Bakris GL, Black HR, et al. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: The JNC 7 Report. *J Am Med Assoc*. 2003;289(19):2560-2572. doi:10.1001/jama.289.19.2560

25. National Department of Health. National Consolidated Guidelines for the Prevention of Mother-To-Child Transmission of HIV (PMTCT) and the Management of HIV in Children, Adolescents and Adults. *Dep Heal Repub South Africa*. 2015.

26. Whelton PK, Carey RM, Aronow WS, et al. 2017 ACC/AHA/AAPA/ABC/ACPM/AGS/APhA/ASH/ASPC/NMA/PCNA Guideline for the Prevention, Detection, Evaluation, and Management of High Blood Pressure in Adults A Report of the American College of Cardiology / American Heart Association Task Force on Clinical P. *Hypertension*. 2017;71(6):e13-e115. doi:10.1161/HYP.0000000000000065/-/DC1.The

27. Witte EC, Heerspink HJL, De Zeeuw D, Bakker SJL, De Jong PE, Gansevoort R. First morning voids are more reliable than spot urine samples to assess microalbuminuria. *J Am Soc Nephrol*. 2009;20(2):436-443. doi:10.1681/ASN.2008030292

28. Zhang WR, Craven TE, Malhotra R, et al. Kidney damage biomarkers and incident chronic kidney disease during blood pressure reduction: A case-control study. *Ann Intern Med*. 2018;169(9):610-618. doi:10.7326/M18-1037

29. KDOQI Clinical Practice Guidelines and Clinical Practice Recommendations for Diabetes and Chronic Kidney Disease. *Am J Kidney Dis*. 2007;47(5 Suppl 3):S11-145. doi:10.1053/j.ajkd.2006.12.005

30. Diana NE, Naicker S. Update on current management of chronic kidney disease in patients with HIV infection. *Int J Nephrol Renovasc Dis*. 2016;9:223-234. doi:10.2147/IJNRD.S93887

31. Stanifer JW, Muiru A, Jafar TH, Patel UD. Chronic kidney disease in low- and middle-income countries. *Nephrol Dial Transplant*. 2016;31(6):868-874. doi:10.1093/ndt/gfv466

32. Jha V, Arici M, Collins AJ, et al. Understanding kidney care needs and implementation strategies in low- and middle-income countries: conclusions from a "Kidney Disease: Improving Global Outcomes" (KDIGO) Controversies Conference. *Kidney Int*. 2016;90(6):1164-1174. doi:10.1016/j.kint.2016.09.009

33. Vivante A, Afek A, Frenkel-Nir Y, et al. Persistent asymptomatic isolated microscopic hematuria in Israeli adolescents and young adults and risk for end-stage renal disease. *J Am Med Assoc*. 2011;306(7):729-736. doi:10.1001/jama.2011.1141

34. Moreno JA, Sevillano Á, Gutiérrez E, et al. Glomerular hematuria: Cause or consequence of renal inflammation? *Int J Mol Sci*. 2019;20(9). doi:10.3390/ijms20092205

35. Bataille A, Wetzstein M, Hertig A, Vimont S, Rondeau E, Galichon P. Evidence of dipstick superiority over urine microscopy analysis for detection of hematuria. *BMC Res Notes*. 2016;9(1):435. doi:10.1186/s13104-016-2240-y

36. Anigilaje EA, Adedoyin OT. Correlation between dipstick urinalysis and urine sediment microscopy in detecting haematuria among children with sickle cell anaemia in steady state in Ilorin, Nigeria. *Pan Afr Med J*. 2013;15:135. doi:10.11604/pamj.2013.15.135.1854

37. Arm JP, Peile EB, Rainford DJ, Strike PW, Tettmar RE. Significance of dipstick haematuria. 1. Correlation with microscopy of the urine. *Br J Urol*. 1986;58(2):211-217. doi:10.1111/j.1464-410x.1986.tb09029.x

38. Arm JP, Peile EB, Rainford DJ. Significance of dipstick haematuria. 2. Correlation with pathology. *Br J Urol*. 1986;58(2):218-223. doi:10.1111/j.1464-410x.1986.tb09030.x

39. Divers J, Núñez M, High KP, et al. JC polyoma virus interacts with APOL1 in African Americans with nondiabetic nephropathy. *Kidney Int*. 2013;84(6):1207-1213. doi:10.1038/ki.2013.173

40. Freedman BI, Kistler AL, Skewes-Cox P, et al. JC polyoma viruria associates with protection from chronic kidney disease independently from apolipoprotein L1 genotype in African Americans. *Nephrol Dial Transplant*. 2018;33(11):1960-1967. doi:10.1093/ndt/gfx368

41. Pires EP, Bernardino-Vallinoto C V., Alves DM, et al. Prevalence of infection by JC and BK polyomaviruses in kidney transplant recipients and patients with chronic renal disease. *Transpl Infect Dis*. 2011;13(6):633-637. doi:10.1111/j.1399-3062.2011.00614.x

42. Kruzel-Davila E, Divers J, Russell GB, et al. JC viruria is associated with reduced risk of diabetic kidney disease. *J Clin Endocrinol Metab*. 2019;104(6):2286-2294. doi:10.1210/jc.2018-02482

43. Nqebelele NU, Dickens C, Dix-Peek T, Duarte R, Naicker S. JC Virus and APOL1 Risk Alleles in Black South Africans With Hypertension-Attributed CKD. *Kidney Int Reports*. 2019;4(7):939-945. doi:10.1016/j.ekir.2019.05.006

44. Simerville JA, Maxted WC, Pahira JJ. Urinalysis: A comprehensive review. *Am Fam Physician*. 2005;71(6):1153-1162.

45. Drain PK, Hyle EP, Noubary F, et al. Diagnostic point-of-care tests in resource-limited settings. *Lancet Infect Dis*. 2014;14(3):239-249. doi:10.1016/S1473-3099(13)70250-0

46. Chapman DP, Gooding KM, McDonald TJ, Shore AC. Stability of urinary albumin and creatinine after 12 months storage at −20 °C and −80 °C. *Pract Lab Med*. 2019;15(March):e00120. doi:10.1016/j.plabm.2019.e00120

47. Kania K, Byrnes EA, Beilby JP, Webb SAR, Strong KJ. Urinary proteases degrade albumin: Implications for measurement of albuminuria in stored samples. *Ann Clin Biochem*. 2010;47(Pt 2):151-157. doi:10.1258/acb.2009.009247

48. Herrington W, Illingworth N, Staplin N, et al. Effect of processing delay and storage conditions on urine albumin-to-creatinine ratio. *Clin J Am Soc Nephrol*. 2016;11(10):1794-1801. doi:10.2215/CJN.13341215

1 49. Osberg I, Chase HP, Garg SK, et al. Effects of storage time and temperature on measurement
2 of small concentrations of albumin in urine. *Clin Chem*. 1990;36(8 Pt 1):1428-1430.
3 doi:10.1093/clinchem/36.8.1428

4 50. Froom P, Bieganiec B, Ehrenrich Z, Barak M. Stability of common analytes in urine
5 refrigerated for 24 h before automated analysis by test strips. *Clin Chem.* 2000;46(9):1384-
6 1386.

7 51. Ekşioğlu MK, Madenci ÖÇ, Yücel N, et al. The effectiveness of BD vacutainer® plus urinalysis
8 preservative tubes in preservation of urine for chemical strip analysis and particle counting.
9 *Biochem Medica*. 2016;26(2):224-232. doi:10.11613/BM.2016.024

10 52. Sysmex Corporation. *MEDITAPE UC-12S (Package Insert)*.; 2017.

11 53. Miller WG, Bruns DE, Hortin GL, et al. Current issues in measurement and reporting of urinary
12 albumin excretion. *Clin Chem.* 2009;55(1):24-38. doi:10.1373/clinchem.2008.106567

13 54. Speeckaert MM, Speeckaert R, Van De Voorde L, Delanghe JR. Immunochemically unreactive
14 albumin in urine: fiction or reality? *Crit Rev Clin Lab Sci*. 2011;48(2):87-96.
15 doi:10.3109/10408363.2011.591366

16 55. Seegmiller JC, Sviridov D, Larson TS, Borland TM, Hortin GL, Lieske JC. Comparison of urinary
17 albumin quantification by immunoturbidimetry, competitive immunoassay, and protein-
18 cleavage liquid chromatography-tandem mass spectrometry. *Clin Chem*. 2009;55(11):1991-
19 1994. doi:10.1373/clinchem.2009.129833

a0044271:BMC.REV.no_mark-up.doc

ORIGINALITY REPORT

14%	11%	12%	4%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

1	academic.oup.com Internet Source	2%
2	www.researchsquare.com Internet Source	2%
3	doaj.org Internet Source	1%
4	synapse.koreamed.org Internet Source	1%
5	journals.lww.com Internet Source	1%
6	Malcolm P. McTaggart, Christopher P. Price, Roger G. Pinnock, Paul E. Stevens, Ronald G. Newall, Edmund J. Lamb. "The Diagnostic Accuracy of a Urine Albumin-Creatinine Ratio Point-of-Care Test for Detection of Albuminuria in Primary Care", American Journal of Kidney Diseases, 2012 Publication	<1%
7	www.ncbi.nlm.nih.gov	

	Internet Source	<1%
8	clearafred.wits.ac.za Internet Source	<1%
9	www.repository.cam.ac.uk Internet Source	<1%
10	Nolubabalo U. Nqebelele, Caroline Dickens, Therese Dix-Peek, Raquel Duarte, Saraladevi Naicker. "JC Virus and APOL1 Risk Alleles in Black South Africans With Hypertension-Attributed CKD", Kidney International Reports, 2019 Publication	<1%
11	www.science.gov Internet Source	<1%
12	Roberta Ricotti, Giulia Genoni, Enza Giglione, Alice Monzani et al. "High-normal estimated glomerular filtration rate and hyperuricemia positively correlate with metabolic impairment in pediatric obese patients", PLOS ONE, 2018 Publication	<1%
13	archsurg.ama-assn.org Internet Source	<1%
14	www.nice.org.uk Internet Source	<1%

15	archive.org Internet Source	<1%
16	reproductive-health-journal.biomedcentral.com Internet Source	<1%
17	"Oral Abstracts", Journal of Paediatrics and Child Health, 4/2008 Publication	<1%
18	M. Yousefzadeh, P. Esfahanian, S. M. S. Movahed, S. Gorgin et al. " : Radiologist-Assistant Deep Learning Framework for COVID-19 Diagnosis in Chest CT Scans ", Cold Spring Harbor Laboratory, 2020 Publication	<1%
19	David Flood, Pablo Garcia, Kate Douglas, Jessica Hawkins, Peter Rohloff. "Screening for chronic kidney disease in a community-based diabetes cohort in rural Guatemala: a cross-sectional study", BMJ Open, 2018 Publication	<1%
20	Submitted to University of Birmingham Student Paper	<1%
21	journals.sagepub.com Internet Source	<1%
22	www.iraqijms.net Internet Source	<1%

23	accesspharmacy.mhmedical.com Internet Source	<1%
24	www.ias2015.org Internet Source	<1%
25	Mark Hensey, Dale J. Murdoch, Janarthanan Sathananthan, David A. Wood, John G. Webb. "Impact of chronic kidney disease on decision-making and management in transcatheter aortic valve interventions", Canadian Journal of Cardiology, 2018 Publication	<1%
26	link.springer.com Internet Source	<1%
27	theses.gla.ac.uk Internet Source	<1%
28	www.dovepress.com Internet Source	<1%
29	"Biomarkers in Kidney Disease", Springer Science and Business Media LLC, 2016 Publication	<1%
30	jasn.asnjournals.org Internet Source	<1%
31	Binqi Li, Anping Wang, Yuxia Wang, Lin Li et al. " A study on the correlation between remnant cholesterol and urinary albumin to creatinine	<1%

ratio in Chinese community adults: A report from the study ", Journal of Diabetes, 2020
Publication

32	Hua-Wei Huang, Guo-Bin Zhang, Hao-Yi Li, Chun-Mei Wang et al. "Development of an Early Prediction Model for Postoperative Delirium in Neurosurgical Patients Admitted to the ICU after Elective Craniotomy (E-PREPOD-NS): A Secondary Analysis of A Prospective Cohort Study", Research Square, 2021 Publication	<1%
33	M. Leahy. "Olfactory detection of human bladder cancer by dogs: Cause or association?", BMJ, 11/27/2004 Publication	<1%
34	N.U. Nqebelele, C. Dickens, T. Dix-Peek, R. Duarte, S. Naicker. "SUN-195 URINARY UROMODULIN LEVELS AND UROMODULIN GENE IN BLACK SOUTH AFRICANS WITH HYPERTENSION-ATTRIBUTED CHRONIC KIDNEY DISEASE", Kidney International Reports, 2019 Publication	<1%
35	bmcnephrol.biomedcentral.com Internet Source	<1%
36	dagensdiabetes.se Internet Source	<1%

37	jamanetwork.com Internet Source	<1%
38	pure.uva.nl Internet Source	<1%
39	rsmedic.com Internet Source	<1%
40	www.biomedcentral.com Internet Source	<1%
41	www.oxford.dec.nihr.ac.uk Internet Source	<1%
42	"Pediatric Kidney Disease", Springer Science and Business Media LLC, 2016 Publication	<1%
43	"Posters", Clinical Microbiology and Infection, 5/2008 Publication	<1%
44	Andrew S. Levey, Cassandra Becker, Lesley A. Inker. "Glomerular Filtration Rate and Albuminuria for Detection and Staging of Acute and Chronic Kidney Disease in Adults", JAMA, 2015 Publication	<1%
45	Benedicte Stengel. "Prediction of Risk and Prognosis: Decision Support for Diagnosis and Management of Chronic Kidney Disease",	<1%

Evidence-Based Nephrology, 12/05/2008
Publication

46 Nikolai C. Hodel, Ali Hamad, Claudia Praehauser, Grace Mwangoka et al. "The epidemiology of chronic kidney disease and the association with non-communicable and communicable disorders in a population of sub-Saharan Africa", PLOS ONE, 2018 <1%
Publication

Exclude quotes	Off	Exclude matches	Off
Exclude bibliography	Off		