Evaluation of the Alere Afinion™ AS100 for measuring the levels of C-Reactive Protein in an aged population

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School of Clinical Medicine, College of Health Sciences
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DECLARATION

I, **Innocentia Mpofana** declare that:

The research reported in this dissertation, except where otherwise indicated, is my original work and the laboratory work was conducted in the HIV Prevention Research Unit, South African Medical Research Council.

This dissertation has not been submitted for any degree or examination at any other university.

This dissertation does not contain other persons' data, pictures, graphs or other information, unless specifically acknowledged as being sourced from other persons.

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Signed:  Date: 26 February 2020

PERMISSION TO SUBMIT

As the candidate's supervisors, we have read the thesis and have given our approval for submission for examination

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……

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ABBREVIATIONS AND ACRONYMS

ACS	Acute Coronary Syndrome
AMI	Acute Myocardial Infarction
BREC	Biomedical Research Ethics Committee
CAD	Chronic Artery Disorder
CHD	Coronary Heart Disease
CP	Cartridge Pack
CRP	C-Reactive Protein
CVDs	Cardiovascular Diseases
EDTA	Ethylenediamine Tetra-acetic Acid
GCLP	Good Clinical Laboratory Practice
HIV	Human Immunodeficiency Virus
HPRU	HIV Prevention Research Unit
hs-CRP	High Sensitivity C-Reactive Protein
IC	Informed Consent
KZN	KwaZulu-Natal
LIS	Laboratory Information System (LIS)
LDL	Low Density Lipoprotein

LLD	Lower Limit of Detection
MG/L	Milligrams Per Litre
MI	Myocardial Infarction
MIL	Minimum Interpretation Limit
NCD's	Non-Communicable Diseases
NO	Nitric Oxide
POCT	Point of Care Testing
SHIOP	A study to investigate Sexual health, HIV and co-morbidity with non-communicable diseases among Older Persons (SHIOP)
SAMRC	South African Medical Research Council
SST	Serum Separator Tube
STIs	Sexually Transmitted Infections
TG	Triglycerides
WHO	World Health Organization

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ABSTRACT

Introduction

The Alere Afinion™ AS100 analyser is a compact bench-top, multi-assay, point-of-care (POC) analyser that provides valuable near patient testing at the point of care. It utilises the latest technology to measure C-Reactive Protein and other analytes to monitor patients' disease progression. The main objective of the study was to evaluate the performance of the Alere Afinion™ AS100 analyser compared with a reference laboratory test method, ABX Pentra 400 for the measurement of C-Reactive Protein (CRP).

Methods

This study was a retrospective analysis in which stored serum samples obtained from a cross-sectional study referred to as Sexual Health, HIV infection and comorbidity with non-communicable diseases among Older Persons (SHIOP) were tested for the quantification of the CRP. The primary aim of SHIOP was to describe sexuality, sexual health and the comorbidity of HIV and sexually transmitted infections with chronic non-communicable diseases in adults aged ≥50 years in a setting of high HIV prevalence. Serum stored at -20 ºC from participants that consented to long term storage(n=183) was used to perform this evaluation. The serum samples were used to measure CRP on the Alere Afinion™ AS100 and ABX Pentra 400, respectively. Lin's correlation coefficient was used to assess the agreement between the two analysers for the measurement of CRP. Risk factors associated with elevated CRP levels were assessed through this study.

Results

A total of 183 serum samples were tested in the study. The mean age of the study participants was 7.62 years (SD 8.15). Male participants were slightly older than female participants (61 vs 58years, $p<0.05$). Approximately 14.2% of study participants were above 70 years of age. The study population consisted of 77/183 (42%) Black South Africans and 106/183 (57.9%) Indians. The Alere Afinion™ AS100 was able to correctly classify (165/183) >90% of the CRP results when compared to the ABX Pentra 400. Bland-Altman analysis and linear regression analysis showed an excellent agreement (correlation concordance 0.97) between the two analysers. This study showed that being obese (Odds Ratio [OR]: 1.98, 95% Confidence Interval [CI]: 1.3616, 2.889, $p<0.001$) and having low HDL levels (OR: 1.64, 95% CI: 1.158, 2.307, $p= 0.005$) were the only significant risk factors that were associated with elevated CRP levels.

Conclusion

This study showed that the Alere Afinion™ AS100 can be used for the measurement of CRP in instances where CRP is greater than 5mg/L and this may enhance the process of patient care and management in low resource settings.

Keywords: C-Reactive Protein (CRP), Affinion AS100, Sexual Health, HIV infection and comorbidity with non-communicable diseases among Older Persons (SHIOP), ABX Pentra 400.

CHAPTER ONE

1.0 INTRODUCTION

Non-Communicable Diseases (NCDs) are by far the leading cause of mortality globally (Gowshall et al., 2018). An even higher proportion (75%) of premature adult deaths (occurring in those aged 30–69 years) were caused by NCDs, demonstrating that NCDs affect a wide age range of the global population (Global Status Report on Non-communicable Diseases, 2018). The global probability of dying from one of the four main NCDs (CVDs e.g. heart attacks and stroke), cancer, chronic respiratory attacks and stroke), cancer, chronic respiratory diseases (such as chronic obstructed pulmonary disease and asthma] and diabetes) in 2016 was 18%, with a slightly higher risk of 22% for males as compared to 15% for females (World Health Organization, Global Status Report on Non-Communicable Diseases, 2018). According to the Global Burden of Diseases, Injuries, and Risk Factors Study GBD, 2016, deaths due to NCDs are projected to rise from 38 million to 52 million by 2030 (Global Status Report on Non-Communicable Diseases, 2014). These statistics demand that focus be directed not only to infectious diseases but to NCDs as well (Global Status Report on Non-Communicable Diseases, 2014).

In 2016, the cause of approximately 17.6 million (95% CI, 17.3–18.1 million) global mortalities were due to CVDs with a documented increase of 14.5% (95% CI, 12.1%–17.1%) over a 10-year period (2006-2016) (Benjamin et al., 2019). Findings from the INTERHEART Africa study indicated that the highest number of premature acute myocardial infarctions (AMI) occurred in sub-Saharan Africa (Steyn et al., 2005). This is due to lack of early detection and effective management of risk factors (South African Dyslipidaemia Guideline Consensus Statement, 2012). Hypertension, diabetes, and hyperlipidaemia were reported to be risk factors for CVDs (Cojocaru et al., 2017). Nearly 50% of deaths resulting from heart disease could be avoided by management of the risk factors (hypertension, hyperlipidaemia and increased body weight (Cojocaru et al., 2017).

In addition to HIV, hypertension is highly prevalent in South Africa (Lloyd-Sherlock, 2014). A past study on hypertension in older individuals reported that 78% of South African adults older than 50 years of age, suffered from hypertension (Lloyd-Sherlock et al., 2014). The study by Peltzer and Mafuya, 2013 on hypertension and associated factors in older adults in South Africa also revealed that older South Africans (50 years and above) are at high risk of CVDs due to high rates of hypertension. According to Peltzer and Mafuya, 2013, of the large proportion of older people who were aware of their condition only a few were treated. Therefore, there is an urgent need for public health education and the implementation of systems to monitor NCDs and their risk factors such as hypertension (Peltzer and Mafuya, 2013).

Evidence has shown that C-Reactive Protein (CRP) levels are able to predict the risk of a variety of cardiovascular outcomes such as AMI, sudden cardiac death and peripheral arterial disease. CRP levels have also been shown to predict the risk of intermittent ischaemia, acute coronary syndrome and percutaneous angioplasty among individuals with stable and unstable angina (Sarkar et al., 2019). Evidence indicates that plasma levels of CRP and other sensitive biomarkers of systemic inflammation may be interrelated with imminent probability of coronary heart

disease (CHD) in the general population (Slaats et al., 2016). Baseline concentrations of CRP and other biomarkers of low-grade inflammation are associated with chronic progressions possibly connected to CHD (Slaats et al., 2016). CRP plays an important role against bacterial infections and during inflammation (Sproston et al., 2018). A previous study from South Africa revealed that there is a close association between obesity and inflammation as well as a close link between cardiovascular function and inflammation (Schutte et al., 2006). Inflammation is thought to contribute to atherogenesis and disease development, and the inflammatory cascade is distinctly vital in the process of atherosclerosis (Welsh et al., 2017). Due to the fundamental role that inflammation plays in disease development and atherogenesis, anti-inflammatory treatments may assist in preventing cardiovascular events (Welsh et al., 2017). Menon et al., 2003 revealed that CRP levels were independently associated with serum albumin level and CVD prevalence.

Studies conducted by Biasucci et al., 2000 and Sakkinen et al., 2002 showed that CRP was a significant predictor of CVDs among participants with or without diabetes. In another study, Jager et al., 1999 investigated the association of CRP with CVDs and all-cause mortality among participants with and without diabetes. This study involved a population of (n=631) participants aged from 50 to 75 years. The study included a prospective follow up for 5 years. The study revealed that after 5 years of follow up, 58 participants had died, and 24 of those deaths were attributed to CVDs. However, Jager et al., 1999 found no association between CRP levels and cardiovascular death in participants with and without diabetes.

A previous study has shown that there is a close link between elevated serum CRP levels and increased vulnerability for disease and mortality in older patients (Velissaris et al., 2017). Due to its involvement in the whole process of CVD from the formation of fatty elements to the actual clinical occurrences, CRP has been found to be comparable to other confirmed cardiovascular risk factors namely, diabetes and hypertension (Bisoendial et al., 2010). CRP has recently been used as the independent tool to enhance certainty in the diagnosis process (Lemiengre et al., 2018). The high mortality rates associated with cardiovascular events is caused by challenges faced by most patients such as access to laboratory testing facilities, or the unavailability of these facilities to quickly identify biomarker risk factors (Sharma et al., 2015). Most developing countries often lack laboratories with state-of-the-art automated analysers that offer laboratory results that are highly reproducible, accurate and highly sensitive (Sharma et al., 2015). Most primary care health centres (PHCs) do not have laboratories. When medical doctors require blood results to make a clinical diagnosis, biological samples are usually sent to a central laboratory that is sometimes a great distance from the clinic (Stone et al., 2007). This leads to delays such as patient counselling and treatment. Clinics in rural areas with limited resources do not house basic diagnostic equipment and there is a shortage of trained staff.

Delivering healthcare in these areas is a major challenge due to unavailability of clean running water and dependable electrical services (Mcnerney and Daley, 2011). In order to overcome these limitations, it is crucial that fast and easy to use point of care tests (POCTs), that can significantly increase clinicians' proficiency to identify patient diseases quickly and accurately be implemented. POCTs have the ability to provide rapid results thus improving patient management (Stone et al., 2007, Mogensen et al., 2011).

Point of care (POC) testing is defined as testing of patient biological specimens in a setting outside a normal clinical laboratory. This is usually near bedside or at the site of patient care. POC testing is usually performed by clinical staff that have not undergone laboratory training, however it can also form part of patient self-monitoring (Fiallos et al., 2001).

The advantages of POCTs over standard laboratory testing is that there is no need for expensive laboratory equipment and highly trained laboratory staff to operate the instrument (Florkowski et al., 2017). Additional advantages include: POC testing also assists in reducing time-dependent variations for certain analytes which can deteriorate due to delays during sample transportation to the clinical laboratory and (smaller sample volumes are used for POC testing when compared to standard laboratory tests (Fiallos et al., 2001). This makes POC testing a method of choice for most clinicians (Kazmierczak, 2011). There is an increase in demand for patients seeking treatment at local clinician's offices and this has contributed to POCTs becoming one of the fastest growing areas of biomedical technology (Kazmierczak, 2011). Jain et al., 2016 confirmed that the Afinion™ AS100 analyser provided a platform for the analysis of multiple analytes with good comparability to the standard laboratory method. Kvam et al., 2009, also found that the Afinion™ AS100 was able to produce a good correlation when compared to another POCT DCA 2000 for Microalbumin/ Creatinine as well as when compared to the automated laboratory modular albumin and creatinine methods. Abbai et al., 2018 recently found a good correlation between the Alere Afinion™ AS100 analyser and the ABX Pentra 400 analyser for the measurement of glycosylated haemoglobin and lipid levels in older adults in Durban, South Africa. This study provided the first report on the diagnostic performance of the Alere Afinion™ AS100 in quantifying CVD biomarkers in a population of older persons aged 50 years and above, which included both HIV uninfected and HIV infected individuals in the KwaZulu-Natal province. This study supported the utilisation of the Alere Afinion™ AS100 as a POCT for quantification of HbA1C, triglycerides and high-density lipoprotein (HDL) in a South African setting.

Of all the above-mentioned studies, none have evaluated the diagnostic performance of the Afinion™ AS100 analyser when compared to standard laboratory tests for the determination of CRP levels in a population of older men and women. Hence the need for the present study. The purpose of the present study was to evaluate the Alere Afinion™ AS100 analyser (Alere, South Africa) against the ABX Pentra 400 analyser for the measurement of CRP levels. In addition, the study will provide data on the prevalence and risk factors of CVDs in older men and women from KwaZulu-Natal.

CHAPTER TWO

2.1 EPIDEMIOLOGY OF NON- COMMUNICABLE DISEASES (NCDs)

Globally, Non-Communicable Diseases (NCDs) are responsible for the largest portion of mortalities, contributing to 73.4% (95% uncertainty interval [UI] 72.5–74.1) of total deaths in 2017 (Roth et al., 2018). The total numbers of deaths due to NCDs increased by 22.7% (21.5–23.9) from 2007 to 2017, indicating a further 7.61 million (7.20–8.01) mortalities in 2017 (Roth et al., 2018).

NCDs are responsible for an estimated 17.0 million (57%) of the 29.8 million deaths in people below 70 years of age, which is the age generally used to identify premature death (Di Cesare et al., 2013). Premature death has become a major challenge when weighing the effect of NCDs on a given population, with nearly 44% of all NCD mortalities occurring prior to the age of 70 years (Ediriweera et al., 2018). A large proportion (82%) of all premature NCD mortalities occur in low- and middle-income countries, (Di Cesare et al., 2013, Nethan et al., 2017, Ediriweera et al. 2018).

In low- and middle-income countries, a higher proportion (48%) of all NCD mortalities are reported in people below the age of 70 years, when compared with high-income countries (26%). Additionally, in low- and middle-income countries, 29% of NCD mortalities occur among people under the age of 60 years, compared to 13% in high-income countries (World Health Organisation, 2011). Low-and lower-middle-income nations are expected to see remarkable rises in the burden of premature mortality and frailty from NCDs by 2040 (Bollyky et al., 2017).

According to the NCD Countdown 2030 collaborators 2018 report, approximately 25% of deaths in persons older than 10 years of age were due to NCDs. More than 50% of these deaths occurred in people older than 40 years of age (Figure 1). Additionally, 13.3 million (56%) deaths attributed to NCDs occurred in people aged 80 years and older (Bennett et al., 2018). According to findings presented in Figure 1, NCDs are responsible for a considerable portion of deaths in both men and women aged 45 years and above with cardiovascular diseases (CVDs) and diabetes as leading causes of deaths across both genders.

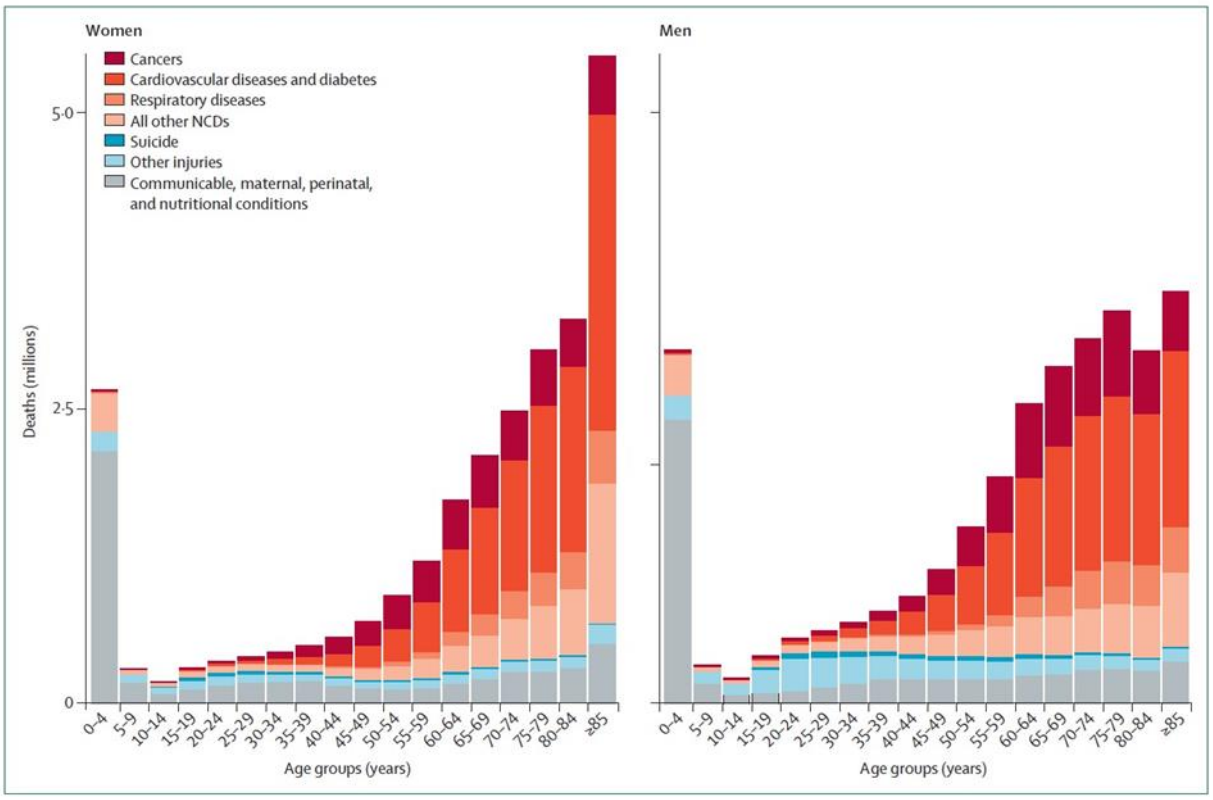


Figure 1: Global number of deaths in 2016 from Non communicable diseases (NCDs), injuries, communicable disease, maternal, perinatal and nutritional conditions (NCD Countdown 2030 collaborators, 2018).

2.2 NON-COMMUNICABLE DISEASES AND CARDIOVASCULAR DISEASES

CVDs remain a key public health challenge globally (Boateng et al., 2018). A large proportion (80%) of mortalities due to CVDs occur in low- and middle-income countries with similar rates in both males and females (Benjamin et al., 2017). In 2013, CVDs were the most common underlying cause of global deaths, accounting for approximately 17.3 million (95% uncertainty interval, 16.5–18.1 million) of the 54 million total mortalities, or 31.5% (95% uncertainty interval, 30.3%-32.9%) of all mortalities globally (Benjamin et al., 2017). According to the Global Status Report on Non-Communicable Diseases, 2014, CVDs accounted for 11.4 million (48%) of the 23.6 million deaths reported for older populations.

A large percentage of the global disease burden is directly from risk factors such as alcohol consumption, obesity, dietary factors and smoking or through other events such as increased blood glucose and cholesterol levels or high blood pressure (Ezzati et al., 2002, Lim et al., 2013). If the major risk factors for NCDs were eliminated, approximately three-quarters of the cases of heart disease, stroke and type 2 diabetes would be prevented; and 40% of cancers would be prevented (World Health Organisation Report, 2015).

The prevalence of CVDs (comprising of cases of Coronary Heart Disease (CHD), Heart Failure (HF), stroke, and hypertension) in adults aged 20 years and older increases with age in both males and females (Benjamin et al., 2017, Benjamin et al., 2019;). There is evidence that CVDs, in approximately 70% of adults between the ages of 60 and 79 years would present itself as either CHD, HF, stroke, or hypertension (Benjamin et al., 2017).

Despite the increase in the prevalence of CVDs the life expectancy in high income and some middle-income countries has increased due to reduced mortality rates in individuals aged 70 years and older (Sherlock et al., 2016; Kontis et al., 2017). Sub-Saharan African countries in the African region are going through numerous stages of advancement related to social, economic, environmental and structural adjustments. Furthermore, as life expectancies increase due to economic advancement, the prevalence of CVDs is rising, producing a new burden on already struggling health systems (Chikafu et al., 2019, Hamid et al., 2019). Men and women in low and middle-income countries have the highest risk of mortality as a result of NCDs (Figure 2). According to Di Cesare et al., 2013, CVDs account for the highest proportions of deaths in both men and women from Central and eastern Europe and central Asia, followed by the Middle East and north Africa. Stratified by gender, a larger proportion of deaths have been reported for men when compared to women for the low-and middle-income countries as well as high income countries (Figure 2) (Di Cesare et al., 2013).

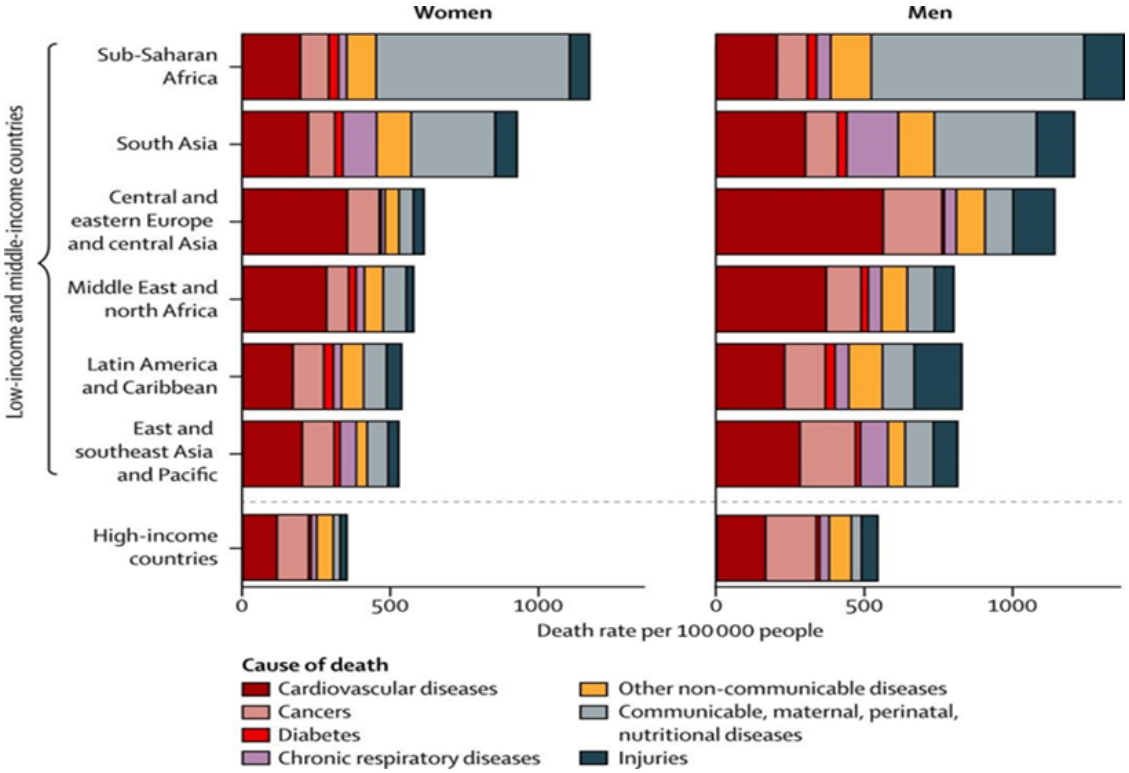


Figure 2 illustrates the worldwide age-standardised death rate by region showing that death rates for most NCDs are lower in high-income countries than in low-income and middle-income countries (Di Cesare et al, 2013).

Sub-Saharan Africa (SSA) is experiencing an epidemic of CVDs on an unconceivable degree (Cappuccio et al., 2016). Frailty and death caused by CVDs and the conventional risk factors, including hypertension, obesity, diabetes mellitus, and dyslipidemia, persist to increase in several SSA countries (Cappuccio et al., 2016, Amegah, 2018,). In 2013, a projection of 1 million mortalities were due to CVDs in SSA, comprising 5.5% CVD-related mortalities worldwide and 11.3% of all mortalities in Africa. Between 1990 and 2013, SSA continued to be the only

geographical region of the world where CVD-correlated mortalities increased (Amegah, 2018). The high mortality rates in Africa due to ischemic heart disease appear to be related to the high prevalence of hypertension, hyperglycemia, and hypercholesterolemia (Fonseca et al., 2016).

2.3 ROLE OF INFLAMMATION IN CORONARY HEART DISEASE (CHD)

Inflammation is essential to the induction and advancement of atherothrombosis leading to CVD events (Yousuf et al., 2013). According to Luan et al., 2018, the response to inflammation is pivotal in most stages of atherosclerosis. Figure 3 illustrates the role of CRP in the pathogenesis of CVDs. Among other proatherogenic effects, CRP upregulates adhesion molecules such as intercellular adhesion molecule-1 [ICAM-1)] Vascular cell adhesion molecule 1(VCAM-1), and E-selectin. The upregulation of adhesion molecules occurs through the up-regulation of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) which facilitates leukocyte transmigration by stimulating the release of Monocyte chemoattractant protein-1MCP-1 (Osman et al., 2006). CRP also upregulates angiotensin type-1 receptor (AT-1) in vascular smooth (ROS) construction (Verma et al., 2002). In addition, CRP inhibits bone marrow derived endothelial progenitor cell survival and differentiation, impairing maintenance of vascular integrity (Figure 3) (Luan et. al, 2018). This occurs at the very beginning with the recruitment of circulating leucocytes to the arterial wall until the unstable plaques become ruptured leading to the clinical manifestation of the actual disease. There is evidence that cardiovascular circumstances are connected to inflammation (Cardoso et al., 2017). Similarly, inflammation plays a vital role in the pathogenesis of atherosclerosis from the formation of the atheriosclerotic lesion caused by lipid deposition to the rupture of fat plaques (Cardoso et al., 2017). This eventually leads to the occurrence of acute cardiovascular events (Habib et al., 2013). Evidence suggests that the atherosclerotic process is characterized by a low-grade inflammation altering the endothelium of the coronary arteries and is associated with increased levels of markers of inflammation (Moore et al., 2011).

To improve global cardiovascular risk prediction, considerable interest has focused on the fact that CRP is not only an excellent biomarker of inflammation, but it is also a direct participant in atherogenesis (Velissaris et al., 2017). Many studies have demonstrated that increased CRP concentrations are associated with an increased risk of Myocardial Infarction (MI), stroke, peripheral arterial disease, and sudden cardiac death (Shrivastava et al., 2015). CHD is the leading cause of death and disability in developed nations and is increasing rapidly in the developing world (Moore et al., 2011). CVDs are the leading cause of mortality worldwide with CHD as the main cause of death in patients with heart conditions followed by stroke, rheumatic heart disease and MI (Sekhri et al., 2014). Almost half of all events associated with CHD are reported to occur in apparently healthy individuals who have few or none of the traditional risk factors, including dyslipidaemia. As a result, attention has increasingly turned to the role of other factors, such as inflammation, in the development of atherosclerosis and CHD (Shi et al., 2016).

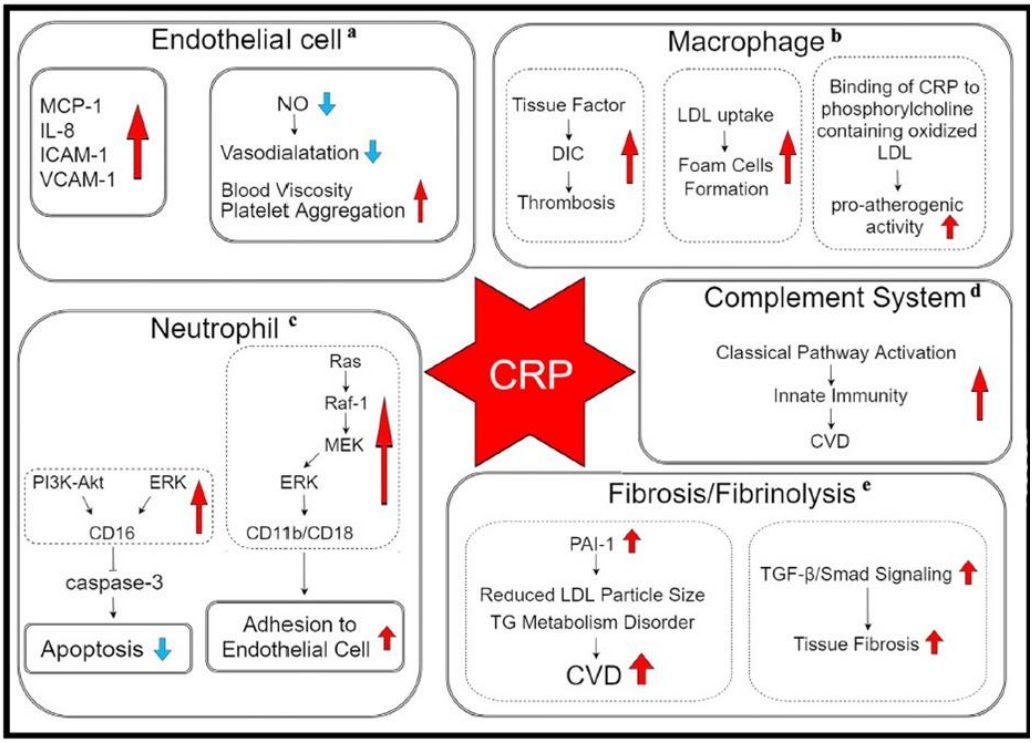


Figure 3: Potential mechanism underlying the role of CRP in the pathogenesis of CVDs (Luan et. al, 2018).

Abbreviations: DIC, disseminated intravascular coagulation; LDL, low-density lipoprotein; CVD, cardiovascular diseases; TG, triglyceride; MCP-1, monocyte chemoattractant protein 1; ICAM-1, intercellular adhesion molecule 1; VCAM-1, vascular cell adhesion molecule 1; NO, nitric oxide; PI3K, phosphatidylinositol 3-kinase; ERK, extracellular signal-regulated kinase; MAPK, mitogen-activated protein kinase; PAI-1, plasminogen activator inhibitor-1; TGF-β, transforming growth factor-β; CRP, C-reactive protein.

2.4 C-REACTIVE PROTEIN (CRP) – HISTORIC PERSPECTIVES

CRP was initially identified in 1930 by William Tillet and Thomas Francis at the Rockefeller Institute for Medical Research, in New York (Ridker, 2009, Shrivastava et al., 2015). Whilst studying the blood samples of patients suffering from acute *Streptococcus pneumonia* infection, it was discovered that serum samples from these patients produced a precipitin with an extract from the streptococcal bacterium. Initially, this extract was given the name, Fraction C, which was later confirmed to be a polysaccharide. As a result of its reactivity with the C polysaccharide of the *Streptococcus* cell wall, the 'substance' in the sera was named CRP (Shrivastava et al., 2015, Sproston et al., 2018). After 100 years, the research team involving Oswald Avery and Maclyn McCarty highlighted that CRP was found to be in high levels in serum of patients suffering from a spectrum of inflammatory stimuli, including myocarditis and the inflammation associated with rheumatic fever (Ridker, 2003).

CRP spreads at low quantities in healthy individuals, its concentrations expand significantly in reaction to infections, tissue injury and inflammation (Boncler et al., 2019). The role of CRP as a marker, specifically for CHD has been studied comprehensively (Biasucci et al., 2000; Sakkinen et al., 2002). CRP has been classified as a strong, independent risk factor for CHD by numerous major prospective clinical case-control studies (Biasucci et al., 2000;

Sakkinen et al., 2002). These studies concluded that CRP is an independent predictor of adverse cardiac events. The advance of high-sensitivity CRP (hs-CRP) assays has been beneficial to studying its role in forecasting initial cardiovascular events (Lubrano et al., 2015).

2.5 C-REACTIVE PROTEIN AS A BIO-MARKER IN CARDIOVASCULAR DISEASES

CRP is a member of the pentraxin superfamily that is extensively recognized as an indicator of inflammatory outcomes and cardiovascular probability in humans (McFadyen et al., 2018). Pentraxins are a superfamily of fluid phase pattern recognition molecules conserved in evolution and characterized by a cyclic multimeric structure. C reactive protein (CRP) and serum amyloid P component (SAP) constitute the short pentraxin arm of the superfamily (Bottazzi et al., 2016). Distinctive characteristics of CRP are its binding specificities and its site of synthesis which places it in a new super family of proteins (Bottazzi et al., 2016). Compared to other markers of inflammation, CRP levels are stable over long periods, have no diurnal changes, can be measured using high-sensitivity assays and have displayed good specificity with regards to predicting the risk of CHD (Burazor, 2004). CRP is an acute phase protein that is synthesized by hepatocytes in response to pro-inflammatory cytokines and interleukin-6(IL-6). CRP is also considered to be a predictor for the occurrence and progression of CVDs (Lee et al., 2014). CRP is considered to be both a marker and a mediator of atherosclerosis and CHD (Sproston et al., 2018). CRP levels have been associated with prognosis in patients with different conditions such as atherosclerotic disease, congestive heart failure, atrial fibrillation, myocarditis, aortic valve disease, and heart transplantation. This link proves that CRP is actively involved in the pathophysiology process of CVDs (Osman et al., 2006).

There is mounting evidence that inflammation is directly associated to atherosclerotic disease at all phases, from silent advancement to clinical expressions (Osman et al., 2006). There is epidemiologic data which suggests that intermediaries of inflammation, particularly hs-CRP, envisage cardiovascular threats autonomously of "traditional" risk factors (Osman et al., 2006). Figure 4 provides a clear description of the role that CRP plays as cardiovascular risk biomarker.

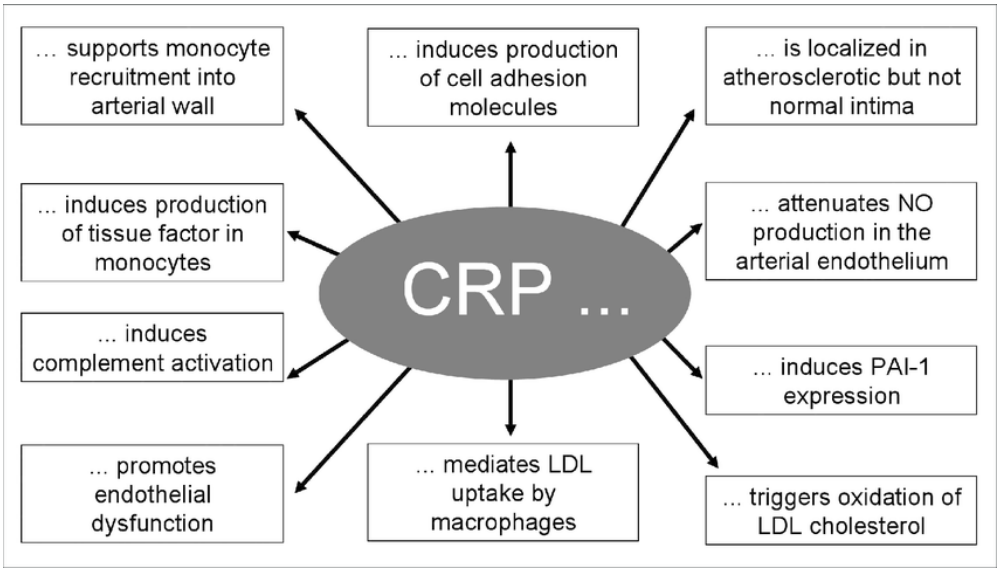


Figure 4: Pathophysiological role of C-Reactive Protein in atherosclerosis, NO, Nitric Oxide, PAI-1, Plasminogen activator Inhibitor -1, LDL -Low-density Lipoprotein: Adapted from Ridker et al., 2003.

CRP attaches itself to the phosphocholine of oxidized low-density lipoprotein (LDL), it also improves LDL uptake into macrophages and enhances the capacity of macrophages to create foam cells (Ridker et al., 2003). CRP impedes endothelial nitric oxide synthase expression in endothelial cells. Nitric oxide has its own important anti-atherogenic effects, including decreased platelet accumulation, vasoconstriction, and smooth muscle cell proliferation. It also improves plasminogen activator inhibitor-1 expression and action. CRP triggers macrophages to secrete tissue factor, a powerful procoagulant. CRP also upregulates the expression of adhesion molecules in endothelial cells that will bring together monocytes to the site of injury. High levels of CRP mRNA have been exhibited to appear in atherosclerotic plaques (Figure 4).

2.6 BIOLOGICAL FUNCTIONS OF C-REACTIVE PROTEIN

The role of CRP in host resistance has been considered to be mainly due to its capability to attach to phosphocholine (PC), stimulate the classical complement cascade, and improve phagocytosis (Simons et al., 2014). The ligand binding physical characteristics of CRP are also essential in realizing its role in inflammation (Simons et al., 2014). In addition to the recognition of microbial antigens, CRP reacts with cells at the sites of tissue injury (Luan et al., 2018). Similarly, to serum amyloid P component (SAP), CRP attaches to nuclear antigens, injured membranes and apoptotic cells, and participates in the clearance of damaged or apoptotic cells, as well as the material distributed from these ravaged cells (Boncler et al., 2019).

During infection, inflammation or injury, CRP inhibits endothelial nitric oxide production and contributes to plaque instability by increasing the expression of endothelial cell adhesion molecules; by promoting monocyte recruitment into the atheromatous plaque and by enzymatically binding to modified LDL. CRP also plays a fundamental role in thrombosis (Badimon et al., 2018). Evidence indicates that CRP contributes to CVDs by several processes, however,

more research in this area is still required (Badimon et al., 2018). The processes are represented in Figure 5 and include endothelial dysfunction, atherosclerotic plaque development and advancement. CRP down-regulates the transcription of endothelial nitric oxide synthase (eNOS) in endothelial cells (EC); which destabilizes eNOS mRNA, leading to a reduction in NO release (Osman et al., 2006). This inhibition of NO production enhances endothelial cell apoptosis and blocks angiogenesis (Verma et al., 2002).

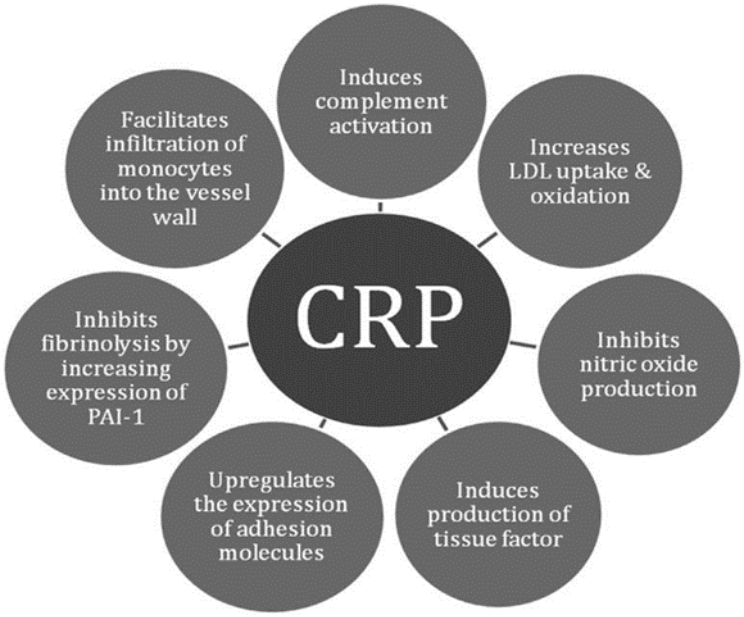


Figure 5: Representation of CRP-mediated effects on atherosclerosis and CHD (Shrivastava et al., 2015).

CRP may also stimulate the construction of endothelin-1(ET-1), a vigorous endogenous vasoconstrictor and intermediary of endothelial dysfunction, leukocyte and platelet stimulation and cellular proliferation (Miyauchi and Masaki, 1999). Finally, CRP stimulates the production of IL-6 in the vasculature (Yudkin et al., 2000). This finding is particularly important since IL- 6 is involved in a positive feedback loop to stimulate CRP production by the liver (Verma et al., 2002). CRP is different to other immunoglobulin molecules; however, it also has some similar functional properties with other immunoglobulins. These similarities include the capability to encourage agglutination, activation of the classical complement pathway, bacterial capsular swelling, phagocytosis and precipitation of poly cationic and poly anionic compounds. By analogy with antibodies, it is therefore possible that CRP might contribute both to host defence against infection and enhancement of inflammatory tissue damage (Luan et al., 2018).

2.7 ATHEROSCLEROSIS, CHRONIC INFLAMMATION, AND C-REACTIVE PROTEIN

Atherosclerosis leads to ischemic stroke; however, modification of lifestyle and control of cardiovascular risk factors can prevent it (Tsivgoulis et al., 2018). The mechanism of inflammation plays a crucial role in all levels of atherosclerosis, from the recruitment of circulating leukocytes to the arterial wall, to the rupture of unstable plaques, which leads to clinical manifestations of the disease (Burazor., 2004).

Chronic inflammation is a well-established background process in many age-related diseases (Rea et al., 2018). Recent studies by Su et al., 2013 and Salazar et al., 2014 investigated the use of various inflammatory biomarkers such as CRP, IL-6, and IL-1 as predictors of physical and cognitive performance amongst the aged. These prospective cohort studies have shown that elevated CRP levels are associated with increased CHD risk in both genders, across a wide age range, and in primary as well as secondary prevention settings (Velissaris et al., 2017). These findings have been consistent in different populations with diverse ethnic backgrounds and in diverse clinical settings, and they have predicted risk for a variety of cardiovascular outcomes, including incident stroke, sudden cardiac death, peripheral artery disease and incident diabetes as well as new onset hypertension (Burazor, 2004). CRP levels have also been shown to predict risk of both recurrent ischemia and death among those with stable and unstable angina, those undergoing percutaneous angioplasty, and those presenting to emergency rooms with acute coronary syndrome (ACS) (Martín-Timón et al., 2014).

Research has revealed that CRP is manufactured by human artery smooth muscle cells of atherosclerotic lesions in reaction to inflammatory cytokines thereby advocating the advancement of cardiovascular difficulties (Pfützner et al., 2010). CRP may be associated at all levels by directly impacting processes such as complement activation, apoptosis, vascular cell activation, monocyte recruitment, lipid accumulation and thrombosis (Luan et al., 2018). This occurs through the complement pathway which consists of 35 plasma or membrane proteins which play a vital role in immunity and acts as a defence mechanism against either, inflammation, infection or injury (Li et al., 2007). The complement pathway has different components that become activated in three different pathways to trigger a cascade of proteins, which are used to bind microbial surfaces, leading to the activation of phagocytosis or cell lysis (Speidl et al., 2011) (Figure 6)

Complement (a major human immune system defence mechanism) together with CRP form part of substances found in the atherosclerotic lesion, explicitly in the vascular intima, where it co-localizes with monocytes, monocyte-derived macrophages and lipoproteins (Moore et al., 2011; Sproston et al., 2018) (Figure 6)

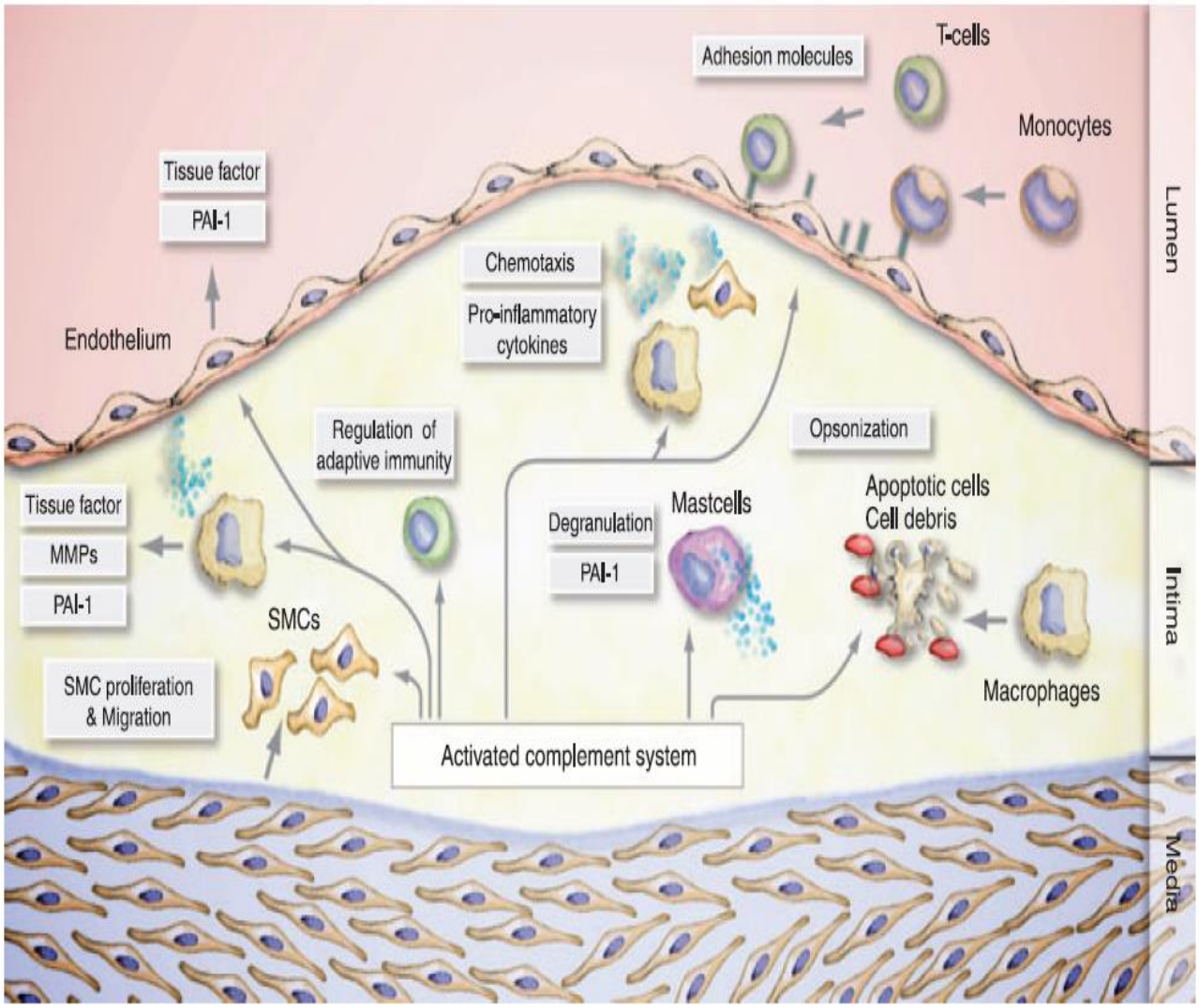


Figure 6: Effects of complement activation in atherosclerosis (Speidl et al., 2011)

The Diagram (Figure 6) illustrates the of effects of complement activation in atherosclerosis in which sub-lytic concentrations of the terminal complement complex induce endothelial and smooth muscle cell proliferation and the release of growth factors. It increases the expression of adhesion molecules on endothelial cells and the release of chemokines and tissue factor from endothelial and smooth muscle cells. The anaphylatoxins C3a and C5a activate and trigger oxidative burst in macrophages and neutrophils and are also regarded as chemoatractants for eosinophils, mast cells, monocytes, and B- as well T-lymphocytes. C5a induces tissue factor, plasminogen activator inhibitor-1 and matrix metalloproteinases in macrophages but acts also on mast cells, endothelial cells and smooth muscle cells. Opsonins promote the clearance of atherosclerotic plaques from apoptotic cells, debris and immune complexes. C1q, C3b and C4b bind to apoptotic cells and induce their phagocytosis by macrophages via complement receptors. PAI-1 (plasminogen activator inhibitor-1); MMPs (matrix metalloproteinases); SMCs (smooth muscle cells) (Speidl et al., 2011).

2.8 CLINICAL IMPORTANCE OF CRP

CRP is useful as a clinical marker for the following reasons: commercially robust assays are available for measuring its levels; the protein is stable in serum or plasma with very marginal fluctuations; and assays for detection are simple and cost effective (Shrivastava et al., 2015). In addition, assay outputs are similar when testing either fresh, stored, or frozen serum or plasma samples. CRP circulating levels has been found to be within similar ranges to other inflammatory markers (Brindle et al., 2010, Doumatey et al., 2014,). Additionally, the intrinsic biological properties of CRP as an acute-phase reactant are especially favorable for its use as a sensitive quantitative systemic measure of the acute-phase response (Shrivastava et al., 2015). CRP has unique properties and can provide closer predictions when compared to other markers (Salazar et al., 2014). Studies suggest that elevated hs-CRP levels can predict poor cardiovascular prognosis over and above to assessing future risk in asymptomatic individuals (Fonseca et al., 2016).

Furthermore, CRP levels are useful in motivating patients to modify their lifestyles more aggressively (Mora et al., 2009). The CRP concentration is therefore a very useful nonspecific biochemical marker of inflammation. (Shrivastava et al., 2015). Determination of CRP levels is important for the following reasons: (a) screening for organic disease (Organic disease is the term used to describe any health condition in which there is an observable and measurable disease process, such as inflammation or tissue damage), (b) monitoring responses to treatment (serial measurements reflects activity and response to treatment and can be used for monitoring), and (c) detection of recurrent infection in immuno-compromised individuals (Shrivastava et al., 2015).

2.9 RATIONALE FOR CURRENT STUDY

Cardiovascular diseases (CVDs) remain a key public health challenge globally (Boateng et al., 2018). POC testing has the ability to provide rapid results thus improving patient management (Stone et al., 2007). Recent studies have shown a number of settings where POC technology is equivalent to gold standard centralized laboratory methods in terms of diagnosis and patient care (Urdea et al., 2006).

Abbai et al., 2018, supported the use of the Alere Afinion™ AS100 Analyser as a POC test to quantify HbA1C, triglycerides and HDL-C in a South African setting. Presently there is lack of data on POC technology for the measurement of CRP levels, especially in older persons. This study will be the first to evaluate the diagnostic performance of the Alere Afinion™ AS100 analyser (Alere, South Africa) when compared to the ABX Pentra 400 analyser (reference test) for measuring the levels of CRP in an older South African population. If the POC test is found to be comparable to the reference test, this will have a positive impact in terms of screening and linkage to care in resource limited settings. In addition, the data generated from this study will provide insights regarding the prevalence of CVDs in the study population as well as to identify risk factors associated with elevated CRP levels which will facilitate better patient management.

2.10 STUDY AIM AND OBJECTIVES

2.10.1 AIM

To evaluate the diagnostic performance of the Alere Afinion™ AS100 Analyser when compared to the ABX Pentra 400 for the determination of C-reactive protein levels in a population of older men and women from KwaZulu-Natal Province, South Africa.

2.10.2 OBJECTIVES OF THE ACCOMPLISHED STUDY

- To compare the sensitivity and specificity of the Alere Afinion™ AS100 analyser to the reference test, ABX Pentra 400 for the measurement of C-reactive protein levels
- To determine the prevalence of risk factors associated with cardiovascular diseases in the studied population
- To identify risk factors associated with elevated CRP levels in the studied population

CHAPTER THREE: METHODS

3.1 STUDY DESIGN AND POPULATION

This study was a retrospective analysis utilizing stored serum samples obtained from a cross-sectional study, Sexual Health, HIV infection and comorbidity with non-communicable diseases among Older Persons (SHIOP). The SHIOP study was a cross-sectional study conducted from February to April 2016. Participants in the SHIOP study were aged 50 years and older, not cognitively impaired, based on observation by the interviewer and willing to provide written informed consent. All participants provided information on their sexual behaviour, health status and were screened for CVD biomarkers. Participants were recruited using a convenient sampling approach from two areas within the eThekwini district, i.e. Chatsworth (urban area) and Botha's Hill (semi-rural). The primary aim of SHIOP was to describe sexuality, sexual health, HIV and comorbidity with chronic non-communicable diseases in adults aged ≥50 years in a setting of high HIV prevalence. The study enrolled n=435 participants across the two study areas. All enrolled participants provided written consent after being provided with information about the study and had to demonstrate adequate understanding of the information before signing the consent form. In cases where the participant was illiterate, they could invite a neutral person who was not a member of the study team or employee of the research organisation to witness the informed consent process. Where a participant was not able to write and sign the consent form, they were provided with ink pads for them to mark the consent form with their thumb print. Further details on the SHIOP can be found elsewhere [Abbai et al., 2018 & Nyirenda et al., 2018].

3.2 STUDY PROCEDURES

All enrolled participants were tested for Human Immunodeficiency Virus (HIV) and Sexually Transmitted Infections (STIs) in the SHIOP study. Additional medical history information was collected via self-reports as well as objectively measured on chronic morbidities (diabetes, hypertension, renal, and cholesterol). In this study, using glycated haemoglobin (HbA1c) levels, participants were categorised as diabetic (HbA1c>6.5%), pre-diabetic (HbA1c 5.7%-6.4%) or normal (HbA1c<5.7%). Spot-collected albumin readings were used to determine renal or chronic kidney disease (CKD). As per Kidney Disease Improving Global Outcomes (KDIGO) guidelines participants were categorised as at severely high risk of renal disease (albuminuria >300mg/g), at moderate risk (albuminuria 30-300mg/g) and normal or mild risk (albuminuria <30mg/g) (Tongma et al., 2013). For hypertension, three readings of blood pressure (BP) were taken using a fully automatic Healthease digital arm BP instrument. Participants were in a sitting position with a one-minute rest between each reading. A mean of the three readings was calculated with participants categorised as hypertensive if their systolic blood pressure was ≥140mm Hg or diastolic blood pressure ≥90 mm Hg, based on the World Health Organization's (WHO) recommended hypertension cut points.

Participants who tested positive for STIs (*Neisseria gonorrhoeae*, *Chlamydia trachomatis* and syphilis), HIV and/or had high HbA1c, albumin, cholesterol or hypertension readings as per cut-points above were referred to local clinics for care. In addition, all participants underwent physical examinations whereby measurements for blood pressure, weight and height were taken. The data obtained from the physical assessments were used to determine the

proportion of participants with diabetes, chronic kidney disease and hypertension, and the risk factors associated with high CRP levels in the studied population.

3.3 SAMPLE COLLECTION AND PROCESSING

Enrolled participants provided venous blood samples for laboratory testing. Blood collection was performed according to Good Clinical Laboratory Practices (GCLP) by a trained nurse where whole blood was drawn aseptically into a serum separator gel (SST) tube and an ethylenediamine tetra-acetic acid (EDTA) tube.

The onsite laboratory at the clinical research site housed all the equipment required to process the samples such as separation of the serum and plasma fractions. The onsite Laboratory was also responsible to process all samples as per laboratory procedures. SST specimens were centrifuged at 3000 rpm for 10 minutes, serum was then separated into an aliquot tube for testing in the central laboratory. Certain tests were performed at the onsite laboratory by qualified, trained Medical Technologists. The remainder of the aliquoted serum samples were shipped at 2-8ºC to the central laboratory for testing and long-term storage. Only the samples of participants who consented to long term storage were stored.

The serum fraction was used for determining the CRP levels. In order to preserve sample integrity, the serum and plasma samples were stored at -80 ºC and -20 ºC for further testing. The samples were stored for approximately 1.5 years (February 2016- August 2017) before being retrieved for testing in this study.

3.4 ETHICAL APPROVAL

This retrospective study was approved by the Biomedical Research Ethics Committee of the University of KwaZulu-Natal (BE074/17), whereas the parent SHIOP study was approved by the South African Medical Council Ethics Committee (ECO30-9-2015).

3.5 SAMPLE RETRIEVAL AND TESTING

Only stored serum samples from participants who consented to storage and future testing during the SHIOP study were utilized in this study. Of the 435 participants enrolled in the parent study, only n=183 participants consented to storage and future testing, hence only 183 samples were available for testing for this study. Samples were retrieved from the Biorepository freezers, allowed to thaw and tested in batches of n=50, to allow for complete testing on both the Alere Afinion™ AS100 and ABX Pentra 400 analyzers.

3.6 LABORATORY TESTING - DETERMINATION OF CRP LEVELS

Samples were tested on both the ABX Pentra 400 and the Alere Afinion™ AS100 for CRP levels. The CRP levels were measured by commercially available turbidimetric and immunochemical methods, respectively.

3.7 ALERE AFINION™ AS100 PRINCIPLE

The principle of this assay is based on a solid phase immunochemical test assay in which the sample is automatically diluted with a liquid (which is the component of the CRP reagent within the cartridge) that also lyses the red cells. The Alere Afinion™ CRP Test Cartridge contains all the reagents necessary for measuring the CRP concentration in serum or plasma. Figure 7 is an illustration of the testing procedure in which the sample mixture was passed through a membrane coated with anti-CRP antibodies; the CRP in the sample was concentrated onto this membrane. A solution containing anti-CRP antibodies conjugated with ultra-small gold particles was passed through the membrane. The gold-antibody conjugate binded to the immobilized CRP on the membrane, which turned reddish brown. Excess gold-antibody conjugate was removed by a washing solution. The Alere Afinion™ AS100 Analyser measured the colour intensity of the membrane, and this was proportional to the amount of CRP in the sample. The CRP concentration was displayed on the Analyser screen (Figure 8) (Afinion™ CRP manual [pamphlet]. Oslo, Norway: Axis Shield PoC AS; 2010). The measurement range used was 5 to 160 mg/L for serum and plasma samples.

Figure 7: The Alere Afinion™ AS100 Analyser instrument

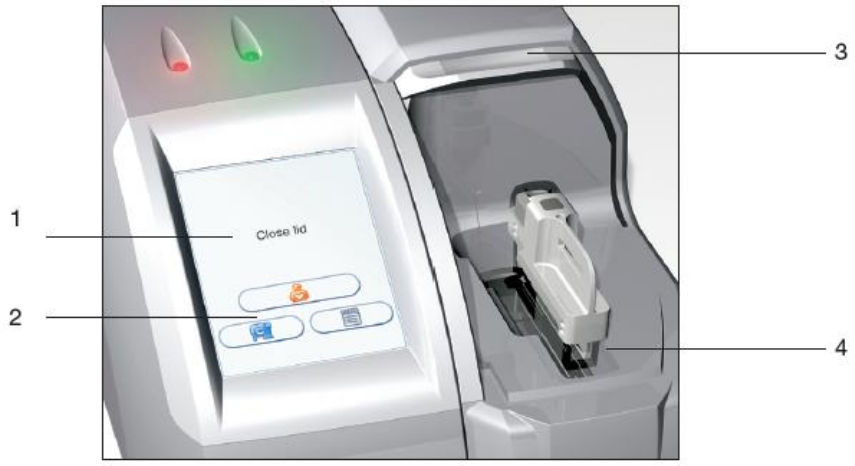


Figure 8: Analyser touch screen and Cartridge chamber.1-Text message, 2- Touch buttons, 3- The lid in open position, 4- The cartridge chamber with a test cartridge

The main components of the Test Cartridge were the sampling device and the reagent container. The Test Cartridge had a handle, a barcode label with lot-specific information and an area for sample ID (Figure 9). Participant ID was recorded on the space provided on the cartridge. Figure 10 provides a quick guide of the test procedure, from sample testing to results generation and cartridge discard after completion of the test procedure (Afinion™ CRP manual [pamphlet]. Oslo, Norway: Axis Shield PoC AS; 2010).

Figure 9: CRP Test Cartridge

Figure 10: Illustration of the test procedure from the quick guide

3.8 ALERE AFINION™ AS100 QUALITY CONTROL

Quality control testing was performed to confirm that the Alere Afinion™ AS100 Analyser system was working properly and providing reliable results. All procedures were performed as per manufacturer's instructions. No problems were experienced with the Controls. The measured values of the CRP were always within acceptable limits (5-160 mg/L) as stated in the CRP control Package inserts. Controls were stored in the fridge (2- 8°C) as per manufacturer's instructions. The control material was used without waiting for it to reach equilibration to room temperature, as indicated on the reagent package insert. The control was mixed well by inverting the vial 8-10 times before the sample was added. Only Afinion™ CRP Controls from Axis-Shield were used as recommended by the manufacturer. The Afinion™ CRP Control kit contained assayed human serum controls at two concentration levels of CRP. The CRP Control kit contained: Liquid human serum with purified CRP at two concentration levels; Afinion™ CRP Control C I and Afinion™ CRP Control C II. These were tested prior to sample testing.

The quality control printout from the Afinion™ AS100 Analyser could not be included in this thesis as the thermal paper on which the results were recorded had faded away. However, the laboratory's internal quality control log has been added as an appendix to show how internal quality control was recorded (Appendix 12).

3.9 ALERE AFINION™ AS100 ANALYSER SAMPLE TESTING

All testing was conducted in accordance with the manufacturer's instructions. The analyser was placed away from the sunlight. As part of routine analyser maintenance, the analyser would first start by performing its self-test upon being switched on. Samples were tested after the analyser had completed the self-test by indicating with a green light that it is was ready for use. Unlike the control material, upon removal from storage, the CRP
Test Cartridge could be allowed to reach a temperature of 15-30°C before use, as indicated by manufacturer's instructions. The cartridge was then labeled with the participant study number. The CRP levels were measured using 1.5 µL of serum. The assay time was ~4 minutes and controls with specific target ranges were included in each assay run. Based on available data, diagnostic cut-off for high sensitivity assays for CRP levels is 1.1mg/L-3mg/L for low to moderate risk and 3mg/L and above correspond to high-risk groups for future cardiovascular events (Ridker, 2003, Pearson et al., 2003). However, due to the Afinion™ AS100 Analysers detection limit of 5mg/L, 5mg/L was used as the diagnostic cut-off in this study.

3.10 THE REFERENCE METHOD – ABX PENTRA 400 CRP TEST

In order to evaluate the performance of the Alere Afinion™ AS100, the ABX Pentra 400 analyser was used as the reference test in this study. All assays were performed according to the manufacturer's instructions. The diagnostic cut-off for CRP used was ≥5 mg/L. This range was used to compare and categorise participants as normal v. abnormal. The ABX Pentra 400 is a compact Clinical Chemistry benchtop analyzer (Figure 11, A). Its great autonomy with continuous loading provides enhanced productivity in a user-friendly environment. The ABX Pentra 400 uses high-quality analytical technology with a single use reaction cuvette to perform the most effective tests with high stability and quality results. It has a high-performance mixer design for optimal mixing in minimum time without risk of cross contamination. It has the optimum test sequence setting program with an incompatibility function.

A **B**

Figure 11: ABX Pentra 400 (A) with 400 Reagent Compartment (B)

3.11 THE PRINCIPLE OF THE ABX PENTRA 400 CRP TEST

The gold standard method is a latex-enhanced immuno-turbidimetric assay developed to accurately measure CRP levels in serum and plasma samples. This assay involves the immune-turbidimetry with 3 stages. During the first stage, blood cells are lysed by Reagent 1. During the second stage of the assay, Reagent 2 which inhibits interference is added and the last stage involves the addition of the Reagent 3 which contains anti-CRP antibodies bound to latex beads. When an antigen-antibody reaction occurs between CRP in a sample and anti CRP antibody which has been sensitized to latex particles, agglutination results. This agglutination is detected as an absorbance change, with the magnitude of the change being proportional to the quantity of CRP in the sample.

The actual concentration is then determined by interpolation from a calibration curve prepared from calibrators of known concentration. Absorbance is then measured at 850nm, and the absorbance is proportional to the CRP concentration of the sample. All this is automatically performed as the sample is analysed by the ABX Pentra 400 analyser (ABX Pentra 400 Package insert). Approximately 4.0 μL of serum is required for this assay. The minimum interpretation limit (MIL) is evaluated using multiple determination of low concentration specimen and equals 3.00 mg/L with a Lower limit of detection (LLOD) of 1.00 mg/L. CRP reagent, with associated calibrators and controls, were used. The ABX Pentra Immuno I Control L/H was used as a quality control to monitor accuracy and precision for CRP. Measurements of CRP ranged from 0.1 mg/L to 43.8 mg/L, with a median of 0.5 mg/L.

3.12 ABX PENTRA 400 REAGENTS

The ABX Pentra CRP (Cartridge Pack: CP) is ready-to-use. In order to maintain the integrity and stability of the reagents, during the testing procedure, reagents cassettes were left in the ABX Pentra 400 refrigerated tray after analysis. Care was taken to ensure reagent caps were not interchanged with other reagent cassettes. Since only183 samples were tested, only one lot number of CRP reagent was used, so there was no risk of interchanging or mixing of different lot numbers. ABX Pentra CRP CP was used according to the package insert provided with the reagent by the supplier. The reagents used in the measurement of the CRP CP included: Reagent 1 Buffer solution which is the Glycine buffer solution and Reagent 2: Latex suspension which is 0.20% w/v suspension of latex particles sensitized with anti-CRP antibodies (rabbit). Both caps of the cassette were placed in the refrigerated ABX Pentra 400 Reagent compartment as per manufacturer's instructions. Reagents were inspected for foam and bubbles, if present this was removed by using a plastic Pasteur pipette.

3.13 ABX PENTRA 400 CALIBRATION

Calibration was not performed as the manufacturer advises that this be done only after a lot number change. Since only one lot number was used, there was no need to perform a calibration as the internal quality Control was checked and verified to be acceptable prior to testing the samples.

3.14 ABX PENTRA 400 INTERNAL QUALITY CONTROL

It is fundamental to ensure Internal Quality Control is optimised in order to ensure that reliable and valid results are produced for proper patient diagnosis, management and care. In order to ensure valid analytical runs on the reference method, ABX Pentra Immuno I Control L/H, Ref. A11A01621 ABX Pentra Low CRP Control, Ref. A11A01731 (low and high controls) were used. Both low and high controls were tested daily prior to testing the samples. Internal Quality Control results were reviewed and checked against expected values.

Only when quality control was within the acceptable range, then samples were tested. Internal Quality Control was performed as per the manufacturer's instructions, ABX Pentra Immuno I Control L/H was tested daily prior to sample processing in order to check the status of the analyser, to verify that it is in good working condition and that it will produce reliable and good quality results. The results were within the range of the defined confidence limits. Calibration was not performed as the testing of the sample occurred within a month. The manufacturer claims that the CRP reference range for adults (20-60 years) is < 5mg/L.

3.15 THE FREQUENCY OF THE PENTRA 400 INTERNAL QUALITY CONTROL

Each control was assayed daily as per manufacturer's instructions. The frequency of controls and the confidence intervals were corresponding to laboratory guidelines and country-specific directives. Samples were tested after the Internal Control results were reviewed and within the range of the defined confidence limits. The package inserts of the ABX Pentra 400 CRP as well as the ABX Pentra 400 instructional manual were followed and used as the Standard Operating Procedures.

3.16 ABX PENTRA 400 TESTING PROCEDURE

All maintenance procedures were completed, internal quality controls were run as per manufacturer's instructions. The CRP Reagents came as a kit that it is ready to use. No preparation/ reconstitution of the reagents was required. During the installation of reagent cartridge, the CRP reagent door which is located on the right-hand side of the ABX Pentra 400 analyser was opened. The CRP Reagent kit consisted of R1, R2 and R3, these were all removed from the refrigerator and added to the analyser. The door was closed and verified that it was properly closed into its locking device as per manufacturer's instruction. The new CRP sensitivity factors were entered on the analyser by following the instruction manual. Single use cuvettes as depicted on Figure 12 were added on the analyser. These cuvettes ensured that there was no risk of cross-contamination.

Figure 12: ABX Pentra 400 sample reaction cuvettes

3.17 COMPARISON OF THE AFINION AS100 AND THE ABX PENTRA 400

3.17.1 WORKING BENCH SPACE

The Pentra 400 system requires approximately 150cm width of bench, which includes enough space for the analyser, printer, keyboard and to access the cuvette carousel for troubleshooting. It also requires a 91cm depth of bench (analyser 80cm deep plus 20cm clearance, 110 cm clearance above bench for the analyser with the lid raised, 70cm (width) x 60cm (depth) x 60cm (height) under the bench to house the cooling unit, the waste and water tanks, and 22cm x 56cm x 22xm (height)for the optional UPS. The Afinion™ Analyser has dimensions of 17cm x 19cm x 34cm therefore requiring much less bench space (Piggott et al., 2005).

3.17.2 DESCRIPTION AND EASE OF USE

The Pentra 400 system is compact although space is required under the bench for a cooling unit, water and waste bottles, and access to the back is required when changing the filter during maintenance. The analyser overhangs a 60cm wide bench. The system software is generally easy to use but some of the icons are confusing at first and require extensive training for the users. Reagents are ready to use and easy to load onto the system, however, bubbles can be difficult to remove. The reagent carousel has a refrigerated section and an ambient temperature section and errors will occur if reagents are loaded into the wrong section; there is no facility to prevent this. User's require extensive training in order to prevent errors (Piggott et al., 2005). The Afinion is simple and easy to use. The touch screen of the Analyzer provides a simple graphical user interface (GUI). Coloured icons are used for language-independent touch buttons while the local language can be chosen for text messages.

The Afinion™ CRP analysis can be done on either the capillary or venous blood sample which takes just approximately 3minutes. A simple sampling procedure, easy touch screen user interface, no manual calibration, no chemistry handling or manual calculation of results makes the Afinion™ Analyser system suitable for both laboratory and non-laboratory users (Afinion™ CRP manual [pamphlet, 2010).

To run an Afinion™ CRP test, there are no additional instruments required apart from the standard capillary or venous puncture equipment. Printer and barcode-scanner (for entering sample ID) can be connected, this is optional. The Test Cartridge is placed in the cartridge chamber and the analysis starts by closing the lid manually. Once loaded with a cartridge, the Afinion™ Analyzer represents a true walk-away system. After analysis the result is stored and displayed on the screen. When the result is accepted or transferred to a connected printer or data system, the lid opens automatically, and the used cartridge can be removed and discarded (Afinion™ CRP manual [pamphlet, 2010).

3.17.3 MAINTENANCE

The Pentra 400 system requires daily, weekly and monthly maintenance for the system to perform optimally. Scheduled maintenance procedures include: a start-up (beginning of the day) and shutdown (end of the day). The procedures are performed on daily basis, requiring approximately 10-15minutes for each process. These steps can only be performed by trained laboratory staff. Whereas the Afinion™ Analyser requires cleaning and disinfecting the exterior of the analyser prior to use and cleaning and disinfecting of the cartridge chamber on a monthly basis (Piggott et al., 2005).

3.17.4 CALIBRATION

The Pentra 400 requires that calibration be performed for each new reagent cassette regardless of lot number change. The packaging of the CRP Reagents which contained the CRP reagent sensitivity factor needs to be retained for the sensitivity factors to be entered on the calibration menu so as to ensure that the result produced by the analyser was reliable (Piggott et al., 2005). While, the Afinion™ Analysers are calibrated against a reference system to ensure that all Analysers operate within identical tolerance limits during manufacturing. Test specific calibration data are established for each lot of test cartridges and then stored in the barcode label. When the cartridge is placed in the Afinion™Analyser, the integrated camera reads the barcode. The calibration data for the actual lot is transferred to the analyzer and used for calculating the results. Calibration by the operator is thus not required (Afinion™ CRP manual [pamphlet, 2010)

TABLE 1: Comparison between Afinion and ABX Pentra 400 systems, (NICE, 2016, Piggott et al., 2005)

	Afinion	**Pentra 400**
Size:	13.4cm x 6.7cm x 7.4 cm	40" x 28" x 25" (W x L x H) Width=101cm, height=63cm, Depth=71cm External Cooling Unit: Width =29cm, height=40cm, depth=42cm
Weight	4Kg	120kg + 35kg (Cooling system)
Users	Suitable for both laboratory and non-laboratory users	Needs trained laboratory personnel
Sample volume/test	1.5 µL of serum	4.0 µL of serum
Cost per test	£3.50 (R67.34) (excluding VAT)	Per test= £1.31=R25.19 (Excluding VAT)
Reagents	All-in-one test cartridge	ABX Pentra CRP CP is ready-to-use.
Measuring range	8 - 200 mg/L (whole blood), 5 - 160 mg/L (serum and plasma)	0.10mg/L-160mg/L (serum and plasma)
Reagent storage and stability	The CRP test cartridge contains all reagents necessary for the measurement of C-reactive protein with 4 weeks room temperature stability Refrigerated (2-8°C) until the expiry date or at room temperature (15-25°C) stable for maximum 4 weeks	Reagents in unopened cassettes are stable up to the expiry date on the label if stored at 2-10 °C and protected from light. Once opened, the reagent cassette placed in the refrigerated ABX Pentra 400 reagent compartment is stable for 64 days
Materials required but not provided	None	NaCl solution: 9 g/L, ABX Pentra Clean-Chem CP, Ref. A11A01755, 30ml
Detection Limit	5-160mg/L	0.10-400mg/L
Maintenance	Cleaning of cartridge chamber with a swab once a month	Daily, weekly, monthly and as required maintenance
Analyser warm-up time	Approximately 3minutes	Start-up procedure from downtime varies from 10-15minutes
Practical aspects of the assay	Auto-self check with integrated error detection. Error codes possible due to small sample volume that may dry out after the 1min limit instructed in the package insert	The sample probe has clot detection but no bubble detection. Operators must remove bubbles from samples before loading on the analyzer. Otherwise erroneous results will be produced

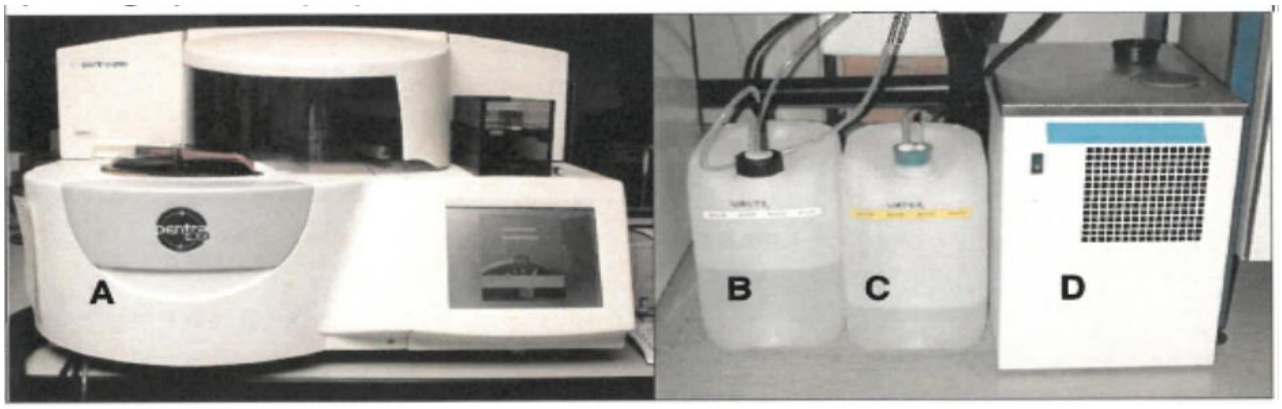


Figure 13: Illustration of the Pentra 400 system (A), separate keyboard and printer (not shown). A floor-standing waste (B, C), water and cooling unit (D), An optional uninterrupted power supply (UPS, not shown)

3.18. DATA ANALYSIS

3.18.1 DESCRIPTION OF VARIABLES

The socio-demographic, behavioural, laboratory and clinical variables analysed in this study were collected during the main SHIOP study. This data was collected using an interview administered questionnaires (see attached appendix 11), clinical and laboratory report forms. The clinical data was obtained after physical examinations were performed such as weight and height measurements. The laboratory data was obtained from the various tests which were conducted by the main study technologists. The socio-demographic variables analysed in this study included age which was collected in single years. It was initially analysed as a continuous variable using means and medians with age range of participants from 50 to 94 years old. Age was then grouped into broad age groups of 50-59 years, 60-69 years and 70+ years for further analyses. Participants were asked to self-identify with a population group and identified as one of three groups: African, Indian or White. The one participant who self-identified as white was excluded from further analyses since no statistical analysis could be performed with just one individual of this population group .

Civil, traditional or religious forms of marriage were all recognised as marriages in this study. Participants therefore had to state their marital status as never been married, married, co-habiting, separated/divorced, or widowed. Regarding religion participants were asked to state their religion from specified options of Christian, Hindu, Muslim, Jewish, none (not belonging to any religious grouping) or to specify if any other. From participant responses religion was then categorised into Christian or Hindu in this analysis. Regarding education, participants were asked to report whether they had no formal education, had completed or not completed primary school, had completed or not completed secondary school, or attained tertiary level (post-matric college or university) education or don't know. Due to the school sample sizes in the study, education level was recategorized as had no formal education, primary (combined completed and not completed), secondary (combined completed and not completed).

Other socio-demographic variables included in the study were employment (formal or informal) status (Yes/No); smoking (Never/Ever smoked); alcohol use (Never/Ever taken an alcoholic beverage); having an income source from self-employment, odd jobs, selling or trading, from formal employment wages; from government grants; from property rentals; from retirement fund; other sources or no income source. Income sources were later recategorized into government grants, other or none due to the small numbers in the initial categories. The other variable included in the descriptive factors of participants was HIV status based on results from the HIV testing conducted in the study. Participants were grouped into HIV positive or HIV negative categories.

3.18.2 STATISTICAL ANALYSIS

Data was analysed using correlation coefficients to assess agreement of CRP readings between the Alere Afinion™ AS100 instrument and the gold standard instrument, ABX Pentra 400. Data was further analyzed using the Bland-Altman analysis method. The Bland-Altman method compared mean differences between the Alere Afinion™ AS100 instrument and standard laboratory method (mean bias). The 95% limits of agreement between the Alere Afinion™ AS100 and laboratory measurements were calculated. The Bland-Altman methods were appropriate for this analysis since there was no repeat measurements on each method per participant, i. e there were two sets of measurements for each participant (one for the Alere Afinion™ AS100 and for the ABX Pentra 400 method). This showed that the two sets of measurements were independent making this approach suitable for data analysis of this evaluation study. Graphical Bland-Altman plots and scatter plots were used to depict the study findings.

Univariable and multi-variable linear regression analyses were used to analyse the data for factors associated with elevated CRP levels. The data are presented as odds ratios, which are exponents of the regression coefficients. The proportion of participants with elevated CRP in the studied population was based on the ABX Pentra 400 results as the gold standard. Regression analysis was used to identify risk factors associated with elevated CRP levels in the studied population, A p-value of <0.05 was regarded as being statistically significant.

CHAPTER FOUR

4.1 RESULTS

4.1.1 CHARACTERISTICS OF THE STUDY POPULATION

The baseline characteristics of the study sample (n=183) are summarized in Table 2. The mean age of the study participants was 7.62 years (SD 8.15). Male participants were slightly older than female participants (61 v. 58 years, $p<0.05$). Approximately 14.2% of study participants were above 70 years of age, and just over half of the participants were in the youngest age group 50-59 (50.3%) p=0.482. The study population consisted of 77/183 (42.0%) Black South Africans and 106/183 (57.9%) Indians Approximately 24.6%(p=0.117) of study participants reported to have never been married, 30.6% were married, and 4.8% were cohabiting, 6.7 % were either separated or divorced and 33.1% were widowed.

A large percentage (70.5%) (p=0.399) of the participants reported to be Christians, with 29.5% belonging to the Hindu religion. Only 12% (p=0.493) had never been to school, 44.8 % had primary school education, and 43.2% had secondary school education. For this analysis there were only 2 participants with tertiary education. These were combined with secondary, which was labelled as secondary or higher. Regarding economic activity, 6.6% (p=0.486) were employed, 88% were unemployed and 5.5% reported to have been retired. In this study we only looked at those who were unemployed at the time of the study. The questionnaire did not probe for whether they had been employed previously.

With regards to smoking habits, 73.2% (p=0.506) reported to be non-smokers, that is, had never smoked in their lives. Just over a quarter, 26.8% had ever smoked, that is, they were currently smoking or previously smoked. The number of ex-smokers was very small. Further because of the long-term effects of smoking we anticipated the risk of developing cardiovascular conditions would be similar between ex-smokers and current smokers compared to never smoked. Hence the smokers were grouped with the ex-smokers for data analysis. A large proportion (80.9%) (p=0.235) never consumed alcohol in their lives, whereas 19.1% currently consumed or had previously been consuming alcohol. In terms of income generation, 71.0%(p=0.984) were receiving a government grant, 23.5% reported to have other sources of income and 5.5% reported to not have any income.

4.1.1.1 CRP LEVELS IN THE STUDIED POPULATION

Table 2 illustrates CRP levels and risk factors associated with elevated CRP levels in the studied population. In the gender group that had elevated CRP levels, females constituted the highest proportion (70.8%) and only 29.2% of the group were males. However, with regards to the group that had normal CRP levels, the larger proportion (54.7%) were males with females accounting for only 45.3% of the group that had normal CRP levels. There was a significant association (p=0.001) between gender and CRP levels. In the group that had normal CRP levels, the highest proportion (47.2%) had a normal BMI. However, 28.3% of the group were overweight and 17% were obese. In the group that had elevated CRP levels, 41.5% were obese and 20.0% had a normal BMI. There was a significant association (p=0.001) between BMI and CRP levels. In the group that had normal CRP levels, the highest proportion (90.6%) had normal total cholesterol. However, 9.4% of the group had high total cholesterol. In the group that had elevated CRP levels, the highest proportion (76.9%) had normal total cholesterol. However, 23.1% of the group had high total cholesterol. This data revealed a significant association (p=0.033) between total cholesterol and CRP levels.

In the group that had normal CRP levels, 81.1% were non-diabetic. However,18.9% of the group were diabetic. In the group that had elevated CRP levels, the highest proportion (65.1%) were non-diabetic whereas 34.9% were diabetic. There was a statistically significant link (p=0.033) between diabetes and CRP levels. A large percentage of the participants (87.4%) (p=0.868) tested HIV negative with 12.6% testing HIV positive in this study. The percentage who tested HIV positive was 14.9% in males and 11.2% in females. However, statistically there was no significant link between HIV status and CRP levels.

Table 2: Demographic and health factors of participants in this study[δ]

	Total	Normal CRP*	High CRP	p-value
Number of participants	183	53 (29%)	130 (71%)	
Gender				0.001
Female	63.4	45.3	70.8	
Male	36.6	54.7	29.2	
Age group				0.482
50-59	50.3	43.4	53.1	
60-69	35.5	39.6	33.8	
70+	14.2	17.0	13.1	
Religion				0.399
Christian	70.5	66.0	72.3	
Non-Christian	29.5	34.0	27.7	
Education				0.493
Had no formal education	12.0	7.5	13.8	
Primary	44.8	47.2	43.8	
Secondary or higher	43.2	45.3	42.3	
Employed				0.486
No	88.0	92.5	86.2	
Yes	6.6	3.8	7.7	
Retired	5.5	3.8	6.2	
Marital status				0.117
Never	20.8	26.4	18.5	
Co-habiting	2.7	3.8	2.3	
Married	34.4	41.5	31.5	
Separate, divorced or widowed	42.1	28.3	47.7	
Smoking				0.506
Never	73.2	69.8	74.6	
Ever	26.8	30.2	25.4	
Alcohol use				0.235
Never	80.9	75.5	83.1	
Ever	19.1	24.5	16.9	
Income				0.984
Other	23.5	22.6	23.8	
Govt grant	71.0	71.7	70.8	
None	5.5	5.7	5.4	
Add salt to food				0.088
Never	35.5	35.8	35.4	
Always	14.8	18.9	13.1	
Rarely	48.6	41.5	51.5	
Don’t know	1.1	3.8	0.0	
Days in a week eat fruits				0.599
0 days	4.9	1.9	6.2	
1-2 days	30.6	35.8	28.5	
3-4 days	38.3	39.6	37.7	
5-7 days	19.1	15.1	20.8	
Don’t know	7.1	7.5	6.9	

Vigorous exercise				0.687
Never	80.9	75.5	83.1	
Always	6.0	7.5	5.4	
Rarely	9.8	13.2	8.5	
Don't know	3.3	3.8	3.1	
Body Mass Index (BMI				0.001
Normal	27.9	47.2	20.0	
Under	6.6	7.5	6.2	
Over	31.1	28.3	32.3	
Obese	34.4	17.0	41.5	
Blood pressure				0.984
Normal	11.5	5.7	13.8	
Prehypertensive	51.9	43.4	55.4	
Hypertensive	36.6	50.9	30.8	
Total Cholesterol				0.033
Normal	80.9	90.6	76.9	
High	19.1	9.4	23.1	
Albuminuria				0.793
Normal	82.0	79.2	83.1	
Micro	13.7	15.1	13.1	
Macro	4.4	5.7	3.8	
Diabetes				0.033
Non-diabetic	69.8	81.1	65.1	
Diabetic	30.2	18.9	34.9	
HIV status				0.868
Negative	87.4	86.8	87.7	
Positive	12.6	13.2	12.3	

$^{\delta}$ Table presents percentage distributions in each column based on the number of participants in the table header as the denominator.

***** CRP levels were categorised as: CRP<1.0 mg/L as low, CRP 1.0-3.0 mg/L intermediate, and CRP>3.0 mg/L high / elevated. These were then recategorized as Normal (low and intermediate) and High (high/elevated), as presented in this table.

4.1.2 COMPARISON OF C-REACTIVE PROTEIN LEVELS

Serum CRP levels on the Alere AfinionTM AS100 ranged from <5mg/L to 45mg/L. The Alere Afinion™ AS100 median values for CRP were 9.5mg/L (25th percentile 6.5, 75th percentile 14.5) and 11.5mg/L (25th percentile 7.0, 75th percentile 19.0) in women and men respectively (p-value = 0.275). The ABX Pentra 400 median levels were lower with 5.6mg/L (25th percentile 3.6, 75th percentile 9.2) and 3.6mg/L (25th percentile 1.6, 75th percentile 8.3) for women and men (p-value = 0.027), respectively. CRP levels, shown as medians with minimum and maximum CRP values, were distributed across a wide range on both the Alere Afinion™ AS100 and ABX Pentra 400 analysers (Figure 14).

Median CRP levels were slightly higher on the Afinion across all ages. However, median values for males on the Afinion were slightly higher compared to median values for males on the ABX Pentra 400. The ABX Pentra 400 showed a wider range of detection and Alere Afinion™ AS100 a narrower range of detection.

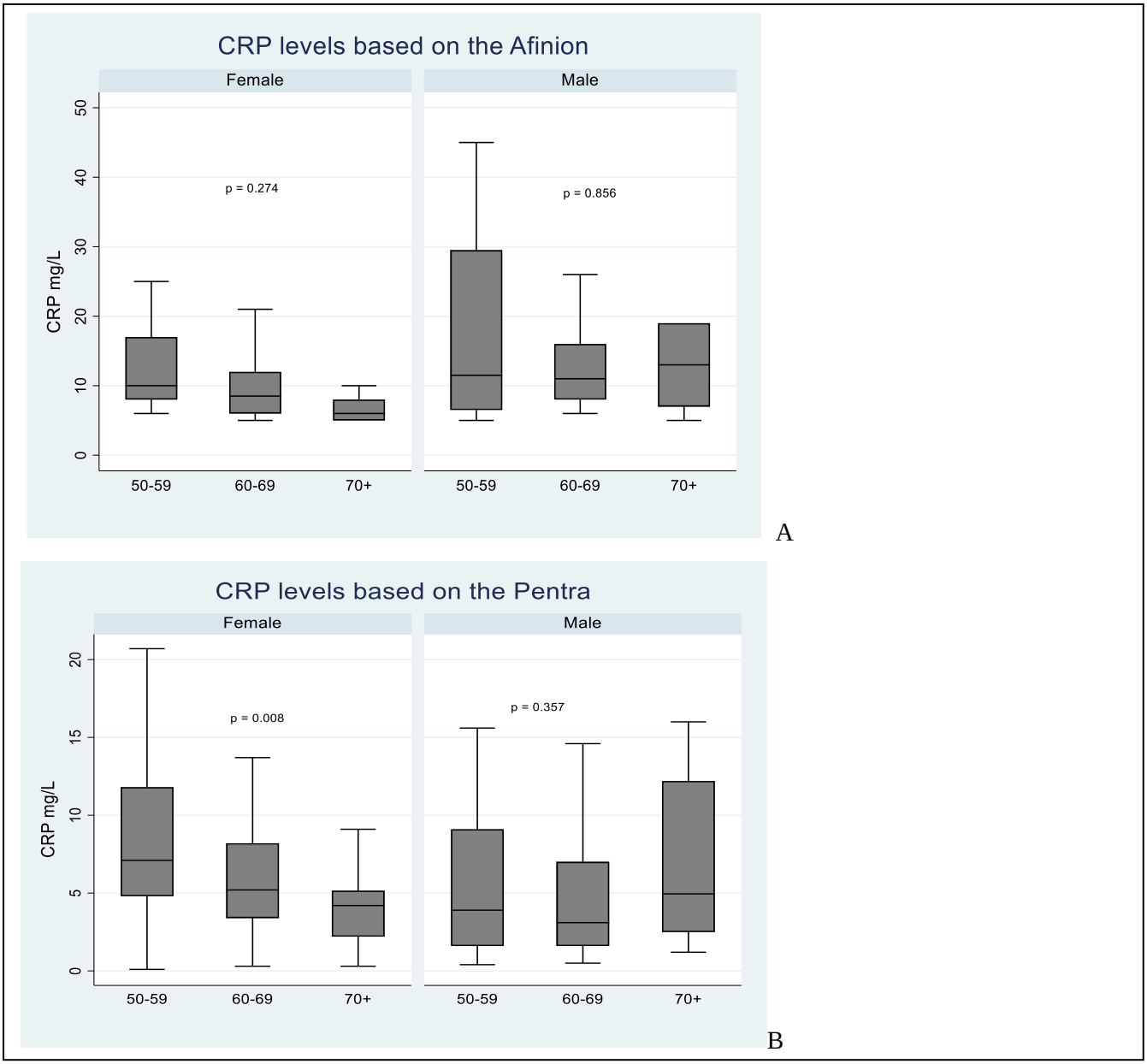
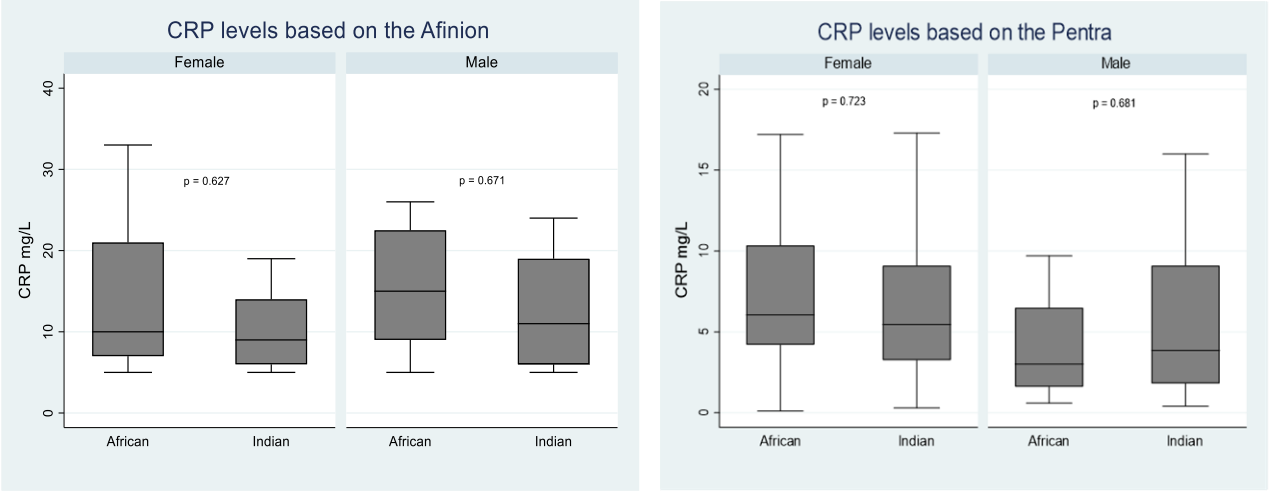


Figure 14: Comparison of C-reactive protein (CRP) levels by age and gender. Data are shown as medians (25th and 75th percentile) with minimum and maximum CRP values.

When comparing CRP levels across different ethnic groups, it was found that the CRP levels on the Alere Afinion™ AS100 were slightly higher in African females compared to Indian females (Figure 15A). Whereas Indian males showed a wider distribution of the CRP levels using the ABX Pentra 400 (Figure15B). African female CRP levels on the Alere Afinion™ AS100 were shown to be on the higher levels whereas there was no difference in median values between Africans and Indians males on the Alere Afinion™ AS100. Both races had a median CRP value of 10mg/L which was similar to median values seen on the ABX Pentra 400 with a 0.3mg/L difference between races. The ABX Pentra 400 had a wider range of detection and the Alere Afinion™ AS100 a narrower range of detection. Figure 14A shows that CRP levels on the Alere Afinion™ AS100 were slightly higher in African Females compared to Indian females. Indian males showed a wider distribution of the CRP levels using the ABX Pentra 400 (Figure 15B).



A **B**

Figure 15: Comparison of C-reactive protein (CRP) levels by race and gender. Data is shown as medians (25th and 75th percentile) with minimum and maximum CRP values.

4.2 DIAGNOSTIC PERFROMANCE OF THE AFINION WHEN COMPARED TO THE PENTRA 400

Lin's correlation coefficients were used to assess the agreement between the Alere Afinion^{TM} AS100 and the ABX Pentra 400 analysers (Table 3). Since the Alere Afinion^{TM} AS100 has a measuring range 5-200mg/L, with any readings below 5mg/L simply labelled as <5mg/L. The Afinion identified 89 samples as low (<5mg/L), these cases with readings marked as <5mg/L on the Alere Afinion^{TM} AS100 were excluded from the correlation analyses reducing the total number of samples to 94.

Table 3: Differences between the Alere Afinion^{TM} AS100 and ABX Pentra 400 CRP measurements using Bland-Altman (BA) Plots and Lin's concordance correlation coefficients

Table 3 presents differences between the Alere Afinion AS100 and ABX Pentra 400 CRP

Abbreviations: SD- Standard Deviation, BA- Bland- Altman

CRP	**N**	**Mean bias (SD)**	**95% BA Limits of Agreement**	**Concordance correlation**	**95% Confidence Interval**
Overall	94	1.323 (1.558)	(-1.731; 4.378)	0.977	(0.969; 0.985)

Mean bias is the average difference between CRP measurements on the Afinion and the Pentra. Most of the differences between the two methods would be expected to be between minus 2 standard deviation (SD) and plus 2 SD. In our analysis, the standard deviation of the mean bias was plus 1.558.

The Bland Altman (BA) limits of agreement gives the differences between the CRP measurement on the Afinion compared to the measurement for the same participant on the Pentra. From the table above, this means 95% of the

measurements on the Afinion were from 1.731 below the Pentra measurement to 4.378 above the Pentra measurements.

Figure 16 shows the Bland Altman plot comparing mean difference in the CRP estimations from the Afinion to the Pentra 400 method. The purple solid line represents the mean difference in CRP concentrations between the two methods, whereas the red lines represent 95% confidence limits of the agreement. The line y=0 is where all values would lie if there was perfect agreement between the Alere Afinion™ AS100 and the ABX Pentra 400 CRP concentrations. The Bland Altman CRP two-way scatter plot shown in Figure 16 indicated that the Alere Afinion™ AS100 gave slightly higher CRP readings than the ABX Pentra 400, with a mean difference of 1.323 (SD 1.558). In only a few cases did the Alere Afinion™ AS100 give a lower reading than the ABX Pentra 400 (scatter points below zero).

The proportion of CRP results from the remaining sample that were correctly classified by the Alere Afinion™ AS100 analyser was 95.07%. Bland-Altman analysis and linear regression analysis showed an excellent correlation between the two analysers, with a correlation concordance 0.977 between the two analysers for the measurement of CRP. This excellent correlation is further illustrated in Figure 17, which shows nearly all the values from the Alere Afinion™ AS100 and the ABX Pentra 400 lying perfectly on the regression line (solid black line) not far away from the line of perfect concordance (dashed red line) (Figure 17). The Alere Afinion™ AS100 was able to identify 44 samples with CRP measurements above 10mg/L compared to the 38 identified by the ABX Pentra 400. Six samples were therefore overestimated by the Alere Afinion™ AS100.

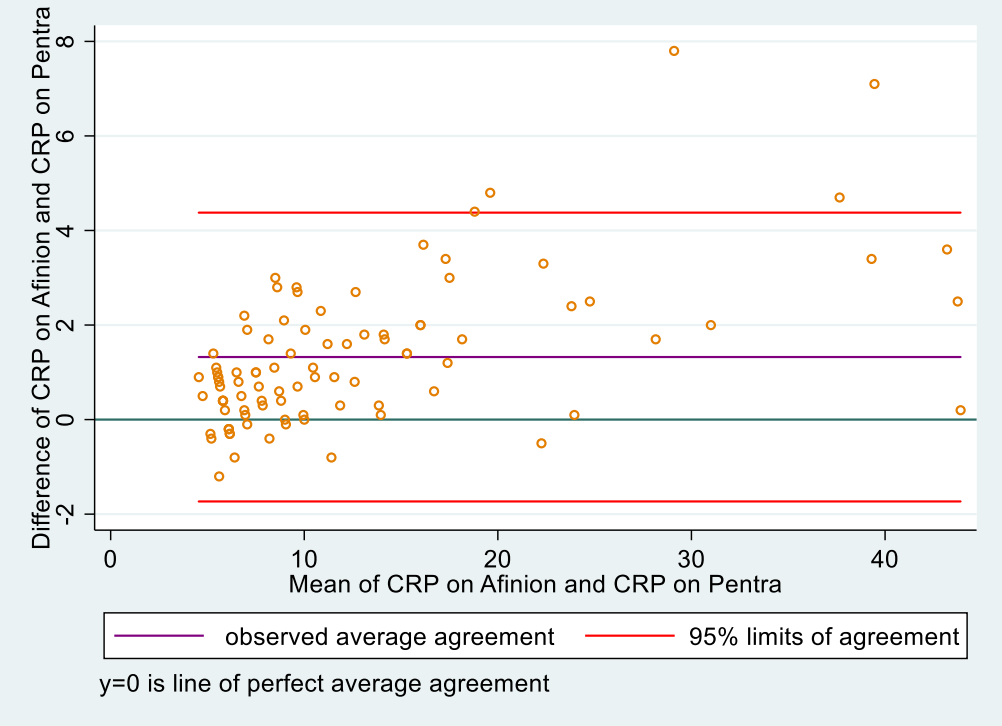


Figure 16: Scatterplot of CRP values comparing the Alere Afinion™ AS100 (A) and ABX Pentra 400 (P) analysers.

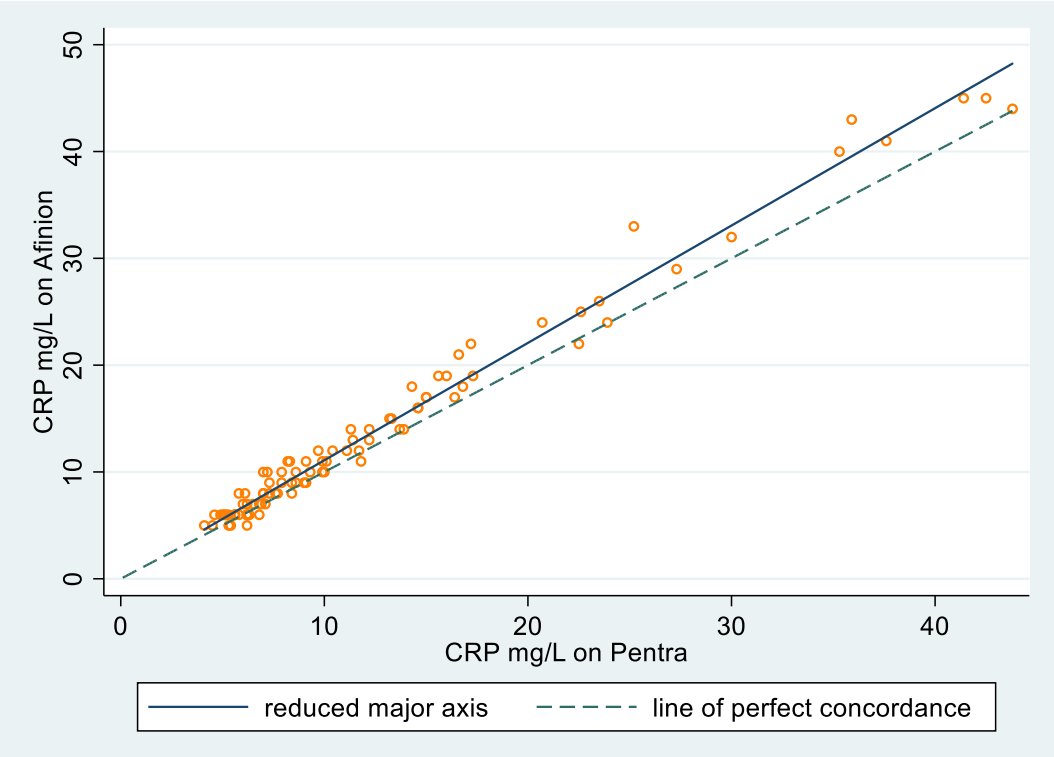


Figure 17: Regression analysis of CRP values produced from the Alere Afinion™ AS100 (A) and ABX Pentra 400 (P) analysers.

4.3 C-REACTIVE PROTEIN – EVALUATION OF BIAS

Although excellent correlation was shown between the Alere Afinion™ AS100 and the ABX Pentra 400 analysers there were some differences in the distribution of the results obtained by the two analysers. The results from the Alere Afinion™ AS100 were from <10mg/L to >40mg/L as compared to ABX Pentra 400 results that were distributed from <10mg/L up to >60mg/L. The mean bias observed for the CRP measurements between the Alere Afinion™ AS100and the ABX Pentra 400 was 1.323 (1.558) with the 95% BA Limits of Agreement of (1.731; 4.37).

4.4 RISK FACTORS ASSOCIATED WITH CRP LEVELS

The data obtained for the risk factors were obtained from the parent study. In the univariate analysis (Table 4), being obese (Odds Ratio [OR]: 1.98, 95% Confidence Interval [CI]: 1.3616, 2.889, p<0.001) and having low HDL levels (OR: 1.64, 95% CI: 1.158, 2.307, p= 0.005) were the only risk factors that were statistically significantly associated with increased likelihood of having elevated CRP levels. Whereas being male (p=0.041); aged ≥60 years (p=0.048); being pre-hypertensive (p=0.018); and being hypertensive (p=0.002) were all associated with statistically significant lower odds ratios of having elevated CRP.

The association with not being at risk of elevated CRP with being pre-hypertensive was also shown in the multivariate analysis (p=OR: 0.52, 95% CI: 0.313, 0.868, p=0.013). Similarly, being hypertensive was significant in the univariate (OR: 0.45, 95% CI: 0.274, 0.752, p=0.002) and multivariate analysis (OR: 0.39, 95% CI: 0.220, 0.680, p=0.001) as a non-risk factor. In the multivariate analysis (Table 5), being obese (OR:
2.58, 95% CI: 1.616, 4.120, p<0.001) and low HDL levels were sustained as risk factors (OR: 1.42, 95% CI:
0.999, 2.016, p=0.050). An additional risk factor identified in the multivariate analysis was HIV positivity (OR: 1.66, 95% CI: 1.004, 2.741, p=0.048). In the univariate analysis, the odds for Indians to have elevated CRP was 1.15 times higher than for black Africans, whereas in the multivariate analysis Indians had an odds ratio of 0.99 compared to black Africans. However, both findings were not statistically significant (p= 0.847 and 0.948, respectively). Associations of age with CRP were conflicting. In the univariate analysis participants aged 60 years and over were less likely to have elevated CRP levels and this was statistically significant (OR: 0.74, 95% CI: 0.545, 0.998, p= 0.048). Although in the multivariate analysis those aged ≥60 years were still less likely to have elevated CRP levels, however, this was not statistically significant (OR: 0.89, 95% CI: 0.640, 1.235, p=0.481).
Age was categorised in order to compare with previously published findings, and to explore any age effects in this group.

Table 4: Univariate analysis of factors associated with Elevated CRP levels in older adults [δ]

	OR	[95 %	CI]	p -value
Gender				
Female	1.00			
Male	0.72	0.5275	0.9871	0.041
Race				
Black	1.00			
Indian	1.15	0.8466	1.5692	0.365
Age group				
<60	1.00			
≥60 years	0.74	0.5453	0.9978	0.048
Education				
Never been to school	1.00			
Primary	0.66	0.4001	1.0728	0.092
Secondary+	0.77	0.4671	1.2574	0.290
Adds salt when cooking				
Never	1.00			
Always	0.93	0.5792	1.4938	0.763
Rarely	1.05	0.7500	1.4732	0.771
Don’t know	0.47	0.1056	2.0592	0.312
Smoking				
Non-smoker	1.00			
Smoker	1.04	0.7346	1.4639	0.836
Alcohol consumption				
Non-drinker	1.00			
Alcohol drinker	0.78	0.5329	1.1546	0.217
Vigorous activity/exercise				
Never	1.00			
Always	0.71	0.3720	1.3501	0.293
Rarely	0.76	0.4518	1.2651	0.285
Don’t know	0.71	0.3003	1.6733	0.430
Hypertension				
Normal	1.00			
Prehypertensive	0.55	0.3403	0.9009	0.018
Hypertensive	0.45	0.2743	0.7529	0.002
BMI				
Normal	1.00			
Underweight	1.18	0.6193	2.2312	0.619
Overweight	1.20	0.8186	1.7679	0.345

Obese	1.98	1.3616	2.8896	<0.001
Total cholesterol				
TC Normal	1.00			
TC elevated	1.01	0.6865	1.4921	0.952
HDL cholesterol				
HDL Normal	1.00			
HDL Low	1.64	1.1586	2.3075	0.005
LDL cholesterol				
LDL Normal	1.00			
LDL elevated	0.92	0.6629	1.2771	0.617
Albuminuria				
Albumin Normal	1.00			
Micro Albuminuria	0.95	0.6091	1.4899	0.831
Macro Albuminuria	1.08	0.5102	2.2928	0.837
HIV status				
HIV negative	1.00			
HIV positive	1.25	0.7924	1.9858	0.332

[δ] Table 4 presents univariate analysis of factors associated with Elevated CRP levels in older adults Factors: Diabetes, high total cholesterol, obesity and hypertension.

Table 5: Multi-variable analysis of factors associated with Elevated CRP levels in older adults δ

	OR	[95 %	CI]	p -value
Gender				
Female				
Male	0.85	0.5606	1.2756	0.422
Race				
Black African				
Indian African	0.99	0.6748	1.4449	0.948
Age group				
<60				
≥60 years	0.89	0.6402	1.2347	0.481
Education				
Never been to school				
Primary	0.81	0.4969	1.3363	0.415
Secondary+	0.92	0.5497	1.5272	0.736
Adds salt when cooking				
Never				
Always	1.03	0.6520	1.6420	0.884
Rarely	1.10	0.7945	1.5293	0.558
Don't know	0.59	0.1331	2.6317	0.489
Smoking				
Non-smoker				
Smoker	1.25	0.8301	1.8779	0.284
Alcohol consumption				
Non-drinker				
Alcohol drinker	0.98	0.6334	1.5234	0.936
Vigorous activity/exercise				
Never				
Always	0.88	0.4552	1.7156	0.713
Rarely	0.89	0.5378	1.4692	0.644
Don't know	0.79	0.3401	1.8458	0.587
Hypertension				
Normal				
Prehypertensive	0.52	0.3137	0.8682	0.013
Hypertensive	0.39	0.2209	0.6809	0.001
Obesity				
Normal				
Underweight	1.07	0.5538	2.0643	0.841
Overweight	1.55	1.0041	2.3997	0.048
Obese	2.58	1.6163	4.1203	<0.001

Total cholesterol				
TC Normal				
TC elevated	0.84	0.5410	1.3180	0.454
HDL cholesterol				
HDL Normal				
HDL Low	1.42	0.9999	2.0164	0.050
LDL cholesterol				
LDL Normal				
LDL elevated	1.00	0.6916	1.4504	0.993
Albuminuria				
Albumin Normal				
Micro Albuminuria	1.00	0.6343	1.5693	0.992
Macro Albuminuria	1.91	0.8883	4.1091	0.097
HIV status				
HIV negative				
HIV positive	1.66	1.0044	2.7419	0.048

[δ] Table 5 presents Multi-variable analysis of factors associated with Elevated CRP levels in older adults

Factors: Diabetes, high total cholesterol, obesity and hypertension.

CHAPTER FIVE

5.1 DISCUSSION

C-reactive protein is a vital biomarker of inflammation which is used to detect cardiovascular diseases in patients at high risk (Lee et al, 2014). CRP has been demonstrated by several studies to be an independent predictor of future risk for cardiovascular events among healthy individuals, including patients with acute coronary syndromes (Shrivastava et al., 2015). Lipid screening is currently used to detect cardiovascular events and to make clinical decisions in terms of patient treatment, care and management (Sproston et al., 2018). However, it has been reported that almost half of cardiovascular events occur in individuals with low to average levels of low-density lipoprotein cholesterol (Clearfield, 2005). The incorporation of CRP levels into standard cholesterol assessment protocols adds value by aiding the clinicians' ability to predict CVD risk (Clearfield, 2005).

The current study evaluated the performance of the Alere Afinion™ AS100 analyser in comparison with the standard laboratory method, the ABX Pentra 400 for the quantification of CRP. This evaluation was performed in a population of HIV positive and negative older adults. The results of the Bland Altman plots showed that overall, the correlation of the Alere Afinion™ AS100 against the Pentra 400 for the measurement of CRP levels was very good, making it a good POC analyser for the measurement of CRP. However, this is only true for CRP levels >5mg/L. In addition, our sample size was very small (about 50% of the original sample) which limits us from making strong recommendations regarding the AS100 Analyser. Our findings are supported by other published studies. A study by Minnaard et al., 2013, evaluated 5 CRP POCT devices (Afinion™, NycoCard™ reader II, Eurolyser Smart 700/340, QuikRead go®, QuikRead®). The study by Minnaard et al., 2013 revealed that at the intermediary concentration of (20-100mg/L), the Afinion™ was the most accurate device. The authors concluded that the Afinion™ showed better agreement when compared to the other 3 POCTs. The agreement between the POCT and the laboratory standard decreased at higher CRP concentrations, resulting in wider confidence intervals around the mean differences at CRP concentrations greater than 100mg/L.

Alere Afinion™ AS100 analyser is capable of testing multiple analytes such as HbA1C and albumin creatinine ratio (ACR). Imprecision results showed the Alere Afinion™ AS100 ACR test to be a precise POCT analysis. Available data revealed that Alere Afinion™ AS100 ACR test provided a three in one POCT for the measurement of albumin, creatinine and ACR in one specimen within only less than an hour 5minutes 35seconds to be precise (Kvam et al., 2009).

Results of the three analytes might be measured concurrently using one patient specimen. The ACR assay presented simultaneously performs the analysis of samples for both albumin and creatinine. The creatinine reaction is completed within 4minutes, utilizing the advanced end point prediction algorithm, while the albumin assay, launched just after the creatinine reaction, takes approximately 3 minutes. Both assays run in parallel and result in a total assay time of 5minutes 35seconds (Kvam et al., 2009).

The Alere Afinion™ AS100 is a simple, robust, convenient and safe analyser (Kvam et al., 2009). This study showed that the Alere Afinion™ AS100 would be able to provide reliable, fast patient results leading to speedy medical decisions taken by clinicians. However, these are our assumptions and yet to be evaluated. A fast turnaround time that would further improve interaction between patients and the clinic staff. Ivaska et al., 2015 compared the Alere Afinion™ AS100 CRP POC analyser to the CRP test (Modular P, Roche Diagnostics). The study focused more on febrile children at the emergency department with median age 2.4 years. In this study, the Alere Afinion™ AS100 showed a good correlation with the reference method. Verbakel et al., 2013 provided further evidence on the analytical accuracy of the Alere Afinion™ AS100 POC CRP test. The Alere Afinion™ AS100 was compared with an immunoturbidimetric CRP test, the Cobas c702 analyser in children and adults. The study confirmed that the few variations observed between the methods did not interfere with the clinical decision on patient care or management. These variations were deemed as of clinical insignificance. CRP measurements using the Alere Afinion™ AS100 CRP showed a high correlation even at high CRP measurements. This analytical accuracy contributed to the consensus amongst all physicians and investigators that had participated in this study to conclude the user-friendliness of the Alere Afinion™ AS100. Results from the study on sexual health and chronic morbidities in people aged ≥50 years which was conducted in the Chatsworth and Botha's Hill areas in Durban, South Africa, revealed that an urgent intervention to address the issue of increasing challenges of HIV and non-communicable disease among older adults is required (Abbai et al., 2018).

In the study by Brouwer et al., 2015, eight analysers (Actim; Cleartest, Afinion™, Eurolyser Smart, Ichroma, Microsemi, AQT90 FLEX) were evaluated for accuracy (agreement, bias, correlation precision (CV) and ease of use. The Alere Afinion™ AS100 was one of the few analysers that showed a good correlation. Brouwer et al., 2015 performed a comparison study of the six quantitative POCT devices (QuikRead go, Smart Eurolyser, Afinion™, ichroma™, Microsemi) and two semi-quantitative methods to measure CRP. A practical evaluation for the six analysers was performed in a laboratory setting, where the main aim of the study was to evaluate the minimum amount of material required, analytical range, pre-analytical handling of the samples and estimated pre-analytical time, if haematocrit (HCT) correction was required, size and weight of the analyzer, and whether the device was also able to measure other analytes. In this evaluation it was concluded that the Alere Afinion™ AS100 required the least pre-analytical handling which was less than a minute and the Alere Afinion™ AS100 and the Eurolyser were concluded as the preferred analysers for CRP POCT (Brouwer et al., 2015). These six devices all use capillary blood samples. Brouwer et al., 2015 concluded that when combining analytical performance and practical evaluation, the Alere Afinion™ AS100 and the Smart Eurolyser gave the best agreement and were concluded to be the preferred POCT analysers.

In this study, we looked at potential risk factors for CVDs in association with CRP levels. We found that 34.4% (P=0.001) of participants were obese according to the BMI calculations taken in this analysis. Evidence that obesity and even excessive weight have a close association with an elevated risk of CVDs has been shown by Imre et al., 2018. According to Lavie et al., 2009, obesity presents many adverse effects on the functioning of the cardiovascular

structure. In addition, obesity tends to increase total blood volume and cardiac input causing cardiac workload to be greater than usual. Evidence from the study on the association between obesity and cardiovascular outcomes by Haris et al., 2018 also revealed that obesity is associated with CVDs. Evidence has also shown that the mortality and morbidity of CVDs is increased in individuals who are overweight (Van Gaal et al., 2006).

Findings from our study are on par with other studies with regards to the association of obesity with the risk of elevated CRP and thus by correlation with increased risk of CVDs. Findings from other studies revealed that high cholesterol was prevalent among adults in Africa (Noubiap et al., 2018). Harris et al., 2017, concluded that increased cholesterol was one of the confirmed risk factors for CVDs. Walker et al., 2001 further confirmed that the prevalence of high total cholesterol and other CVD risk factors in the South African population was evident and there is a high probability that the growing urbanisation of the black African population may cause this prevalence to increase. Seedat et al., 2011 also confirmed that high total cholesterol is a major CVD risk factor in South Africans and that it is found in all population groups in South Africa. Our study was able to demonstrate that being obese (Odds Ratio [OR]: 1.98, 95% Confidence Interval [CI]: 1.3616, 2.889, $p<0.001$) and having low HDL levels (OR: 1.64, 95% CI: 1.158, 2.307, $p= 0.005$) were the only significant risk factors that were associated with elevated CRP levels in the studied population. Something that has been previously shown, which our results were unable to demonstrate, was that having a low level of the good high-density lipoprotein (HDL) was a significant risk fact for having elevated CRP. However, further studies in the older population are needed to collaborate these findings for a nuanced understanding of the contribution of low HDL to cardiovascular disease risk in adult populations.

The study by Matthew et al., 2008 suggested that the presence of atherosclerosis caused by a condition such as HIV and its treatment have a high effect in increasing the risk of CVDs among people who are infected with HIV. Some observational cohort studies have also demonstrated increased levels of CVDs such as CHD or AMI in HIV-infected versus control patients, with an approximate 1.5 to 2-fold increased relative risk (Klein et al., 2002; Currier et al., 2003; Triant et al., 2007; Lang et al., 2010; Durand et al., 2011).

A study on HIV, hypertension and CVDs by Spencer, 2016 revealed that there is a close link between HIV infection and increased risk of CVDs. Other studies have also revealed that specific antiretroviral drugs, such as abacavir, indinavir and lopinavir may increase risk for CVDs (Bavinger et al., 2013). These studies further confirmed that HIV infected individuals are at high risk of developing CVDs (Bavinger et al., 2013; Triant, 2013). The study investigated the link between hospitalization for severe infection and consequent risk of CVD in a large, well-characterized cohort of HIV-infected individuals in a middle-income country.

The study also revealed that after controlling for traditional and HIV-related CVD risk factors, the incidence of severe infection was associated with an elevated CVD risk in the year subsequent to hospital discharge. Furthermore, the study detected that this link was time-dependent, greater within the first 3 months after hospital discharge (Bavinger et al., 2013; Triant, 2013).

5.2 LIMITATIONS OF THE STUDY

The manufacturer claims that the CRP reference range for adults (20-60 years) is < 5mg/L. This reference range did not cater for 14.2% of the study population of participants aged above 70 years of age. Stored samples were used for the evaluation of the Alere Afinion™ AS100 for the measurement of the CRP, which may not provide as similar readings as using fresh samples. Our findings, however, showed a good correlation between the Alere Afinion™ AS100 analyser and the standard laboratory analyser, the ABX Pentra 400, suggesting the reliability of these findings. Abdominal obesity has been indicated to be a worldwide risk factor for CVDs. In addition, over and above CVD, obesity has been revealed to increase the risk of high blood pressure (Din-Dzietham et al., 2007). However, in the parent study, no data was collected on waist circumference and this was another limitation in the parent study. Our study did not have a good representation with regards to HIV infection as this was a population with only 12.6% HIV-positive individuals, so the prevalence is not significantly high.

There is thus sufficient evidence to show that the Alere Afinion™ AS100 may be a good POC analyser for screening in older adults within a high HIV prevalence setting. However, another major limitation of this study is the fact that nearly 50% of the sample could not be used in the concordance correlations due to the Afinion™ not being able to detect values lower than 5mg/L.This substantially reduces statistical power, making it extremely difficult to draw conclusions.

5.3 CONCLUSION

Presently there is lack of data on POCTs for the measurement of CRP levels, especially in older South African persons. This study was the first to evaluate the performance of the Alere Afinion™ AS100 in the quantification of CRP in a population of HIV negative and HIV-positive adults from the age of 50 years and above in a high HIV setting. It was also the first study to evaluate the diagnostic performance of the Alere Afinion™ AS100 analyser compared to the ABX Pentra 400 analyser (reference test) in measuring levels of CRP in an older South African population. Though the Alere Afinion™ AS100 was found to be comparable to the reference test, it would not add much value in detecting high risk patients as it can only detect CRP levels from 5mg/L and above. Patients with CRP levels below 5mg/L would be missed despite possibly being at high risk

In addition, the data generated from this study provided insights regarding the association of elevated CRP and various CVD risk factor conditions which will facilitate better patient management. The results of the study revealed that the Alere Afinion™ AS100 can be used as a POCT for the measurement of CRP to detect CRP levels from 5mg/L and above.

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APPENDICES

This section contains examples of documents used during the study, Specimen Storage Protocols, Pictorial views of equipment used during the study, results of the samples tested during the study. It also contains copies of the informed consents, ethics approval letters, examples of documents completed during sample retrieval process

APPENDIX 1: INFORMED CONSENT (STORAGE AND FUTURE TESTING OF SPECIMENS)

SOUTH AFRICAN MEDICAL RESEARCH COUNCIL,
HIV PREVENTION RESEARCH UNIT

INFORMED CONSENT (STORAGE AND FUTURE TESTING OF SPECIMENS)

A Study to investigate Sexual health, HIV and co-morbidity with non-communicable infections among Older Persons (SHIOP)

Version 1.2, 21 January 2016

PRINCIPAL INVESTIGATOR: Dr. Makandwe Nyirenda
PHONE: 031 242 3671 / 073 806 2660

INTRODUCTION

You have decided to take part in the *study to investigate Sexual health, HIV and co-infection with non-communicable diseases (high blood pressure, diabetes, kidney and heart diseases) among Older Persons (SHIOP)*, which is funded by the South African Medical Research Council (SAMRC). While you are in the SHIOP study, there may be some biological specimens (blood and urine) taken from you that might be useful for future related studies (refer to paragraph three: "How will you use my biological specimens"). You are being asked to agree to the storage of these biological specimens. This consent form gives you information about the collection, storage, and use of your biological specimens. The study staff will talk with you about this information. Please ask study staff any questions you may have. You will be asked to sign or make your mark on this form to indicate whether you agree to have your biological specimens stored and tested in the future. You will be offered a copy of this form to keep.

HOW WILL YOU GET THE BIOLOGICAL SPECIMENS FROM ME?

You have agreed to have biological specimens collected and tested as part of the SHIOP study. During the study, your stored biological specimens will be tested to check on your health and to see if you have diseases common in older adults like diabetes, hypertension, heart and kidney diseases and diseases that can be passed on through sex like HIV. The study staff would like to keep any biological specimens that are leftover, after the SHIOP study is done, to use for future testing. If you agree to this, no additional biological specimens will be taken from you. Only left over biological specimens will be kept and used for future testing.

HOW WILL YOU USE MY BIOLOGICAL SPECIMENS?

Your biological specimens will only be used to look for additional evidence of infection with HIV, diabetes, hypertension, heart and kidney diseases and diseases that can be passed on through sex or damage caused by any of these infections; or your body's response to these infections. For instance, researchers may look at your blood cells and substances in your biological specimens called proteins and chemicals. They also may look at your genes (DNA), since your genes might affect your response to disease in important ways. Your genes might make you more likely or less likely to becoming infected, make your responses to infection or to treatment either stronger or weaker, or make HIV progress either more rapidly or more slowly. No other kinds of genetic test will be done by anyone on your stored blood without first explaining the test to you and obtaining your permission.

The researchers do not plan to contact you or your regular doctor with any results from tests done on your stored biological specimens. This is because research tests are often done using ways that are experimental, so the results do not usually help doctors manage your health. If a rare situation comes up in which the researchers decide that a test result is important for your health, the researchers will notify your study doctor and your study doctor will try to contact you. If you wish to be contacted with this type of test result, you must give the study doctor or nurse any change to your contact information. If you want your regular doctor to be told about this type of test result, you must provide the study doctor or nurse with your regular doctor's name and contact information.

Your biological specimens will not be sold or used directly to produce commercial products. Research studies wishing to use your biological specimens will be reviewed by the South African Medical Research Council Ethics Committee. The role of this committee is to protect you and other research volunteers from harm.

HOW LONG WILL YOU KEEP MY BIOLOGICAL SPECIMENS?
Your blood and urine specimen will be stored for 15 years with the option to renew for another 15 years.

HOW WILL MY BIOLOGICAL SPECIMENS BE STORED?
Your biological specimens will be stored in South Africa at facilities that are designed to store samples safely and securely. The storage facilities are designed so that only approved researchers will have access to the samples. Your biological specimens will be stored at:

- MRC Specimen Repository
  HIV Prevention Research Unit,
  Medical Research Council (SA),
  Central Routine Laboratory 1ST Floor,
  Westville Village Market ,
  123 Jan Hofmeyer Road,
  Westville, 3630
  KwaZulu Natal, South Africa

- MRC Specimen Repository
  HIV Prevention Research Unit,
  Medical Research Council (SA)
  Basement Level, Shop Number B01,
  Westville Village Market ,
  123 Jan Hofmeyer Road,
  Westville, 3630
  KwaZulu Natal, South Africa

DOES STORAGE OF MY BIOLOGICAL SPECIMENS BENEFIT ME?
There are no direct benefits to you. The benefit of doing research on stored biological specimens includes learning more about HIV, diabetes, hypertension, heart and kidney diseases and diseases that can be passed on through sex.

WHAT ARE THE RISKS?
There are few risks related to storing your biological specimens. When tests are done on the stored biological specimens, there is a small but possible risk to your privacy. It is possible that if others found

out information about you that is learned from tests (such as information about your genes), it could cause you problems with your family (having a family member learn about a disease that may be passed on in families or learning who is the true parent of a child) or problems getting a job or insurance.

WHAT ABOUT CONFIDENTIALITY?

To keep your information private, your biological specimens will be labeled with a code that can only be traced back to your research clinic. Your name and other personal information will be protected by the research clinic. When researchers are given your stored biological specimens to study, they will not be given your personal information.

The results of future tests will not be included in your health records. Any publication about the results of future tests will not use your name or identify you personally. The researchers will do everything they can to protect your privacy. Every effort will be made to keep your personal information confidential. However, it is not always possible to guarantee confidentiality. Your personal information may be disclosed if required:

- A subpoena or court order;
- To protect the safety of any individual or the general public; or
- To comply with any law

Your records may be reviewed by:

- The South African Medicines Control Council
- The Medical Research Council of South Africa Ethics Committee
- Study monitors
- Study staff

WHAT ARE MY RIGHTS?

Allowing your biological specimens to be stored is completely voluntary. If you decide not to have any biological specimens stored other than what is needed to complete the SHIOP study, you can still remain in the SHIOP study, and your leftover biological specimens will be destroyed. If you decide now that your biological specimens can be stored for future research, you may change your mind at any time. However, you must contact your study doctor or nurse and let them know that you no longer want your samples used for future research. Your biological specimens will then not be used and will be destroyed.

WHAT DO I DO IF I HAVE QUESTIONS?

The South African Medical Research Council Ethics Committee has approved this study.

If you have questions about your rights related to the storage and future testing of your biological specimens for research, contact the chairperson of MRC Ethics Committee for reporting any complaints/problems:

Prof. Danie du Toit
MRC Ethics Committee
P.O. Box 19070
Tygerberg, Cape Town
Tel: (021) 938-0341
Fax: 0866 854 023
E-mail: adri.labuschagne@mrc.ac.za

If you have questions about the storage and future testing of you biological specimens or if you ever have any questions about the research site, you should contact:

Principal Investigator	Address	Telephone Number
Dr. Makandwe Nyirenda	123 Jan Hofmeyer Rd, Village Market, Westville, 3630 Durban, KwaZulu-Natal, South Africa.	031-242-3671 / 073-806-2660

STORAGE CONSENT

Please carefully read the statements below and think about your choice. No matter what you decide it will not affect your participation in the SHIOP study or your medical care. Please initial or mark your choice and sign or make your mark below.

☐ I DO agree to allow my biological specimens and health data to be stored and used in future research studies.

_____
Participant Signature/ Initial

OR

☐ I DO NOT agree to allow my biological specimens and health data to be stored and used in future research studies.

_____
Participant Signature/ Initial

GENETIC TESTING

☐ I DO agree to allow my biological specimens to be genetically tested

_____
Participant Signature/ Initial

OR

☐ I DO NOT agree to allow my biological specimens to be genetically tested.

_____
Participant Signature/ Initial

SIGNATURES

_____	_____	_____
Participant Name (print)	Participant Signature	Date
_____	_____	_____
Study Staff Conducting Consent Discussion (print)	Study Staff Signature	Date
_____	_____	_____
*Witness Name (print)	*Witness Signature	*Date

* Witness name, signature and date are required on this consent form only when the consenting participant is not able to read (illiterate).

APPENDIX 2: INFORMED CONSENT (IC) (STORAGE AND FUTURE TESTING OF SPECIMENS)- ISIZULU 1.2

SOUTH AFRICAN MEDICAL RESEARCH COUNCIL,
HIV PREVENTION RESEARCH UNIT

IMVUME UNOLWAZI (UKUGCINWA NOKUHLOLWA KWESIKHATHI ESIZAYO KWAMANEMBE)

Ucwaningo lokubheka Impilo yezocansi, isandulela ngculaza nokutheleleka okungahambisana nezifo ezingathathelani kubantu abadala (SHIOP)

Version 1.2, 21 January 2016

PRINCIPAL INVESTIGATOR: Dr. Makandwe Nyirenda
PHONE: 031 242 3671 / 073 806 2660

ISINGENISO

Usunqume ukubamba iqhaza *Ucwaningo lokubheka Impilo yezocansi, isandulela ngculaza nokutheleleka okungahambisana nezifo ezingathathelani njengo (ukushaya komfutho wegazi okuphezulu, ushukela, izifo zezinso kanye nezenhliziyo) kubantu abadala (SHIOP)*, oluxhaswe i-South African Medical Research Council (SAMRC). Ngesikhathi usocwaningweni i-SHIOP, kungenzeka kube namanye amanembe omzimba (igazi nomchamo) athathwe kuwena angase abe usizo ocwaningweni lwesikhathi esizayo oluhlobene (bheka indima yesithathu: "Nizowasebenzisa kanjani amanembe omzimba wami"). Uyacelwa ukuba uvume ukugcinwa kwalamanembe omzimba. Lelifomu lemvume likunikeza imininingwane mayelana nokuthathwa, ukugcinwa, kanye nokusetshenziswa kwamanembe omzimba akho. Abasebenzi bocwaningo bazokhuluma nawe mayelana nalemininingwane. Sicela ubuze abasebenzi bocwaningo nanoma imiphi imibuzo ongase ube nayo. Uzocelwa ukuba usayine noma wenze umaka wakho kulelifomu ukukhombisa ukuthi uyavuma ukuthi amanembe omzimba akho agcinwe nokuthi ahlolwe esikhathini esizayo. Uzonikezwa umfanekiso walelifomu ukuthi uwugcine.

NIZOWATHOLA KANJANI AMANEMBE OMZIMBA KUMINA?

Usuvumile ukuthi amanembe omzimba athathwe futhi ahlolwe njengengxenye yocwaningo i-SHIOP. Ngesikhathi socwaningo, amanembe omzimba akho agciniwe azohlolwa ukubheka impilo yakho nokubona ukuthi unazo yini izifo ezivamise kubantu abadala njengo shukela, ukushaya komfutho wegazi okuphezulu, izifo zenhliziyo nezinso kanye nezifo ezingadluliselwa ngokocansi njengegciwane lesandulela ngculaza. Abasebenzi bocwaningo bazofisa ukugcina nanoma imaphi amanembe omzimba akho asele, emva kokuba ucwaningo i-SHIOP selwenziwe, ukusetshenziselwa ukuhlolwa esikhathini esizayo. Uma uvuma lokhu, awekho amasanembe omzimba wakho engeziwe azothathwa kuwena. Ilawo manembe omzimba kuphela asalile azogcinwa futhi asetshenziselwe ukuhlola esikhathini esizayo.

NIZOWASEBENZISA KANJANI AMANEMBE OMZIMBA WAMI?

Amanembe omzimba wakho azosetshenziswa kuphela ukubheka ubufakazi obengeziwe bokutheleleka ngegciwane lesandulela ngculaza, ushukela, ukushaya komfutho wegazi, izifo zenhliziyo nezinso kanye nezinye izifo ezingadluliselwa ngokocansi noma umonakalo odalwe inoma iziphi kulezi zifo, noma umzimba wakho omelana ngayo nalezi zifo.Isibonelo, abacwaningi bangabheka izicubu zakho zegazi nezinto ezikumanembe omzimba wakho ezibizwa ngokuthi amaphrotheyni noma amakhemikhali. Kungenzeka futhi baphinde babheke ufuzo (DNA), njengoba ufuzo lwakho kungenzeka lube nomthelela ekumelaneni nezifo ngezindlela ezibalulekile. Ufuzo lwakho lungakwenza ukuthi ube namathuba amaningi noma amancane ukuthi utheleleke, lungenza ukumelana nezifo noma ukwelashwa kuqine okanye kube ntekenteke, noma lwenze ukudlondlobala kwegciwane lesandulela ngculaza kusheshe

kakhulu okanye kuhambe kancane kakhulu. Azikho ezinye izindlela zokuhlolwa kofuzo ezizokwenziwa inoma ubani kumanembe akho agciniwe ngaphandle kokuqala uchazelwe ngokuhlolwa kanye nokuthola imvume yakho.

Abacwaningi abahleli ukuxhumana nawe noma udokotela wakho ojwayelekile nganoma imiphi imiphumela esuselwa ekuhlolweni okwenziwe emanembeni omzimba wakho agciniwe. Lokhu kungenxa yokuthi lokhu kuhlola kocwaningo kujwayele ukwenziwa ngezindlela ezisahlolwa, ngakho ke imiphumela ayijwayele ukuthi isize odokotela ukumelana nempilo yakho. Uma isimo esingajwayelekile siqhamuka lapho abacwaningi benquma ukuthi umphumela wokuhlola ubalulekile ngempilo yakho, abacwaningi bazokwazisa udokotela wakho wocwaningo bese udokotela wakho wocwaningo ezama ukuxhumana nawe. Uma ufisa ukuthi kuxhunyanwe nawe ngaloluhlobo lwemiphumela yokuhlola, kufanele unikeze udokotela wocwaningo noma umhlengikazi noma iluphi ushintsho kwimininingwane yakho yokuxhumana. Uma ufuna udokotela wakho ojwayelekile atshelwe ngaloluhlobo lwemiphumela wokuhlolwa, kufanele unikeze udokotela wocwaningo noma umhlengikazi igama nemininingwane yokuxhumana kadokotela wakho ojwayelekile.

Amanembe omzimba wakho angeke adayiswe noma asetshenziswe ngqo ukukhiqiza imikhiqizo yokwenza inzuzo. Ucwaningo olufisa ukusebenzisa amanembe omzimba akho luzobuyekezwa i-South African Medical Research Council Ethics Committee. Indima yalelikomiti ukuvikela wena namanye amavolontiya ocwaningo ebungozini.

NIZOWAGCINA ISIKHATHI ESINGAKANANI AMANEMBE OMZIMBA WAMI?
Amanembe egazi lakho nomchamo wakho kuzogcinwa iminyaka engu 15, ungaba nokukhetha ukuvuselela eminye iminyaka engu 15.

NIZOWAGCINA KANJANI AMANEMBE OMZIMBA WAMI?
Amanembe omzimba wakho azogcinwa ezikhungweni ezikhethekile ezakhelwe ukugcinwa kwamanembe ngokuphepha ngangokuvikeleka e-South Africa. Izikhungo zokugcina zakhiwe ngendlela yokuthi kube abacwaningi abavumelekile kuphela abangafinyelela kumanembe. Amanembe omzimba wakho azogcinwa e:

- MRC Specimen Repository
  HIV Prevention Research Unit,
  Medical Research Council (SA),
  Central Routine Laboratory 1ST Floor,
  Westville Village Market ,
  123 Jan Hofmeyer Road,
  Westville, 3630,
  KwaZulu-Natal, South Africa

- MRC Specimen Repository
  HIV Prevention Research Unit,
  Medical Research Council (SA)
  Basement Level, Shop Number B01,
  Westville Village Market ,
  123 Jan Hofmeyer Road,
  Westville, 3630,
  KwaZulu-Natal, South Africa

NGABE UKUGCINWA KWAMANEMBE OMZIMBA WAMI KUNENZUZO KUMINA?
Azikho izinzuzo eziqonde kuwena. Inzuzo yokwenza ucwaningo kumanembe omzimba agciniwe kuhlanganisa ukufunda kabanzi mayelana negciwane lesandulela ngculaza, ushukela, ukushaya komfutho wegazi okuphezulu, izifo zenhliziyo nezinso kanye nezifo ezingadluliselwa ngokocansi.

IBUPHI UBUNGOZI?
Buncane ubungozi obuhlobene nokugcinwa kwamanembe omzimba wakho. Uma ukuhlolwa kwenziwa kumanembe omzimba agciniwe, kukhona ubungozi kodwa obuncane obungenzeka kubumfihlo bakho. Kungenzeka ukuthi uma abanye bethola imininingwane mayelana nawe olutholwe ekuhlolweni (njengemininingwane mayelana nofuzo lwakho), kungakudalela izinkinga nomndeni wakho (ukuthola ukuthi ilunga lomndeni elingazi mayelana neesifo esingadluliseleka emndenini noma ukwazi ukuthi ubani umzali wangempela wengane) noma izinkinga ukuthola umsebenzi noma umshwalense.

NITHINI NGOBUMFIHLO?
Ukugcina imininingwane yakho iyimfihlo, amanembe omzimba akho azobekwa uphawu oluyi-khodi engakwazi ukuthi iqondaniswe neklinikhi lakho locwaningo kuphela. Igama lakho neminye imininingwane eqondene nawe luzovikelwa iklinikhi locwaningo. Uma ngabe abacwaningi benikezwa amanembe omzimba wakho agcininiwe ukuwacwaninga, angeke banikezwe imininingwane eqondene nawe.

Imiphumela yokuhlolwa kwesikhathi esizayo angeke ifakwe kwifayela lakho le-rekhodi lezempilo. Noma ikuphi ukushicilelwa mayelana nemiphumela yokuhlolwa kwesikhathi esizayo angeke kusebenzise igama lakho noma kukhombe wena uqobo. Abacwaningi bazokwenza konke ukuvikela ingasese lakho. Kuzokwenziwa konke okusemandleni ukuvikela imininingwane yakho. Kepha, kungangenzeka njalo ukuqinisekisa ubumfihlo futhi kungenzeka kumele sidalule imininingwane yakho uma kudingeka ngenxa:

- Yokujutshwa noma umyalelo wenkantolo;
- Ukuvikela ukuphepha kwanoma ubani noma umphakathi wonkana ; noma
- Ukuvumelana nanoma imuphi umthetho

Amarekhodi akho angabuyekezwa:

- I- South African Medicines Control Council
- I- Medical Research Council of South Africa Ethics Committee
- Abahloli bocwaningo
- Abasebenzi bocwaningo

IMAPHI AMALUNGELO AMI?
Ukuvumela amanembe omzimba wakho ukuthi agcinwe kungukuzinikela okuphelele. Uma unquma ukuthi ukungabi nanoma imaphi amanembe omzimba wakho agcinwe ngaphandle kwalokhu okudingekayo ukuqedela ucwaningo i-SHIOP, usangaqhubeka ube socwaningweni i-SHIOP, futhi amanembe omzimba wakho asalile azolahlwa. Uma unquma manje ukuthi amanembe omzimba wakho angagcinelwa ucwaningo lwesikhatho esizayo, ungawuguqula umqondo wakho nanoma ingasiphi isikhathi. Kodwa ke kumele uxhumane nodokotela wakho wocwaningo noma umhlengikazi ubazise ukuthi awusathandi ukuthi amasampula akho asetshenziselwe ucwaningo lwesikhathi esizayo. Amanembe omzimba wakho azobe engeke esasetshenziswa futhi ayolahlwa.

NGENZENJANI UMA NGINEMIBUZO?
I-South African Medical Research Council Ethics Committee iluvumelile lolucwaningo.

Uma unemibuzo mayelana namalungelo akho ahlobene nokugcinwa kanye nokuhlolwa kwesikhathi esizayo kwamanembe omzimba akho kucwaningo, xhumana nosihlalo we-MRC Ethics Committee ukubika noma iziphi izikhalazo/imibuzo:

Prof. Danie du Toit
MRC Ethics Committee
P.O. Box 19070
Tygerberg, Cape Town
Tel: (021) 938-0687
Fax: 0866 854 023
E-mail: adri.labuschagne@mrc.ac.za

Uma unemibuzo mayelana nokugcinwa kanye nokuhlolwa kwesikhathi esizayo kwamanembe omzimba wakho noma uba nanoma imiphi imibuzo mayelana nesikhungo socwaningo, kumele uxhumane no:

Principal Investigator	Address	Telephone Number
Dr. Makandwe Nyirenda	123 Jan Hofmeyer Rd, Village Market, Westville, 3630 Durban, KwaZulu-Natal, South Africa.	031-242-3671 / 073-806-2660

UKUGCINWA KWAMANEMBE
Sicela ufunde ngokucophelela lezi zitatimende ezingezansi bese ucabanga ngokukhetha kwakho. Noma ngabe ikuphi okunqumayo lokho ngeke kube nomthelela ekubambeni kwakho iqhaza ocwaningweni i-SHIOP noma ukunakekelwa kwakho kwezempilo. Sicela ufake iziqalo magama noma umake okukhethayo futhi usayine noma wenze umaka wakho ngezansi.

☐ NGIYAVUMA ukuba amanembe omzimba kanye nemininingwane yezempilo yami ukuthi kugcinwe futhi kusetshenziswe ocwaningweni lwesikhathi esizayo.

_____
Isishicilelo sombambiqhaza/ Isiqalomagama

NOMA

☐ ANGIVUMI ukuba amanembe omzimba kanye nemininingwane yezempilo yami ukuthi kugcinwe futhi kusetshenziswe ocwaningweni lwesikhathi esizayo.

_____
Isishicilelo sombambiqhaza/ Isiqalo magama

UKUHLOLWA KOFUZO

☐ NGIYAVUMA ukuba amanembe omzimba ami ahlolelwe ufuzo.

_____
Isishicilelo sombambiqhaza/ Isiqalo magama

NOMA

☐ ANGIVUMI ukuba amanembe omzimba ami ahlolelwe ufuzo.

_____
Isishicilelo sombambiqhaza/ Isiqalo magama

IZISHICILELO

Igama Lombambiqhaza (hlukanisa)	Isishicilelo sombambiqhaza	Usuku
Umsebenzi wocwaningo ophethe izingxoxo zemvume (hlukanisa)	Isishicilelo somsebenzi wocwaningo	Usuku
*Igama likafakazi (hlukanisa)	*Isishicilelo sikafakazi	*Usuku

*Igama likafakazi, isishicilelo kanye nosuku kudingeka kulelifomu lemvume, kuphela uma umbambiqhaza onikezela imvume engakwazi ukufunda (engafundile)

APPENDIX 3: INITIAL BREC APPROVAL LETTER

UNIVERSITY OF KWAZULU-NATAL
INYUVESI YAKWAZULU-NATALI

31 August 2017

Ms IB Mpofana (216076315)
Discipline of Medical Microbiology
School of Laboratory Medicine and Medical Sciences
xoli.mpofana@gmail.com

Dear Mpofana

Protocol: Evaluation of the afinion As100 analyser for measuring the levels of C-Reactive protein in an aged population.
Degree: MMedSc
BREC reference number: BE074/17

EXPEDITED APPROVAL

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 13 February 2017.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 03 August 2017 to BREC letter dated 26 April 2017 have been noted by a sub-committee of the Biomedical Research Ethics Committee. The conditions have now been met and the study is given **full ethics approval** and may begin as from 31 August 2017.

This approval is valid for one year from **31 August 2017**. To ensure uninterrupted approval of this study beyond the approval expiry date, an application for recertification must be submitted to BREC on the appropriate BREC form 2-3 months before the expiry date.

Any amendments to this study, unless urgently required to ensure safety of participants, must be approved by BREC prior to implementation.

Your acceptance of this approval denotes your compliance with South African National Research Ethics Guidelines (2015), South African National Good Clinical Practice Guidelines (2006) (if applicable) and with UKZN BREC ethics requirements as contained in the UKZN BREC Terms of Reference and Standard Operating Procedures, all available at http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx.

BREC is registered with the South African National Health Research Ethics Council (REC-290408-009). BREC has US Office for Human Research Protections (OHRP) Federal-wide Assurance (FWA 678).

The sub-committee's decision will be **RATIFIED** by a full Committee at its next meeting taking place on **10 October 2017.**

We wish you well with this study. We would appreciate receiving copies of all publications arising out of this study.

Yours sincerely

Professor V Rambiritch
Deputy Chair: Biomedical Research Ethics Committee

cc postgraduate administrator: konan@ukzn.ac.za
cc: Administrator: konan@ukzn.ac.za

Biomedical Research Ethics Committee
Professor J Tsoka-Gwegweni (Chair)
Westville Campus, Govan Mbeki Building
Postal Address: Private Bag X54001, Durban 4000
Telephone: +27 (0) 31 260 2486 **Facsimile:** +27 (0) 31 260 4609 **Email:** brec@ukzn.ac.za
Website: http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

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APPENDIX 4: RECERTIFICATION ETHICS APPROVAL LETTER

UNIVERSITY OF
KWAZULU-NATAL
INYUVESI
YAKWAZULU-NATALI
RESEARCH OFFICE
Biomedical Research Ethics Administration
Westville Campus, Govan Mbeki Building
Private Bag X 54001
Durban
4000
KwaZulu-Natal, SOUTH AFRICA
Tel: 27 31 2604769 - Fax: 27 31 2604609
Email: BREC@ukzn.ac.za
Website http://research.ukzn.ac.za/Research-Ethics/Biomedical-Research-Ethics.aspx

17 July 2018

Ms IB Mpofana (216076315)
Discipline of Medical Microbiology
School of Laboratory Medicine and Medical Sciences
xoli.mpofana@gmail.com

Dear Mpofana

Protocol: Evaluation of the afinion As100 analyser for measuring the levels of C-Reactive protein in an aged population.
Degree: MMedSc
BREC reference number: BE074/17

RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved:	31 August 2018
Expiration of Ethical Approval:	30 August 2019

I wish to advise you that your application for Recertification received on 21 June 2018 for the above protocol has been **noted and approved** by a sub-committee of the Biomedical Research Ethics Committee (BREC) for another approval period. The start and end dates of this period are indicated above.

If any modifications or adverse events occur in the project before your next scheduled review, you must submit them to BREC for review. Except in emergency situations, no change to the protocol may be implemented until you have received written BREC approval for the change.

The committee will be notified of the above approval at its next meeting to be held on 14 August 2018.

Yours sincerely

Prof V Rambiritch
Chair: Biomedical Research Ethics Committee

cc supervisor: abbain@ukzn.ac.za
cc postgraduate administrator: konar@ukzn.ac.za

APPENDIX 5: MEDICAL RESEARCH COUNCIL ACCESS TO STORED SAMPLES APPROVAL

HIV PREVENTION RESEARCH UNIT

03 August 2017

Biomedical Research Ethics Committee
University of KwaZulu-Natal

RE: Access to stored samples for research project

Dear Committee

This letter serves to confirm that the Research Laboratory based at the South African Medical Research Council, HIV Prevention Research Unit has agreed to provide access to stored samples for the project titled: ***Evaluation of the Afinion AS100 Analyser for measuring the levels of C-Reactive Protein in an aged population*** (PI: Ms I.B. Mpofana, Student no. 216076315).

Should you require any further information please feel free to contact the PI on the parent study: Dr. M. Nyirenda, on 0312423600 or makandwe.nyirenda@mrc.ac.za.

Yours sincerely

Gita Ramjee

Professor Gita Ramjee MSc, PhD, FRCPE
Director, HIV Prevention Research Unit
South African Medical Research Council, Durban
Professor: Department of Epidemiology and Population Health
London School of Hygiene & Tropical Medicine, London UK
Clinical Professor, Department of Global Health, University of Washington, Seattle USA

Tel office: +27 31 242 3631/3635
Mobile: +27 824588453
Email: gita.ramjee@mrc.ac.za
Skype:gitaram70

APPENDIX 6: SAMRC GUIDELINES FOR REQUESTING HUMAN BIOLOGICAL MATERIAL

HIV PREVENTION RESEARCH UNIT

HPRU Central Biorepository

5.5.3. GUIDELINES FOR REQUESTING HUMAN BIOLOGICAL MATERIAL

Below are the guidelines to follow for those requesting Human Biological Material from the South African Medical Research Council HIV Prevention Research Unit (SAMRC HPRU):

Submit concept using the SAMRC HPRU concept form to the HPRU Director

↓

If the concept is approved by the HPRU Director, approval will need to be sought, via the unit director, from the relevant sponsors

↓

The Biorepository team will check the Laboratory Data Management System (LDMS) system to verify that the human biological material /specimens, being requested are still available

↓

The necessary transportation requirements for Human Biological Material to be discussed and finalized by both parties

NOTE:

- The use of all samples must be in line with guidelines for human biological material use, as specified by the sponsor and participant informed consent.
- The mandatory Human Biological Material /Specimen Request Form must be submitted together with the Concept Form
- The human biological material /specimen request, must be for one concept, at a time.
- Human biological material /specimens can only be used for the concept that they have been approved for.
- Before human biological material /specimens can be made available, the following mandatory documents are required:

a. Approved Concept Form – signed by all parties (SAMRC HPRU Director and collaborator)
b. Ethics clearance documentation
c. Import/export permits – if applicable

Document Title	5.5.3. Guidelines for Requesting Human Biological Material
Document Number	BF004
Version Number	01
Effective Date	29 Jul 16
Date of Next Review	29 Jul 17

APPENDIX 7: SAMRC HUMAN BIOLOGICAL MATERIAL(HBM) REQUEST FORM

HIV PREVENTION RESEARCH UNIT

HPRU Central Biorepository

5.4.3. HUMAN BIOLOGICAL MATERIAL(HBM) REQUEST FORM

STUDY	PTID	VISIT CODE	HBM TYPE	HBM VOLUME	TYPE OF ANALYSIS OR TESTING

Document Title	5.4.3. Human Biological Material Request Form
Document Number	BF002
Version Number	01
Effective Date	10 May 16
Date of Next Review	10 May 17

APPENDIX 8: SAMRC HPRU CONCEPT FORM

HIV PREVENTION RESEARCH UNIT

HPRU Central Biorepository

5.4.3. SAMRC HPRU - CONCEPT FORM
REQUEST FOR HUMAN BIOLOGICAL MATERIAL FROM HPRU BIOREPOSITORY

Name of Person submitting the concept: _____

Concept is proposed for:	☐ Abstract for conference submission: *[add specific conference here, REQUIRED]* ☐ Manuscript: *[add target journal here if known]* ☐ Honors Project ☐- Masters Study ☐- PhD Study

1. Title:	
2. Lead author/PI name, qualification and institutional affiliation:	
3. Co-author/ Co-PI name, qualification and institutional affiliation:	
4. Mentor (if applicable):	
5. Study Design:	
6. Sample Size:	
7. List Primary Objectives:	

HPRU Central Biorepository

8. List Secondary Objectives:	
9. What are the research questions? Provide a brief a rational and a brief overview of what the project/paper will demonstrate:	
10. Funding - has funding been secured:	
11. Name of Funder:	
12. Can the project proceed without funding:	
13. Describe proposed statistical analysis:	
14. Proposed timeline for completion of analysis.	

15. For samples required from the SAMRC HPRU BioRepository- please provide information pertaining to the study that the sample was collected in, sample identification number, visit code of sample, sample type, volume, and the testing that will be conducted on the sample. Please complete attached 5.4.3. Human Biological Material Request Form (Example below).

HPRU Central Biorepository

SIGNATURE PAGE

Details of person submitting concept

Signature : _____

Institutional affiliation : _____

Date : _____

Details of SAMRC HPRU Director

Signature : _____

Date : _____

APPENDIX 9: STUDENT SPECIFIC STUDY CHECKLIST

HIV PREVENTION RESEARCH UNIT

HPRU Laboratory

Student Name: _____
Student Project Title: _____
Student's Supervisor Name: _____
Date form completed: _____

The following documents are required to be submitted for the degree SAMRC HPRU support is been provided for:

Item	Status
1. Acceptance/Referral letter :	**to be provided**
2. Protocol :	**received**
3. Supervisor Name :	**Dr Nathlee Abbai , Dr Makandwe Nyirenda**
4. SOPs/forms of test analyte applicable:	**To be provided**
5. Ethics approval :	**received**
6. Student work plan:	**to be provided**
7. total number of Samples required:	**to be provided**
8. total retrieval of samples from LDMS:	**to be provided**
9. template and final Sample storage database:	**to be provided on completion**
10. template and final Results database:	**to be provided on completion**
11. Email communication to HPRU supervisors:	**to be provided**
12. Shipment of samples if required:	**lab name, locate, address, contact info, courier info, budget total**
13. Progress reports to Supervisors and Prof Ramjee/Neetha Morar:	**to be provided**
14. CRFs or forms or questionnaires used in the study:	**to be provided**
15. Budget :	**please confirm costs is correct and student to purchase the kits and place the Affinion**
16. Application for re-certification:	**to be provided if required**
17. list of participants who consented for long term storage and those	**to be provided**

who did not [so we complete sample destruction for SHIOP]	
18. Verification/Validation of the test analyte:	Testing query: please may you follow-up with the student on the following:
a. Pentra 400- 200 tests were ordered and tested for CRP for pentra 400 in 2016 –	**can this results be provided to write up CRP validation**
b. Affinion- 29 kits of CRP test and controls were ordered and tested in 2016-	**can this results be provided to write up CRP validation**
19. Access to lab:	**provide schedule and frequency**
20. Access to freezers:	**provide schedule and frequency**
21. Documents for sample access:	**attached to sign off and submit**
22. Confidentiality agreement:	**student to sign**
23. Monthly reports of progress to Prof Ramjee:	**to be provided**
24. Qualifications: copies of honors degree for staff file	**to be provided**
25. Completion of attached documents to be signed and provided:	**5.5.3 BF004, 5.4.3 BF002, 5.4.3 BF001**
26. Certified copy of degree upon receipt	**To be provided**

Student signature: _____
Date: _____

APPENDIX 10: LABORATORY RESULTS Table CRP TEST RESULTS–SAMPLES 001- 184

CRP test results for older adults, South Africa

PTID	*CRP_Pentra*	*CRP_Afinion*	*Mean CRP*	*Mean difference*
30816253	6.2	5	5.6	0.6
30816254	6.9	7	6.95	-0.05
30816257	12.2	14	13.1	-0.9
30816259	5.1	6	5.55	-0.45
30816260	11.3	14	12.65	-1.35
30816261	35.9	43	39.45	-3.55
30816265	9	9	9	0
30816266	15	17	16	-1
30816267	5.8	8	6.9	-1.1
30816268	14.6	16	15.3	-0.7
30816269	8.4	8	8.2	0.2
30816270	11.7	12	11.85	-0.15
30816271	17.2	22	19.6	-2.4
30816273	22.5	22	22.25	0.25
30816274	27.3	29	28.15	-0.85
30816275	9.9	11	10.45	-0.55
30816279	5.6	6	5.8	-0.2
30816281	9.9	10	9.95	-0.05
30816282	10	10	10	0
30816285	7.7	8	7.85	-0.15
30816289	4.6	6	5.3	-0.7
30816291	5.1	6	5.55	-0.45
30816293	6.5	7	6.75	-0.25
30816294	16.6	21	18.8	-2.2
30816295	25.2	33	29.1	-3.9
30816296	6.1	8	7.05	-0.95
30816297	8.2	11	9.6	-1.4
30816298	7.9	10	8.95	-1.05
30816300	7.2	10	8.6	-1.4
30816301	9.1	11	10.05	-0.95
30816303	5	6	5.5	-0.5
30816305	4.1	5	4.55	-0.45
30816308	8.3	11	9.65	-1.35
30816309	43.8	44	43.9	-0.1
30816310	16	19	17.5	-1.5
30816311	4.9	6	5.45	-0.55
30816312	4.5	5	4.75	-0.25
30816313	17.3	19	18.15	-0.85
30816314	11.1	12	11.55	-0.45
30816319	6	7	6.5	-0.5
30816320	42.5	45	43.75	-1.25

30816323	13.9	14	13.95	-0.05
30816325	41.4	45	43.2	-1.8
30816329	37.6	41	39.3	-1.7
30816331	16.8	18	17.4	-0.6
30816332	7.3	8	7.65	-0.35
30816333	5.8	6	5.9	-0.1
30816336	6.2	6	6.1	0.1
30816339	5.6	6	5.8	-0.2
30816341	14.6	16	15.3	-0.7
30816345	8.4	9	8.7	-0.3
30816348	7.1	7	7.05	0.05
30816350	20.7	24	22.35	-1.65
30816356	7.9	9	8.45	-0.55
30816357	15	17	16	-1
30816358	11.8	11	11.4	0.4
30816359	8.6	9	8.8	-0.2
30816362	5.6	6	5.8	-0.2
30816363	6.3	6	6.15	0.15
30816366	5.3	5	5.15	0.15
30816368	11.4	13	12.2	-0.8
30816369	6.2	6	6.1	0.1
30816370	9.3	10	9.65	-0.35
30816371	7	8	7.5	-0.5
30816372	6.8	7	6.9	-0.1
30816377	7.3	9	8.15	-0.85
30816379	6.2	6	6.1	0.1
30816382	13.7	14	13.85	-0.15
30816383	6.8	6	6.4	0.4
30816387	12.2	13	12.6	-0.4
30816389	6.3	6	6.15	0.15
30816390	23.9	24	23.95	-0.05
30816392	5.3	6	5.65	-0.35
30816394	6.2	7	6.6	-0.4
30816396	15.6	19	17.3	-1.7
30816400	7	8	7.5	-0.5
30816401	35.3	40	37.65	-2.35
30816406	13.3	15	14.15	-0.85
30816407	5.6	6	5.8	-0.2
30816408	9.7	12	10.85	-1.15
30816411	7	10	8.5	-1.5
30816415	23.5	26	24.75	-1.25
30816416	10.4	12	11.2	-0.8
30816417	10.1	11	10.55	-0.45
30816418	5.2	6	5.6	-0.4
30816420	8.6	10	9.3	-0.7

30816421	9.1	9	9.05	0.05
30816422	3	32	17.5	-14.5
30816425	16.4	17	16.7	-0.3
30816427	5.4	5	5.2	0.2
30816429	22.6	25	23.8	-1.2
30816430	7.6	8	7.8	-0.2
30816431	14.3	18	16.15	-1.85
30816432	13.2	15	14.1	-0.9
Total mean	**12.02**	**13.63**		

NB: For samples where CRP_Afinion is greater or equal to five (measuring range of Afinion AS100 analyser).

CRP test results for older adults, South Africa Continued

PTID	*CRP_Pentra*	*CRP_Afinion*	*Mean CRP*	*Mean difference*
30816252	1.9	<5	1.9	0
30816255	2.6	<5	2.6	0
30816258	0.4	<5	0.4	0
30816262	0.5	<5	0.5	0
30816263	3.2	<5	3.2	0
30816264	4.2	<5	4.2	0
30816272	5.3	<5	5.3	0
30816276	3.8	<5	3.8	0
30816277	2.5	<5	2.5	0
30816278	1.3	<5	1.3	0
30816280	2	<5	2	0
30816283	3.4	<5	3.4	0
30816284	2.8	<5	2.8	0
30816286	1.6	<5	1.6	0
30816287	2.1	<5	2.1	0
30816288	3.8	<5	3.8	0
30816290	4.3	<5	4.3	0
30816292	0.7	<5	0.7	0
30816299	1.8	<5	1.8	0
30816302	9.1	<5	9.1	0
30816304	2.3	<5	2.3	0
30816306	3.4	<5	3.4	0
30816307	0.1	<5	0.1	0
30816315	1.3	<5	1.3	0
30816316	1.7	<5	1.7	0
30816317	1.5	<5	1.5	0
30816318	1.2	<5	1.2	0
30816321	1.9	<5	1.9	0
30816322	1.6	<5	1.6	0
30816324	4	<5	4	0
30816326	2.2	<5	2.2	0
30816327	4.4	<5	4.4	0
30816328	1.6	<5	1.6	0
30816330	4.9	<5	4.9	0
30816334	1.1	<5	1.1	0
30816335	0.3	<5	0.3	0
30816337	3.3	<5	3.3	0
30816338	5.3	<5	5.3	0
30816340	3.5	<5	3.5	0
30816342	2.3	<5	2.3	0
30816343	2.2	<5	2.2	0
30816344	1.9	<5	1.9	0
30816346	3.6	<5	3.6	0
30816347	3.8	<5	3.8	0

30816349	1.5	<5	1.5	0
30816351	2.9	<5	2.9	0
30816352	5.4	<5	5.4	0
30816353	2.4	<5	2.4	0
30816354	2.1	<5	2.1	0
30816355	4.4	<5	4.4	0
30816360	1.7	<5	1.7	0
30816361	3.9	<5	3.9	0
30816364	1	<5	1	0
30816365	0.3	<5	0.3	0
30816367	2	<5	2	0
30816373	1.4	<5	1.4	0
30816374	1.8	<5	1.8	0
30816375	1.4	<5	1.4	0
30816376	3.3	<5	3.3	0
30816378	2.1	<5	2.1	0
30816380	2.3	<5	2.3	0
30816381	5	<5	5	0
30816384	4	<5	4	0
30816385	0.3	<5	0.3	0
30816386	4.1	<5	4.1	0
30816388	4.7	<5	4.7	0
30816391	4.5	<5	4.5	0
30816393	0.7	<5	0.7	0
30816395	3.4	<5	3.4	0
30816397	0.5	<5	0.5	0
30816398	1.6	<5	1.6	0
30816399	2.1	<5	2.1	0
30816402	3.1	<5	3.1	0
30816403	2.5	<5	2.5	0
30816404	3.9	<5	3.9	0
30816405	3.1	<5	3.1	0
30816409	0.6	<5	0.6	0
30816410	1.3	<5	1.3	0
30816412	4.1	<5	4.1	0
30816413	4.8	<5	4.8	0
30816414	0.8	<5	0.8	0
30816419	2.4	<5	2.4	0
30816423	2.2	<5	2.2	0
30816424	1	<5	1	0
30816426	4.2	<5	4.2	0
30816428	4.9	<5	4.9	0
30816433	4	<5	4	0
30816434	3	<5	3	0
30816435	4.5	<5	4.5	0
Total mean	**2.67**	**NA**		

APPENDIX 11: QUESTIONAIRE FROM PARENT STUDY

HIV PREVENTION RESEARCH UNIT, MRC

A Study to investigate Sexual health, HIV and co-morbidity with non-communicable infections among Older Persons (SHIOP)

Questionnaire guide – Version 1.2

Participant Id: ☐☐☐☐☐☐☐☐ **Visit Date:** D D / M M M / Y Y

Section 1: Respondent and household characteristics

I will start by asking you some general questions about yourself and your household.

101	Sex *(Tick only one)*	Male ○ Female ○ *(Tick only one)*
102	What is your ethnic group? *(Tick only one)*	☐ African ☐ Indian ☐ Coloured ☐ White ☐ Other, specify:_____
103	Date of birth	Date of birth D D / M M M / Y Y
104	*If complete date of birth is not available:* How old are you in completed years?	Age (years) ☐☐
105	What is your religion? *(Tick only one)*	Christian ○ Hindu ○ Muslim ○ Jewish ○ *None* ○ Other, specify:_____
106	What is the highest level of education you attained? *(Tick only one)*	Never been to school ○ Primary incomplete ○ Primary completed ○ Secondary incomplete ○ Secondary completed ○ Tertiary ○ Don't know ○
107	Are you currently in employment?	Yes, Full time ○ Yes, Part time ○ Self-employed ○ Retired ○ No ○
108	Do you own the house you live in?	Yes ○ No ○
109	Who is the head of your household? *(Tick only one)*	Self ○ Spouse ○ Sibling ○ Own child ○ Grandchild ○ Don't know ○ Other, specify _____
110	How many rooms are in your house?	Rooms ☐☐
111	What is the main source of drinking water for members of your household? *(Tick only one)*	Piped - in yard ○ Piped - public tap ○ Borehole/Well ○ Rainwater ○ Neighbour's tap ○ Flowing river/stream ○ Dam/Stagnant water ○ Other, specify _____
112	Is your house connected to electricity?	Yes ○ No ○
113	[Please tell me] what is your household's main source of income, by that I mean from which source does most of the money used in your household come from? *(Tick All that apply)*	Self-employed, odd jobs, selling or trading ○ Wages/salary from formal employment ○ Government grants ○ Income from rental property ○ Retirement fund ○ No source of income ○ Other, specify _____

Section 2: Tobacco use

Now I would like to ask you some questions about tobacco use.

201	Do you **currently** smoke any tobacco products, such as cigarettes, cigars or pipes? *Probe for frequency*	Yes, daily ○ Yes, but not daily ○ No, quit ○ Never smoked ○	*If 'No' skip to Q208.* *If 'Never' skip to Q209.*
202	Do you **currently** smoke cigarettes, cigars or pipes **on a daily basis**?	Yes ○ No ○	
203	On average, how many cigarettes, cigars or pipes do you smoke each day or week?	Daily ☐☐ Weekly ☐☐	
204	In the last 12 months, have you been advised by a doctor or health worker to quit smoking?	Yes ○ No ○	
205	In the last 12 months, have you tried on your own to quit smoking?	Yes ○ No ○	

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206	***Skip Q206 if Q201 is 'Never smoked'*** How old were you when you **first started** to smoke cigarettes, cigars or pipes?	Age (years) ☐☐ Don't know ○
207	In the past, did you ever smoke cigarettes, cigars or pipes **on a daily basis**?	Yes ○ No ○
208	***Skip Q208 if Q201 is 'Yes, ...' or 'Never smoked'*** If **quit smoking**, how old were you when you **stopped smoking**?	Age (years) ☐☐ Don't know ○
209	Do you **currently use** any **smokeless tobacco** products such as snuff, snus, or chewing tobacco?	Yes, daily ○ No, quit ○ ***If 'No' skip to Q210.*** Yes, but not daily ○ Never used ○ ***If 'Never' skip to Q211.***
210	***Skip Q20810 if Q209 is 'Yes, ...' or 'Never smoked'*** If **quit using smokeless tobacco**, how old were you when you **stopped using smokeless tobacco**?	Age (years) ☐☐ Don't know ○
211	In the **last 30 days**, did you live with anyone in your house who smoked?	Yes ○ No ○ Don't know ○
212	Do you **currently** use any homemade or grown smoking products such as *whoonga, dagga, rock, sugars, tash*?	Yes, daily ○ No, quit ○ Yes, but not daily ○ Never used ○

Section 3: Alcohol consumption

Now I would like to ask you some questions about alcohol consumption.

301	Do you consume any alcoholic drinks such as beer, wine, spirits or ciders? ***Probe for frequency. If 'Never' skip to Q306***	Yes, daily ○ No, quit ○ Yes, but not daily ○ Never ○
302	In the **last 12 months**, have you consumed any alcoholic drink?	Yes ○ No ○ ***If 'No' skip to Q306***
303	In the **last 12 months**, how often have you had at least one **standard alcoholic drink**? ***Show card*** ***Read responses and tick only one***	Daily ○ 5-6 days per week ○ 3-4 days per week ○ 1-2 days per week ○ 1-3 days per month ○ Less than once per month ○
304	In the **last 30 days**, how often have you had at least one **standard alcoholic drink**? ***Read responses and tick only one***	Daily ○ 5-6 days per week ○ 3-4 days per week ○ 1-2 days per week ○ None ○ ***If 'None' skip to Q306***
305	During the **last 7 days**, how many standard drinks did you have each day? **Show card**	☐☐ Monday ☐☐ Tuesday ☐☐ Wednesday ☐☐ Thursday ☐☐ Friday ☐☐ Saturday ☐☐ Sunday
306	During the **last 7 days**, did you consume any homebrewed or traditional alcohol?	Yes ○ No ○ ***If 'No' skip to Q308***
307	In the **last 7 days**, on average how many standard drinks of homebrewed or traditional alcohol did you drink?	Number ☐☐ Don't know ○
308	In the **last 12 months**, have you been advised by a doctor or health worker to **stop drinking**?	Yes ○ No ○ N/A ○
309	In the **last 12 months**, have you ever tried on your own to **stop drinking**?	Yes ○ No ○ N/A ○

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Section 4: Diet and nutrition

Now I would like to ask you some questions about the consumption of fruits, vegetables and salt in your diet.

401	In a typical week, on **how many days** do you eat fruit?	Number of days __ Don't know ○
402	On average, how many **servings** of fruit did you eat on each of those days?	Number of servings __ Don't know ○
403	During the **last 7 days**, how many fruits did you have each day? ***Read out each day and enter number of fruits eaten. If none eaten enter 00***	__ Monday __ Tuesday __ Wednesday __ Thursday __ Friday __ Saturday __ Sunday
404	In a typical week, on **how many days** do you eat vegetables?	Number (days) __ Don't know ○
405	On average, how many **servings** of vegetables did you eat on each of those days?	Number (servings) __ Don't know ○
	With the next questions, we would like to learn more about salt in your diet. Dietary salt includes ordinary table salt, unrefined salt such as sea salt, iodized salt, salty stock cubes and powders, and salty sauces such as, mayonnaise, tomato sauce, sweet chilli sauce, soy sauce or fish sauce. The following questions are on adding salt to the food right before you eat it, on how food is prepared in your home, on eating processed foods that are high in salt such as chips, nandos, KFC, and questions on controlling your salt intake. Please answer the questions even if you consider yourself to eat a diet low in salt	
406	How often do you add salt or a salty sauce such as mayonnaise, tomato, fish or sweet chilli sauce or fish sauce to your food right before you eat it?	Always ○ Often ○ Sometimes ○ Rarely ○ Never ○ Don't know ○
407	How often is salt, salty seasoning or a salty sauce added in cooking or preparing foods in your household?	Always ○ Often ○ Sometimes ○ Rarely ○ Never ○ Don't know ○
408	How often do you eat processed food high in salt? ***Foods such as cheese, bacon, packaged snacks (chips, salted nuts), or canned food such as pickles, chakalaka, fruits, meat or take-away food.***	Always ○ Often ○ Sometimes ○ Rarely ○ Never ○ Don't know ○
409	How much salt do you think you consume?	Far too much ○ Too much ○ Just the right amount ○ Too little ○ Far too little ○ Don't know ○
410	How important to you is lowering the salt in your diet?	Very important ○ Somewhat important ○ Not at all ○ Don't know ○
411	Do you think that too much salt or salty sauce in your diet could cause a health problem?	Yes ○ No ○
412	On average, how many times per week do you eat food from restaurants?	Number __ Don't know ○

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Section 5: Physical activity

Now I would like to ask you some questions about the time you spend doing physical activities during a typical week. Please answer these questions even if you do not consider yourself as a physically active person.

501	Does your daily routine involve physical activity like carrying or lifting heavy loads, gardening, street cleaning, or construction work for at least 10 minutes?	Yes ○ No ○ Don't know ○
502	In a typical week, on how many days do you do physical activities like carrying or lifting heavy loads, gardening, street cleaning, or construction work? (*Vigorous activities require hard physical effort and cause large increases in breathing or heart rate*)	Number ☐☐ Don't know ○
503	On average, in a typical week on **how many days** do you go for a walk, run or ride a bicycle? ***If none enter 00, then Skip Q504***	Number ☐☐ Don't know ○
504	How much time do you usually spend walking, running or riding a bicycle on a typical day?	Hours : minutes ☐☐ : ☐☐ hrs mins
	With the next questions, we would like to learn more about leisure physical activity	
505	Do you do any vigorous sports, fitness or recreational (leisure) activities like running, swimming, bowling, playing football, or cricket for at least 10 minutes that cause large increases in breathing or heart rate? ***If 'Never' or 'Don't know' Skip to Q508***	Always ○ Often ○ Sometimes ○ Rarely ○ Never ○ Don't know ○
506	In a typical week, on how many days do you do vigorous sports, fitness or recreational (leisure) activities?	Number ☐☐ Don't know ○
507	How much time do you spend doing vigorous sports, fitness or recreational activities on a typical day?	Hours : minutes ☐☐ : ☐☐ hrs mins
508	How much time do you usually spend sitting or reclining on a typical day?	Hours : minutes ☐☐ : ☐☐ hrs mins

Section 6: General health status and health care utilisation

I will now ask you a few questions about your general health status and health care utilisation.

601	In general, how would you rate your health today? **Read responses**	Very Good ○ Good ○ Moderate ○ Bad ○ Very Bad ○
602	Overall, in the last 30 days/month, how much difficulty did you have with work or household activities? **Read responses**	None ○ Mild ○ Moderate ○ Severe ○ Extreme/cannot do ○
603	How was your health during the last two weeks? **Read responses**	Very Good ○ Good ○ Moderate ○ Bad ○ Very Bad ○
604	How would you rate your overall quality of life? **Read responses**	Very Good ○ Good ○ Moderate ○ Bad ○ Very Bad ○
605	When was the last time that you felt sick or needed to consult someone about your health?	Weeks ago ☐☐ Months ago ☐☐ Years ago ☐☐ Never ○ ***If 'Never' skip to Q613***
606	What sickness did you have or the main reason you needed to consult someone about your health? ****See options. Specify only one (main reason).***	Sickness code ☐☐ **1=** communicable diseases, infections, malaria, infection TB, HIV; **2=** nutritional deficiencies **3=** acute conditions, (diarrhoea, flu, headaches, fever, cough and others); **4=** injury; **5=** surgery; **6=** sleep problem; **7=** occupational /work related condition/injury; **8=** chronic pain in joints/arthritis (joints, back, neck); **9=** diabetes or related complications; **10=** problems with heart including unexplained pain in chest; **11=** problems with mouth, teeth, swallowing; **12=** problems with breathing; **13=** high blood pressure, hypertension; **14=** stroke/ sudden paralysis of one side of body; **15=** generalized pain(stomach, muscle or other nonspecific pain); **16=** depression, anxiety; **17=** cancer; **87=** Other, specify

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607	The last time you felt sick or needed to consult someone about your health, did you get the needed health care?	Yes ○ No ○ *If 'Yes' skip to Q609*
608	Which reason(s) best explains why you did not get the needed health care? ***Tick all that apply***	Had no transport available/could not afford cost ☐ You did not know where to go ☐ You tried but were denied health care ☐ You were previously badly treated ☐ Had work or other commitments ☐ You thought you were not sick enough ☐ Drugs from health centre not effective ☐ Could not afford medical fees ☐ *Other, specify:*_____
609	Where did you go first? ***Tick only one*** ***Skip Q609 if Q607 is 'No'***	Traditional healer/herbalist/shrine ○ Pharmacy/chemist/shop ○ Government clinic/health centre ○ Government hospital ○ Private clinic ○ Private hospital ○ Others Specify
610	What signs of illness did you experience in the last two weeks? ***Tick all that respondent mentions then read the others and tick all that apply***	Bed sores ☐ Constipation ☐ Bowel/bladder control loss ☐ Fatigue ☐ Dehydration ☐ Appetite loss ☐ Bodily pain ☐ Painful wounds ☐ Not able to sleep ☐ Mouth & throat discomfort ☐ Cough/chest pain ☐ Memory loss/forgetfulness ☐ Dry/Itchy skin ☐ Nausea & vomiting ☐ None ☐ Others Specify
611	For those symptoms, what did you do….? ***Tick all that apply (Skip if Q610 is "None")***	Visited government clinic ☐ Visited private clinic ☐ Used own herbal medicine ☐ Saw a traditional healer/ herbalist ☐ Took medicine (self-treatment) ☐ isited the Pharmacy/chemist/shop ☐ Admitted to a government hospital ☐ Admitted to a private hospital ☐ Did nothing about the symptoms ☐ Other Specify
612	Overall, are you satisfied with the services at health facilities?	Satisfied ○ Somewhat satisfied ○ Dissatisfied ○
613	Do you ever go to traditional healers for treatment?	Yes ○ Never goes to traditional healer ○ *If 'Never' go to Q615*
614	What are the reason(s) that you go to traditional healers for? ***Tick all that apply***	Traditional healers allow you to pay in goods ☐ Closer distance to homestead ☐ Traditional healers will wait for your payment ☐ Traditional healers are cheaper ☐ Traditional healers give better treatment ☐ Other Specify _____

Chronic conditions history

Now I would like to ask you some questions about some diseases common in older adults

615	Have you ever been told by a doctor or other health worker that you have ……?	**HEART DISEASE**	Yes ○	No ○		
		ARTHRITIS	Yes ○	No ○		
		HYPERTENSION	Yes ○	No ○		
		DIABETES	Yes ○	No ○		
		RAISED CHOLESTEROL	Yes ○	No ○		
616	How long ago were you told you have……?	**HEART DISEASE**	0-12 months ○	>12 months ○	Don't know ○	NA ○
		ARTHRITIS	0-12 months ○	>12 months ○	Don't know ○	NA ○
		HYPERTENSION	0-12 months ○	>12 months ○	Don't know ○	NA ○
		DIABETES	0-12 months ○	>12 months ○	Don't know ○	NA ○
		RAISED CHOLESTEROL	0-12 months ○	>12 months ○	Don't know ○	NA ○
617	In the past two weeks, have you taken any medication prescribed by a doctor or other health worker for …….?	**HEART DISEASE**	Yes ○	No ○	NA ○	
		ARTHRITIS	Yes ○	No ○	NA ○	

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		HYPERTENSION	Yes ○	No ○	NA ○
		DIABETES	Yes ○	No ○	NA ○
		RAISED CHOLESTEROL	Yes ○	No ○	NA ○
618	Have you ever seen a traditional healer for .....?	HEART DISEASE	Yes ○	No ○	NA ○
		ARTHRITIS	Yes ○	No ○	NA ○
		HYPERTENSION	Yes ○	No ○	NA ○
		DIABETES	Yes ○	No ○	NA ○
		RAISED CHOLESTEROL	Yes ○	No ○	NA ○
619	Are you currently taking any herbal or traditional remedy for .....?	HEART DISEASE	Yes ○	No ○	NA ○
		ARTHRITIS	Yes ○	No ○	NA ○
		HYPERTENSION	Yes ○	No ○	NA ○
		DIABETES	Yes ○	No ○	NA ○
		RAISED CHOLESTEROL	Yes ○	No ○	NA ○
620	Are you currently taking insulin for diabetes prescribed by a doctor or other health worker?		Yes ○	No ○	Don't know ○
621	Have you ever had a heart attack or chest pain from heart disease (angina) or a stroke (cerebrovascular accident or incident)?		Yes ○	No ○	Don't know ○
622	Are you currently taking any medication regularly to prevent or treat heart disease? ***(such as Aspirin, Lovastatin, Simvastatin, Atorvastatin or other statin)***		Yes ○	No ○	Don't know ○
623	Have you ever been told by a doctor or other health worker that you have arthritis (joint pain, swelling, stiffness or inflammation)?		Yes ○	No ○	Don't know ○

Mental health status

Now I would like to ask you some questions about feelings of sadness or depression

624	Have you ever been diagnosed with depression?	Yes ○ No ○
625	During **the last 2 weeks** have you been taking any medications or other treatment for depression? (Other treatment can include attending therapy or counselling sessions.)	Yes ○ No ○
626	During the last 12 months, have you had a period lasting several days when you felt **sad, empty or depressed**?	Yes ○ No ○
627	During the last 12 months, have you had a period lasting several days when you **lost interest** in most things you usually enjoy doing such as personal relationships, work or hobbies/recreation?	Yes ○ No ○
628	During the last 12 months, have you had a period lasting several days when you have been feeling your **energy decreased** or that you **are tired all the time**?	Yes ○ No ○
	INTERVIEWER: IF ANY ONE OF Q626, Q627 OR Q628 IS "YES", CONTINUE TO Q629. IF ALL 3 ARE "NO", GO TO Q701	
629	Was this period [of sadness/loss of interest/low energy] for more than 2 weeks?	Yes ○ No ○
630	Was this period [of sadness/loss of interest/low energy] most of the day, nearly every day?	Yes ○ No ○
631	During this period, did you lose your appetite?	Yes ○ No ○
632	Did you notice any slowing down in your thinking?	Yes ○ No ○
633	Did you notice any problems falling asleep?	Yes ○ No ○
634	Did you notice any problems waking up too early?	Yes ○ No ○
635	During this period, did you have any difficulties concentrating; for example, listening to others, working, watching TV, listening to the radio?	Yes ○ No ○
636	Did you notice any slowing down in your moving around?	Yes ○ No ○
637	During this period, did you feel anxious and worried most days?	Yes ○ No ○
638	During this period, were you so restless or jittery nearly every day that you paced up and down and couldn't sit still?	Yes ○ No ○
639	During this period, did you feel negative about yourself or like you had lost confidence?	Yes ○ No ○
640	Did you frequently feel hopeless - that there was no way to improve things?	Yes ○ No ○
641	During this period, did your interest in sex decrease?	Yes ○ No ○
642	Did you think of death, or wish you were dead?	Yes ○ No ○
643	During this period, did you ever try to end your life?	Yes ○ No ○

Participant Id: _____ **Visit Date:** D D / M M M / Y Y

Section 9: Anthropometric measurements & clinical screening

Now we would like to ask you to participate in a few tests to determine your health status. We would like to measure a few things, like your blood pressure, your weight, height and STI screening. We will start with taking your blood pressure

Ask the respondent to release the arm and relax.

901	**Time 1:** Systolic ___	Diastolic ___	Pulse rate ___
	Ask the respondent to release the arm and relax. Wait for one minute before time 2. Do not ask the respondent questions.		
902	**Time 2:** Systolic ___	Diastolic ___	Pulse rate ___
	Again, remind the respondent to relax and wait. Wait for one minute before time 3. Do not ask the respondent questions.		
903	**Time 3:** Systolic ___	Diastolic ___	Pulse rate ___

904	***Interviewer: Can respondent stand up?***	Yes ○ No ○
I would now like to measure how tall you are. To measure your height I need you to please take off your shoes. Put your feet and heels close together, stand straight and look forward standing with your back, head and heels touching the wall. Look straight ahead		
905	Measured height in centimetres	Height ___ Not able to measure ○ Refused ○
Now we want to measure your weight – could you please keep your shoes off and step on the scale.		
906	Measured weight in kilograms	Weight ___.___ Not able to measure ○ Refused ○

Specimen collection

	Specimen type	Qty	Collection date
907	Whole blood	4ml EDTA	D D / M M M / Y Y
908	Whole blood	5ml SST	D D / M M M / Y Y
909	Urine	15-60ml	D D / M M M / Y Y

Participant Id: ☐☐☐☐☐☐☐☐ **Visit Date:** D D / M M M / Y Y

Section 7: Sexual behaviour and partnership patterns

Now I would like to ask you some questions about sexual relationships and behaviours. Some of the questions may be very sensitive, but please answer as honestly as you can to help us understand challenges older adults like you may face with regard to Sexual health and HIV.

701	What is your **current** marital status? ***(Tick only one)*** ***If Q701 is 'Never married' skip to Q704***	Never been married ○ Married ○ Co-habiting ○ Separated/Divorced ○ Widowed ○ Don't know ○ Other, specify_____
702	**If married**, are you currently living with your spouse?	Yes ○ No ○
703	How old were you when you first married?	Age ☐☐ Don't know ○ Age range (if cannot remember precisely) <15 ○ 15-19 ○ 20-24 ○ 25+ ○
704	Are you currently sexually active?	Yes ○ No ○ ***If 'Yes' skip Q706***
705	**If not currently sexually active**, why not? ***Tick all that apply, then skip to Q709***	Too old for that ☐ Cannot find a suitable partner ☐ Fear to contract HIV or STIs ☐ Unable to perform due to health reasons, specify:_____ Other, specify:_____
706	In the **last 12 months**, how many different sexual partners have you had?	Number ☐☐ None ○ Prefers not to say ○ Don't know ○
707	What was your relationship to your most recent sexual partner? ***(Tick only one)***	Spouse ○ Current regular partner ○ Previous spouse/regular partner ○ Casual partner ○ *Other, specify:*_____
708	How old were you when you first had sexual activity?	Age ☐☐ Don't know ○ Age range (if cannot remember precisely) <15 ○ 15-19 ○ 20-24 ○ 25+ ○
709	How many sexual partners have you ever had in your life (including current partner if any)?	Number (partners) ☐☐ Don't know ○ Prefers not to say ○
710	When was the last time you had sexual activity? ***(Enter number of weeks, months or years ago)***	Weeks ago ☐☐ Months ago ☐☐ Years ago ☐☐ Days ago ☐☐ Prefers not to say ○
711	Have you ever used condoms?	Yes ○ No ○ ***If 'No skip Q713***
712	Did you use condoms with your most recent partner?	Yes ○ No ○
713	Have you ever consulted with a health care worker (nurse/ doctor/counsellor) about your sexual health or needs?	Yes ○ No ○ Prefers not to say ○ ***If 'No' skip to Q716***
714	When was the last time you consulted a health care worker about your sexual health or needs?	Weeks ago ☐☐ Months ago ☐☐ Years ago ☐☐ Never ○
715	When was the last time you had a disease that can be transmitted through sex?	Weeks ago ☐☐ Months ago ☐☐ Years ago ☐☐ Never ○ ***If 'Never' skip to Q717***
716	Have you ever been treated by a health care worker for a disease that can be transmitted through sex?	Yes ○ No ○
717	Have you ever consulted with a traditional healer or herbalist about your sexual health or needs?	Yes ○ No ○ Prefers not to say ○ ***If 'No' skip to Q719***
718	When was the last time you consulted a traditional healer or herbalist about your sexual health or needs?	Weeks ago ☐☐ Months ago ☐☐ Years ago ☐☐
719	Have you ever been treated by a traditional healer or herbalist for a disease that can be transmitted through sex?	Yes ○ No ○ ***If 'No' skip to Q801***
720	How many times have you been treated by a traditional healer or herbalist for a disease that can be transmitted through sex in your life?	Number of times ☐☐

Participant Id: | | | | | | | | **Visit Date:** D D / M M M / Y Y

Section 8: HIV knowledge and attitudes

Now I would like to ask you some questions about your knowledge about HIV.

801	Have you ever heard about HIV	Yes ○ No ○ Don't know ○ ***If 'No' or 'don't know' skip to Q807***
802	What are the ways a person could get HIV infection? ***Read out and tick for each item***	Through sex with an HIV infected person Yes ○ No ○ Through touching an HIV infected person Yes ○ No ○ Through kissing an HIV infected person Yes ○ No ○ Through blood transfusion Yes ○ No ○ Through mosquito bites Yes ○ No ○ Through injection with a contaminated needle Yes ○ No ○ Through sharing utensils with an HIV infected person Yes ○ No ○ From HIV infected mother to child Yes ○ No ○ *Other, specify:*_____
803	Have you ever been tested for HIV?	Yes ○ No ○ Prefers not to say ○
804	Do you know your HIV status?	Yes ○ No ○ Prefers not to say ○ ***If 'No' or 'Prefers not to say' skip to Q806***
805	Do you know your sexual partner's HIV status?	Yes ○ No ○ Prefers not to say ○ NA ○ ***Not applicable if Q704 is 'No'***
806	Are a lot of people in your community at risk of becoming HIV infected?	Yes ○ No ○ Don't know ○
807	Are older adults aged 50 years and above also at risk of getting HIV?	Strongly agree ○ Agree ○ Neither agree or disagree ○ Disagree ○ Strongly disagree ○
808	How would you rate your risk of HIV?	Not at risk ○ At low risk ○ At High risk ○ Don't know ○
809	What can you do to protect yourself from getting HIV during sexual activity?	Do nothing ○ Use condoms ○ Use herbal remedies/immune boosters ○ Seek spiritual intervention ○ *Other, specify:* ○ _____
810	Is there anything that has happened to you in the past that may have put you at risk of becoming HIV infected?	Yes ○ No ○ Don't know ○ Prefers not to say ○
811	Are you currently in a situation where you may be at risk of becoming HIV infected?	Yes ○ No ○ Don't know ○ Prefers not to say ○
812	If you were to become infected with HIV, do you think you would be able to get help and acceptance within your community?	Yes ○ No ○ Don't know ○ Prefers not to say ○
813	Do older adults in your age group know where to go for help or information on HIV infection?	Yes ○ No ○ Don't know ○ Prefers not to say ○
814	Do you know of anyone **in your community** who has died or is living with HIV infection?	Yes ○ No ○ Don't know ○ Prefers not to say ○
815	Do you know of anyone **in your family** who has died of or is living with HIV infection?	Yes ○ No ○ Don't know ○ Prefers not to say ○

APPENDIX 12: AFINION AS100 INTERNAL QUALITY CONTROL FORM

HIV PREVENTION RESEARCH UNIT

HPRU Central Routine Laboratory

5.6.2 AFINION AS100 INTERNAL QUALITY CONTROL

Month / Year:_____Afinion Serial Number: _____

Test Kit name_____Kit lot number: _____Kit expiry date: _____

Date Tested	Control 1 Lot Number	Control 1 expiry date	Within acceptable range (Yes/No)	Comments on control out of range	Control 2 Lot Number	Control 2 expiry date	Within acceptable range (Yes/No)	Comments on control out of range	TESTED BY (INITIALS)

Document Title	5.6.2. Afinion AS100 Internal Quality control Form
Document Number	F002
Version Number	01
Effective Date	01 Sep 16
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