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Evaluation of the impact of delayed centrifugation on the diagnostic performance of serum creatinine as a baseline measure of renal function before antiretroviral treatment

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Evaluation of the impact of delayed centrifugation on the diagnostic performance of serum creatinine as a baseline measure of renal function before antiretroviral treatment

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Student number: 1577728

A dissertation submitted to the Faculty of Health Sciences, University of Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the Degree of Master of Medicine (MMed)

Johannesburg, 20 November 2020

DECLARATION

I, Chemedzai Chikomba, declare that this dissertation is my own, unaided work. This dissertation is being submitted to the Faculty of Health Sciences, University of Witwatersrand, Johannesburg, in partial fulfilment of the requirements for the Degree of Master of Medicine (MMed).

Johannesburg 2020

Candidate Name: C.E Chikomba

Signature: 

Date: 20/11/2020

ABSTRACT

Background: The measurement of serum creatinine is a standard requirement of the medical management of people living with HIV. Renal dysfunction is common, both as a complication of HIV-infection and as a result of its treatment. The detection of abnormal renal function before the start of antiretroviral therapy will impact patient management and the outcome of treatment.

Objectives: This study aimed to determine if a time delay in the centrifugation of serum samples affected the creatinine level and the estimated glomerular filtration rate as recorded on the analytical platforms used in the laboratory.

Methods: Twenty-two (n = 22) HIV-positive, newly diagnosed and treatment-naïve patients were randomly recruited from Alexandra Health Clinic, Johannesburg, South Africa. Serum samples were centrifuged at six time intervals following receipt of the sample viz. < 4 h (baseline), 6 h, 24 h, 48 h, 72 h and 96 h. Creatinine concentrations were measured on the Roche platform utilizing the enzymatic and kinetic Jaffe methods. Whole blood samples were also analysed with the Abbott i-STAT point-of-care instrument. The estimated glomerular filtration rate was calculated using the Cockcroft Gault, CKD–Epidemiology Collaboration and Modified Diet and Renal Disease v3/4 equations.

Results: At baseline (< 4 h) there was good agreement between the enzymatic and kinetic Jaffe methods: bias 1.7 µmol/l. The enzymatic and i-STAT creatinine concentrations were stable over 96 h viz. changes of 1.8% and 5.7%. However, from 24 h onwards agreement between the enzymatic and kinetic Jaffe methods was poor with the latter measuring 43.7 µmol/l higher than the enzymatic method at 96 h. Creatinine concentrations measured with the kinetic Jaffe method increased significantly in samples centrifuged after 6 h (p < 0.001, 61.7% change), and resulted in

a 95% decline in eGFR at 96 h as determined with the CKD–Epidemiology Collaboration equation.

Conclusion: The analysis of serum creatinine using the isotope dilution mass spectrometry traceable kinetic Jaffe method is unreliable if performed on samples centrifuged ≥ 6 h after collection. The raised creatinine concentration can affect clinical decisions such as renal functional assessment, choice of antiretroviral drug or regimen, and the dose and frequency of medication.

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- Mr. Philani Buthelezi from the Department of Health (DOH) for liaising with the DOH Gauteng Region E.
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PUBLICATIONS EMANATING FROM THIS MMED RESEARCH PROJECT

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. S Afr J HIV Med. 2020;21(1), a1056.

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NOTES ON THE RESEARCH SUBMISSION

This submission serves as the final research report for my MMed project. This research report consists of the paper published in the Southern African Journal of HIV Medicine. The format of this research report is different from the published paper; however it is a direct copy of the publication. The published article, in the format in which it was published, is available in Appendix 1.

The research protocol for this project was assessed and approved by the assessors panel from the School of Pathology at the University of the Witwatersrand, Faculty of Health Sciences. A more comprehensive literature review was done during protocol writing and will provide a broader review of the topic of this research report. The approved protocol can be found in Appendix 2. However, the title of this study was subsequently changed from 'Evaluation of the diagnostic performance of serum creatinine as a baseline measure of function before antiretroviral (ARV) treatment', to 'Evaluation of the impact of delayed centrifugation on the diagnostic performance of serum creatinine as a baseline measure of renal function before antiretroviral treatment'. The title was changed to allow for a more precise (specific) title as delay in separation was the only attribute that varied in this study, and thus the only element of the diagnostic performance evaluated. The approval for the change of title is presented in Appendix 3.

Ethics clearance was obtained from the University of the Witwatersrand, Human Research Ethics Committee (MEDICAL) (Appendix 4).

This research report follows the "Publication Model" format as stipulated by the University of the Witwatersrand for published articles.

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LIST OF ABBREVIATIONS

DOH	Department of Health
NHLS	National Health Laboratory Service
KDIGO	Kidney Disease Improving Global Outcomes
NICE	National Institute for Health and Care Excellence
WHO	World Health Organisation
CLIA	Clinical Laboratory Improvement Amendments
MMed	Masters in Medicine
HIV	Human immunodeficiency virus
AIDS	Acquired immune deficiency syndrome
ART	Antiretroviral therapy
TDF	Tenofovir disoproxil fumarate
CKD	Chronic kidney disease
GFR	Glomerular filtration rate
eGFR	Estimated glomerular filtration rate
CKD EPI	Chronic Kidney Disease Epidemiology Collaboration
MDRD	Modified Diet and Renal Disease
CG	Cockcroft Gault
MPI	Maximum permissible instability

SL	Stability limit
IDMS	Isotope dilution mass spectrometry
UOM	Uncertainty of measurement

1. INTRODUCTION

South Africa (SA) has one of the highest HIV infection rates in the world. In 2016, the overall prevalence of HIV infection in SA was 12.7%.[1, 2] The use of antiretroviral drugs (ARVs) has slowed the progression of the infection and increased the life expectancy of people living with HIV.[3, 4, 5] In SA's public health sector, patients are started on antiretroviral treatment (ART) with combinations of different ARV drug classes, prescribed as a single, once-a-day tablet.[6] This has led to a reduction of the pill-burden of therapy and to improved adherence.[7, 8] In the southern African region the most frequently prescribed initial combination is the nucleotide reverse transcriptase inhibitor (NtRTI), tenofovir disoproxil fumarate (TDF), the nucleoside reverse transcriptase inhibitors (NRTIs) emtricitabine (FTC) or lamivudine (3TC) and the non- nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz (EFV). This is first-line ART.[6] Current SA national ART guidelines indicate that the integrase strand transfer inhibitor (INSTI), dolutegravir (DTG), has replaced efavirenz for all except women of child-bearing age who may fall pregnant or those initiating ART in the first trimester of pregnancy.[9, 10] Tenofovir, however, remains the standard NRTI-backbone of all first-line ART in southern Africa.

TDF is an acyclic nucleoside phosphate prodrug. Its long half-life permits once-daily dosing. Although the drug is extensively filtered by the kidneys, 20% – 30% is actively reabsorbed at the proximal tubule.[11] TDF is an occasional cause of renal injury viz. a proximal renal tubulopathy and a salt-wasting syndrome (Fanconi Syndrome and renal tubular acidosis), drug-induced acute kidney injury and a slower, general decline in glomerular filtration, diabetes insipidus, and a dysregulation of the kidney's calcium and phosphorus metabolism.[11, 12] Impaired renal function may also result from the use of other drugs, such as aminoglycosides, sulphonamides, and amphotericin B, the presence of HIV-associated nephropathy (HIVAN), blood-borne infection, for example tuberculosis, bacteraemia and fungaemia, HIV-associated immune-complex kidney

disease and common comorbidities such as hypertension and diabetes mellitus.[13, 14] Pre-existing mild renal dysfunction may increase susceptibility to the toxicity of TDF. Kidney function must be evaluated before starting ART: the use of TDF is contraindicated if the estimated glomerular filtration rate (eGFR) is < 50 ml/min. Patients on TDF have serum creatinine measured at 3 and 6 months following the initiation of ART and biannually thereafter. In high-risk patients, for example with coexistent hypertension or diabetes, the creatinine is measured more frequently.[9]

The measurement of the GFR confirms and stages the degree of renal impairment and provides a platform for the ongoing monitoring of kidney function. Although the urinary clearance of inulin is the gold standard of GFR measurement,[15] the method is time-consuming, expensive and impractical in most clinical settings. Endogenous substances such as serum creatinine and cystatin C are cheaper, provide more rapid results and are widely available.[16] Although the National Institute for Health and Care Excellence (NICE) and the Kidney Disease Improving Global Outcomes (KDIGO) groups both base their eGFR assessment (equations) on cystatin C, its cost and lack of standardisation excludes its general use.[17, 18] Creatinine is produced at a constant rate, is present in all body fluids, and is filtered by the glomerulus. Its serum level is influenced by several biological factors, such as active secretion by the renal tubules in the presence of declining renal function, diet, extremes of muscle mass, age, gender, drugs and the use of creative supplements.[19] Several equations control for some of these variables.[20] The SA National Department of Health (SANDOH) 2019 guidelines recommend, therefore, that the Counahan-Barratt equation be used to measure the eGFR of youths aged 10–16 years and the Modifications of Diet in Renal Disease (MDRD) equation be used for adolescents and adults 16 years of age and older.[9]

The Cockcroft-Gault (CG) equation incorporates the serum creatinine level and the variables of weight, age and sex in the measurement of the eGFR.[21] The equation has limitations: in pregnancy, at the extremes of weight, and in those on dialysis for acute renal failure. Its principal

use is in drug dosing and pharmacokinetic studies.[20] The MDRD equation adds a fourth variable: race.[22] This ethnicity adjustment-factor is based on African Americans and tends to overestimate the GFR in sub-Saharan (African) populations.[23] The CKD–Epidemiology Collaboration (EPI) equation is recommended by the KDIGO guidelines group and has been validated in participants with and without impaired renal function with eGFR < 90 ml/ min/1.73 min^2.[17, 24] The SA National Health Laboratory Service (SA-NHLS) currently reports both the MDRD and CKD-EPI eGFR without ethnic adjustment. The adoption of the CKD-EPI equation in SA has been delayed due to limited local data.

Routine laboratory serum creatinine measurements are analysed on Jaffe and enzymatic assays. These methods should be traceable to isotope dilution mass spectrometry (IDMS) method and the Standard Reference Material for creatinine in serum (SRM 967). NHLS laboratories in SA generally use the modified kinetic Jaffe method, as opposed to the enzymatic assay, as it is more affordable. The World Health Organization's (WHO) guidelines indicate that at room temperature, creatinine is stable for 2–3 days.[25] However, a report from Shepherd et al. [26] noted a significant increase in creatinine levels measured on the kinetic Jaffe method when processing occurred later than 24 h.[26] This impacted the study's eGFR results and had caused the renal misclassification of patients.[26] A turnaround time for serum creatinine measurements from local clinics in Gauteng of 12–72 h has been observed at NHLS laboratories, as reported in the internal TAT reports. This delay increases with the remoteness of clinics due to the poor transport networks, the restricted working hours at referral laboratories, and the greater workload of regional laboratories. The aim of this study was to determine, in HIV-positive black South Africans not on ART, the stability limit (SL), that is, the time at room temperature, when the serum creatinine results are still within the maximum permissible instability (MPI) range.[27]

2. METHODS

2.1. Study population

Twenty-two (n = 22) newly diagnosed and treatment-naïve people living with HIV between the ages of 18 and 70 years were randomly recruited from the Alexandra Health Community Centre in Alexandra, Johannesburg, SA. The minimum number of participants required for testing the stability of biochemical analytes is 10.[27] Furthermore, multiple samples were required from each participant for analysis on three analysers at six time points, providing 396 creatinine measurement data points. Patients with comorbidities such as diabetes mellitus, pregnancy, urinary tract infection, hypertension and proteinuria were excluded from the study. De-identified demographic and anthropometric data were transcribed from the patient files.

2.2. Sample collection and processing

Each patient provided 13 tubes of blood: 12 × approximately 2 ml in 5 ml serum separator-tubes (SST) and 1 × 5 ml in a heparin tube (Becton Dickenson, Plymouth, UK). Serum was isolated from SST tubes following centrifugation at 1370 × g for 5 min at defined time intervals viz. < 4 h, 6 h, 24 h, 48 h, 72 h and 96 h. Prior to centrifugation, samples were stored at room temperature. Whole blood heparin tubes were stored at room temperature until required.

2.3. Measurement of creatinine concentrations

Serum creatinine concentrations were measured immediately after each centrifugation (as above) using the IDMS-traceable enzymatic and IDMS-traceable kinetic Jaffe method on the Roche COBAS 8000 module 702 and Roche COBAS 6000 module 502 instruments at the NHLS laboratory of the Chris Hani Baragwanath Hospital, Soweto, SA. The enzymatic method test principle is based on a series of coupled reactions that result in the conversion of creatinine to hydrogen peroxide. In the final reaction, catalysed by peroxidase, a quinone imine chromogen is formed. The colour intensity of this chromogen is measured at 550 nm and is directly proportional

to the concentration of creatinine in the sample. The kinetic Jaffe method test principle is based on the reaction of creatinine and picrate under alkaline conditions forming a yellow-orange complex. The rate of formation of this complex is proportional to the amount of creatinine in the sample. The kinetic Jaffe method is prone to interference from non- creatinine chromogens such as proteins and ketones. A correction factor of -26 µmol/l is, therefore, universally applied to correct for these inherent chromogens. Bilirubin is the major negative interferent in the kinetic Jaffe method, and this is overcome by rate blanking. The internal quality control for creatinine in our laboratory is performed twice daily. The laboratory adheres to the Royal College of Pathologists of Australasia (RCPA) quality assurance programmes. External quality assurance submissions during the study period were within allowable limits.

Creatinine concentrations were also measured using whole blood samples on the Abbott i-STAT point-of-care instrument immediately after collection and at 6 h, 24 h, 48 h, 72 h and 96 h. This method is based on the conversion of creatinine to hydrogen peroxide, via hydrolysis and oxidation. The hydrogen peroxide is oxidized (at the platinum electrode) to produce a current that is proportional to the concentration of creatinine in the sample.

2.4. Estimated glomerular filtration rate measurements

eGFR was calculated using the MDRDv3 *without ethnic adjustment* (age, sex, and serum creatinine), MDRDv4 (age, sex, ethnicity, and serum creatinine), CG and CKD-EPI equations *with ethnic adjustment.*

2.5. Statistical analysis

All analyses were performed using Medcalc software. The Shapiro-Wilks test was used to test for normality. Normally distributed continuous variables (creatinine) were presented as mean ± standard deviation and continuous variables that were not normally distributed (age, weight) were presented as median [interquartile range]. Categorical variables are presented as proportions and

percentages. The Student paired t-test was used to compare baseline creatinine concentrations between the three different methods (Bonferroni corrected *p*-value). The serum creatinine results were evaluated for analytically significant changes using the uncertainty of measurement (UOM; calculated for the laboratory quality control data in the preceding 6 months), total allowable error (TAE) of 15% (Clinical Laboratory Improvement Amendments [CLIA] and coefficient of variation [CV]). Passing-Bablok linear regression and Bland- Altman plots were generated to evaluate correlation and method agreement over time, using the enzymatic method as the reference method. For all analyses, $p < 0.05$ was considered statistically significant.

2.6. Ethical consideration

Ethical approval was obtained from the University of the Witwatersrand Human Research Ethic Committee, reference number: M1711109. R14/49.

3. RESULTS

3.1. Characteristics of the study population

A total of 22 randomly recruited HIV-positive, treatment-naïve black South African individuals consented to participate in this study. The median age of the participants was 37 years. The majority (54.5%; 12 of 22) of participants were younger than 40 years of age. The age distribution is consistent with the current demographics published by Statistics South Africa with respect to individuals mostly affected by HIV/AIDS. All participants were black Africans (ethnicity self-reported). The median weight for the participants was 70.0 kg (range 45–110 kg) and 59.1% (13 of 22) of participants were male.

3.2. Creatinine assay performance

The mean baseline (< 4 h) creatinine concentrations for the study cohort obtained using the enzymatic, kinetic Jaffe and i-STAT methods were 78.73 µmol/l ± 14.63 µmol/l, 77.05

µmol/l ± 13.12 µmol/l and 70.14 µmol/l ± 15.3 µmol/l: $p > 0.05$ for all comparisons (Table 1). The mean creatinine concentrations analysed using the kinetic Jaffe method were significantly higher than baseline when blood samples were processed after 6 h: $p \leq 0.001$ for all comparisons. Creatinine concentrations did not change significantly over the 96 h when measured using the enzymatic method or i-STAT system: $p > 0.05$ for all comparisons (Table 1).

Table 1: Creatinine concentrations for the study cohort (n = 22) obtained using the enzymatic, kinetic Jaffe and i-STAT methods at six different time intervals

Time interval (hours)	Creatinine concentration					
	Enzymatic		Kinetic Jaffe		i-STAT	
	µmol/l	*p	µmol/l	*p	µmol/l	*p
< 4 h	78.73 ± 14.63	-	77.05 ± 13.12	-	70.14 ± 15.35	-
6 h	74.55 ± 14.11	0.340	78.41 ± 15.19	0.752	69.91 ± 16.00	0.962
24 h	78.91 ± 14.64	0.967	95.23 ± 20.26	0.001	67.59 ± 14.72	0.578
48 h	79.86 ± 15.70	0.805	117.64 ± 19.82	< 0.001	68.18 ± 14.02	0.662
72 h	76.79 ± 14.82†	0.676	121.29 ± 20.97§	< 0.001	67.59 ± 14.71	0.577
96 h	77.31 ± 10.45‡	0.743	124.56 ± 18.49¶	< 0.001	66.14 ± 15.82††	0.406

Results are expressed as mean ± standard deviation, missing data due to insufficient volume or high haemolysis index (> 1000 mg/dL) for †, $n = 3$; ‡, $n = 6$; §, $n = 1$; ¶, $n = 4$; ††, $n = 1$.
* p-value for results compared to 4 h creatinine concentration

The kinetic Jaffe method exhibited the widest intra- individual CV (range: 12% – 40% vs. 1.2% – 12% enzymatic and 1.9% – 12% i-STAT method) as well as the greatest inter- assay variation (22.7% vs. 6.0% enzymatic and 6.6% i-STAT method), as seen in Figure 1. When creatinine results were assessed against the 10% UOM for each respective assay, 8.9% of the enzymatic creatinine results fell outside the UOM for the duration of the study compared to 65% of the results for the kinetic Jaffe method. The i-STAT results could not be evaluated as UOM has not been established for this assay.

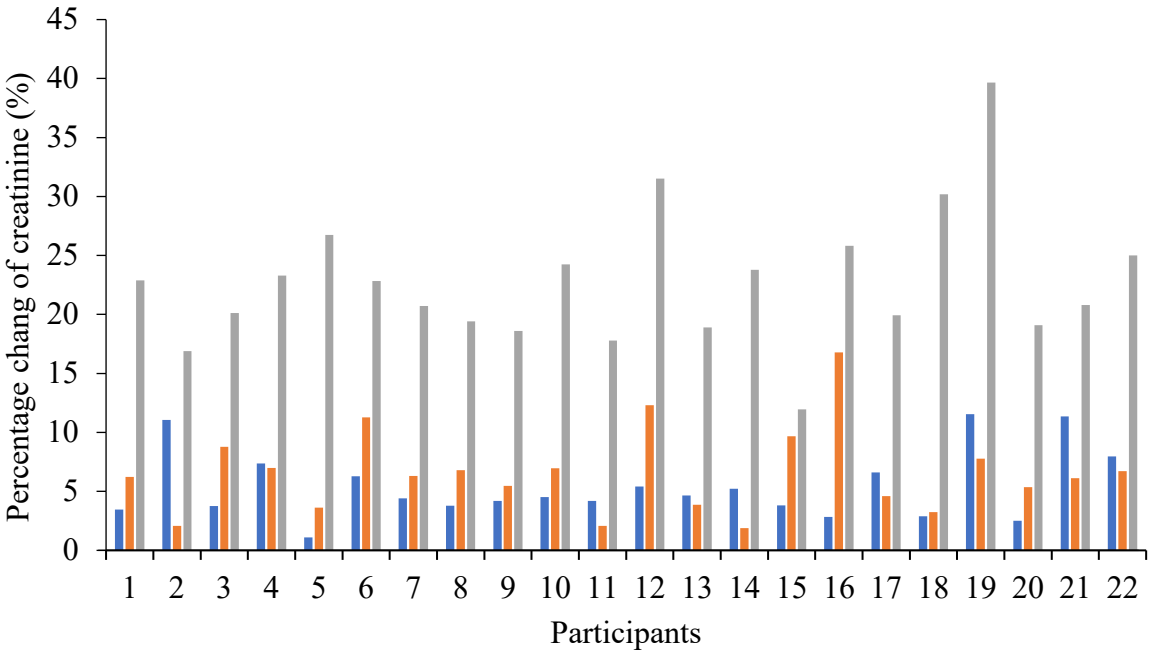


Figure 1: Intra-individual coefficient of variation for 22 participants for the enzymatic, kinetic Jaffe and i-STAT creatinine methods over the 96h study period.
Blue bars represent results obtained for the enzymatic method, orange bars for the i-STAT method and grey bars for the kinetic Jaffe method.

Based on the positive trend of increasing creatinine concentrations over time observed for the Jaffe method, we explored the data further to determine if a correction factor could be established to adjust creatinine results obtained from samples with delayed processing time. The percentage change of the serum creatinine results for the participants over the 96 h time frame was inconsistent and a correction factor could, therefore, not be established based on the current sample size (Figure 2).

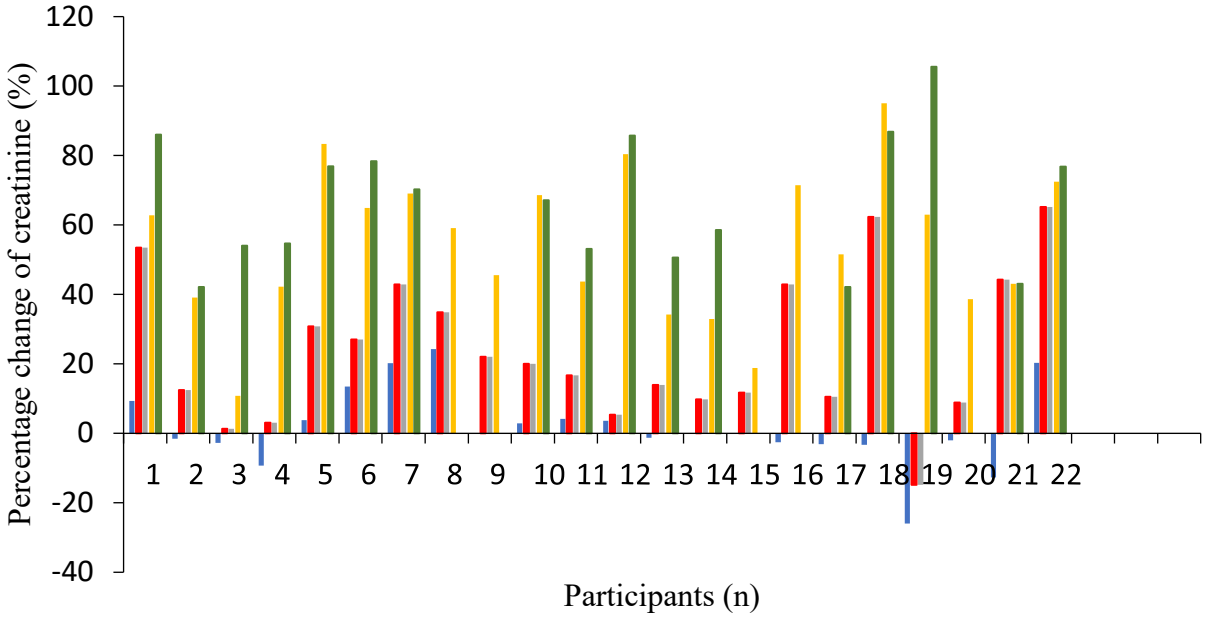


Figure 2: Percentage change in serum creatinine concentrations obtained using the kinetic Jaffe method over 96 h for 22 participants relative to the serum creatinine results at 4 h. Percentage change to baseline (4 h):
Blue bars = 4–6 h, Orange = 4–24 h, Grey = 4–48 h, Yellow = 4–72 h, Green = 4–96 h.

3.3. Method comparison

Passing-Bablok regression and Bland-Altman plots were used to determine the correlation and agreement of the kinetic Jaffe method and i-STAT creatinine results compared to the enzymatic creatinine results at different time intervals (see Table 2, Figure 3 and Figure 4). The enzymatic method was chosen as the reference method as it had the best analytical CV in our study and previous studies have shown that this method correlated well with the standard reference method (isotopic dilution mass spectrometry).[27,28,29]

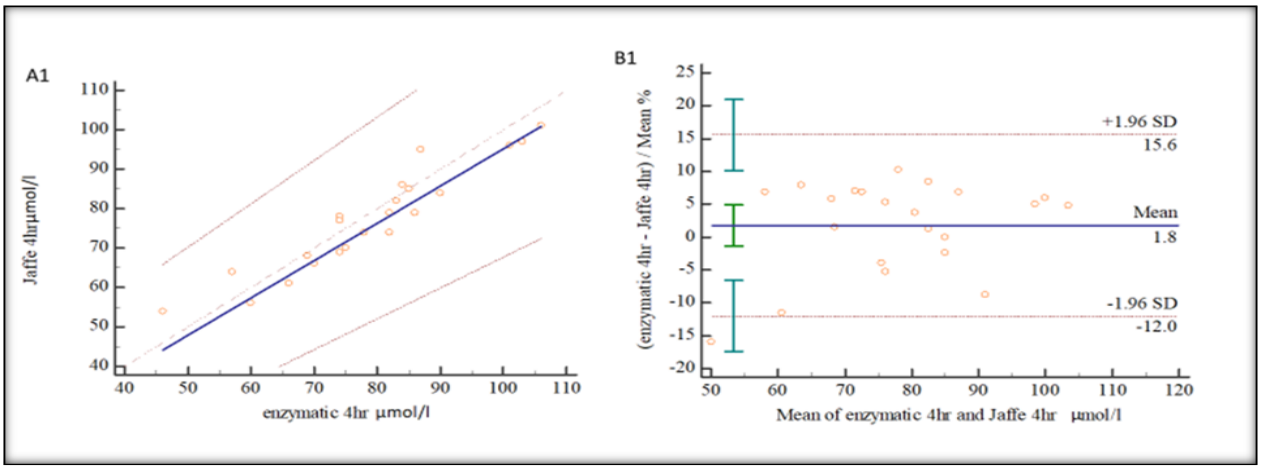
Table 2: Comparison of serum creatinine levels from kinetic Jaffe method and i-STAT method to the enzymatic method using Passing-Bablok and Bland-Altman analysis

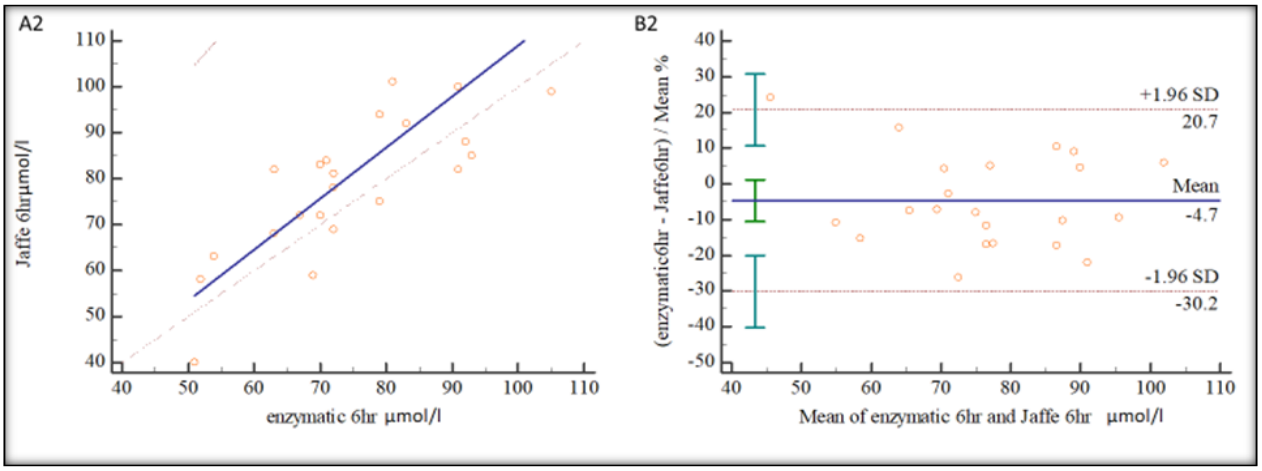
Analytical method	Time (hours)	Passing–Bablok parameters regression				Bland-Altman			
		Slope	95% CI	Intercept (95% CI)	95% CI	*r*	Mean bias	%	Mean bias (µmol/l)
Kinetic Jaffe Method	< 4 h	0.95	0.78–1.1	0.54	-10.74–15.54	0.953	1.8	-1.31–4.93	1.7
	6 h	1.10	0.75–1.64	-2.00	-41.18–21.25	0.803	-4.7	-10.47–1.04	3.9
	24 h	1.6	0.95–2.50	-27.83	-88.50–19.65	0.596	-17.8	-25.20–10.40	16.3
	48 h	1.26	0.83–2.23	16.84	-56.85–52.75	0.582	-38.6	-45.66–-31.51	37.8
	72 h	1.33	1.06–2.00	18.90	-32.00–40.13	0.858†	-44.7	-50.24–39.18	44.0
	96 h	2.25	1.13–5.50	-23.82	152.40–43.94	0.855‡	-48.6	-53.41–43.88	49.9
i-STAT	< 4 h	1.01	0.83–1.38	-10.53	-38.23–3.50	0.836	12.2	7.38–16.96	8.6
	6 h	1.13	0.91–1.40	-13.24	-33.00–2.50	0.900	7.4	2.89–11.84	4.6
	24 h	1.04	0.86–1.20	-16.30	-28.50–-0.59	0.923	16.0	12.48–19.65	11.7
	48 h	0.88	0.67–1.12	-1.35	-21.54–13.67	0.893	15.8	11.69–19.95	11.6
	72 h	1.08	0.86–1.37	-14.61	-38.41–2.57	0.948§	12.3	7.93–16.74	8.6
	96 h	1.35	1.00–2.00	-38.02	-90.00–10.00	0.847¶	18.0	10.86–25.16	12.0

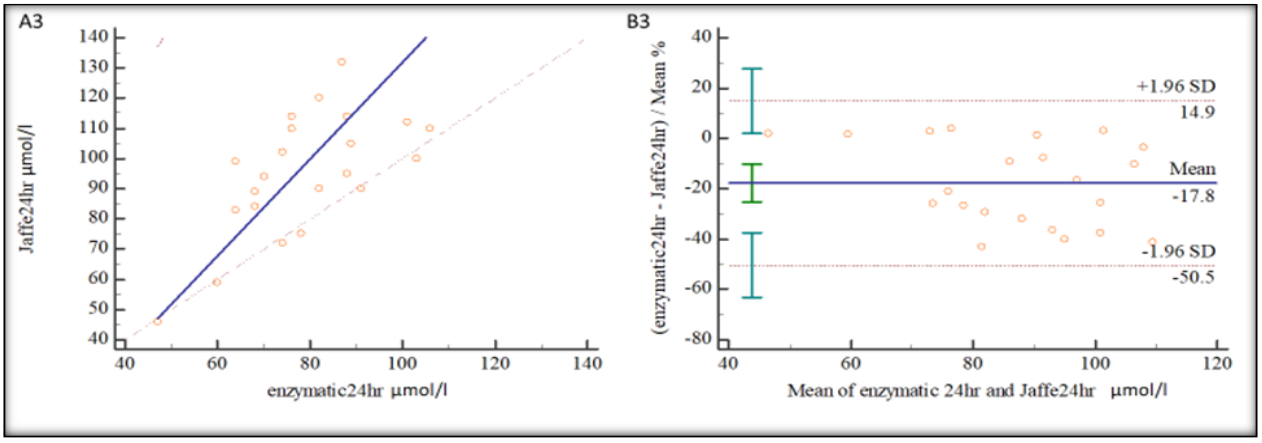
Legend: CI= Confidence Interval, r = correlation coefficient, linear regression line $y = mx + c$, c = intercept (constant error), m = slope (proportional error), number of participants results analysed. †, $n = 19$; ‡, $n = 16$; §, $n = 19$; ¶, $n = 16$.

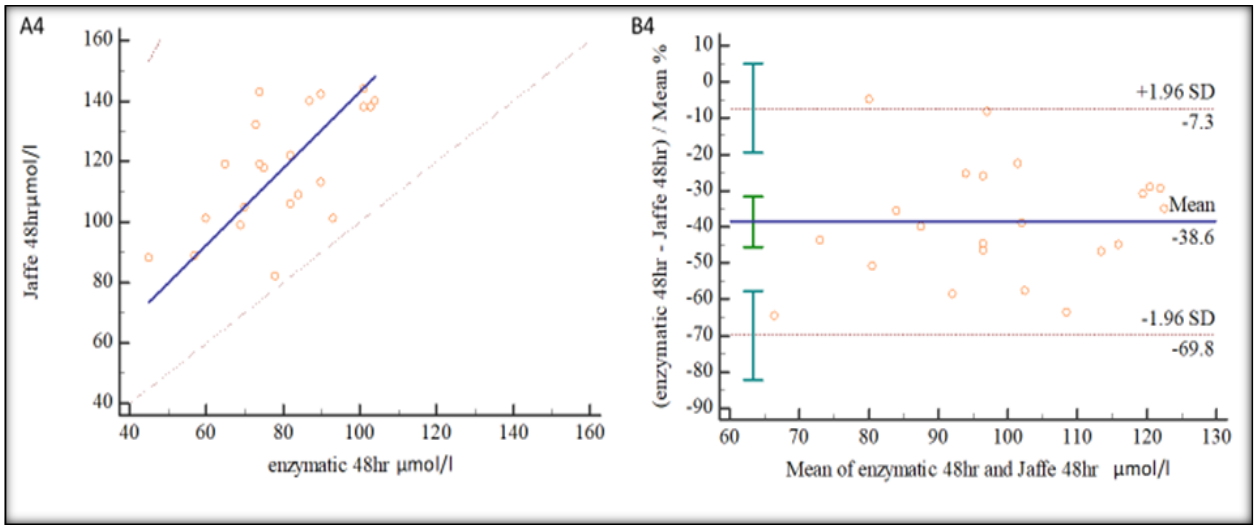
The Jaffe and enzymatic method showed a strong correlation at < 4 h (r = 0.953); however, this correlation became weaker with time. The higher r values at 72 h and 96 h may be due to the missing creatinine data (enzymatic 72 h n = 3, enzymatic 96 h n = 6, Jaffe 72 h n = 1, Jaffe 96 h n = 4) for participants at these time intervals. The slope and the intercept increased over the time interval (Table 2), and this demonstrated the increase in the magnitude of the systematic error. At 4 h, there was a strong agreement between the kinetic Jaffe method and enzymatic methods with a small negative bias of 1.8% (1.7 µmol/l. With an increased delay in sample separation, the kinetic Jaffe method resulted in an overestimation of creatinine concentrations with a positive bias of 48.6% (49.9 µmol/l) at 96 h (Figure 3).

Creatinine results from the i-STAT correlated well with the enzymatic creatinine results over the six time intervals used in the study: r-value, 0.836–0.948. The i-STAT method had a negative bias ranging from 7.4% to 18.0% throughout the study. The i-STAT method had a negative bias of 12.2% (8.6 µmol/l) at 4 h and 18.0% (12.0 µmol/l) at 96 h.


A1
Jaffe 4hrμmol/l
enzymatic 4hr μmol/l
B1
(enzymatic 4hr - Jaffe 4hr) / Mean %
+1.96 SD
15.6
Mean
1.8
-1.96 SD
-12.0
Mean of enzymatic 4hr and Jaffe 4hr μmol/l


A2
Jaffe 6hrμmol/l
enzymatic 6hr μmol/l
B2
(enzymatic6hr - Jaffe6hr) / Mean %
+1.96 SD
20.7
Mean
-4.7
-1.96 SD
-30.2
Mean of enzymatic 6hr and Jaffe 6hr μmol/l


A3
Jaffe24hr μmol/l
enzymatic24hr μmol/l
B3
(enzymatic24hr - Jaffe24hr) / Mean %
+1.96 SD
14.9
Mean
-17.8
-1.96 SD
-50.5
Mean of enzymatic 24hr and Jaffe24hr μmol/l


A4
Jaffe 48hrμmol/l
enzymatic 48hr μmol/l
B4
(enzymatic 48hr - Jaffe 48hr) / Mean %
+1.96 SD
-7.3
Mean
-38.6
-1.96 SD
-69.8
Mean of enzymatic 48hr and Jaffe 48hr μmol/l

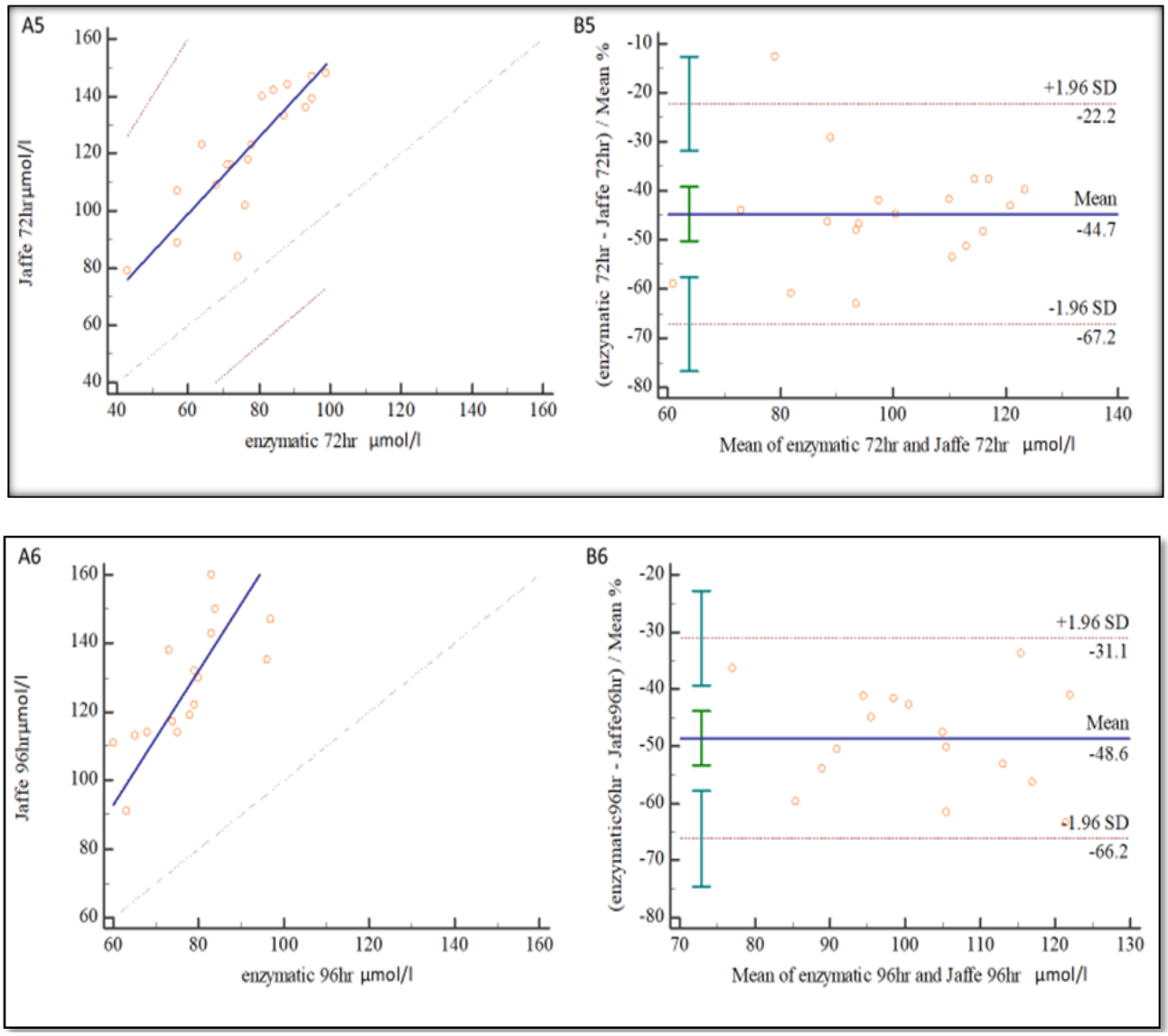
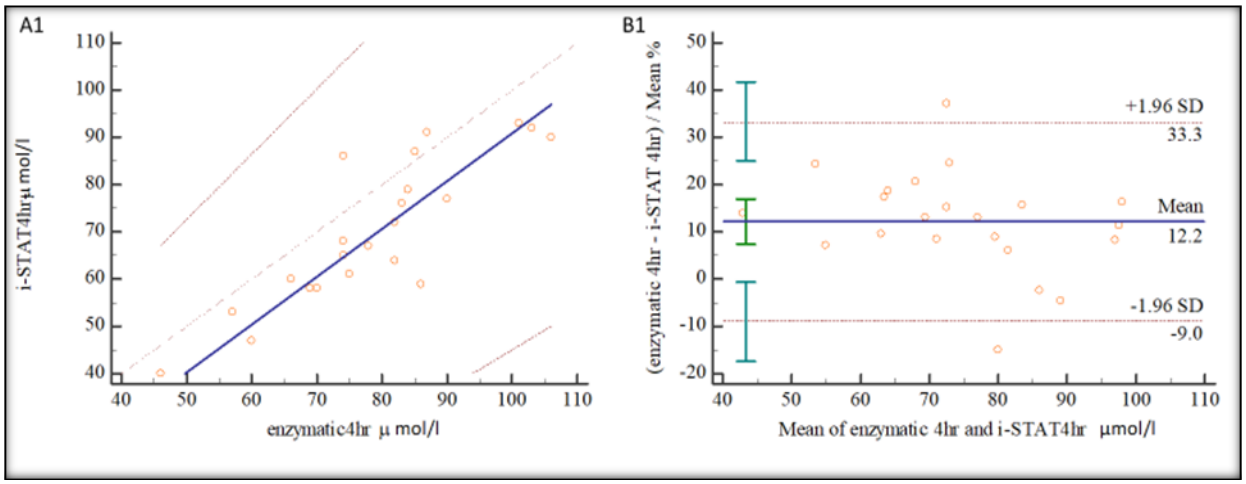
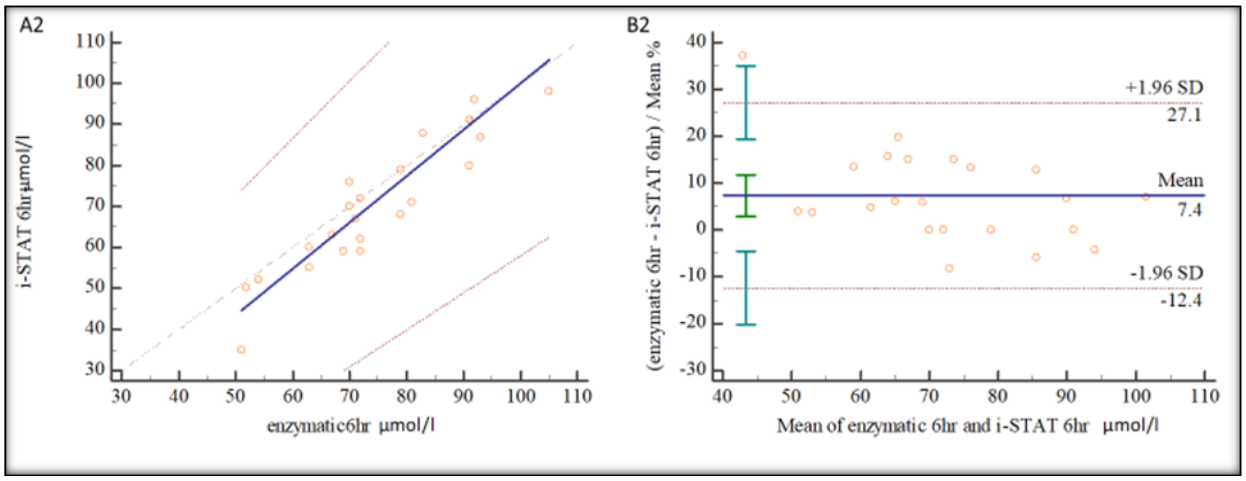
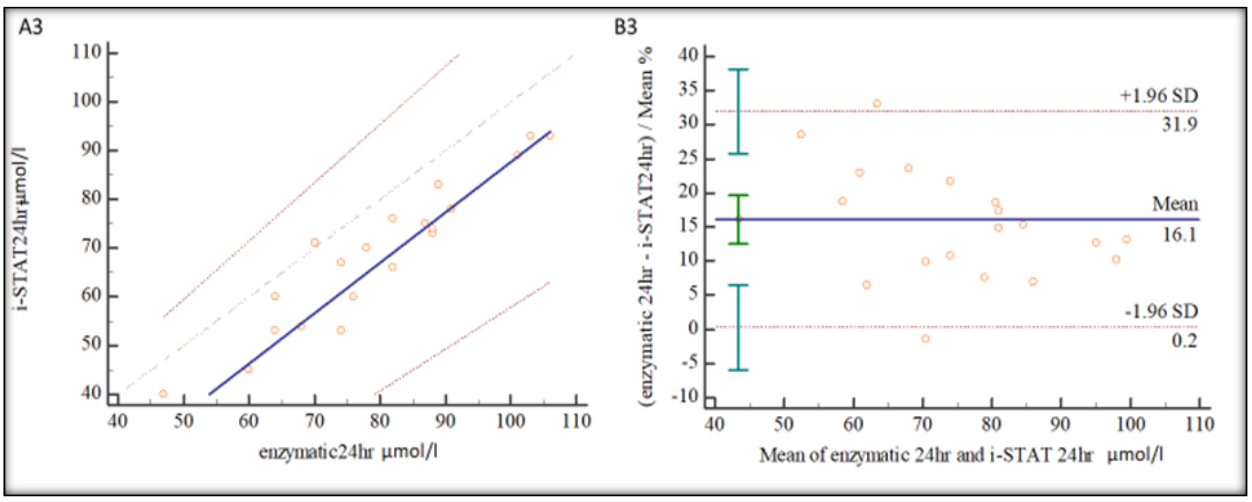
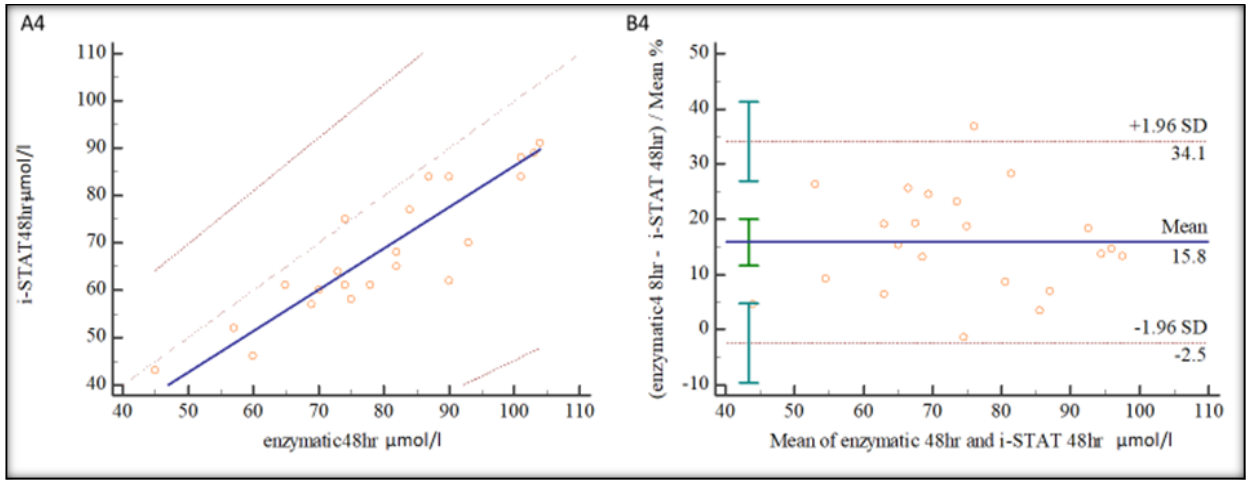


Figure 3: Passing-Bablok regression curves (A) and Bland-Altman plots (B) comparing creatinine concentrations from the kinetic Jaffe method with those from the enzymatic method. Creatinine concentrations at (1) < 4 h, (2) 6 h, (3) 24 h, (4) 48 h, (5) 72 h and (6) 96 h.


A1
i-STAT4hr µ mol/l
enzymatic4hr µ mol/l
B1
(enzymatic 4hr - i-STAT 4hr) / Mean %
+1.96 SD
33.3
Mean
12.2
-1.96 SD
-9.0
Mean of enzymatic 4hr and i-STAT4hr µmol/l


A2
i-STAT 6hr µmol/l
enzymatic6hr µmol/l
B2
(enzymatic 6hr - i-STAT 6hr) / Mean %
+1.96 SD
27.1
Mean
7.4
-1.96 SD
-12.4
Mean of enzymatic 6hr and i-STAT 6hr µmol/l


A3
i-STAT24hr µmol/l
enzymatic24hr µmol/l
B3
(enzymatic 24hr - i-STAT24hr) / Mean %
+1.96 SD
31.9
Mean
16.1
-1.96 SD
0.2
Mean of enzymatic 24hr and i-STAT 24hr µmol/l


A4
i-STAT48hr µmol/l
enzymatic48hr µmol/l
B4
(enzymatic4 8hr - i-STAT 48hr) / Mean %
+1.96 SD
34.1
Mean
15.8
-1.96 SD
-2.5
Mean of enzymatic 48hr and i-STAT 48hr µmol/l

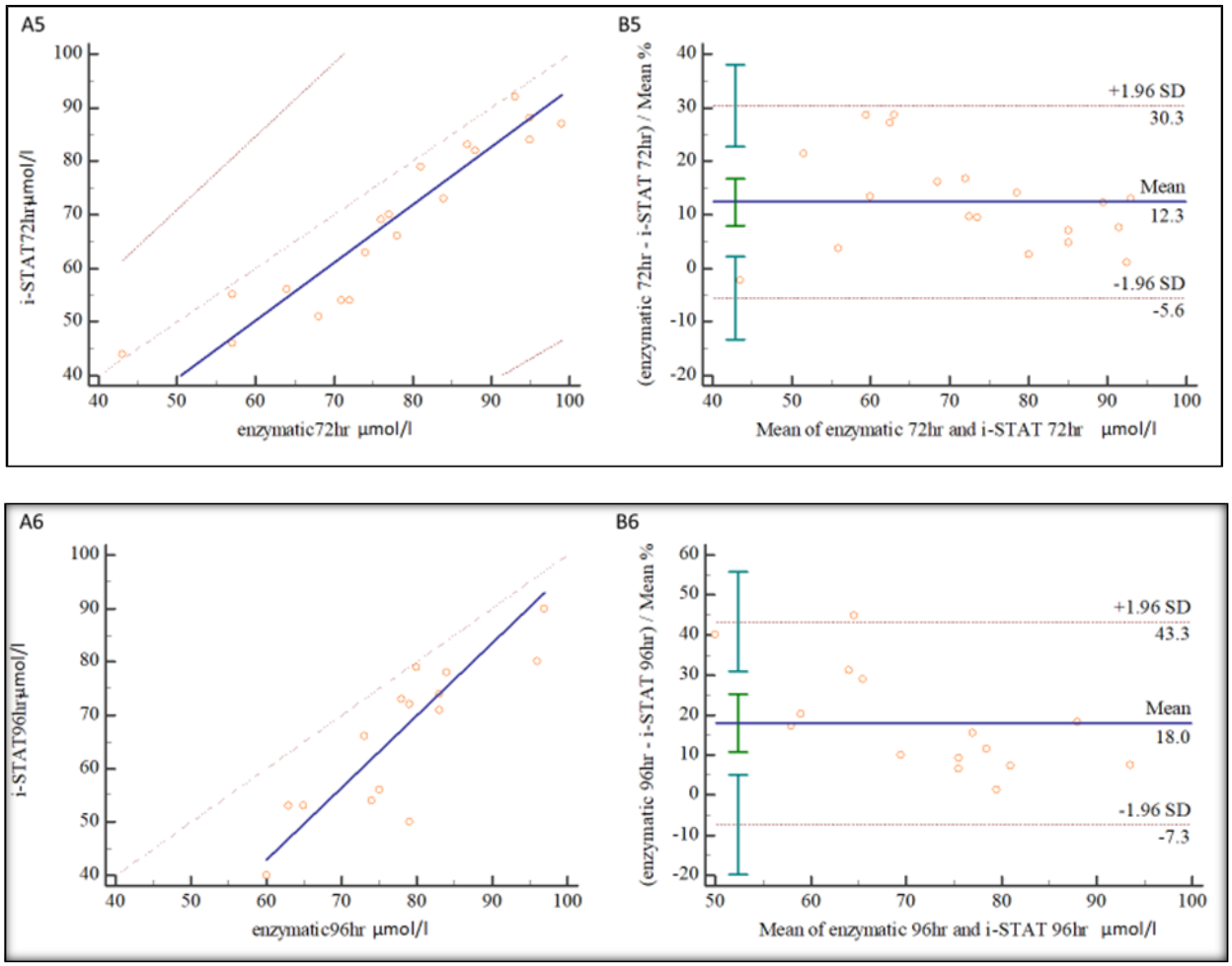


Figure 4: Passing-Bablok regression curves (A) and Bland-Altman plots (B) comparing creatinine concentrations from the i-STAT device with those obtained from the enzymatic method. Creatinine concentrations at (1) < 4 h, (2) 6 h, (3) 24 h, (4) 48 h, (5) 72 h and (6) 96 h.

3.4. Comparison of estimated glomerular filtration rate equation performance

The impact on the classification of renal dysfunction of the delay in centrifugation of blood and serum samples measured for creatinine levels by means of three different laboratory methods was evaluated with four eGFR equations viz. CG, MDRD v4, MDRD v3, and CKD-EPI. The serum creatinine eGFR results of the enzymatic and i-STAT methods performed well (to within the 10% TAE for eGFR throughout the study). However, the eGFR from the Jaffe data decreased over time. At baseline, viz. < 4 h, there was consensus among all four equations. All 22 participants had an eGFR > 60 ml/min per 1.73 m^2 using the CG and MDRD v4 equation. Similarly, 21 participants had an eGFR > 60 ml/min per 1.73 m^2 with the MDRD v3 and the CKD-EPI equations (Table 3). One participant had an eGFR of < 60 ml/min per 1.73 m^2. This person was classified as having stage 3A renal failure with an eGFR 45 ml/min – 59 ml/min per 1.73 m^2, according to the KDIGO guidelines.

Table 3: Chronic Kidney Disease staging using the four equations based on creatinine results obtained from the enzymatic, Jaffe and i-STAT methods.

Measure	Enzymatic method			Kinetic Jaffe method			i-STAT method		
	4 h	24 h	96 h	4 h	24 h	96 h	4 h	24 h	96 h
CKD-EPI eGFR mean (ml/min per 1.73 m²)	118 ± 36	118 ± 37	114 ± 28	100 ± 26	75± 33	45 ± 10	147 ± 50	152 ± 56	161 ± 68
Range	53–217	53–210	73–148	48–150	34–181	24–63	63–269	62–269	81–269
15–29, *n* (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1(5.5)	0 (0)	0 (0)	0 (0)
30–44, *n* (%)	0 (0)	0(0)	0 (0)	0 (0)	2 (9)	9 (50)	0 (0)	0 (0)	0 (0)
45–59, *n* (%)	1 (4.5)	1(4.5)	0 (0)	1 (0,5)	6 (27)	7 (39)	0 (0)	0 (0)	0 (0)
60–89, *n* (%)	5 (23)	4 (18)	5 (31)	7 (32)	10 (45)	1 (5.5)	2 (9)	2 (9)	2 (10)
≥ 90, *n* (%)	16 (73)	17 (77)	11 (69)	14 (64)	4 (18)	0 (0)	20 (91)	20 (91)	19 (90)
MDRDv3 eGFR mean (ml/min per 1.73 m²)	89 ± 20	89 ± 21	89 ± 15	91 ± 18	83 ± 31	51 ± 9	104 ± 126	107 ± 27	112 ± 34
Range	49–141	40–137	62–108	52–125	55–190	31–67	55–166	55–166	68–166
15–29, *n* (%) 30–44, *n* (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
45–59, *n* (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	4 (22)	0 (0)	0 (0)	0 (0)
>60	1 (4.5)	2 (9)	0 (0)	1 (4.5)	3 (14)	12 (67)	1 (4.5)	1 (4.5)	0 (0)
	21 (95.5)	20 (91)	16 (100)	21 (95.5)	19 (86)	2 (11)	21(95.5)	21 (95.5)	21 (100)
MDRDv4 eGFR mean (ml/min per 1.73 m²)	108 ± 25	108 ± 26	108 ± 18	100 ± 22	69 ± 26	42 ± 7	125 ± 32	130 ± 32	136 ± 41
Range	59–171	59–166	75–133	63–151	45–157	26–55	67–200	66–209	82–201
15–29, *n* (%) 30–44, *n* (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.5)	0 (0)	0 (0)	0 (0)
45–59, *n* (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	10 (55.5)	0 (0)	0 (0)	0 (0)
>60, *n* (%)	1 (4.5)	2 (9)	0 (0)	0 (0)	11 (9)	7 (39)	0 (0)	1 (4.5)	0 (0)
	21 (95.5)	20 (91)	16 (100)	22 (100)	11 (8)	0 (0)	22 (100)	21 (95.5)	21(100)
CG eGFR mean (ml/min per 1.73 m²)	129 ± 66	128 ± 64	125 ± 51	129 ± 54	111 ± 69	79 ± 28	125 ± 52	130 ± 51	137 ± 57
Range	66–391	66–382	79–295	69–333	54–391	47–162	78–328	76–324	83–324
15–29, *n* (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
30–44, *n* (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
45–59, *n* (%)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.5)	2 (11)	0 (0)	0 (0)	0 (0)
>60, *n* (%)	22 (100)	22 (100)	16 (100)	22 (100)	21 (95.5)	16 (89)	22 (100)	22 (100)	21 (100)

CKD-EPI, Chronic Kidney Disease-Epidemiology; eGFR, estimated glomerular filtration rate; MDRD, Modifications of Diet in Renal Disease; CG, Cockcroft-Gault.

The change in eGFR varied over time among the different equations. The CG equation was the least sensitive to the increase in serum creatinine: only 27% (n = 6/22) of eGFR- based 96-h creatinine concentrations resulted in a change in the Chronic Kidney Disease (CKD) stage when compared with baseline (i.e. the < 4 h eGFR; see Figure 5). The MDRD v4 equation gave the greatest change in eGFR over 96 h: 50% (n = 11/22) of participants at 24 h and 100% (n = 22/22) at 96 h showed an eGFR decline compared to baseline. The most significant change of eGFR was noted with the MDRDv3 equation: 25% eGFR at 48 h and 10% at 96 h compared to the baseline eGFR.

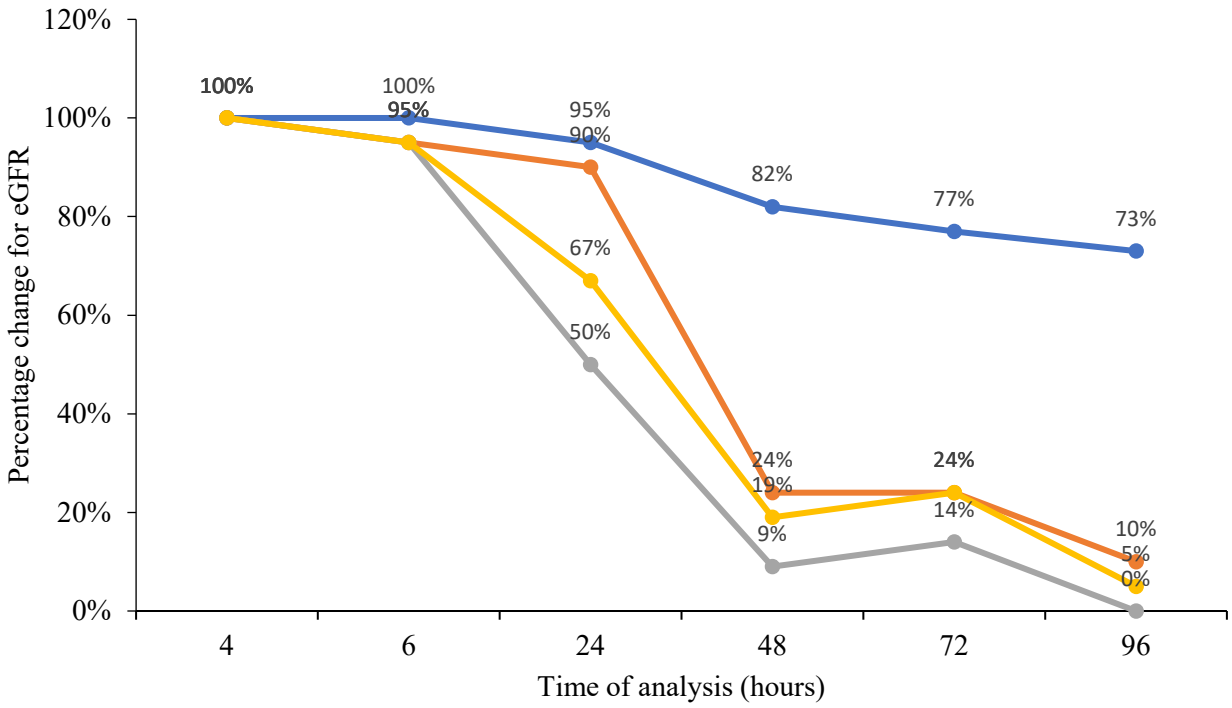


Figure 5: Percentage change of estimated glomerular filtration rate classification based on kinetic Jaffe creatinine concentrations over the 96 h study period compared to estimated glomerular filtration rate obtained within 4 h using the Cockcroft-Gault, Modifications of Diet in Renal Disease v3, Modifications of Diet in Renal Disease v4, and Chronic Kidney Disease-Epidemiology equations.
Blue = CG, Orange = MDRD3, Purple = CKD, Green = MDRD4.

Using the CKD-EPI equation as recommended by the KDIGO guidelines, enzymatic and i-STAT renal classification was noted at different time intervals with changes between stages 1 and 2, which are not clinically significant for ARV drug choice. Using the kinetic Jaffe method however, changes in the renal classification were observed for 21 participants over the 96 h study period.

At baseline, < 4 h, 21 of 22 (95%) participants had an eGFR > 60 ml/min/1.73 m² (renal stage 1 and 2) while at 96 h only 1 of 18 (6%) participant was classified as stage 2. The remaining 17 participants (94%) were classified as stage 3a or higher (eGFR < 60 ml/min/1.73 m²). Figure 6 demonstrates that renal staging deteriorated over the study period, with all participants (100%) eligible for the tenofovir based regimen with an eGFR > 50 ml/min/1.73 m² at 4 h while only four participants (22%) would have been eligible for this regimen at 96 h.

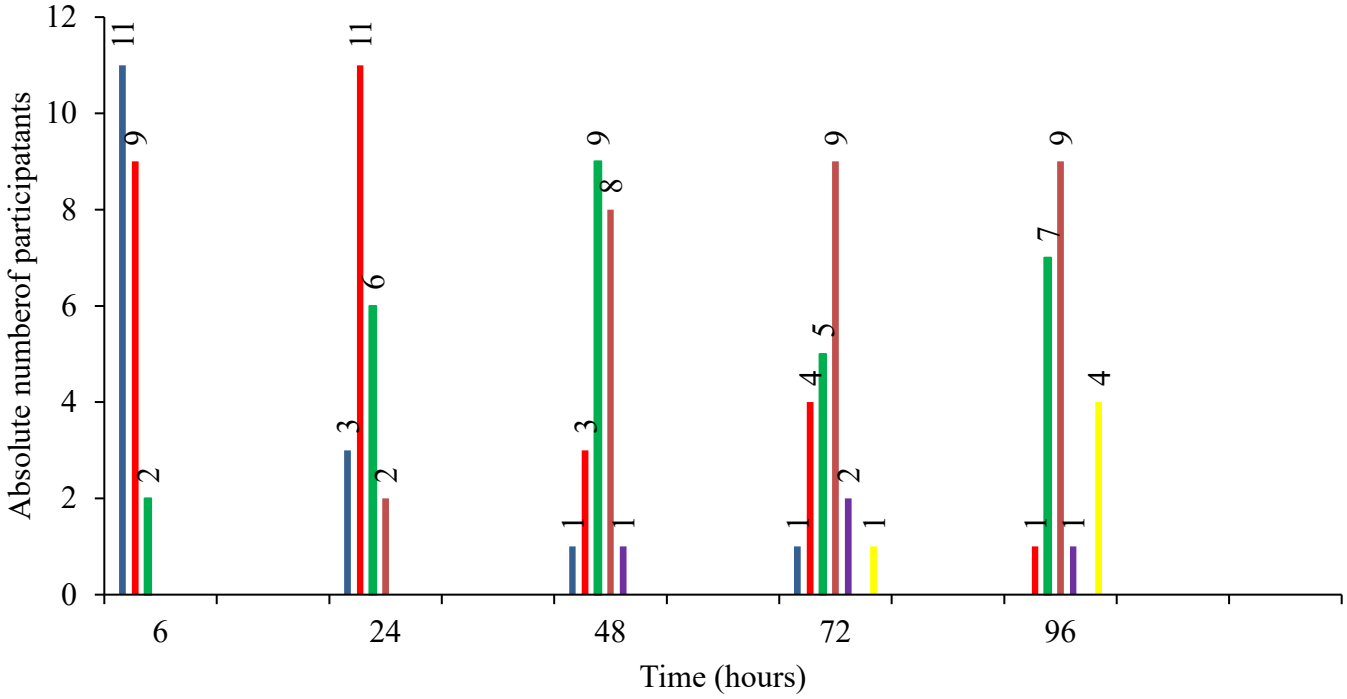


Figure 6: The number of participants classified per renal stage at each time interval using the serum creatinine results obtained from the kinetic Jaffe method to calculate estimated glomerular filtration rate using the Chronic Kidney Disease-Epidemiology equation.
Blue bars = stage 1 (eGFR ≥ 90 ml/min per 1.73 m²), Red bars = stage 2 (eGFR 60– 89 ml/min per 1.73 m²), Green bars = stage 3a (eGFR 45– 59 ml/min per 1.73 m²), Orange bars = stage 3b (eGFR 30 – 44 ml/min per 1.73 m²), Purple bars = stage 4 (eGFR 15 – 29 eGFR ml/min per 1.73 m²), Missing data due to hemolysis or insufficient sample = yellow bars.

4. DISCUSSION

The accurate measurement of serum and whole-blood creatinine is essential to the determination of the eGFR. Laboratory methods have been standardised, but concern remains regarding the impact on results of nonspecific analytical biases.[30, 31] This study from a clinical laboratory based at a large public hospital in SA reports levels of serum creatinine as assessed by three standard methods of measurement, following a delay in sample separation and centrifugation. The clinical significance of the delay was evaluated by calculating the eGFR of each of the 22 patient's serum samples by means of the following four equations: the CG, MDRD v4, MDRD v3, and CKD-EPI. Serum and whole blood creatinine as assessed by the enzymatic and i-STAT methods were stable throughout the study. However, we found that a delay in centrifugation and sample separation of > 6 h resulted in significantly raised creatinine concentrations when using the kinetic Jaffe method. This led to the misclassification of patients when the eGFR was determined using all four formulas (equations), and was consistent with previous studies.[29, 32]

The Roche enzymatic creatinine results were stable over the duration of the study, with a CV of 6%, which was consistent with a previous study which showed that the enzymatic method meets the analytical performance specifications as stipulated by the National Kidney Disease Education Program (NKDEP) (USA).[33, 34] The i-STAT method showed a mean negative bias of 13.6 µmol/l compared to the enzymatic method. Studies comparing the performance of the i-STAT device to other platforms have been inconsistent.[35, 36, 37, 38] One study showed that the i-STAT overestimated creatinine results by 3.88 µmol/l in comparison to the Roche enzymatic creatinine results. This overestimation occurred predominantly at higher creatinine concentrations. In contrast, a study conducted on oncology patients found that mean creatinine concentrations obtained on the i-STAT system were 42.4 µmol/l lower than those obtained using the core laboratory method.[35] The negative bias may be attributed to whole blood creatinine negative

interferences.[39] POCT creatinine whole blood samples are affected by the matrix effect (such as hematocrit) and are prone to negative bias.[40]

Despite the negative bias seen with the i-STAT device, the creatinine results were sufficiently sensitive to detect the participant with renal dysfunction using the MDRD v3 and CKD-EPI equations. There are currently no POCT analytical goals for creatinine and no UOM was available as this was a new platform. However, the negative bias and the CV were within the acceptable 'Laboratory Working Group of the NKDEP' total error goal of less than 10% for the eGFR (CV < 8% and an analytical bias relative to IDMS < 5%).[41] Previous studies have cautioned utilisation of POCT creatinine due to failure to detect renal dysfunction; however, in our study the i-STAT did not erroneously classify any of the participants.[42] It is worth noting that the equations for eGFR were derived using serum samples, therefore utilisation of these equations with whole blood samples should still be validated using measured GFR.

While standardisation of creatinine methods has addressed calibration biases, method-specific interferences remain problematic.[30] In the current study, the Roche kinetic Jaffe method showed analytical, clinically significant imprecision and a positive bias for creatinine results when analysed 24 h after collection. This finding is consistent with previous studies that showed delayed separation of serum from cellular components following specimen collection resulted in a positive interference with different Jaffe methods and could be attributed to the accumulation of pyruvate and other non-creatinine chromogens.[26] Ford et al.[32] established that on the Roche kinetic Jaffe method the serum creatinine results were significantly increased from 16 h and recommended that creatinine results with a delay in separation should not be reported as they impacted CKD staging. Similarly, Shepherd et al.[26]showed an overestimation of Jaffe creatinine results when assessing the impact of delayed sample separation using five different analytical platforms.[26] These results are in contrast to the WHO guidelines that state that creatinine in whole blood is stable at room

temperature for 2–3 days.[25] In the current study, the overestimation by the kinetic Jaffe method over time was clinically significant as this led to a higher CKD staging (i.e. lower eGFR). This is consistent with a study by Drion et al. where they found that creatinine results from Jaffe techniques were more biased, imprecise and overestimated serum creatinine concentrations, especially at low concentrations compared to enzymatic techniques.[29] The authors recommended that clinical laboratories consider the health costs associated with inappropriate referral and further suggested using the enzymatic assay that had a minimal bias.

The inconsistencies observed in renal classification using the four GFR equations shown in this study highlight the fact that these equations cannot be used interchangeably for identifying kidney disease, monitoring of renal function, drug dosing and selection of ARV drug regimens. Measured GFR was not required for this study as ascertaining the GFR was not pertinent, but rather the study sought to quantify the clinical significance of the impact of the instability on the calculated GFR. The CG equation was the least sensitive to the effect of the increased creatinine concentrations on eGFR in our study. This is consistent with a previous finding that demonstrated that the CG equation overestimated the eGFR in a large cohort of Europeans, resulting in the misclassification of 29.2% of individuals.[43] In contrast, the CKD-EPI equation was sensitive to the serum creatinine changes of the kinetic Jaffe method, and the impact on the eGFR was noted at 24 h. This result is consistent with a previous study by Seape and colleagues who found that the CKD-EPI equation performed better than other creatinine- based eGFR equations when assessing renal function in 100 treatment-naïve HIV-positive individuals.[44]

4.1. Strengths and limitations of the study

This study was able to show the impact of delayed processing of samples on creatinine concentrations on three platforms with a larger sample size than previous studies. In addition, blood samples were taken from participants at a single time point and the pre-analytical conditions were standardised. A limitation of this study is that serum indices (such as bilirubin which can falsely elevate creatinine results) were not considered when evaluating the creatinine result. Furthermore, we did not exclude all confounders to creatinine measurements such as drugs and pre-existing renal dysfunction which may have adversely affected the creatinine results at all-time intervals. In addition, we did not use the reference creatinine method (IDMS) for comparison purposes; we used an enzymatic method traceable to IDMS. A further limitation is that we did not have a wide range of creatinine concentrations to see how a delay in centrifugation would affect the higher concentrations. Furthermore, this study did not explore all the risks associated with POCT devices such as lot-to-lot variation (one lot number was used), operator variability, quality control, analytical traceability, impact of temperature, humidity, different sample types, and the different analytical performance of different POCT devices which have previously been shown to have a significant impact on the eGFR.[45]

5. CONCLUSION

This study demonstrated that the Roche kinetic Jaffe method creatinine assay gave falsely elevated serum creatinine levels if samples were not separated and analysed within 24 h of collection. The increase in the serum creatinine concentration over time was clinically significant resulting in misclassification of renal status which would, in turn, lead to incorrect clinical decisions regarding ARV regimen choice. Based on the outputs of this study, and previously published literature, [26, 44] laboratories currently analysing creatinine on any Jaffe method should reject (not analyse) samples reaching the laboratory after the stability index time from collection (< 24 h), or if these

laboratories decide to analyse these serum creatinine samples an appropriate interpretative comment should be appended to the result as done for other biochemical analytes such as potassium, glucose, and phosphate. The enzymatic creatinine method is highly recommended in clinical laboratories that analyse samples more than 24 h after collection due to transport delays and excessive workload. A major deterrent concerning the utility of the enzymatic method is its high cost. However, the health costs associated with the misclassification of patients using the Jaffe method may outweigh the analytical costs of the enzymatic method. Increased demand for the enzymatic method will result in competition between suppliers and ultimately reduce the cost. This study demonstrated that although creatinine assays have been standardised the extra analytical considerations which are not standardised may result in clinically significant creatinine results, thus the use of POCT, which performed well in this study, in remote clinics may avert the need to perform the laboratory-based kinetic Jaffe.

Measurement of creatinine with a POCT provides an accurate, timely result, and has eliminated the pre-analytical problems noted with the kinetic Jaffe method; therefore, adopting of POCT creatinine should be envisaged. The i-STAT POCT creatinine, however, had a negative bias which resulted in the misdiagnosis of a renal dysfunction for one participant with stage 3a renal failure while the eGFR of the other 21 participants was not affected. Its utilisation in HIV/AIDS programmes could, therefore, be adopted once the eGFR equations have been validated for the sample type, confounders of POCT mentioned previously and infrastructural issues such as integration with the laboratory information systems have been addressed. POCT and enzymatic assays will reduce unnecessary clinic visits due to renal misdiagnoses (false positives) and importantly reduce the pill burden, thereby ultimately reducing health costs to both the government and the patient.

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7. APPENDICES

7.1. Appendix 1: Published article

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Evaluation of the impact of delayed centrifugation on the diagnostic performance of serum creatinine as a baseline measure of renal function before antiretroviral treatment

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Background: The measurement of serum creatinine is a standard requirement of the medical management of people living with HIV. Renal dysfunction is common, both as a complication of HIV-infection and as a result of its treatment. The detection of abnormal renal function before the start of antiretroviral therapy will impact patient management and the outcome of treatment.

Objectives: This study aimed to determine if a time delay in the centrifugation of serum samples affected the creatinine level and the estimated glomerular filtration rate as recorded on the analytical platforms used in the laboratory.

Methods: Twenty-two (n = 22) HIV-positive, newly diagnosed and treatment-naïve patients were randomly recruited from Alexandra Health Clinic, Johannesburg, South Africa. Serum samples were centrifuged at six time intervals following receipt of the sample viz. < 4 h (baseline), 6 h, 24 h, 48 h, 72 h and 96 h. Creatinine concentrations were measured on the Roche platform utilising the enzymatic and kinetic Jaffe methods. Whole blood samples were also analysed with the Abbott i-STAT point-of-care instrument. The estimated glomerular filtration rate was calculated using the Cockcroft Gault, CKD–Epidemiology Collaboration and Modified Diet and Renal Disease v3/4 equations.

Results: At baseline (< 4 h) there was good agreement between the enzymatic and kinetic Jaffe methods: bias 1.7 µmol/l. The enzymatic and i-STAT creatinine concentrations were stable over 96 h viz. changes of 1.8% and 5.7%. However, from 24 h onwards agreement between the enzymatic and kinetic Jaffe methods was poor with the latter measuring 43.7 µmol/l higher than the enzymatic method at 96 h. Creatinine concentrations measured with the kinetic Jaffe method increased significantly in samples centrifuged after 6 h (p < 0.001, 61.7% change), and resulted in a 95% decline in eGFR at 96 h as determined with the CKD–Epidemiology Collaboration equation.

Conclusion: The analysis of serum creatinine using the isotope dilution mass spectrometry traceable kinetic Jaffe method is unreliable if performed on samples centrifuged ≥ 6 h after collection. The raised creatinine concentration can affect clinical decisions such as renal functional assessment, choice of antiretroviral drug or regimen, and the dose and frequency of medication.

Keywords: Kidney function; serum creatinine; antiretroviral; estimated GFR; kinetic Jaffe; i-STAT; ROCHE.

Introduction

South Africa (SA) has one of the highest HIV infection rates in the world. In 2016, the overall prevalence of HIV infection in SA was 12.7%.[1,2] The use of antiretroviral drugs (ARVs) has slowed the progression of the infection and increased the life expectancy of people living with HIV.[3,4,5] In SA's public health sector, patients are started on antiretroviral treatment (ART) with combinations of different ARV drug classes, prescribed as a single, once-a-day tablet.[6] This has led to a reduction of the pill-burden of therapy and to improved adherence.[7,8] In the southern African region the most frequently prescribed initial combination is the **nucleotide** reverse transcriptase

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inhibitor (NtRTI), tenofovir disoproxil fumarate (TDF), the **nucleoside** reverse transcriptase inhibitors (NRTIs) emtricitabine (FTC) or lamivudine (3TC) and the non-nucleoside reverse transcriptase inhibitor (NNRTI), efavirenz (EFV). This is first-line ART.[6] Current SA national ART guidelines indicate that the integrase strand transfer inhibitor (INSTI), dolutegravir (DTG), has replaced efavirenz for all except women of child-bearing age who may fall pregnant or those initiating ART in the first trimester of pregnancy.[9,10] Tenofovir however remains the standard NRTI-backbone of all first-line ART in southern Africa.

TDF is an acyclic nucleoside phosphate prodrug. Its long half-life permits once-daily dosing. Although the drug is extensively filtered by the kidneys, 20% – 30% is actively reabsorbed at the proximal tubule.[11] TDF is an occasional cause of renal injury viz. a proximal renal tubulopathy and a salt-wasting syndrome (Fanconi Syndrome and renal tubular acidosis), drug-induced acute kidney injury and a slower, general decline in glomerular filtration, diabetes insipidus, and a dysregulation of the kidney's calcium and phosphorus metabolism.[11,12] Impaired renal function may also result from the use of other drugs, such as aminoglycosides, sulphonamides, and amphotericin B, the presence of HIV-associated nephropathy (HIVAN), blood-borne infection, for example tuberculosis, bacteraemia and fungaemia, HIV-associated immune-complex kidney disease and common comorbidities such as hypertension and diabetes mellitus.[13,14] Pre-existing mild renal dysfunction may increase susceptibility to the toxicity of TDF. Kidney function must be evaluated before starting ART: the use of TDF is contraindicated if the estimated glomerular filtration rate (eGFR) is < 50 mL/min. Patients on TDF have serum creatinine measured at 3 and 6 months following the initiation of ART and biannually thereafter. In high-risk patients, for example with coexistent hypertension or diabetes, the creatinine is measured more frequently.[9]

The measurement of the GFR confirms and stages the degree of renal impairment and provides a platform for the ongoing monitoring of kidney function. Although the urinary clearance of inulin is the gold standard of GFR measurement,[15] the method is time-consuming, expensive and impractical in most clinical settings. Endogenous substances such as serum creatinine and cystatin C are cheaper, provide more rapid results and are widely available.[16] Although the National Institute for Health and Care Excellence (NICE) and the Kidney Disease Improving Global Outcomes (KDIGO) groups both base their eGFR assessment (equations) on cystatin C, its cost and lack of standardisation excludes its general use.[17,18] Creatinine is produced at a constant rate, is present in all body fluids, and is filtered by the glomerulus. Its serum level is influenced by several biological factors, such as active secretion by the renal tubules in the presence of declining renal function, diet, extremes of muscle mass, age, gender, drugs and the use of creatine supplements.[19] Several equations control for some of these variables.[20] The SA National Department of Health (SANDOH) 2019 guidelines recommend therefore that the Counahan-Barratt equation be used to measure the eGFR of youths aged 10–16 years and the Modifications of Diet in Renal Disease (MDRD) equation be used for adolescents and adults 16 years of age and older.[9]

The Cockcroft-Gault (CG) equation incorporates the serum creatinine level and the variables of weight, age and sex in the measurement of the eGFR.[21] The equation has limitations: in pregnancy, at the extremes of weight, and in those on dialysis for acute renal failure. Its principal use is in drug dosing and pharmacokinetic studies.[20] The MDRD equation adds a fourth variable: race.[22] This ethnicity adjustment-factor is based on African Americans and tends to overestimate the GFR in sub-Saharan (African) populations.[23] The CKD–Epidemiology Collaboration (EPI) equation is recommended by the KDIGO guidelines group and has been validated in participants with and without impaired renal function with eGFR < 90 mL/min/1.73 min².[17,24] The SA National Health Laboratory Service (SA-NHLS) currently reports both the MDRD and CKD-EPI eGFR without ethnic adjustment. The adoption of the CKD-EPI equation in SA has been delayed due to limited local data.

Routine laboratory serum creatinine measurements are analysed on Jaffe and enzymatic assays. These methods should be traceable to isotope dilution mass spectrometry (IDMS) method and the Standard Reference Material for creatinine in serum (SRM 967). NHLS laboratories in SA generally use the modified kinetic Jaffe method, as opposed to the enzymatic assay, as it is more affordable. The World Health Organization's (WHO) guidelines indicate that at room temperature, creatinine is stable for 2–3 days.[25] However, a report from Shepherd et al.[26] noted a significant increase in creatinine levels measured on the kinetic Jaffe method when processing occurred later than 24 h.[26] This impacted the study's eGFR results and had caused the renal misclassification of patients.[26] A turnaround time for serum creatinine measurements from local clinics in Gauteng of 12–72 h has been observed at NHLS laboratories, as reported in the internal TAT reports. This delay increases with the remoteness of clinics due to the poor transport networks, the restricted working hours at referral laboratories, and the greater workload of regional laboratories. The aim of this study was to determine, in HIV-positive black South Africans not on ART, the stability limit (SL), that is, the time at room temperature, when the serum creatinine results are still within the maximum permissible instability (MPI) range.[27]

Methods

Study population

Twenty-two (n = 22) newly diagnosed and treatment-naïve people living with HIV between the ages of 18 and 70 years were randomly recruited from the Alexandra Health Community Centre in Alexandra, Johannesburg, SA. The minimum number of participants required for testing

the stability of biochemical analytes is 10.[27] Furthermore, multiple samples were required from each participant for analysis on three analysers at six time points, providing 396 creatinine measurement data points. Patients with comorbidities such as diabetes mellitus, pregnancy, urinary tract infection, hypertension and proteinuria were excluded from the study. De-identified demographic and anthropometric data were transcribed from the patient files.

Sample collection and processing

Each patient provided 13 tubes of blood: 12 × approximately 2 mL in 5 mL serum separator-tubes (SST) and 1 × 5 mL in a heparin tube (Becton Dickenson, Plymouth, UK). Serum was isolated from SST tubes following centrifugation at 1370 × g for 5 min at defined time intervals viz. < 4 h, 6 h, 24 h, 48 h, 72 h and 96 h. Prior to centrifugation, samples were stored at room temperature. Whole blood heparin tubes were stored at room temperature until required.

Measurement of creatinine concentrations

Serum creatinine concentrations were measured immediately after each centrifugation (as above) using the IDMS-traceable enzymatic and IDMS-traceable kinetic Jaffe method on the Roche COBAS 8000 module 702 and Roche COBAS 6000 module 502 instruments at the NHLS laboratory of the Chris Hani Baragwanath Hospital, Soweto, SA. The enzymatic method test principle is based on a series of coupled reactions that result in the conversion of creatinine to hydrogen peroxide. In the final reaction, catalysed by peroxidase, a quinone imine chromogen is formed. The colour intensity of this chromogen is measured at 550 nm and is directly proportional to the concentration of creatinine in the sample. The kinetic Jaffe method test principle is based on the reaction of creatinine and picrate under alkaline conditions forming a yellow-orange complex. The rate of formation of this complex is proportional to the amount of creatinine in the sample. The kinetic Jaffe method is prone to interference from non-creatinine chromogens such as proteins and ketones. A correction factor of -26 µmol/L is, therefore, universally applied to correct for these inherent chromogens. Bilirubin is the major negative interferent in the kinetic Jaffe method, and this is overcome by rate blanking. The internal quality control for creatinine in our laboratory is performed twice daily. The laboratory adheres to the Royal College of Pathologists of Australasia (RCPA) quality assurance programmes. External quality assurance submissions during the study period were within allowable limits.

Creatinine concentrations were also measured using whole blood samples on the Abbott i-STAT point-of-care instrument immediately after collection and at 6 h, 24 h, 48 h, 72 h and 96 h. This method is based on the conversion of creatinine to hydrogen peroxide, via hydrolysis and oxidation. The hydrogen peroxide is oxidized (at the platinum electrode) to produce a current that is proportional to the concentration of creatinine in the sample.

Estimated glomerular filtration rate measurements

eGFR was calculated using the MDRDv3 *without ethnic adjustment* (age, sex, and serum creatinine), MDRDv4 (age, sex, ethnicity, and serum creatinine), CG and CKD-EPI equations *with ethnic adjustment.*

Statistical analysis

All analyses were performed using Medcalc software. The Shapiro-Wilks test was used to test for normality. Normally distributed continuous variables (creatinine) were presented as mean ± standard deviation and continuous variables that were not normally distributed (age, weight) were presented as median [interquartile range]. Categorical variables are presented as proportions and percentages. The Student paired t-test was used to compare baseline creatinine concentrations between the three different methods (Bonferroni corrected *p*-value). The serum creatinine results were evaluated for analytically significant changes using the uncertainty of measurement (UOM; calculated for the laboratory quality control data in the preceding 6 months), total allowable error (TAE) of 15% (Clinical Laboratory Improvement Amendments [CLIA] and coefficient of variation [CV]). Passing-Bablok linear regression and Bland-Altman plots were generated to evaluate correlation and method agreement over time, using the enzymatic method as the reference method. For all analyses, $p < 0.05$ was considered statistically significant.

Ethical consideration

Ethical approval was obtained from the University of the Witwatersrand Human Research Ethic Committee, reference number: M1711109. R14/49.

Results

Characteristics of the study population

A total of 22 randomly recruited HIV-positive, treatment-naïve black South African individuals consented to participate in this study. The median age of the participants was 37 years. The majority (54.5%; 12 of 22) of participants were younger than 40 years of age. The age distribution is consistent with the current demographics published by Statistics South Africa with respect to individuals mostly affected by HIV/AIDS. All participants were black Africans (ethnicity self-reported). The median weight for the participants was 70.0 kg (range 45–110 kg) and 59.1% (13 of 22) of participants were male.

Creatinine assay performance

The mean baseline (< 4 h) creatinine concentrations for the study cohort obtained using the enzymatic, kinetic Jaffe method and i-STAT methods were 78.73 µmol/L ±14.63 µmol/L, 77.05 µmol/L ± 13.12 µmol/L and

70.14 µmol/L ± 15.3 µmol/L: $p > 0.05$ for all comparisons (Table 1). The mean creatinine concentrations analysed using the kinetic Jaffe method were significantly higher than baseline when blood samples were processed after 6 h: $p \leq 0.001$ for all comparisons. Creatinine concentrations did not change significantly over the 96 h when measured using the enzymatic method or i-STAT system: $p > 0.05$ for all comparisons (Table 1).

The kinetic Jaffe method exhibited the widest intra-individual CV (range: 12% – 40% vs 1.2% – 12% enzymatic and 1.9% – 12% i-STAT method) as well as the greatest inter-assay variation (22.7% vs 6.0% enzymatic and 6.6% i-STAT method), as seen in Figure 1. When creatinine results were assessed against the 10% UOM for each respective assay, 8.9% of the enzymatic creatinine results fell outside the UOM for the duration of the study compared to 65% of the results for the kinetic Jaffe method. The i-STAT results could not be evaluated as UOM has not been established for this assay.

Based on the positive trend of increasing creatinine concentrations over time observed for the Jaffe method, we explored the data further to determine if a correction factor could be established to adjust creatinine results obtained from samples with delayed processing time. The percentage change of the serum creatinine results for the participants over the 96-h time frame was inconsistent and a correction factor could, therefore, not be established based on the current sample size (Figure 2).

TABLE 1: Creatinine concentrations for the study cohort (n = 22) obtained using the enzymatic, kinetic Jaffe method and i-STAT methods at six different time intervals.

Time interval (hours)	Creatinine concentration					
	Enzymatic		Kinetic Jaffe		i-STAT	
	µmol/l	*p	µmol/L	*p	µmol/L	*p
< 4 h	78.73 ± 14.63	-	77.05 ± 13.12	-	70.14 ± 15.35	-
6 h	74.55 ± 14.11	0.340	78.41 ± 15.19	0.752	69.91 ± 16.00	0.962
24 h	78.91 ± 14.64	0.967	95.23 ± 20.26	0.001	67.59 ± 14.72	0.578
48 h	79.86 ± 15.70	0.805	117.64 ± 19.82	< 0.001	68.18 ± 14.02	0.662
72 h	76.79 ± 14.82†	0.676	121.29 ± 20.97§	< 0.001	67.59 ± 14.71	0.577
96 h	77.31 ± 10.45‡	0.743	124.56 ± 18.49¶	< 0.001	66.14 ± 15.82††	0.406

Results are expressed as mean ± standard deviation, missing data due to insufficient volume or high haemolysis index (> 1000 mg/dL) for

†, n = 3.

‡, n = 6.

§, n = 1.

¶, n = 4.

††, n =1.

*, p-value for results compared to 4 h creatinine concentration.

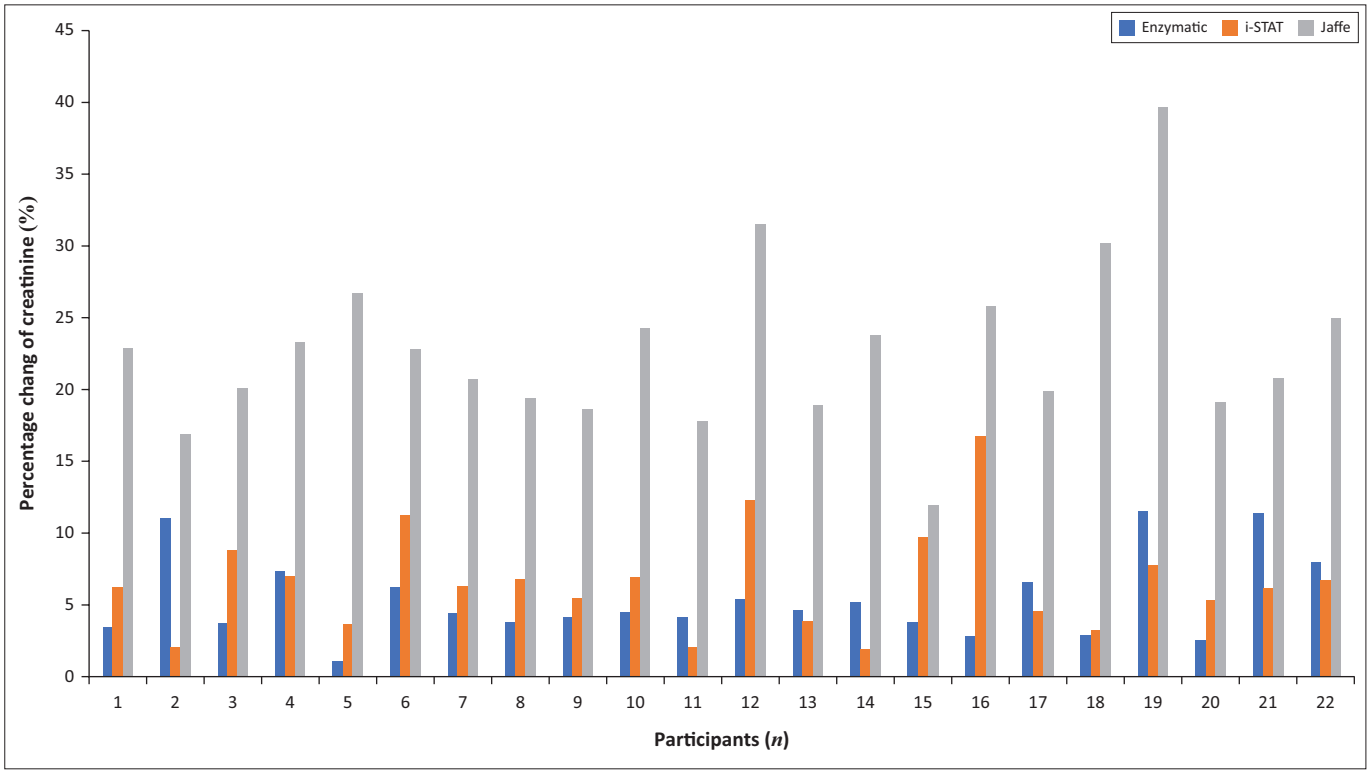

FIGURE 1: Intra-individual coefficient of variation for 22 participants for the enzymatic, kinetic Jaffe and i-STAT creatinine methods over the 96-h study period. Blue bars represent results obtained for the enzymatic method, orange bars for the i-STAT method and grey bars for the kinetic Jaffe method.

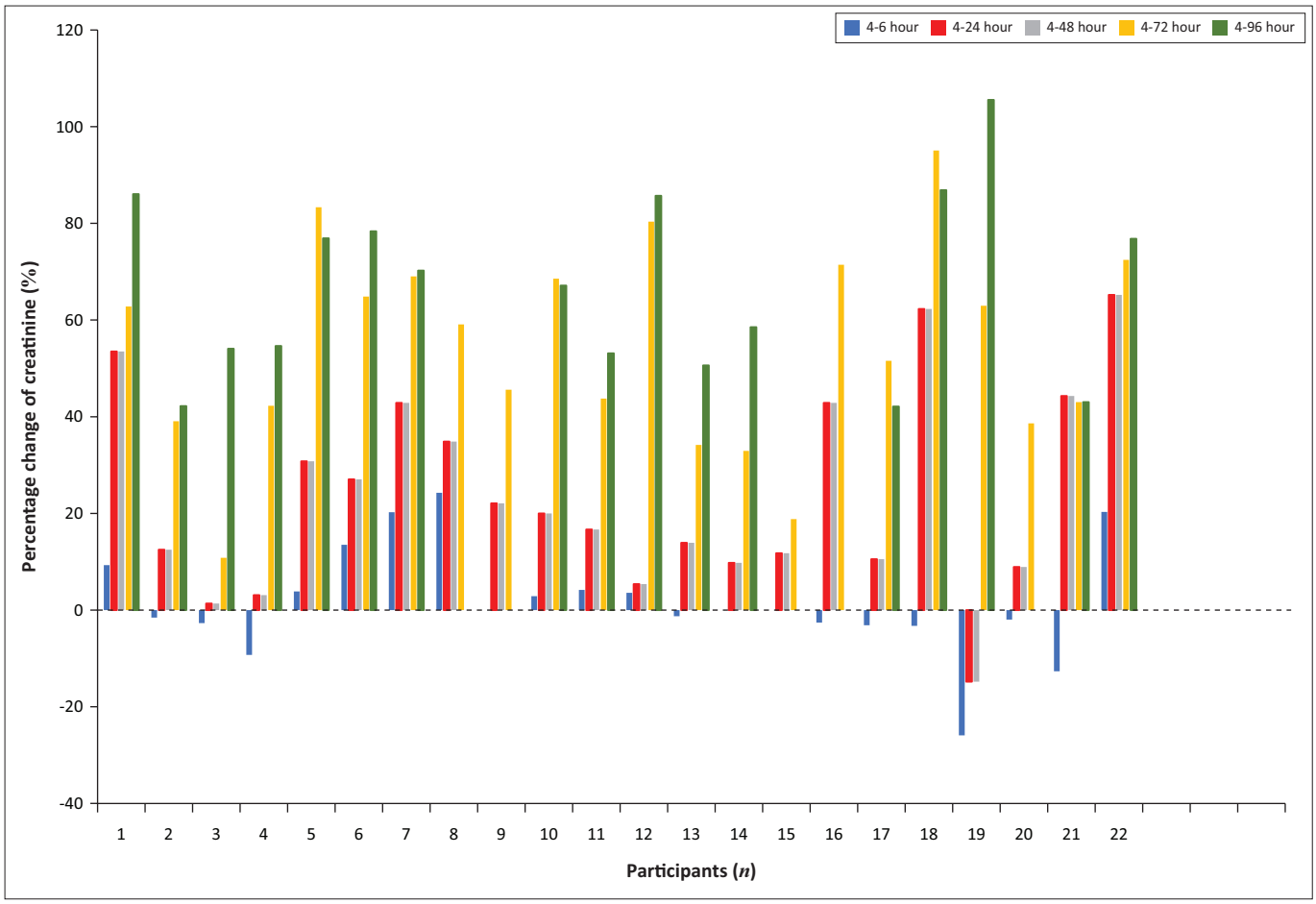

FIGURE 2: Percentage change in serum creatinine concentrations obtained using the kinetic Jaffe method over 96 h for 22 participants relative to the serum creatinine results at 4 h. Percentage change to baseline (4 h): blue bars = 4–6 h, orange = 4–24 h, grey = 4–48 h, yellow = 4–72 h, green = 4–96 h.

Method comparison

Passing-Bablok regression and Bland-Altman plots were used to determine the correlation and agreement of the kinetic Jaffe method and i-STAT creatinine results compared to the enzymatic creatinine results at different time intervals (see Table 2, Figure 3 and Figure 4). The enzymatic method was chosen as the reference method as it had the best analytical CV in our study and previous studies have shown that this method correlated well with the standard reference method (isotopic dilution mass spectrometry).[27,28,29]

The Jaffe and enzymatic method showed a strong correlation at < 4 h (r = 0.953); however, this correlation became weaker with time. The higher r values at 72 h and 96 h may be due to the missing creatinine data (enzymatic 72 h n = 3, enzymatic 96 h n = 6, Jaffe 72 h n = 1, Jaffe 96 h n = 4) for participants at these time intervals. The slope and the intercept increased over the time interval (Table 2), and this demonstrated the increase in the magnitude of the systematic error. At 4 h, there was a strong agreement between the kinetic Jaffe method and enzymatic methods with a small negative bias of 1.8% (1.7 µmol/L). With an increased delay in sample separation, the kinetic Jaffe method resulted in an overestimation of creatinine concentrations with a positive bias of 48.6% (49.9 µmol/l) at 96 h (Figure 3).

Creatinine results from the i-STAT correlated well with the enzymatic creatinine results over the six time intervals used in the study: r-value, 0.836–0.948. The i-STAT method had a negative bias ranging from 7.4% to 18.0% throughout the study. The i-STAT method had a negative bias of 12.2% (8.6 µmol/L) at 4 h and 18.0% (12.0 µmol/L) at 96 h.

Comparison of estimated glomerular filtration rate equation performance

The impact on the classification of renal dysfunction of the delay in centrifugation of blood and serum samples measured for creatinine levels by means of three different laboratory methsods was evaluated with four eGFR equations viz. CG, MDRD v4, MDRD v3, and CKD-EPI. The serum creatinine eGFR results of the enzymatic and i-STAT methods performed well (to within the 10% TAE for eGFR throughout the study). However, the eGFR from the Jaffe data decreased over time. At baseline, viz. < 4 h, there was consensus among all four equations. All 22 participants had an eGFR > 60 mL/min per 1.73 m² using the CG and MDRD v4 equation. Similarly, 21 participants had an eGFR > 60 mL/min per 1.73 m² with the MDRD v3 and the CKD-EPI equations (Table 3). One participant had an eGFR of < 60 mL/min per 1.73 m². This person was classified as having stage 3A renal failure with an eGFR 45 mL/min – 59 mL/min per 1.73 m², according to the KDIGO guidelines.

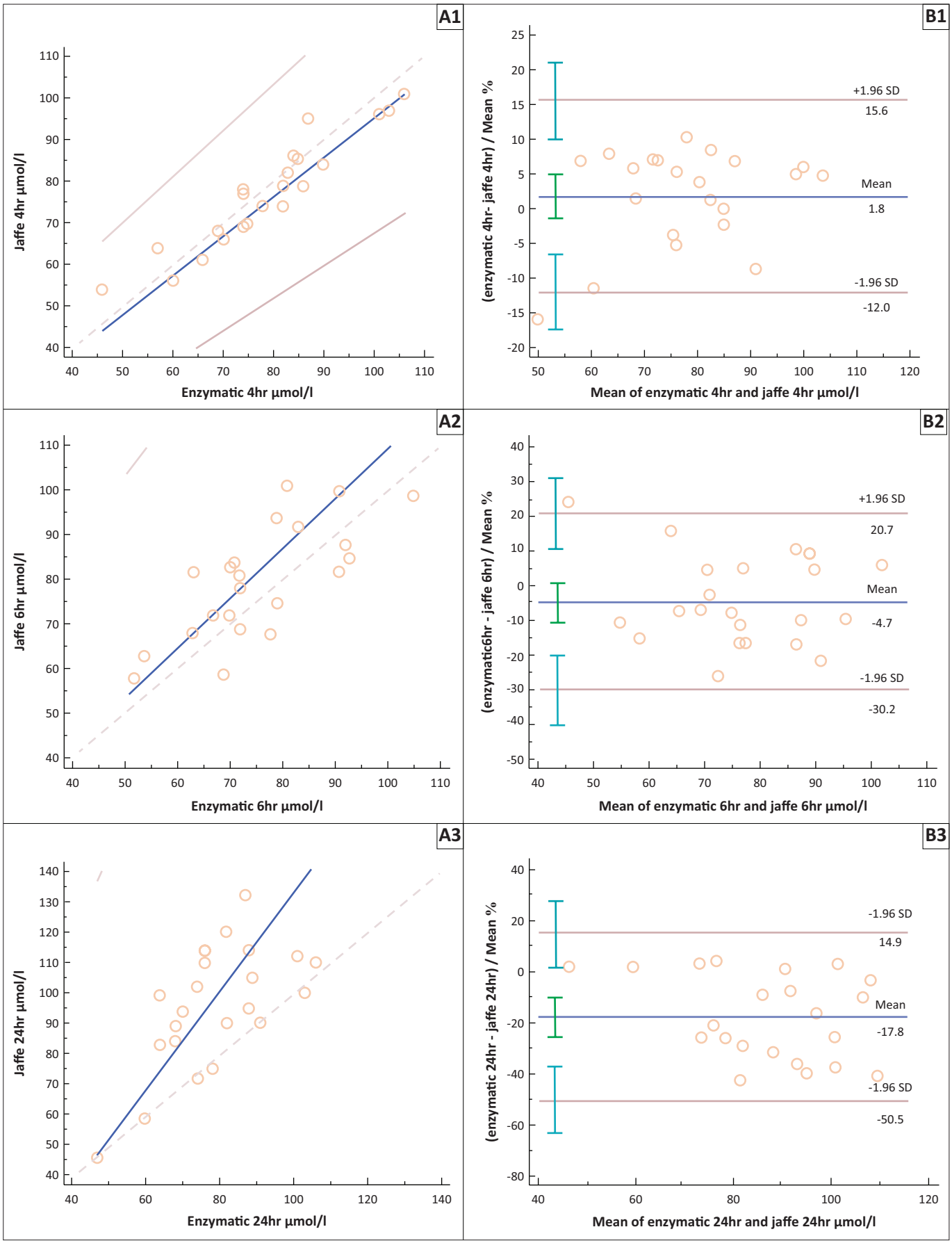

FIGURE 3: Passing-Bablok regression curves (A) and Bland-Altman plots (B) comparing creatinine concentrations from the kinetic Jaffe method with those from the enzymatic method. Creatinine concentrations at (1) < 4 h, (2) 6 h, (3) 24 h, (4) 48 h, (5) 72 h and (6) 96 h.

Figure 3 continues on the next page →

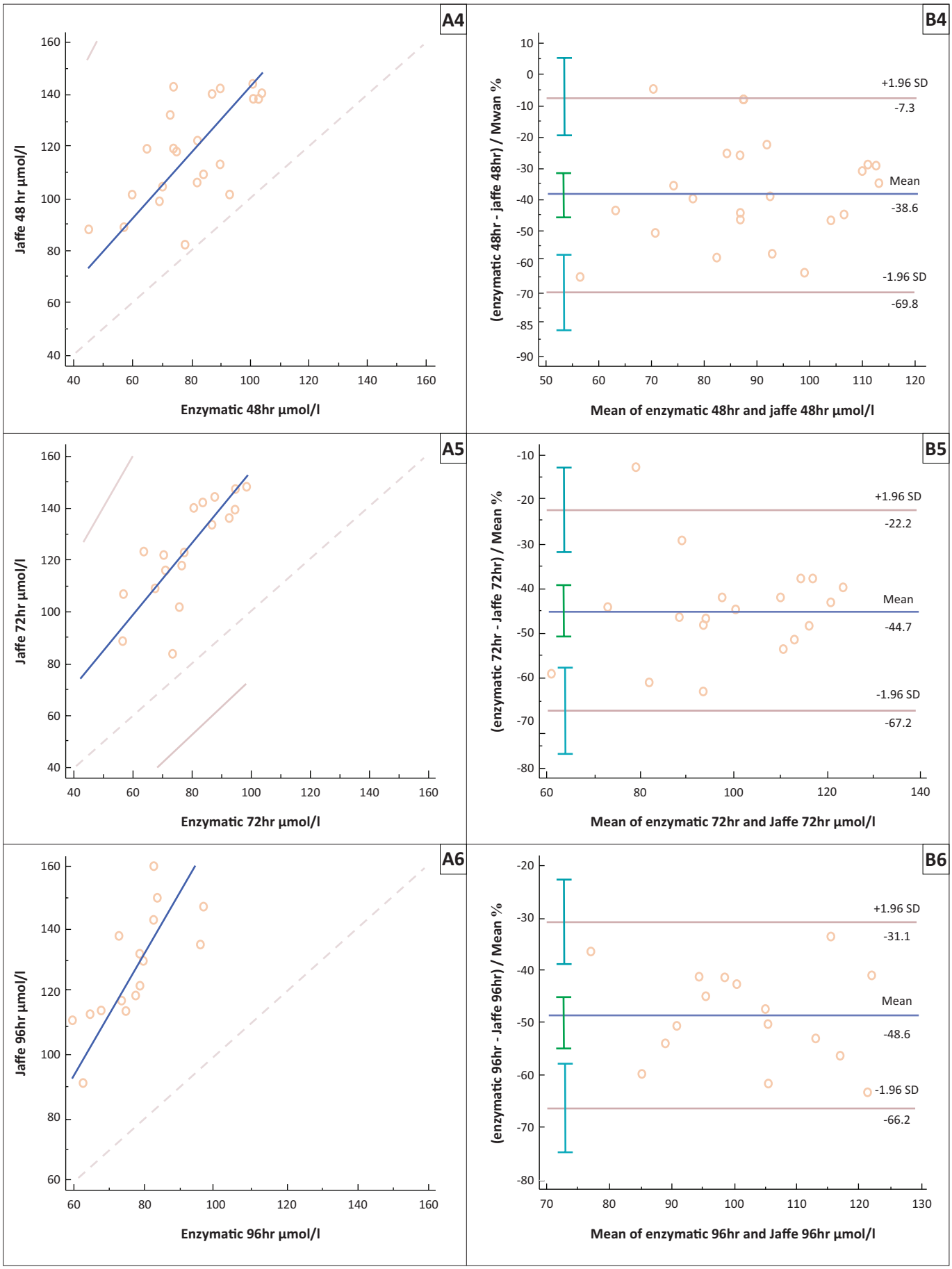

FIGURE 3 (Continues...): Passing-Bablok regression curves (A) and Bland-Altman plots (B) comparing creatinine concentrations from the kinetic Jaffe method with those from the enzymatic method. Creatinine concentrations at (1) < 4 h, (2) 6 h, (3) 24 h, (4) 48 h, (5) 72 h and (6) 96 h.

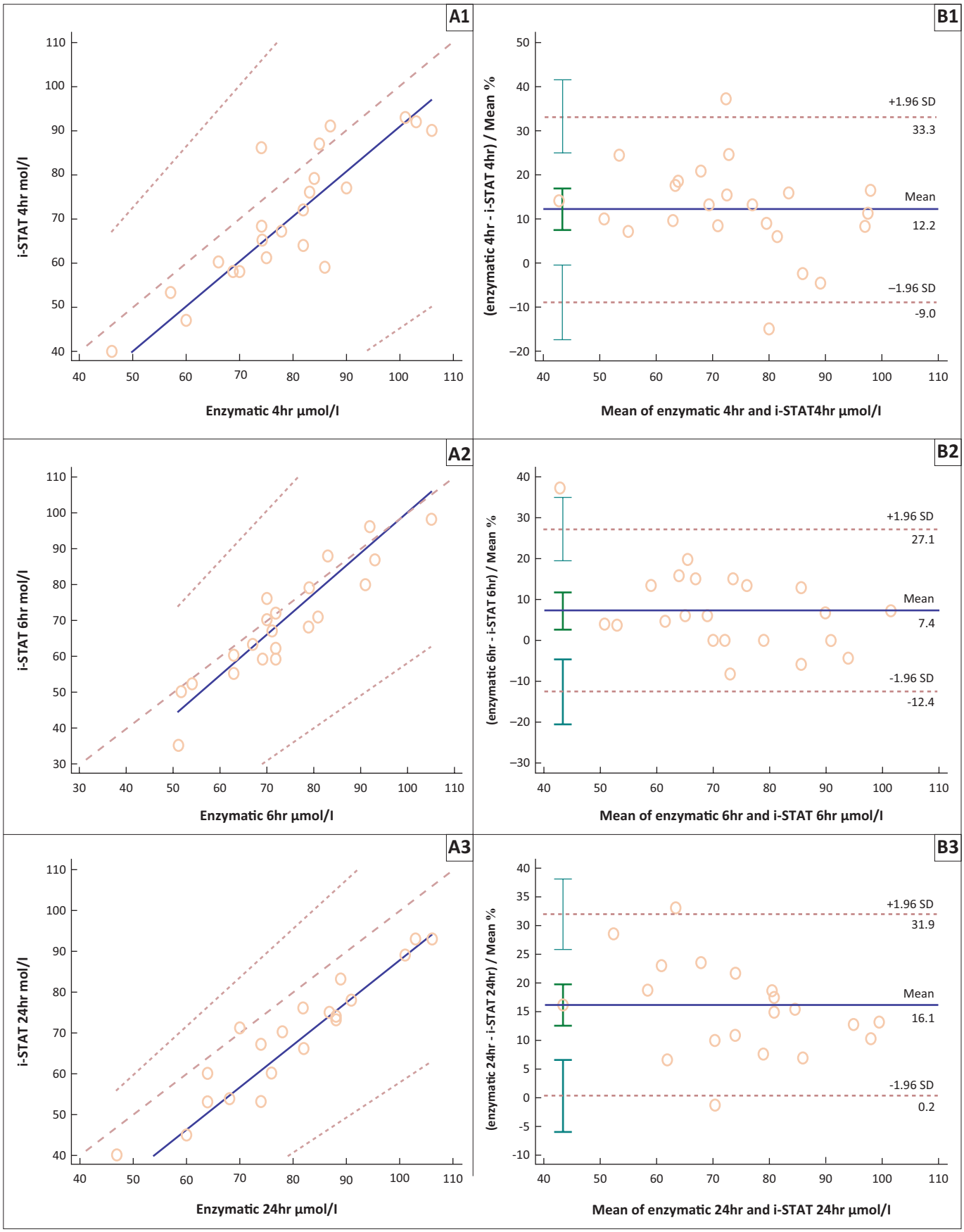

FIGURE 4: Passing-Bablok regression curves (A) and Bland-Altman plots (B) comparing creatinine concentrations from the i-STAT device with those obtained from the enzymatic method. Creatinine concentrations at (1) < 4 h, (2) 6 h, (3) 24 h, (4) 48 h, (5) 72 h and (6) 96 h.

Figure 4 continues on the next page →

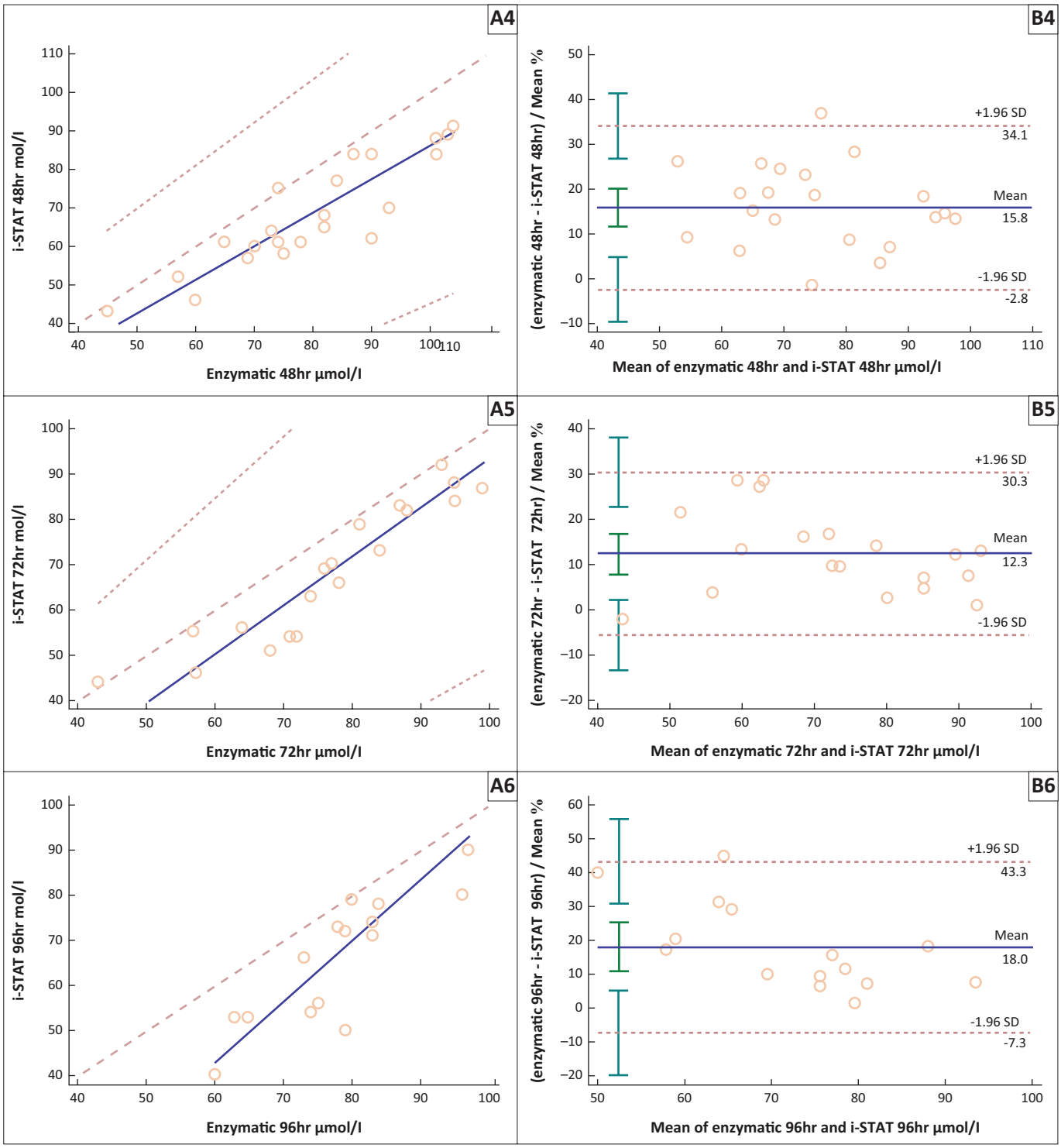

FIGURE 4 (Continues...): Passing-Bablok regression curves (A) and Bland-Altman plots (B) comparing creatinine concentrations from the i-STAT device with those obtained from the enzymatic method. Creatinine concentrations at (1) < 4 h, (2) 6 h, (3) 24 h, (4) 48 h, (5) 72 h and (6) 96 h.

The change in eGFR varied over time among the different equations. The CG equation was the least sensitive to the increase in serum creatinine: only 27% (n = 6/22) of eGFR-based 96-h creatinine concentrations resulted in a change in the Chronic Kidney Disease (CKD) stage when compared with baseline (i.e. the < 4-h eGFR; see Figure 5). The MDRD v4 equation gave the greatest change in eGFR over 96 h: 50% (n = 11/22) of participants at 24 h and 100% (n = 22/22) at 96 h showed an eGFR decline compared to baseline. The most significant change of eGFR was noted with the MDRDv3 equation: 25% eGFR at 48 h and 10% at 96 h compared to the baseline eGFR.

Using the CKD-EPI equation as recommended by the KDIGO guidelines, enzymatic and i-STAT renal classification was noted at different time intervals with changes between stages 1 and 2, which are not clinically significant for ARV drug choice. Using the kinetic Jaffe method however, changes in the renal classification were observed for 21 participants over the 96-h study period.

TABLE 2: Comparison of serum creatinine levels from kinetic Jaffe method and i-STAT method to the enzymatic method using Passing-Bablok and Bland-Altman analysis.

Analytical method	Time (hours)	Passing–Bablok regression parameters				Bland-Altman			
		Slope	95% CI	Intercept (95% CI)	95% CI	*r*	Mean bias	%	Mean bias, µmol/l
Kinetic Jaffe Method	< 4 h	0.95	0.78–1.1	0.54	-10.74–15.54	0.953	1.8	-1.31–4.93	1.7
	6 h	1.10	0.75–1.64	-2.00	-41.18–21.25	0.803	-4.7	-10.47–1.04	3.9
	24 h	1.6	0.95–2.50	-27.83	-88.50–19.65	0.596	-17.8	-25.20–10.40	16.3
	48 h	1.26	0.83–2.23	16.84	-56.85–52.75	0.582	-38.6	-45.66–-31.51	37.8
	72 h	1.33	1.06–2.00	18.90	-32.00–40.13	0.858†	-44.7	-50.24–39.18	44.0
	96 h	2.25	1.13–5.50	-23.82	152.40–43.94	0.855‡	-48.6	-53.41–43.88	49.9
i-STAT	< 4 h	1.01	0.83–1.38	-10.53	-38.23–3.50	0.836	12.2	7.38–16.96	8.6
	6 h	1.13	0.91–1.40	-13.24	-33.00–2.50	0.900	7.4	2.89–11.84	4.6
	24 h	1.04	0.86–1.20	-16.30	-28.50–-0.59	0.923	16.0	12.48–19.65	11.7
	48 h	0.88	0.67–1.12	-1.35	-21.54–13.67	0.893	15.8	11.69–19.95	11.6
	72 h	1.08	0.86–1.37	-14.61	-38.41–2.57	0.948§	12.3	7.93–16.74	8.6
	96 h	1.35	1.00–2.00	-38.02	-90.00–10.00	0.847¶	18.0	10.86–25.16	12.0

Legend: r = correlation coefficient, linear regression line $y = mx + c$, c = intercept (constant error), m = slope (proportional error), number of participants results analysed.

†, n = 19.

‡, n = 16.

§, n = 19.

¶, n = 16.

At baseline, < 4 h, 21 of 22 (95%) participants had an eGFR > 60 mL/min/1.73 m² (renal stage 1 and 2) while at 96 h only 1 of 18 (6%) participant was classified as stage 2. The remaining 17 participants (94%) were classified as stage 3a or higher (eGFR < 60 mL/min/1.73 m²). Figure 6 demonstrates that renal staging deteriorated over the study period, with all participants (100%) eligible for the tenofovir based regimen with an eGFR > 50 mL/min/1.73 m² at 4 h while only four participants (22%) would have been eligible for this regimen at 96 h.

Discussion

The accurate measurement of serum and whole-blood creatinine is essential to the determination of the eGFR. Laboratory methods have been standardised, but concern remains regarding the impact on results of nonspecific analytical biases.[30,31] This study from a clinical laboratory based at a large public hospital in SA reports levels of serum creatinine as assessed by three standard methods of measurement, following a delay in sample separation and centrifugation. The clinical significance of the delay was evaluated by calculating the eGFR of each of the 22 patient's serum samples by means of the following four equations: the CG, MDRD v4, MDRD v3, and CKD-EPI. Serum and whole blood creatinine as assessed by the enzymatic and i-STAT methods were stable throughout the study. However, we found that a delay in centrifugation and sample separation of > 6 h resulted in significantly raised creatinine concentrations when using the kinetic Jaffe method. This led to the misclassification of patients when the eGFR was determined using all four formulas (equations), and was consistent with previous studies.[29,32]

The Roche enzymatic creatinine results were stable over the duration of the study, with a CV of 6%, which was consistent with a previous study which showed that the enzymatic method meets the analytical performance specifications as stipulated by the National Kidney Disease Education Program (NKDEP) (USA).[33,34] The i-STAT method showed a mean negative bias of 13.6 µmol/L compared to the enzymatic method. Studies comparing the performance of the i-STAT device to other platforms have been inconsistent.[35,36,37,38] One study showed that the i-STAT overestimated creatinine results by 3.88 µmol/L in comparison to the Roche enzymatic creatinine results. This overestimation occurred predominantly at higher creatinine concentrations. In contrast, a study conducted on oncology patients found that mean creatinine concentrations obtained on the i-STAT system were 42.4 µmol/lower than those obtained using the core laboratory method.[35] The negative bias may be attributed to whole blood creatinine negative interferences.[39] POCT creatinine whole blood samples are affected by the matrix effect (such as haematocrit) and are prone to negative bias.[40]

Despite the negative bias seen with the i-STAT device, the creatinine results were sufficiently sensitive to detect the participant with renal dysfunction using the MDRD v3 and CKD-EPI equations. There are currently no POCT analytical goals for creatinine and no UOM was available as this was a new platform. However, the negative bias and the CV were within the acceptable 'Laboratory Working Group of the NKDEP' total error goal of less than 10% for the eGFR (CV < 8% and an analytical bias relative to IDMS < 5%).[41] Previous studies have cautioned utilisation of POCT creatinine due to failure to detect renal dysfunction; however, in our study the i-STAT did not erroneously classify any of the participants.[42] It is worth noting that the equations for eGFR were derived using serum samples, therefore utilisation of these equations with whole blood samples should still be validated using measured GFR.

While standardisation of creatinine methods has addressed calibration biases, method-specific interferences remain problematic.[30] In the current study, the Roche kinetic Jaffe

TABLE 3: Chronic Kidney Disease staging using the four equations based on creatinine results obtained from the enzymatic, kinetic Jaffe and i-STAT methods.

Measure	Enzymatic						Kinetic Jaffe						i-STAT					
	4 h		24 h		96 h		4 h		24 h		96 h		4 h		24 h		96 h	
	n	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%	*n*	%
CKD-EPI																		
eGFR mean (mL/min per 1.73 m²)	118 ± 36		118 ± 37		114 ± 28		100 ± 26		75± 33		45 ± 10		147 ± 50		152 ± 56		161 ± 68	
Range	53–217		53–210		73–148		48–150		34–181		24–63		63–269		62–269		81–269	
15–29	0	0	0	0	0	0	0	0	0	0	1	5.5	0	0	0	0	0	0
30–44	0	0	0	0	0	0	0	0	2	9	9	50	0	0	0	0	0	0
45–59	1	4.5	1	4.5	0	0	1	0.5	6	27	7	39	0	0	0	0	0	0
60–89	5	23	4	18	5	31	7	32	10	45	1	5.5	2	9	2	9	2	10
≥ 90	16	73	17	77	11	69	14	64	4	18	0	0	20	91	20	91	19	90
MDRDv3																		
eGFR mean (mL/min per 1.73 m²)	89 ± 20		89 ± 21		89 ± 15		91 ± 18		83 ± 31		51 ± 9		104 ± 126		107 ± 27		112 ± 34	
Range	49–141		40–137		62–108		52–125		55–190		31–67		55–166		55–166		68–166	
15–29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30–44	0	0	0	0	0	0	0	0	0	0	4	22	0	0	0	0	0	0
45–59	1	4.5	2	9	0	0	1	4.5	3	14	12	67	1	4.5	1	4.5	0	0
> 60	21	95.5	20	91	16	100	21	95.5	19	86	2	11	21	95.5	21	95.5	21	100
MDRDv4																		
eGFR mean (mL/min per 1.73 m²)	108 ± 25		108 ± 26		108 ± 18		100 ± 22		69 ± 26		42 ± 7		125 ± 32		130 ± 32		136 ± 41	
Range	59–171		59–166		75–133		63–151		45–157		26–55		67–200		66–209		82–201	
15–29	0	0	0	0	0	0	0	0	0	0	1	5.5	0	0	0	0	0	0
30–44	0	0	0	0	0	0	0	0	0	0	10	5.5	0	0	0	0	0	0
45–59	1	4.5	2	9	0	0	0	0	11	9	7	39	0	0	1	4.5	0	0
> 60	21	95.5	20	91	16	100	22	100	11	8	0	0	22	100	21	-	21	100
CG																		
eGFR mean (mL/min per 1.73 m²)	129 ± 66		128 ± 64		125 ± 51		129 ± 54		111 ± 69		79 ± 28		125 ± 52		130 ± 51		137 ± 57	
Range	66–391		66–382		79–295		69–333		54–391		47–162		78–328		76–324		83–324	
15–29	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
30–44	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
45–59	0	0	0	0	0	0	0	0	1	4.5	2	11	0	0	0	0	0	0
> 60	22	100	22	100	16	100	22	100	21	95.5	16	89	22	100	22	100	21	100

CKD-EPI, Chronic Kidney Disease-Epidemiology; eGFR, estimated glomerular filtration rate; MDRD, Modifications of Diet in Renal Disease; CG, Cockcroft-Gault.

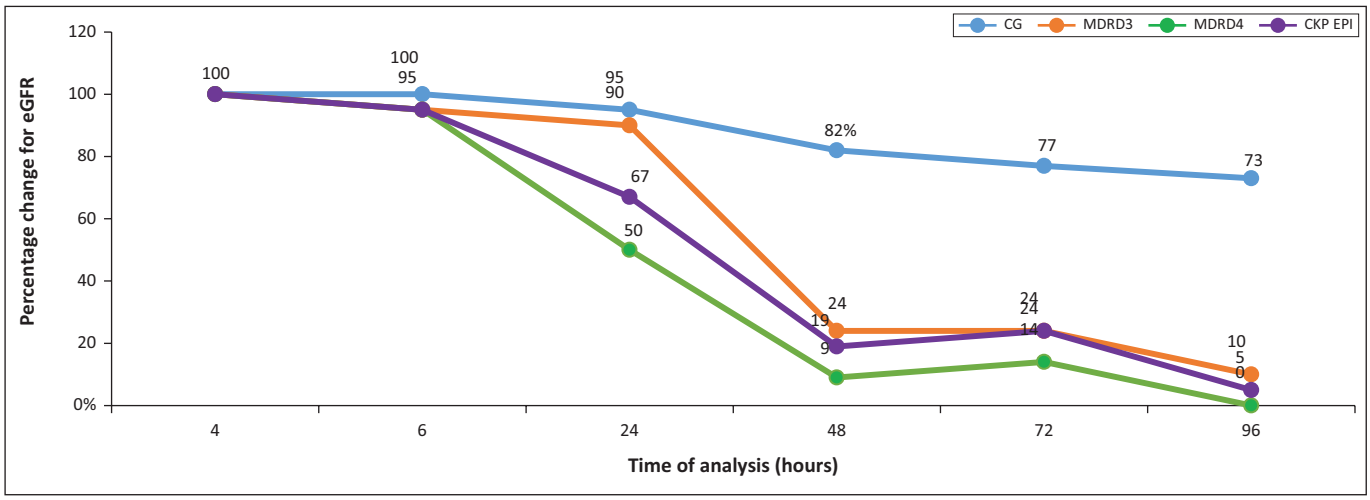

FIGURE 5: Percentage change of estimated glomerular filtration rate classification based on kinetic Jaffe creatinine concentrations over the 96-h study period compared to estimated glomerular filtration rate obtained within 4 h using the Cockcroft-Gault, Modifications of Diet in Renal Disease v3, Modifications of Diet in Renal Disease v4, and Chronic Kidney Disease-Epidemiology equations. blue = CG, orange = MDRD3, purple = CKD, green = MDRD4.

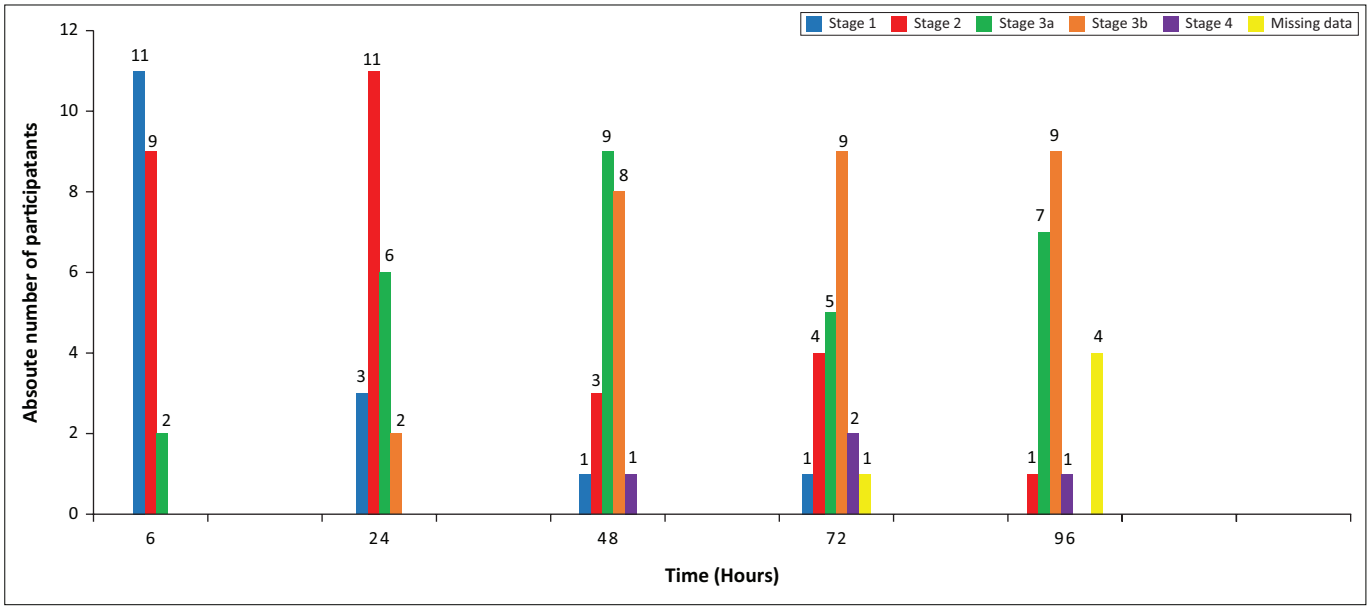

FIGURE 6: The number of participants classified per renal stage at each time interval using the serum creatinine results obtained from the kinetic Jaffe method to calculate estimated glomerular filtration rate using the Chronic Kidney Disease-Epidemiology equation. Blue bars = stage 1 (eGFR ≥ 90 mL/min per 1.73 m²), Red bars = stage 2 (eGFR 60 mL/min per 1.73 m² – 89 mL/min per 1.73 m²), Green bars = stage 3a (eGFR 45 mL/min per 1.73 m² – 59 mL/min per 1.73 m²), Orange bars = stage 3b (eGFR 30 mL/min per 1.73 m² – 44 mL/min per 1.73 m²), Purple bars = stage 4 (15 eGFR mL/min per 1.73 m²– 29 eGFR mL/min per 1.73 m²), Missing data due to haemolysis or insufficient sample = yellow bars.

method showed analytical, clinically significant imprecision and a positive bias for creatinine results when analysed 24 h after collection. This finding is consistent with previous studies that showed delayed separation of serum from cellular components following specimen collection resulted in a positive interference with different Jaffe methods and could be attributed to the accumulation of pyruvate and other non-creatinine chromogens.[26] Ford et al.[32] established that on the Roche kinetic Jaffe method the serum creatinine results were significantly increased from 16 h and recommended that creatinine results with a delay in separation should not be reported as they impacted CKD staging. Similarly, Shepherd et al.[26]showed an overestimation of Jaffe creatinine results when assessing the impact of delayed sample separation using five different analytical platforms.[26] These results are in contrast to the WHO guidelines that state that creatinine in whole blood is stable at room temperature for 2–3 days.[25] In the current study, the overestimation by the kinetic Jaffe method over time was clinically significant as this led to a higher CKD staging (i.e. lower eGFR). This is consistent with a study by Drion et al. where they found that creatinine results from Jaffe techniques were more biased, imprecise and overestimated serum creatinine concentrations, especially at low concentrations compared to enzymatic techniques.[29] The authors recommended that clinical laboratories consider the health costs associated with inappropriate referral and further suggested using the enzymatic assay that had a minimal bias.

The inconsistencies observed in renal classification using the four GFR equations shown in this study highlight the fact that these equations cannot be used interchangeably for

identifying kidney disease, monitoring of renal function, drug dosing and selection of ARV drug regimens. Measured GFR was not required for this study as ascertaining the GFR was not pertinent, but rather the study sought to quantify the clinical significance of the impact of the instability on the calculated GFR. The CG equation was the least sensitive to the effect of the increased creatinine concentrations on eGFR in our study. This is consistent with a previous finding that demonstrated that the CG equation overestimated the eGFR in a large cohort of Europeans, resulting in the misclassification of 29.2% of individuals.[43] In contrast, the CKD-EPI equation was sensitive to the serum creatinine changes of the kinetic Jaffe method, and the impact on the eGFR was noted at 24 h. This result is consistent with a previous study by Seape and colleagues who found that the CKD-EPI equation performed better than other creatinine-based eGFR equations when assessing renal function in 100 treatment-naïve HIV-positive individuals.[44]

Strengths and limitations

This study was able to show the impact of delayed processing of samples on creatinine concentrations on three platforms with a larger sample size than previous studies. In addition, blood samples were taken from participants at a single time point and the pre-analytical conditions were standardised. A limitation of this study is that serum indices (such as bilirubin which can falsely elevate creatinine results) were not considered when evaluating the creatinine result. Furthermore, we did not exclude all confounders to creatinine measurements such as drugs and pre-existing renal dysfunction which may have adversely affected the creatinine results at all time intervals. In addition, we did not use the reference creatinine method (IDMS) for comparison purposes; we used an enzymatic method traceable to IDMS. A further limitation is that we did not have a wide range of creatinine concentrations to see how a delay in centrifugation would affect the higher concentrations. Furthermore, this study did not explore all the risks associated with POCT devices such as lot-to-lot variation (one lot number was used), operator variability, quality control, analytical traceability, impact of temperature, humidity, different sample types, and the different analytical performance of different POCT devices which have previously been shown to have a significant impact on the eGFR.[45]

Conclusion

This study demonstrated that the Roche kinetic Jaffe method creatinine assay gave falsely elevated serum creatinine levels if samples were not separated and analysed within 24 h of collection. The increase in the serum creatinine concentration over time was clinically significant resulting in misclassification of renal status which would, in turn, lead to incorrect clinical decisions regarding ARV regimen choice. Based on the outputs of this study, and previously published literature,[26,44] laboratories currently analysing creatinine on any Jaffe method should reject (not analyse) samples reaching the laboratory after the stability index time from collection (< 24 h), or if these laboratories decide to analyse these serum creatinine samples an appropriate interpretative comment should be appended to the result as done for other biochemical analytes such as potassium, glucose, and phosphate. The enzymatic creatinine method is highly recommended in clinical laboratories that analyse samples more than 24 h after collection due to transport delays and excessive workload. A major deterrent concerning the utility of the enzymatic method is its high cost. However, the health costs associated with the misclassification of patients using the Jaffe method may outweigh the analytical costs of the enzymatic method. Increased demand for the enzymatic method will result in competition between suppliers and ultimately reduce the cost. This study demonstrated that although creatinine assays have been standardised the extra analytical considerations which are not standardised may result in clinically significant creatinine results, thus the use of POCT, which performed well in this study, in remote clinics may avert the need to perform the laboratory-based kinetic Jaffe.

Measurement of creatinine with a POCT provides an accurate, timely result, and has eliminated the pre-analytical problems noted with the kinetic Jaffe method; therefore, adopting of POCT creatinine should be envisaged. The i-STAT POCT creatinine, however, had a negative bias which resulted in the misdiagnosis of a renal dysfunction for one participant with stage 3a renal failure while the eGFR of the other 21 participants was not affected. Its utilisation in HIV/AIDS programmes could, therefore, be adopted once the eGFR equations have been validated for the sample type, confounders of POCT mentioned previously and infrastructural issues such as integration with the laboratory information systems have been addressed. POCT and enzymatic assays will reduce unnecessary clinic visits due to renal misdiagnoses (false positives) and importantly reduce the pill burden, thereby ultimately reducing health costs to both the government and the patient.

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Competing interests

The authors have declared that no competing interest exists.

Authors' contributions

C.E.C. recruited patients, collected data, obtained ethics approval and wrote the first draft. C.P. reviewed and edited the manuscript. D.T. conceptualised the study, reviewed the statistical analysis, reviewed and edited the manuscript and approved the final version of the manuscript.

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We received the i-STAT machine and the cartridges from Obsidian Health (Pty) Ltd the official distributor of Abbott Point of Care South Africa. The company did not influence the protocol formulation, recruitment of participants, data analysis or the final write-up.

Data availability statement

Data from this study is available.

Disclaimer

The views and opinions expressed in this article are those of the authors and do not necessarily reflect the official policy or position of any affiliated agency of the authors.

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7.2. Appendix 2: Research protocol

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

Evaluation of the diagnostic performance of serum creatinine as a baseline measure of renal function before antiretroviral (ARV) treatment

Research Proposal



OCTOBER 1, 2017

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DEPARTMENT: Chemical Pathology				
TITLE OF PROPOSED RESEARCH: Evaluation of the diagnostic performance of serum creatinine as a baseline measure of renal function before antiretroviral (ARV) treatment				
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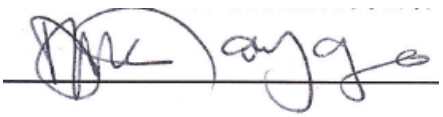
SYNOPSIS OF RESEARCH:

This study seeks to determine the effectiveness of the sole measurement of serum creatinine is in assessing kidney function before commencing antiretroviral therapy in the South African public health clinics. Prior research has shown that delayed separation of serum creatinine samples results in falsely elevated creatinine results. In the South African public health sector serum creatinine taken for the assessment of HIV patients before initiation of the Tenofovir based regimens takes up to 5 days before they can be processed. Based on the aforementioned facts it is important to evaluate if the delays in creatinine analysis result in substantially high creatinine results that will impact on the renal disease classification of the patients and hence their ARV treatment regime.

ETHICS PENDING: √ ETHICS APPROVED: (circle appropriate symbol)	IF Y SUPPLY ETHICS CLEARANCE No:
SIGNATURE OF SUPERVISOR/S: 	

Title of study:

Evaluation of the diagnostic performance of serum creatinine as a baseline measure of renal function before ARV treatment

Background and Literature Review

Human immunodeficiency virus (HIV) is a lentivirus that destroys the immune system and over time the HIV infection culminates into the acquired immunodeficiency syndrome (AIDS). South Africa still harbours one of the largest HIV burdens, with an HIV prevalence of approximately 12,7% in 2016. The total number of people living with HIV in South Africa was estimated at approximately 7 million in 2016. (StatsSA, 2017). The introduction of antiretroviral drugs has drastically slowed the progression of the infection to AIDS and has seen an increase in the life expectancy of these patients.

There are five different classes of antiretroviral (ARV) drugs in South Africa namely nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), integrase inhibitors (INSTIs) and fusion inhibitors (FIs). ARV's are usually prescribed as a three drug combination, sometimes referred to as the highly active antiretroviral drugs (HAART). A number of three drug dose combinations are used and in our setting and the preferred first line combination, is the Tenofovir based regimen. The fixed drug combination of Tenofovir (TDF), Emtricitabine (FTC) and Efavirenz (EFV) is the preferred combination in the public health sector (Davies, 201.3). The selection of the regimen is based on patient co-morbities (i.e. psychiatric conditions), and biochemical markers (i.e. creatinine and alanine transferase).

TDF is an oral prodrug of Tenofovir, an acyclic nucleoside phosphate and was the first NRTI approved for treatment of HIV. TDF has a long half-life that permits once daily dosing thus reducing the pill burden and improving compliance. It is a very well tolerated drug and can be used to treat hepatitis B virus coinfection. TDF is extensively excreted by the kidneys by filtration and 20–30% is actively reabsorbed by the proximal renal tubules via the organic anion transporter 1 (OAT-1). TDF is associated with proximal renal tubulopathy, (tubular wasting syndromes) like Fanconi syndrome, and other related nephrotoxicity's like the decline in glomerular filtration, acute kidney injury, diabetes insipidus and calcium and phosphorus dysregulation associated with bone pathology. It is therefore important to investigate the renal function of patients prior to the initiation of therapy as borderline or mild renal dysfunction increases the risk of nephrotoxicity from TDF. (Tourret, 2013).

HIV patients are prone to an impaired renal function because of many factors such as the use of nephrotoxic ARV's, nephrotoxic opportunistic infection therapy (amphotericin and HIV associated nephropathy. Renal function can be assessed by determining the glomerular filtration rate (GFR) or estimating the glomerular filtration rate (eGFR). GFR is used for classification of chronic kidney disease (CKD) and monitoring of CKD patients. The gold standard for determining the GFR is the use of bolus inulin infusion. This method requires timed urine collection, is tedious and has poor analytical specificity. Silver exogenous methods such as the Inulin (sinistrin) single-bolus plasma clearance method or radio isotopic methods (51Cr-EDTA,99mTc-DTPA,125I-iothalamate) and Iohexol are used in first world countries but cannot be used in our routine clinical practices because they are time-consuming, expensive, have a risk of ionising radiation from isotopes and are not readily available in our country (Tietz, 2012).

Using methods of estimating GFR in our settings by use of endogenous methods such as creatinine and cystatin tend to offer a more practical approach for the day to day clinical utilisation. Creatinine is readily used in state laboratories because it is more accessible and cheaper compared to Cystatin C. The serum creatinine result and the patient demographic details (age, gender, and race) are used to calculate the eGFR using the Modification of Renal Disease MDRD equation. The serum creatinine result and the eGFR have an inverse relationship. Alternatively, the Cockcroft Gault equation can be used to estimate the GFR. (Froissart, 2005).

Creatinine is an endogenous marker synthesised in the kidneys, liver and pancreas by two spontaneous reactions from high energy creatine phosphate or dehydration of creatine. Creatinine is produced at a constant rate, present in all body fluids, easily filtered by the glomerulus, easy and cheap to analyse. Its biggest drawbacks are that it is affected by diet, muscle mass extremes, age, gender, certain drugs such as cimetidine, creatine supplements; it is reabsorbed in the renal tubules and secreted in the renal tubules (Tietz, 2012).

Creatinine was first identified in 1847 by Lieberg as a substance that is obtained from creatine heating. The measurement of creatinine started in 1886 after Jaffe noted a red colour when creatinine reacted with picric acid in an alkaline solution (Blijenberg, 1994). Introduction of the kinetic methods for creatinine measurements were introduced to overcome the interferences on the endpoint assay. The kinetic methods exploit the fact that creatinine and non- creatinine chromogens have a differential rate of colour development (Kaplan, 2010). In the public-sector laboratories, creatinine measurements are predominantly performed using the cost effective Kinetic Jaffe method. Enzymatic methods for creatinine analysis are based on degradation of creatinine to provide measurable products, such as nicotinamide adenine dinucleotide - hydrogen (NADH),

hydrogen peroxide and ammonia selective electrodes. The enzymatic method uses coupled reactions using creatininase, creatinase and creatinine deaminase in the initial reactions (Kaplan, 2010).

The 2017 South African HIV guidelines recommend the use of creatinine as a marker of renal function, to help ascertain the suitability of using TDF based regimens and for early detection of renal disease in HIV populations. These complications inform the treatment guidelines that state that the creatinine clearance (CrCl) should be estimated before commencing TDF, and further elucidate that TDF should not be used if the eGFR or CrCl is < 50 mL/min. The patients on TDF should have their creatinine monitored at three months, six months and then 6-monthly thereafter. In high risk patients (particularly those with coexistent hypertension or diabetes), creatinine should also be checked at one and two months (Meintjes, 2017). The creatinine clearance is derived from the Cockcroft Gault equation or Modification of Renal Disease (MDRD) equation.

Serum creatinine samples are analysed by the National Health Laboratory Services Laboratories (NHLS) for the government HIV clinics. The majority of these laboratories run the Jaffe kinetic methods. The average delay from sample collection to arrival at the Charlotte Maxeke Academic Hospital NHLS for the year 2016 was 9.4 hours. The clinic samples are not prioritised at the academic laboratory, resulting in further delays in sample registration and analysis. This time delay increases in remote clinics because of distances between laboratories and clinics, poor transport network, restricted working hours at referral laboratories and an increased workload at regional laboratories.

The usefulness of using serum creatinine as a sole determinant for the renal function for any clinical decisions in antiretroviral treatment in the South African Public Health clinics should be evaluated as previous studies such as Shepherd *et al*. (2007), stated that the delays in sample separation could lead to significant increases in measured creatinine and misclassification of CKD on the Jaffe assay

over the 30 hours of their study. The enzymatic creatinine assays however showed reliable results despite the delay in sample separation. Tietz *et al*. (2012) also states that delayed separation of serum beyond 14 hours will result in elevated creatinine results on some kinetic Jaffe creatinine methods most likely due to the release of non-creatinine chromogens from red cells. The abovementioned fact does infer that the delay in sample separation in the analyses of serum creatinine can give a falsely high result thus lowering eGFR, which might result in misclassification of patients and the unnecessary use of other ARV drug regimens.

Problem Statement

Kidney disease was reported as a complication of the acquired immunodeficiency syndrome before the advent of antiretroviral therapy. Prior to initiation of HAART, biochemical tests are done to exclude renal complications of HIV and to help in the selection of the appropriate ARV drug regimens. Creatinine is used as the sole renal marker in South Africa to ascertain presence of HIV related nephropathy (HIVAN) or to the suitability of using Tenofovir based ARV drugs. Delay in separation of serum Creatinine measured on the jaffe platforms has been reported to cause falsely elevated creatinine results and thus may lead to misclassification of the renal pathology. This study hence seeks to ascertain if this delay indeed affects the classification of HIV patients, thus resulting in them not qualifying for user friendly and cost effective Tenofovir drug regimens.

Research Question

How effective is the sole measurement of serum creatinine in assessing kidney function before commencing antiretroviral therapy in South African public health clinics.

Hypothesis

Null hypothesis: Delayed sample separation does not significantly affect creatinine levels to result in altered ARV therapy.

Alternative: Delayed separation of samples can result in increased creatinine levels that prevent the use of preferred ARV therapy.

Aim of study

To evaluate the impact of delayed serum separation on the diagnostic performance of serum creatinine as a baseline measure of renal function before ARV treatment.

Specific objectives

1. To compare the analytical stability of serum creatinine in HIV positive patients.
2. To compare the impact of delaying sample separation of serum creatinine on different analytical methods (enzymatic, kinetic Jaffe and i-STAT methods)
3. To determine if changes in the serum creatinine results obtained on the different assays have an impact on eGFR results
4. To establish the effect of delayed separation of serum creatinine on renal function classification and treatment choice of ARV naïve patients
5. To establish a sample separation cut off time where the serum creatinine result can still be deemed reliable based on the analytical platform used (Jaffe / enzymatic / i-STAT)
6. To establish a percentage deterioration of serum creatinine, to establish a mathematical model that can be used on a creatinine analysed after determined stability index time.

Materials and Methods

Study design:

Experimental study design in which serum creatinine samples will be analysed at different time intervals, using three different methods.

Study population:

Recruitment of participants: 20 HIV positive, treatment naïve, patients will be randomly selected from the Alexandra Health clinic. These are patients that have recently tested positive for HIV and are awaiting initiation of ARVs. Twelve 3.5ml yellow serum separator tubes (SST) will be collected from each consenting patient during a single venesection procedure using the push button 21 gauge (green) butterfly by the primary investigator. The samples will be split into two groups for analysis of serum creatinine at CHBAH (group B) and CMJAH (group J). The J group will be analysed on the Roche enzymatic platform and the B group will be analysed on the Roche kinetic and point of care I stat machine.

All tubes labelled 1 will be spun within 4 hours post venesection and will be treated as the baseline samples. Tubes labelled 2 will be retrieved 6 hours post centrifugation of the baseline samples. Samples labelled 3 to 6 will be retrieved on subsequent days from day 2 until day 5. All centrifugation steps and analysis will be done between 08:00 – 10:00 on the respective days to allow for the consistency in the 24-hour periods between samples being analysed. A further creatinine measurement will be performed on day 7 on all the samples analysed above (i.e. 144 samples) to determine if there are any significant variations in serum creatinine results post centrifugation. Refer to Figure 3 (Appendix 1) for a schematic representation of the process.

Anthropometric measurements, age and gender will be obtained for each patient and recorded on the data collection information sheet (see Appendix 2).

Sample size

The required number of samples was determined from the following equation:

$n = [Z2 (100-p) pd] \div Ɛ2$

n = required sample number Z = Critical value

We will use a 95% confidence interval (95% – Z score = 1.96)

P = proportion, based on 97% of creatinine samples received at CMJAH and CHBAH from the HIV clinics

Ɛ= Margin of error (4%) d = design effect, in our case will be 1 (awaiting HAART).

Therefore

$n = [Z2 (100-p) pd] \div Ɛ2$

$n = [1.96 (100-97) 97x1] \div (4\%)2$

$n= [3.8416x3 x 97] \div 16$

n = 70

We will recruit 20 participants which will give us 240 samples. Previous studies such as Sheperd *et al*. (2007) and Devgun (1989) used 5 and 20 participants, respectively.

Inclusion criteria:

HIV patients randomly selected from the Alexandra Health Clinic:

- HIV positive adults >18 years
- HIV positive adults < 70 years. This age range is ideal because one of the eGFR equations, namely MDRD is validated in this age range
- ARV naïve
- patients with no other co-morbities i.e. DM

Exclusion Criteria

- History of renal disease:
  - Acute kidney injury - this renders creatinine unstable thus cannot be used in eGFR
  - Urinary tract infection
  - Chronic kidney disease i.e. secondary to hypertension or diabetes mellitus
- Patients on any nephrotoxic drugs i.e. amphotericin
- Samples that have noted haemolysis, lipaemia, icterus that are remarkable and affect the creatinine analysis on Jaffe and enzymatic method (obtained from the serum indexes report).
- Any patients on antibiotics containing cephalosporin as this can give significant false positives on the Jaffe method.
- Patients on any vitamin C supplements as this affects the hydrogen peroxidase indicator reaction on the enzymatic platform.
- Patients with any other conditions that can affect creatinine i.e. Recent amputation
- Samples that do not have the baseline analysis done within the 30 minutes to 4 hours prescribed time.

- Results that are obtained when the daily quality control has failed.

Sample storage

All samples will be stored at ambient temperature to mimic the current analytical environment available in most NHLS laboratories. Ambient temperature storage can be found at all laboratories from small to tertiary laboratories and during sample transportation.

Analytical Methods

Creatinine Enzymatic method:

This method will be run on the Roche cobas 8000 at CMJAH. The enzymatic method test principle is a coupled reaction with the initial reaction based on the conversion of creatinine to creatine in the presence of creatininase (Figure 1). Vitamin C and rifampicin will result in a negative interference on this assay (see appendix 4)

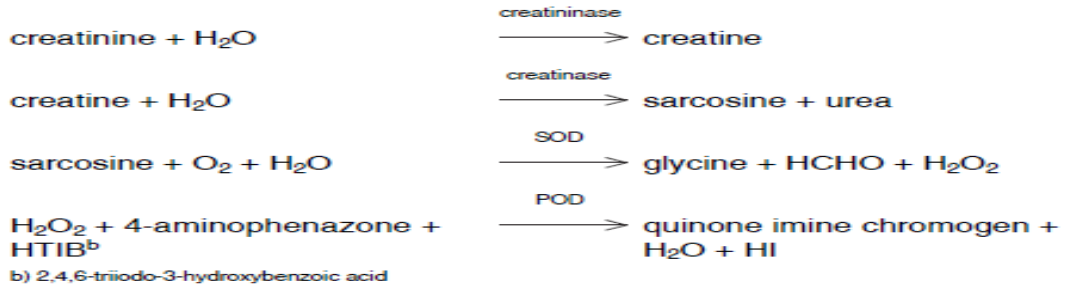


Figure 1: Enzymatic colorimetric method for the measurement of creatinine (see appendix 4)

Kinetic Jaffe method:

This method will be run on the cobas 8000 at Chris Hani Baragwanath Hospital NHLS Laboratory. The test principle is based on the reaction of creatinine and picrate under alkaline conditions which forms a yellow orange complex. The Jaffe method is prone to interference from non-creatinine

chromogens such as protein and ketones, and this method uses a correction factor of -26 mmol/L to account for these chromogens. Bilirubin is the most predominant negative interference on the Jaffe reaction and this is overcome by rate blanking. (See appendix 3)

POCT device

An I-STAT Creatinine point of care device will be used at CHBAH NHLS laboratory. The creatinine will be measured by hydrolyzing creatinine to creatine in the presence of creatinine amidohydrolase. Multiple coupled reactions occur to give hydrogen peroxide as an end product. Hydrogen peroxide is oxidized by the platinum electrode to produce current that is proportional to the serum sample concentration.

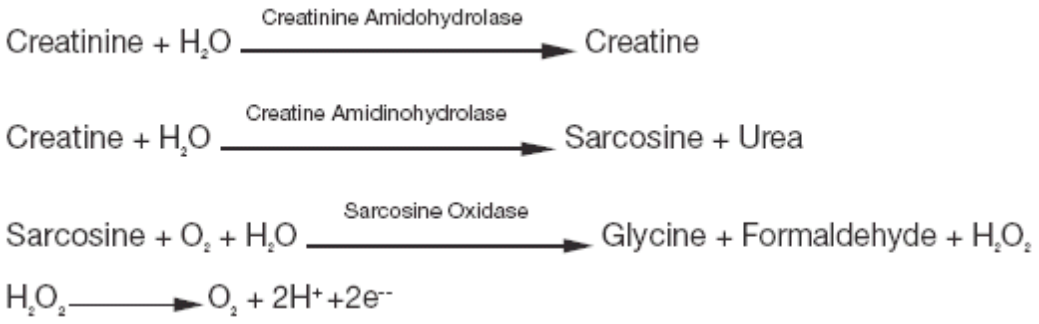


Figure 2: Enzymatic amperometric method (See Appendix 5)

Statistical Analysis

All the information collected on the information sheet will be used for statistical analysis. The significance levels of the tests to be used will be set at the 5% level.

Data analysis techniques

- Coefficient of variation will be calculated to assess the consistency and precision of the results over time.
- The measurement of uncertainty of creatinine for each platform will be calculated, to identify the significant change
- Bland-Altman plot will be used to compare the methods
- A two sample T-test will be used to compare the difference in the mean serum results between days.
- An agreement test will be carried out to assess the significant difference in the serum levels detected across the methods.
- A paired student t- test will be used to test the consistency of results across the varying sample replicates.
- Categorical tests using methods such as Chi-square tests will be used to measure the degree of misclassification of renal disease.
- The Kaplan- Meier survival estimate will be calculated to determine the extent of renal classification over varying waiting times.
- A regression model to estimate a mathematical model that can be applied to the creatinine under analysis at varying time periods will be done if the creatinine variation is uniformly distributed.

Ethical considerations

Signed informed consent will be obtained from the participants and any detection of renal impairment will be reported to the treating clinician, to give the participant timeous appropriate medical care. I will obtain ethical clearance from the University of the Witwatersrand Human Research Ethics Committee (Medical) before collection of any blood samples from patients. Permission will be sort from the Gauteng Department of Health to allow us to conduct our study at the Alexandra Health Clinic.

Time frame

Task	Time
Protocol presentation to department	17 October 2017
Ethics submission	4 November 2017
Protocol submission	18 October 2017
Protocol assessor meeting	7 Nov 2017
Funding application	November 2017
Data collection and sample analysis	Dec to March 2018
Data analysis	March to June 2018
Write up of research	June to December 2018

Table 1: Outline of Time budget

Budget and Funding: The budget for this study is illustrated in Table 2. The NHLS tariffs of R30.02 per creatinine analysed is based on an all-inclusive itemised costing which includes all the pre-analytical phlebotomy instrumentation required i.e. cotton wool, swabs and needles. (Tariff

code 2960). I intend on applying for funding from the NHLS K funding and Roche diagnostics. If no funding can be accessed for this project, the study will proceed.

BUDGET		
Quantifiable items		Rands
Creatinine tests @ R 30.02	240 *2	R 14 409.60
I –stat Creatinine	20	R 720.00
Sub Total		R 15 139.60
Grand Total		R 15 139.60

Table 2: Budget

Limitations

- Our research is limited to one geographical region which might be a confounding factor in this instance as the analysis will be restricted to a single clinic which might attract a population of a specific profile.
- We are only analysing the impact of delay in separation on one manufactures platform for the Enzymatic and Jaffe reaction.

References

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   DOI: 10.4102/sajhivmed. v18i1.776
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12. Stats SA. Mid-year population

Available: http://www.statssa.gov.za/publications/P0302/P0302201

Appendix 1

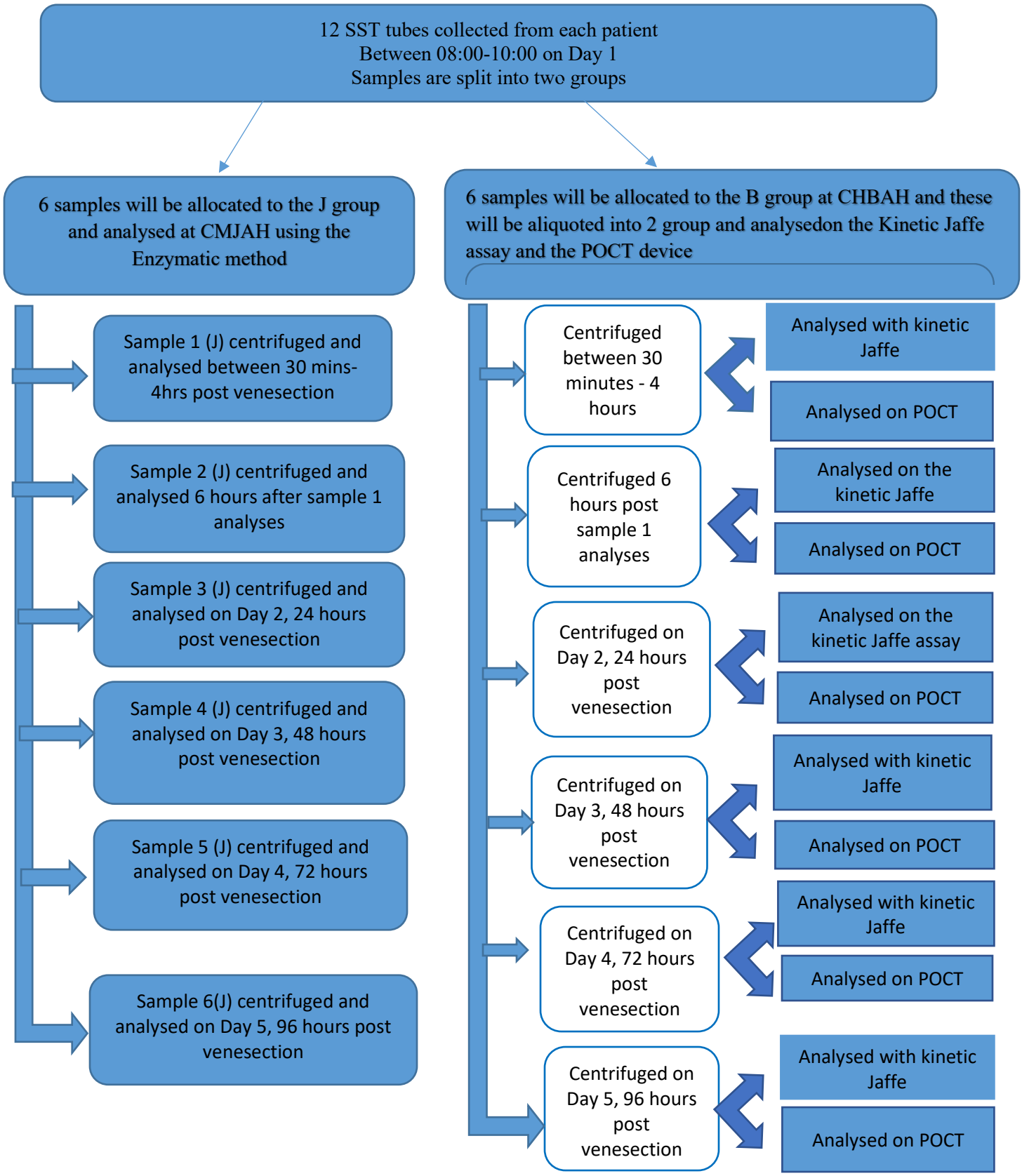


Figure 3: Schematic representation of analytical process

Appendix 2

ARV-Creatinine Stability Project
Data Collection Schedule

Patient Identity	1					
Date of birth		Gender		Female	Male	
Folder Number		Project ID		HV/Creat---/2017		
Blood pressure		Weight	kg		Height	cm
Urinalysis		Protein				

Sample	Analysis schedule	Time	Date	Creat Roche E	Creat Jaffe	Creat I-stat
1	Baseline (< 4 hours)					
2	6 hours					
3	24 h					
4	48 h					
5	72 h					
6	96 h					

Appendix 3. Roche Diagnostics. Cobas package insert CREJ 2017: 1.0

00049107181900201V17.0

CREJ2

cobas®

Creatinine Jaffé Gen.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
04810716 190	Creatinine Jaffé Gen.2 700 tests	System ID 07 6928 2	Roche/Hitachi **cobas c** 311, **cobas c** 501/502
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
12149443 160	Precipath U plus (10 x 3 mL, for USA)	Code 301	
10171743 122	Precinorm U (20 x 5 mL)	Code 300	
10171778 122	Precipath U (20 x 5 mL)	Code 301	
03121313 122	Precinorm PUC (4 x 3 mL)	Code 240	
03121291 122	Precipath PUC (4 x 3 mL)	Code 241	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947526 160	PreciControl ClinChem Multi 1 (4 x 5 mL, for USA)	Code 391	
05947526 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, for USA)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
04489357 190	Diluent NaCl 9 % (50 mL)	System-ID 07 6869 3	

English

System information

For **cobas c** 311/501 analyzers:

CREJ2: ACN 690 (Rate blanked, compensated, serum and plasma)

CRJ2U: ACN 691 (Rate blanked, urine)

SCRE2: ACN 773 (STAT, compensated, serum and plasma, reaction time: 4)

SCR2U: ACN 774 (STAT, urine, reaction time: 4)

For **cobas c** 502 analyzer:

CREJ2: ACN 8690 (Rate blanked, compensated, serum and plasma)

CRJ2U: ACN 8691 (Rate blanked, urine)

SCRE2: ACN 8773 (STAT, compensated, serum and plasma, reaction time: 4)

SCR2U: ACN 8774 (STAT, urine, reaction time: 4)

Intended use

In vitro test for the quantitative determination of creatinine in human serum, plasma and urine on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5]

Chronic kidney disease is a worldwide problem that carries a substantial risk for cardiovascular morbidity and death. Current guidelines define chronic kidney disease as kidney damage or glomerular filtration rate (GFR) less than 60 mL/min per 1.73 m^2 for three months or more, regardless of cause.

The assay of creatinine in serum or plasma is the most commonly used test to assess renal function. Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). It is freely filtered by the glomeruli and, under normal conditions, is not re-absorbed by the tubules to any appreciable extent. A small but significant amount is also actively secreted.

Since a rise in blood creatinine is observed only with marked damage of the nephrons, it is not suited to detect early stage kidney disease. A considerably more sensitive test and better estimation of glomerular filtration rate (GFR) is given by the creatinine clearance test based on creatinine's concentration in urine and serum or plasma, and urine flow rate. For this test a precisely timed urine collection (usually 24 hours) and a blood sample are needed. However, since this test is prone to error due to the inconvenient collection of timed urine, mathematical attempts to estimate GFR based only on the creatinine concentration in serum or plasma have been made. Among the various approaches suggested, two have found wide recognition: that of Cockroft and Gault and that based on the results of the MDRD trial. While the first equation was derived from data obtained with the conventional Jaffé method, a newer version of the second is usable for IDMS-traceable creatinine methods. Both are applicable for adults. In children, the Bedside Schwartz formula should be used.[5,7,8,9]

In addition to the diagnosis and treatment of renal disease, the monitoring of renal dialysis, creatinine measurements are used for the calculation of the fractional excretion of other urine analytes (e. g., albumin, α-amylase). Numerous methods were described for determining creatinine. Automated assays established in the routine laboratory include the Jaffé alkaline picrate method in various modifications, as well as enzymatic tests.

Test principle[10,11,12]

This kinetic colorimetric assay is based on the Jaffé method. In alkaline solution, creatinine forms a yellow-orange complex with picrate. The rate of dye formation is proportional to the creatinine concentration in the specimen. The assay uses "rate-blanking" to minimize interference by bilirubin. To correct for non-specific reaction caused by serum/plasma pseudo-creatinine chromogens, including proteins and ketones, the results for serum or plasma are corrected by -26 µmol/L (-0.3 mg/dL).

Creatinine + picric acid $\xrightarrow{\textit{Alkaline pH}}$ yellow-orange complex

Reagents - working solutions

R1	Potassium hydroxide: 900 mmol/L; phosphate: 135 mmol/L; pH ≥ 13.5; preservative; stabilizer
R3 (STAT R2)	Picric acid: 38 mmol/L; pH 6.5; non reactive buffer

R1 is in position B and R3 (STAT R2) is in position C.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

For USA: For prescription use only.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Appendix 4: Roche **Diagnostics. Cobas package insert CREP2 2016-02: 8.0**

CREP2

cobas®

Creatinine plus ver.2

Order information

REF	CONTENT		Analyzer(s) on which **cobas c** pack(s) can be used
05168589 190	Creatinine plus ver.2 600 tests	System-ID 05 6612 7	Roche/Hitachi **cobas c** 701/702
10759350 190	Calibrator f.a.s. (12 x 3 mL)	Code 401	
10759350 360	Calibrator f.a.s. (12 x 3 mL, for USA)	Code 401	
12149435 122	Precinorm U plus (10 x 3 mL)	Code 300	
12149435 160	Precinorm U plus (10 x 3 mL, for USA)	Code 300	
12149443 122	Precipath U plus (10 x 3 mL)	Code 301	
12149443 160	Precipath U plus (10 x 3 mL, for USA)	Code 301	
10171743 122	Precinorm U (20 x 5 mL)	Code 300	
10171778 122	Precipath U (20 x 5 mL)	Code 301	
03121313 122	Precinorm PUC (4 x 3 mL)	Code 240	
03121291 122	Precipath PUC (4 x 3 mL)	Code 241	
05117003 190	PreciControl ClinChem Multi 1 (20 x 5 mL)	Code 391	
05947626 190	PreciControl ClinChem Multi 1 (4 x 5 mL)	Code 391	
05947626 160	PreciControl ClinChem Multi 1 (4 x 5 mL, USA)	Code 391	
05117216 190	PreciControl ClinChem Multi 2 (20 x 5 mL)	Code 392	
05947774 190	PreciControl ClinChem Multi 2 (4 x 5 mL)	Code 392	
05947774 160	PreciControl ClinChem Multi 2 (4 x 5 mL, USA)	Code 392	
05172152 190	Diluent NaCl 9 % (119 mL)	System-ID 08 6869 3	

English

System information

CREA2: ACN 8452 (serum and plasma)

CRE2U: ACN 8152 (urine)

Intended use

In vitro assay for the quantitative determination of creatinine in human serum, plasma and urine on Roche/Hitachi **cobas c** systems.

Summary[1,2,3,4,5]

Chronic kidney disease is a worldwide problem that carries a substantial risk for cardiovascular morbidity and death. Current guidelines define chronic kidney disease as kidney damage or glomerular filtration rate (GFR) less than 60 mL/min per 1.73 m^2 for three months or more, regardless of cause.

The assay of creatinine in serum or plasma is the most commonly used test to assess renal function. Creatinine is a break-down product of creatine phosphate in muscle, and is usually produced at a fairly constant rate by the body (depending on muscle mass). It is freely filtered by the glomeruli and, under normal conditions, is not re-absorbed by the tubules to any appreciable extent. A small but significant amount is also actively secreted.

Since a rise in blood creatinine is observed only with marked damage of the nephrons, it is not suited to detect early stage kidney disease. A considerably more sensitive test and better estimation of glomerular filtration rate (GFR) is given by the creatinine clearance test based on creatinine's concentration in urine and serum or plasma, and urine flow rate. For this test a precisely timed urine collection (usually 24 hours) and a blood sample are needed. However, since this test is prone to error due to the inconvenient collection of timed urine, mathematical attempts to estimate GFR based only on the creatinine concentration in serum or plasma have been made. Among the various approaches suggested, two have found wide recognition: that of Cockroft and Gault and that based on the results of the MDRD trial. While the first equation was derived from data obtained with the conventional Jaffé method, a newer version of the second is usable for IDMS-traceable creatinine methods. Both are applicable for adults. In children, the Bedside Schwartz formula is used.[6,7,8,9]

In addition to the diagnosis and treatment of renal disease, the monitoring of renal dialysis, creatinine measurements are used for the calculation of the fractional excretion of other urine analytes (e. g., albumin, α-amylase). Numerous methods were described for determining creatinine. Automated assays established in the routine laboratory include the Jaffé alkaline picrate method in various modifications, as well as enzymatic tests.

Test principle

This enzymatic method is based on the conversion of creatinine with the aid of creatininase, creatinase, and sarcosine oxidase to glycine, formaldehyde and hydrogen peroxide. Catalyzed by peroxidase the liberated hydrogen peroxide reacts with 4-aminophenazone and HTIB[a] to form a quinone imine chromogen. The color intensity of the quinone imine chromogen formed is directly proportional to the creatinine concentration in the reaction mixture.

a) 2,4,6-triiodo-3-hydroxybenzoic acid

$$\text{creatinine} + H_2O \xrightarrow{\text{creatininase}} \text{creatine}$$

$$\text{creatine} + H_2O \xrightarrow{\text{creatinase}} \text{sarcosine} + \text{urea}$$

$$\text{sarcosine} + O_2 + H_2O \xrightarrow{\text{SOD}} \text{glycine} + HCHO + H_2O_2$$

$$H_2O_2 + \text{4-aminophenazone} + \text{HTIB}^a \xrightarrow{\text{POD}} \text{quinone imine chromogen} + H_2O + HI$$

a) 2,4,6-triiodo-3-hydroxybenzoic acid

Creatine of the sample is destroyed by creatinase, SOD and catalase during incubation in R1.

Reagents - working solutions

R1 TAPS buffer (N-Tris(hydroxymethyl)methyl-3-aminopropanesulfonic acid): 30 mmol/L, pH 8.1; creatinase (microorganisms): ≥ 332 µkat/L; sarcosine oxidase (microorganisms): ≥ 132 µkat/L; ascorbate oxidase (microorganisms): ≥ 33 µkat/L; catalase (microorganisms): ≥ 1.67 µkat/L; HTIB: 1.2 g/L; detergents; preservative

R3 TAPS buffer: 50 mmol/L, pH 8.0; creatininase (microorganisms): ≥ 498 µkat/L; peroxidase (horseradish): ≥ 16.6 µkat/L; 4-aminophenazone: 0.5 g/L; potassium hexacyanoferrate (II): 60 mg/L; detergent; preservative

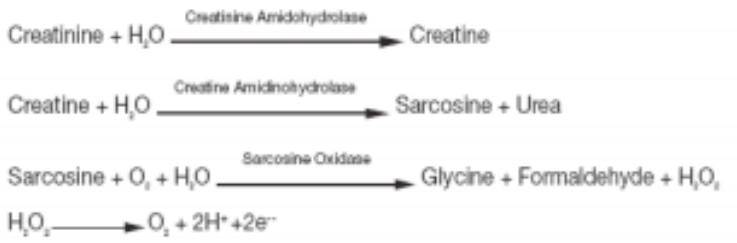
R1 is in position B and R3 is in position C.

Appendix 5. Chemistry China Basin. I-STAT Creatinine. UCSF Clinical Labs

i-STAT Creatinine

Chemistry China Basin

Creatinine is measured amperometrically. Creatinine is hydrolyzed to creatine in a reaction catalyzed by the enzyme creatinine amidohydrolase. Creatine is then hydrolyzed to sarcosine in a reaction catalyzed by the enzyme creatine amidinohydrolase. The oxidation of sarcosine, catalyzed by the enzyme sarcosine oxidase, produces hydrogen peroxide (H2O2). The liberated hydrogen peroxide is oxidized at the platinum electrode to produce a current which is proportional to the sample creatinine concentration.



See below for information on factors affecting results. Certain substances, such as drugs, may affect analyte levels *in vivo*.1

If results appear inconsistent with the clinical assessment, the patient sample should be retested using another cartridge.

INTENDED USE

The test for creatinine, as part of the i-STAT System, is intended for use in the *in vitro* quantification of creatinine in arterial, venous, or capillary whole blood.

Contents

Each i-STAT cartridge contains one reference electrode (when potentiometric sensors are included in the cartridge configuration), sensors for the measurement of specific analytes, and a buffered aqueous calibrant solution that contains known concentrations of analytes and preservatives. For cartridges that contain a sensor for the measurement of creatinine, a list of reactive ingredients is indicated below:

Reactive Ingredient	Biological Source
Creatinine	N/A
Creatine Amidinohydrolase	*Actinobacillus sp.*
Creatinine Amidohydrolase	Microbial
Sarcosine Oxidase	Microbial

7.3. Appendix 3: Approval of title change

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

Private Bag 3 Wits, 2050

Tel: 02711 7172076

Reference: Mrs Sandra Benn
E-mail: sandra.benn@wits.ac.za

11 September 2020
Person No: 1577728
TAA

Dr CE Chikomba
10 Mimosa
Midstream Ridge
Midstream
1692
South Africa

Dear Dr Chemedzai Chikomba

Master of Medicine: Change of title of research

I am pleased to inform you that the following change in the title of your Research Report for the degree of **Master of Medicine** has been approved:

From:	**Evaluation of the diagnostic performance of serum creatinine as a baseline measure of function before antiretroviral (ARV) treatment**
To:	**Evaluation of the impact of delayed centrifugation on the diagnostic performance of serum creatinine as a baseline measure of renal function before antiretroviral treatment**

Yours sincerely

Mrs Sandra Benn
Faculty Registrar
Faculty of Health Sciences

7.4. Appendix 4: Ethics clearance certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

Office of the Deputy Vice-Chancellor (Research & Post Graduate Affairs)

TO: Dr CE Chikomba
School of Pathology
Department of Chemical Pathology
Medical School
University

E-mail: drcheme@gmail.com

CC: Supervisor: Dr DM Tanyanyiwa <Donald.Tanyanyiwa@wits.ac.za>
and <HREC-Medical.ResearchOffice@wits.ac.za>

FROM: Iain Burns
Human Research Ethics Committee (Medical)
Tel: 011 717 1252

E-mail: Iain.Burns@wits.ac.za

DATE: 07/05/2018

REF: *R14/49*

PROTOCOL NO: **M1711109** *(This is your ethics application study reference number. Please quote this reference number in all correspondence relating to this study)*

PROJECT TITLE: *Evaluation of the diagnostic performance of serum creatine as a baseline measure of renal function before antiretroviral (ARV) treatment*

Please find attached the Clearance Certificate for the above project. I hope it goes well and that an article in a recognized publication comes out of it. This will reflect well on your professional standing and contribute to the Government funding of the University.

MSWorks2000/Iain0007/Clearscan.wps

UNIVERSITY OF THE
WITWATERSRAND,
JOHANNESBURG

R14/49 Dr CE Chikomba

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)
CLEARANCE CERTIFICATE NO. M1711109

NAME: (Principal Investigator)	Dr CE Chikomba
DEPARTMENT:	School of Pathology Department of Chemical Pathology Medical School University
PROJECT TITLE:	Evaluation of the diagnostic performance of serum creatine as a baseline measure of renal function before antiretroviral (ARV) treatment
DATE CONSIDERED:	24/11/2017
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Dr DM Tanyanyiwa
APPROVED BY:	Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	07/05/2018

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 3rd floor, Phillip V Tobias Building, Parktown, University of the Witwatersrand, Johannesburg.

I/We fully understand the conditions under which I am/we are authorised 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 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 **November** and will therefore be due in the month of **November** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature

Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

7.5. Appendix 5: Turnitin Report

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Dr. C Padoa
22/09/2020

