Clinical Research Application Form

Last updated: 09/30/2015

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Section A: General Project Information

Geographical information

In which country does your project take place?	South Africa
In which ISN region does your project take place?	Africa

What is the title of your project?

Please choose a short but descriptive title: e.g.: "CKD screening in Abuja, Nigeria"

Validation of eGFR equations in South Africans

Name and address of the coordinating Institution

Legal name:	University of the Witwatersrand
Address:	Faculty of Health Sciences Division of Chemical Pathology 7 York Rd Parktown 2193 Gauteng South Africa
Head of the Institute:	Professor Jaya George

Name of the local coordinator of the project

Full Name:	June Fabian
Position:	Honorary Lecturer Division of Nephrology Department of Medicine University of Witwatersrand
Email:	june.fabian@mweb.co.za
Phone no:	+27827853579
Fax no:	+27113566395

Duration of the project (in months- max 36)

36

Co-Investigators / Collaborators

Name	Professor Saraladevi Naicker
Role in project	Supervision

Name of the Regional Coordinator who was consulted about the project

Omar Abboud

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Section B: Concept summary (maximum 300 words)

What (intervention/exposure, outcome) will this project study, why (rationale), in whom (population), how (what design will be used), over how long (duration) and what impact will it have (significance)?

This project aims to investigate currently available methods to assess kidney function in Africans. Early detection of chronic kidney disease (CKD) is based on simple routine laboratory tests such as urine protein and serum creatinine. A meta-analysis of data from several African countries showed the overall prevalence of CKD from 21 medium and high quality studies was 13.9%[1]. They noted an absence of validated and reliable measures of kidney function for Africans in Africa and noted that the prevalence estimates varied substantially even within similar populations depending on the definition of CKD used and the method of measurement.

Serum creatinine levels have been shown to be higher in African Americans regardless of kidney function. In response to this, adjustments for ethnicity have been included in the 4-variable MDRD and the CKD-EPI equations to better approximate GFR among African Americans[2]. Data from our centre has shown that these equations perform better without the adjustment for ethnicity[3-5]. This has been also been demonstrated in two studies from Kwa-Zulu Natal and the Western Cape in South Africa[6,7]. Studies from Kenya and Ghana also showed an over-estimation of the GFR with the adjustment for ethnicity [8,9]. In all of these sub-Saharan African studies, the BMI and weight of all participants was lower than that seen in studies from Europe and the USA, suggesting that lower muscle mass may contribute to this phenomenon. The single limiting factor however, for all these studies has been in the small sample size. To date, there have been no population-based studies that have validated the use of eGFR equations in South Africans.

In this study we will determine how best to measure kidney function accurately using serum creatinine and cystatin C (cysC). We will do this by comparing the accuracy of different equations for calculating glomerular filtration rate (eGFR) to a directly measured GFR using iohexol (iGFR).

We will use an appropriately large sample size that will allow for the derivation of a new equation that accurately predicts kidney function in black Africans. This will be central to future diagnosis and management of patients with CKD in South Africa as well as the conduct of future epidemiological studies.

We will also assess the accuracy and precision of a point of care device for determination of estimated GFR. This is essential to determine whether existing devices are sufficiently accurate for assessing kidney function in resource-limited areas.

Section C: Project description (maximum 6 pages, single spaced)

Please prepare and upload a document with the following information:

1. **Specific aims of the program (approximately 0.5 pages):** What are the objectives of your project? Please provie 1-4 objectives:

fewer is better. Each objective should be specific, measurable and linked to a hypothesis. See Appendix for examples.

2. **Background and rationale (approximately 1 page):** What is the previous work that has led you to contemplate this study? Why is this project important? Why is it important for the investigator's country/region? Why is this work novel or how will it extend what has already been done? How will the results be used?

3. **Methods (approximately 2-3 pages):** Provide a detailed description of how the work will be done. Try to address the following as appropriate:
   • **Primary and secondary outcomes**: What outcomes will you study? How will these outcomes be defined? Who will assess them and how?
   • **Inclusion criteria/exclusion criteria:** who will be eligible and ineligble to participate, and why?
   • **Confounders/covariates:** how will you account for the possibility of confounding and bias? If there are potential confounders, how will these be defined/measured and how will you account for them in analysis?
   • **Recruitment/sampling method; sample size calculation:** how will you identify participants? How many participants do you need to make the study worthwhile and is this feasible?
   • **Statistical methods:** how will you analyze the data? Do you have the skills to do the analysis yourself, or will you involve a statistician colleague?
   • **Ethical considerations:** have you obtained/will you obtain ethics approval for this study? Are there any other ethical considerations to be addressed?
   • **Knowledge translation:** who needs to know about the results of your study, and how will you ensure that they are aware of the findings once the study is finished?

4. **Research team (approximately 0.5 pages):** who are the members of your team, why are their qualifications/experience relevant for this study? What will each member of the team do? Does the team have the necessary skills and experience to do the work?

5. **Institutional environment (approximately 0.5 pages):** provide a few details about your institution, and why it is a suitable environment do this work. Does it have the necessary facilities/patient population to do the work?

6. **Significance (approximately 0.25 pages):** what do you expect to find, and how will this make a difference? What impact will it have scientifically, or to healthcare in your country/region? What are the next steps once this study is finished?

http://cr.theisn.org/media/assets/survey-uploads/29711/4304994-8dtRBRtpNz/June%20Fabian%20Application%20ISN.pdf

Section D: timelines (Gantt chart)

Upload a planning or gantt chart with a timeline, milestones and deliverables.

http://cr.theisn.org/media/assets/survey-uploads/29711/4304994-XCnZzGg4pV/June%20Fabian%20Gantt%20Chart%20ISN.pdf

Section E: Relevant references

References

1. Stanifer JW, Jing B, Tolan S, Helmke N, Mukerjee R, et al. (2014) The epidemiology of chronic kidney disease in sub-Saharan Africa: a systematic review and meta-analysis. Lancet Glob Health 2: e174-181.

2. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, et al. (2006) Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. Ann Intern Med 145: 247-254.

3. van Deventer HE, George JA, Paiker JE, Becker PJ, Katz IJ (2008) Estimating glomerular filtration rate in black South Africans by use of the modification of diet in renal disease and Cockcroft-Gault equations. Clin Chem 54: 1197-1202.

4. van Deventer HE, Paiker JE, Katz IJ, George JA (2011) A comparison of cystatin C- and creatinine-based prediction equations for the estimation of glomerular filtration rate in black South Africans. Nephrol Dialy Transplant 26: 1553-1558.

5. Seape T, Gounden V, van Deventer HE, Candy GP, George JA (2015) Cystatin C- and creatinine-based equations in the assessment of renal function in HIV-positive patients prior to commencing Highly Active Antiretroviral Therapy. Ann Clin Biochem.

6. Matsha TE, Yako YY, Rensburg MA, Hassan MS, Kengne AP, et al. (2013) Chronic kidney diseases in mixed ancestry south African populations: prevalence, determinants and concordance between kidney function estimators. BMC nephrology 14: 75.

7. Madala ND, Nkwanyana N, Dubula T, Naiker IP (2012) Predictive performance of eGFR equations in South Africans of African and Indian ancestry compared with (9)(9)mTc-DTPA imaging. Int Urol Nephrol 44: 847-855.

8. Wyatt CM, Schwartz GJ, Owino Ong'or W, Abuya J, Abraham AG, et al. (2013) Estimating kidney function in HIV-infected adults in Kenya: comparison to a direct measure of glomerular filtration rate by iohexol clearance. PloS one 8: e69601.

9. Eastwood JB, Kerry SM, Plange-Rhule J, Micah FB, Antwi S, et al. (2010) Assessment of GFR by four methods in adults in Ashanti, Ghana: the need for an eGFR equation for lean African populations. Nephrol Dial Transplant 25: 2178-2187.

10. National Kidney Foundation (2002) K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. Am J Kidney Dis 39: S1-266.

11. Ninomiya T, Perkovic V, de Galan BE, Zoungas S, Pillai A, et al. (2009) Albuminuria and kidney function independently predict cardiovascular and renal outcomes in diabetes. J Am Soc Nephrol 20: 1813-1821.

12. Astor BC, Hallan SI, Miller ER, 3rd, Yeung E, Coresh J (2008) Glomerular filtration rate, albuminuria, and risk of cardiovascular and all-cause mortality in the US population. Am J Epidemiol 167: 1226-1234.

13. Herget-Rosenthal S, Bokenkamp A, Hofmann W (2007) How to estimate GFR-serum creatinine, serum cystatin C or equations? Clin Biochem 40: 153-161.

14. Levey AS (1990) Measurement of renal function in chronic renal disease. Kidney Int 38: 167-184.

15. Poggio ED, Wang X, Greene T, Van Lente F, Hall PM (2005) Performance of the modification of diet in renal disease and Cockcroft-Gault equations in the estimation of GFR in health and in chronic kidney disease. J Am Soc Nephrol 16: 459-466.

16. Wyatt CM, Schwartz GJ, Owino Ong'or W, Abuya J, Abraham AG, et al. (2013) Estimating kidney function in HIV-infected adults in Kenya: comparison to a direct measure of glomerular filtration rate by iohexol clearance. PloS one: e69601.

17. Stevens LA, Coresh J, Greene T, Levey AS (2006) Assessing kidney function--measured and estimated glomerular filtration rate. N Engl J Med 354: 2473-2483.

18. Levey AS, Bosch JP, Lewis JB, Greene T, Rogers N, et al. (1999) A more accurate method to estimate glomerular filtration rate from serum creatinine: a new prediction equation. Modification of Diet in Renal Disease Study Group. Ann Intern Med 130: 461-470.

19. Levey AS, Stevens LA, Schmid CH, Zhang Y, Castro IIIAF, et al. (2009) A New Equation to Estimate Glomerular Filtration Rate. Ann Intern Med 150: 604-612.

20. Myers GL, Miller WG, Coresh J, Fleming J, Greenberg N, et al. (2006) Recommendations for improving serum creatinine measurement: a report from the Laboratory Working Group of the National Kidney Disease Education Program. Clin Chem 52: 5-18.

21. Filler G, Bokenkamp A, Hofmann W, Le Bricon T, Martinez-Bru C, et al. (2005) Cystatin C as a marker of GFR--history, indications, and future research. Clin Biochem 38: 1-8.

22. Knight EL, Verhave JC, Spiegelman D, Hillege HL, de Zeeuw D, et al. (2004) Factors influencing serum cystatin C levels other than renal function and the impact on renal function measurement. Kidney Int 65: 1416-1421.

23. Kahn K, Collinson MA, Gomez-Olive FX, Mokoena O, Twine R, et al. (2012) Profile: Agincourt health and socio-demographic surveillance system. Int J Epidemiol 41: 988-1001.

24. Nilsson-Ehle P (2006) Iohexol clearance for the determination of glomerular filtration rate: 15 years' experience in clinical practice. eJIFCC 13.

25. Gaspari F, Perico N, Ruggenenti P, Mosconi L, Amuchastegui CS, et al. (1995) Plasma clearance of nonradioactive iohexol as a measure of glomerular filtration rate. JASN 6: 257-263.

26. National Health Service, UK (2010) Evaluation report Point of care tests for the measurement of creatinine. UK: Centre for Evidence-based Purchasing.

27. Bland JM, Altman DG (1986) Statistical methods for assessing agreement between two methods of clinical measurement. Lancet 1: 307-310.

28. Spinler SA, Nawarskas JJ, Boyce EG, Connors JE, Charland SL, et al. (1998) Predictive performance of ten equations for estimating creatinine clearance in cardiac patients. Iohexol Cooperative Study Group. Ann Pharmacother 32: 1275-1283.

29. Bland JM, Altman DG (1997) Difference versus mean plots. Ann Clin Biochem 34 (Pt 5): 570-571.

Section F: Detailed budget in local currency and USD

What funds do you need and why? is the budget sufficient to complete the project? If not, where will additional funds come from? How did you estimate the costs?

Description of funds	Budget
	Total cost of this project : USD 26 343.79 (ZAR 339 132.56) The National Health Laboratory Services, South Africa has partially funded USD 6 730.00 (ZAR 90 000.00) for this project already. The outstanding amount required would therefore be: USD 2 6343.79 – 6730.00 = USD 19613.79
Budget requested to ISN in USD	19613.79

Upload a detailed budget

http://cr.theisn.org/media/assets/survey-uploads/29711/4304994-piNWJbrTRE/June%20Fabian%20Budget%20ISN.pdf

Section G: Patient Information Sheet and Informed Consent Form

Please upload the form: a standard form can be found in the Guidelines for Applicants

http://cr.theisn.org/media/assets/survey-uploads/29711/4304994-HtvwS6527K/June%20Fabian%20Informed%20consent%20ISN.pdf

To: 18th September 2015

Dr Marcello Tonelli
**ISN Clinical Research Program Chair**
& ISN Clinical Research Program Committee

Subject: **Letter of approval of the application of Dr June Fabian**

"Validation of eGFR formulae and Point-of-Care Creatinine
measurements in the Assessment of kidney function in Africans"

This is to confirm to you that the above-mentioned application was initially submitted to me by the applicant, Dr June Fabian.

I would like to convey to you that the topic is suitable and that the application submitted by the candidate was in a competitive form without requiring any amendments.

Kind regards
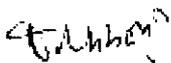
Omar Abboud
ISN Clinical Research Coordinator for Africa Region

Omar Abboud
Professor of Medicine
University of Khartoum
P O Box 102
Khartoum
Sudan.

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M150558

NAME:
(Principal Investigator)   Prof Jaya George

DEPARTMENT: Chemical Pathology

PROJECT TITLE: Assesment of Kidney Function in Africans
(Previously M10410, Title changed 29/07/2015)

DATE CONSIDERED: 29/05/2015

DECISION: Renewal approved

CONDITIONS:

SUPERVISOR:

APPROVED BY:

Professor Angela Woodiwiss, Co-Chairperson, HREC (Medical)

DATE OF APPROVAL: 29/05/2015

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 Secretary in Room 10004, 10th floor, Senate House, University.
I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report**.

Principal Investigator Signature   Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Project title: Validation of eGFR formulae and Point-of-Care Creatinine measurements in the Assessment of kidney function in Africans

The primary aim of this study is to compare the accuracy and precision of the MDRD and CKD-EPI (with/without adjustments for ethnicity and with/without cysC) to a gold standard method for measuring GFR measured using iohexol (iGFR).

If indicated, we will develop an accurate prediction equation for estimating GFR in black Africans We hypothesize that the current MDRD and CKD-EPI equations as recommended by KDIGO are not accurate or precise for use in Black Africans.

The second aim will be to compare the accuracy of a point of care device measurement of creatinine with estimation of GFR to iGFR
We hypothesize that using an IDMS-traceable device for creatinine measurement in resource-limited settings is sufficiently accurate to diagnose CKD

Objectives
1. To evaluate the accuracy and precision of the MDRD and the CKD-EPI with and without the inclusion of the ethnicity factor to iGFR.
2. To compare the accuracy and the precision of the CKD-EPI cysC and combined cysC, creatinine equation to iGFR.
3. To develop a new equation for use in black Africans that may include the use of other variables such as height.
4. To assess the accuracy of a point of care device(POC) for the estimation of GFR

Background
With the serious burden of CKD in Africans it is essential to be able to accurately assess renal function in these populations. Glomerular filtration rate (GFR) is the best overall marker of renal function in people with healthy or diseased kidneys[9,10] and is an independent predictor of renal and cardiovascular disease[11,12]. GFR can be measured as the renal clearance of exogenous markers such as inulin, [51]chromium ethylenediaminetetraacetic acid (^{51}Cr-EDTA),technetium-labelled diethylene-triamine-pentacetate (^{99m}Tc-DTPA) and iohexol. However, these exogenous markers are impractical for routine clinical use due to their limited access and high cost. Endogenous GFR markers include serum creatinine (S-Cr) and cysC.

S-Cr is the most commonly used marker in the clinical laboratory to assess GFR but it has multiple limitations [13]. For example, creatinine concentration is not only determined by GFR but also affected by factors such as muscle mass, diet, gender and age [14]. This results in a large intra-individual variation in creatinine production. The four-variable Modification of Diet in Renal Disease (4-v MDRD) and Cockcroft–Gault (CG) equations, two S-Cr-based equations commonly used for estimating GFR, account for some of these factors. The Modification of Diet in Renal Disease 4-variable formula (MDRD) was derived from a study population with impaired renal function; and thus is less accurate within a healthy range GFR and has been shown to have varying accuracy in different population groups [3,15,16].This has been attributed to variations in non-GFR determinants of S-Cr such as muscle mass and diet which may be affected by acute and chronic disease[17]. The MDRD is based on four variables namely age, sex, S-CR and ethnicity. An ethnicity factor of 1.212 was established in African-Americans[18]. A study from our group showed that the MDRD without the ethnicity factor can be used in black South Africans [3]. More

recently the creatinine based CKD-EPI equation (CKD-EPI) was shown to estimate GFR more accurately than the MDRD equation [19] and has replaced the MDRD equation for estimating GFR in some laboratories, however we have shown that the accuracy within 30% of measured GFR was less than 90% and in HIV infected patients it is less than 50% for all creatinine based equations[5].

The measurement of creatinine has also been the subject of considerable recent international attention to align all current methods (including point-of-care) to isotope dilution mass spectrometry (IDMS) equivalent standards[20]. With the prevalence of CKD increasing across the world, the need for good screening methods for identifying CKD risk is becoming more important. A POC device fit-for purpose in this setting requires capillary sampling, fast turnaround of result and automatic eGFR calculation, as well as good analytical performance specifications to correctly categorise CKD risk. The use of POC creatinine would allow for early diagnosis and intervention for patients with CKD, which would offset the increased cost of the test.

CysC is a low molecular weight (13kD) non-glycosylated basic protein produced by all nucleated cells and production is independent of muscle mass and dietary influences. While cysC based prediction equations are not subject to some of the limitations of serum creatinine-based eGFR equations[21] cysC may be influenced by inflammation[22]. A study from our centre showed that cysC based prediction equations are more precise than serum creatinine based equations for patients in predicting eGFR in patients with measured GFR > 60 ml/min/1.73m^2[4].
This study will allow us to validate current equations and/or develop an equation that accurately predicts GFR in our black African population. This will allow for early detection of CKD that is accurate as well as further epidemiological studies of CKD[20].

Detailed methodology
Study Site
This work will be based in the rural Agincourt-Bushbuckridge subdistrict of the Mpumalanga province of South Africa, adjacent to Mozambique. The area includes a high-functioning health and socio-demographic surveillance system (HDSS) which covers approximately 115 000 people. It is supported by the SA MRC/Wits University Rural Public Health and Health Transitions Unit and has an extensive portfolio of observational and intervention research over the last 20 years with a deep understanding of the contextual factors in this community[23]. The study setting also serves as a national pilot site for the development of integrated chronic disease care. As an established Health and socio-Demographic Surveillance site, there is a complete enumeration of the population annually, allowing the selection of a representative sample for this study. Trained fieldworkers and nurses have extensive experience in administering questionnaires, performing clinical assessments and obtaining biological specimens. An on-site research laboratory, equipped with a centrifuge and -80 °C freezers will enable the processing and storage of venous samples. The School of Public Health at Wits provides a further managerial base for Agincourt. Modern IT facilities ensure effective and frequent communication between the sites.

Primary Outcome:
Validation of accuracy and precision of eGFR equations in comparison to mGFR using iohexol as the gold standard

Sample

Inclusion criteria: Black African adults [≥ 18years], males and females, who have given written informed consent; with an eGFR based on the MDRD to allow for categorisation.

Exclusion criteria: Age <18 years, failure to give written informed consent, ethnicity other than African, pregnant or breast feeding, receiving dialysis

The sample size was determined set for two groups as follows:

Group 1: a mean of 65ml/min/1.73 m2 and a standard deviation(SD) of 50. The minimum sample size is 411

Group 2: mean of 45ml/min/1.73m2 and an SD of 45 the minimum sample size is 435.

The participants will be divided into 4 groups with 435 participants in each group:

1. Males with eGFR <60 ml/min/1.73m^2

2. Males with eGFR >60ml/min/1.73m^2

3. Females with eGFR <60ml/min/1.73m^2

4. Females with eGFR >60ml/min/1.73m^2

Total sample size 1740

Methods

Following confirmation of contact details and eligibility and after getting informed consent we will carry out a health examination, and then draw blood for a laboratory measurement of serum creatinine as well as point of care creatinine, and serum cystatin C. This will be followed by the reference measurement of GFR. The health examination will include weight, height measurement and the administration of a questionnaire (see appendix). Thereafter, the GFR measurement will be conducted over a four hour period. Participants will be observed closely for the first 15 minutes after intravenous administration of IVI iohexol for adverse effects, as per previously published protocols [24].

Measured GFR

GFR will be measured by iohexol clearance as previously described [25]. Briefly, a single dose of 5cc of iohexol (Omnipaque 300mhI/mL, GE Healthcare) will be administered over 30 seconds through a butterfly needle inserted in the arm contralateral to the IV, followed by a 10cc normal saline flush. The syringe containing iohexol will be weighed to the nearest 0.1 g prior to and immediately after injection to more accurately calculate the dose of iohexol administered. Five mls of blood will be drawn from the IV at the time of insertion for an iohexol blank, for determination of haematocrit, serum creatinine and cystatin C. Blood will be drawn again and at approximately 120, and 240 minutes after injection of iohexol, with exact times recorded. The GFR is calculated using a single compartment model based on iohexol clearance between 120 and 240 min. Analysis will be carried out on plasma by HPLC. Plasma samples will be extracted using a solution of 5% perchloric acid containing internal standard. Chromatographic separation will use a C18 5 µ column with detection at 244 nm. Analyte concentrations will be calculated by comparison to multipoint linear standard curves derived from plasma samples spiked to contain between 3.125 and 800µg/mL Iohexol.

Serum creatinine measurement

Serum creatinine will be measured using the Jaffe method on the ADVIA 1800.

Serum cystatin C

Will be measured on will be measured using Cystatin C (Dako, Glostrup, Denmark) by immunoturbidimetry. Blood will be collected for Cystatin C analyses and stored until additional funding has been obtained.

Estimated GFR will be calculated using:

1. MDRD formula (for creatinine measurements traceable to the IDMS reference method and reported in umol/L) both with and without the use of the ethnicity factor [3].

$$eGFR = 175 * [(Scr*0.0113)^{-1.154}] * (age)^{-0.203} * (0.742 \text{ if female}) * (1.212 \text{ if African})$$

2. The CKD-EPI formula [19]

$$GFR = 14.1 * \min(Scr*0.0113/k, l)^{\alpha} * \max(SCr*0.0113/l, l)^{-1.209} * 0.993^{Age} * 1.018(\text{if female}) * 1.159$$
(if black).

3.CKD -EPI combined

$$eGFR = 135 \times \min(SCr/k, 1)^{a} \times \max(SCr/k, 1)^{-0.601} \times \min(SCysC/0.8, 1)^{-0.375} \times \max(SCysC/0.8, 1)^{-0.711} \times 0.995^{Age} [\times 0.969 \text{ if female}] [\times 1.08 \text{ if black}],$$ where SCr is serum creatinine (in mg/dL), CysC is serum cystatin C (in mg/L), k is 0.7 for females and 0.9 for males, a is 0.248 for females and 0.207 for males. This will be done o n subjects with eGFR of $>60 \text{mL/min}/1.73\text{m}^2$
This analyses will be done after additional funding has been obtained.

<u>**Anthropometry**</u>
Weight (digital scale, Dismed, USA) to the nearest 100g, and height (stadiometer, Holtaine, UK) to the nearest millimetre will be measured with subjects wearing light clothing and no shoes. Body mass index (BMI) will be calculated as weight in kilograms divided by the square of the height in meters (kg/m^2).

<u>**Secondary Outcome:**</u>
Comparison of the accuracy and precision of a point of care device for the estimation of GFR
Point of care creatinine will be measured using either the ABL 700 or the Abbot iSTAT. These uses whole blood and the creatinine assays are IDMS aligned. eGFR will be determined by point of care creatinine measurement . These instruments have been validated using a CLSI-EP15-A protocol [26] by the National Health Service, UK.

Statistical analysis
The predictive performance of the various formulae to estimate GFR will be compared to the reference GFR in terms of bias, precision, and accuracy according to the methods of Bland-Altman[27]. Bias will be defined as the median of the individual differences between eGFR and mGFR(median percentage difference). Bias of <10% of iGFR is considered clinically acceptable[28]. Precision will be assessed as the interquartile range(IQR) of the differences eGFR-iGFR and expressed in mL/min/1.73m2. Accuracy will be assessed from the absolute difference (eGFR-iGFR) and expressed as percent of iGFR. The percentage of estimates within 10%, 15% and 30% of iGFR will be calculated. The proportions of overestimations > +30% and underestimations < -30% will also be evaluated. The same analyses will be carried out for eGFR as determined by POC in comparison to iGFR. In addition we will take a subset of 40 samples across a wide range of creatinine and calculate correlation of POC creatinine with laboratory creatinine, and comparison of mean differences using Bland-Altman method[29]

Research Team
A multidisciplinary team will ensure the goals of this project are achieved. June Fabian is a nephrologist and will be undertaking the project as part of a doctoral thesis; she will manage the project day-to-day and take overall responsibility. Saraladevi Naicker will supervise the project. Jaya George, a chemical pathologist with expertise in testing of renal function will oversee the analysis of samples. The department of chemical pathology has two liquid chromatography mass spectrometers which can be used for the iohexol analysis. The department also has three -70 freezers and a numbers of centrifuges. Jonathan Levin will contribute the statistical analysis for the determination of the accuracy and precision of iGFR with current eGFR equations and the point of care creatinine measurements. If indicated, he will conduct the statistical analysis for development of a prediction equation for eGFR. Stephen Tollman and Kathleen Kahn will facilitate access to the Agincourt site for conducting the study and will provide support for the project through the School of Public Health at Wits and their experience with epidemiological studies at the Agincourt HDSS.

Institutional environment
To base the study within the Faculty of Health Sciences, University of Witwatersrand, is perfectly suited to address the needs of the study. Access to the Agincourt HDSS, with the managerial component based within the School of Public Health will allow for access to an appropriate sample during their annual census in 2016 and the appropriate statistical support. Working collaboratively with the School of Chemical Pathology will allow for processing/analysis of the samples. Supervision from Nephrology with Saraladevi Naicker will enable a strong team effort to ensure completion of the study.

Significance
This study will contribute significantly to the evaluation of kidney function in black Africans. Without a validated, population-specific eGFR equation, no accurate diagnoses of CKD can be made. This impacts upon public health policy regarding the implementation of large scale screening and prevention programs for Southern Africa and is particularly relevant because of the rising prevalence of CKD in low to middle income countries. The necessity of early screening is accentuated by the resource-poor setting in which we find ourselves which precludes the majority of those with end stage kidney disease from any treatment.

The evaluation of the accuracy of a point of care device will also determine whether existing devices can safely be used in resource-poor setting where performance of laboratory-based testing for screening of CKD is unavailable.

Ethical committee approval and informed consent
The Wits Human Research Ethics Committee (Medical) has approved this study. (see attached pdf document). Since the approval, we have changed to study site to the Wits MRC Agincourt HDSS and we will also include the assessment of the point of care device. As such, we will submit an amendment to the Ethics committee for these changes.

Informed consent

INFORMATION SHEET AND CONSENT FORM
TITLE: ASSESSMENT OF KIDNEY FUNCTION IN AFRICANS

Good morning Patient

My name is Dr June Fabian and I am a nephrologist. I would like to welcome you and request your participation in our study.

The information here explains the study and what will be needed from you. It is important to assess kidney function properly. This can be done by measuring Creatinine or cystatin C (a substance in the blood). The concentrations are then used in a formula to assess kidney function. It has been shown in other countries that this formula is accurate. However we do not know if we can use this formula in African patients.

If you agree to take part in the study we will place a cannula (small plastic tube)in your arm use it to draw bloods until the end of the study. Five ml of blood (1 teaspoon) will be drawn at 5 minutes. This blood will be tested for Creatinine and Cystatin C. After that we will inject 5mls of iohexol slowly into your arm followed by 5 ml normal saline.

Iohexol is a pharmaceutical commonly used in the assessment of renal function. The dose injected is extremely small and is not dangerous. However if you are allergic to iodine you may not participate in this study.

We will then again take 5 ml of blood (2 teaspoon) from you at 2 hours and 4 hours afterwards. In this blood we will measure the iohexol level to see how long it takes for your kidneys to excrete the iohexol. The blood taken will not be used for any other tests.

Your participation in this study is voluntary and if you should choose not to participate this will in no way change your treatment at the hospital. Your identity will remain anonymous at all times and only your doctor will know the outcomes of the tests. You will be identified for the purposes of this study by a unique patient number.

I (Dr -----------------------) have described the tests we wish to carry out and have explained the reasons for it. I have also asked whether there were any questions about the tests and have answered them the best I can.

Date: _____   Doctor: _____

Consent form (please sign this form)

I agree to take part in the above clinical study. The procedures to be carried out have been explained to me. The possible discomforts, risks and benefits involved in taking part in the test have also been described to me. I understand that I can stop the test at any point. I also understand that if I have any questions concerning the test then the clinician will explain these to me.

Date: _____   Patient: _____

Gantt Chart

Time lines	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec
2016												
Human Research Ethics Committee	█	█										
Screening of participants in the Agincourt HDSS annual census (eGFR)			█	█	█							
Iohexol GFR testing in Agincourt HDSS						█	█	█	█	█	█	█
2017												
Iohexol GFR testing in Agincourt HDSS	█	█	█	█	█	█	█	█	█	█	█	█
2018												
Data preparation and statistical analysis	█	█	█	█								
Write up and publication of paper					█	█	█	█	█	█		