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**Spatial analysis of HIV infections in high burden sub-districts in  
KwaZulu-Natal, South Africa**

By

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*BSc Biomedical Sciences, BMedScHons (UKZN)*

**Submitted in partial fulfilment of the requirements for the degree of  
Master of Medical Sciences  
in  
Medical Microbiology  
School of Laboratory Medicine and Medical Sciences  
College of Health Sciences  
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## Preface

This study represents original work by the author and has not been submitted in any other form to another University. Where use was made of the work of others, it has been duly acknowledged in the text.

The research described in this dissertation was carried out in the Centre for the AIDS Programme of Research in South Africa (CAPRISA), College of Health Sciences School of Laboratory Medicine and Medical Sciences, University of KwaZulu-Natal, Durban, South Africa, under the supervision of Professor Ayesha B.M. Kharsany and Professor Frank Tanser.



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## Declaration: Plagiarism

I, **Usangiphile Evile Buthelezi** declare that:

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## **Publications and Conference presentations**

The publications (published or submitted) that form part of this dissertation and the contribution I made to each of the manuscripts are presented below.

**Publication 1:** Usangiphile E Buthelezi, Candace L Davidson & Ayesha BM Kharsany (2016) Strengthening HIV surveillance: measurements to track the epidemic in real time, African Journal of AIDS Research, 15:2, 89-98, DOI: 10.2989/16085906.2016.1196223.

Authors contributions:

I and my supervisor (Professor Ayesha B.M Kharsany) and the co-author Ms Candace Davidson conceptualised the idea and I performed the literature review and the co-authors reviewed the manuscript.

### **Conference presentations:**

Usangiphile Evile Buthelezi<sup>1</sup>, Ayesha BM Kharsany, Cherie Cawood, David Khanyile, Anneke Grobler, Candace Davidson, Lorna Madurai, Tulio de Oliveira, Sabelo Ntuli and Frank Tanser (2016): **Spatial analysis of HIV infections in high burden sub-districts in KwaZulu-Natal, South Africa.**

- Poster presentation at the 21<sup>st</sup> International AIDS conference, Durban, South Africa (17-22 July 2016). Abstract number: TUPEC223.

Signed:



Date: 14 December 2017

## **Dedication**

This work is dedicated to my family, especially my mother Mrs E.R.K. Buthelezi and my late father Mr A.N. Buthelezi.

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## **List of abbreviations**

<b>AGYW</b>	Adolescent Girls and Young Women
<b>AIDS</b>	Acquired Immunodeficiency Syndrome
<b>ART</b>	Antiretroviral Therapy
<b>CI</b>	Confidence Interval
<b>EAs</b>	Enumeration Areas
<b>ELISA</b>	Enzyme-Linked Immunosorbent Assay
<b>GIS</b>	Geographic Information System
<b>GPS</b>	Global Positioning System
<b>HCT</b>	HIV Counselling and Testing
<b>HIPSS</b>	HIV Incidence Provincial Surveillance System
<b>HIV</b>	Human Immunodeficiency Virus
<b>HSV-2</b>	Herpes Simplex Virus type 2
<b>LLR</b>	Log Likelihood Ratio
<b>MAT</b>	Medically Assisted Therapy
<b>MMC</b>	Medical Male Circumcision
<b>MSM</b>	Men who have Sex with Men
<b>NSP</b>	Needle and Syringe Programs
<b>PDA</b>	Personal Digital Assistant
<b>PHC</b>	Public Health Clinic
<b>PMTCT</b>	Prevention of Mother to Child Transmission
<b>PWID</b>	People Who Inject Drugs
<b>PrEP</b>	Pre-Exposure Prophylaxis
<b>RNA</b>	Ribonucleic Acid
<b>SaTScan</b>	Spatial Scan Statistics
<b>STIs</b>	Sexually Transmitted Infections
<b>SHR</b>	Sexual Reproductive Health
<b>SSA</b>	sub-Saharan Africa
<b>SW</b>	Sex Workers
<b>TB</b>	Tuberculosis
<b>TRIs</b>	Test for Recent Infection
<b>UNAIDS</b>	Joint United Nations Programme on HIV/AIDS
<b>WHO</b>	World Health Organisation

## Abstract

**Background:** Substantial spatial variations in HIV prevalence and incidence at a global, national and district levels have been shown to occur. However, only a few studies have assessed variability of these infections at a highly localised level.

**Aim:** The aim of the study was to assess the spatial variability of HIV prevalence and HIV-1 RNA viral load in two areas within the uMgungundlovu district, KwaZulu-Natal, South Africa.

**Methods:** The data source for this study was from the HIV Incidence Provincial Surveillance System (HIPSS), a multi-stage random sampling of enumeration areas (EAs), households and individuals. From June 2014 to June 2015, HIPSS enrolled 9812 household-representative sample of men and women aged 15-49 years from 221 of the 591 randomly selected EAs. Briefly, the randomly selected households were identified through the global positioning system (GPS) co-ordinates. The head or designate of the selected household was provided with detailed study information, followed by verbal consent, collection of basic sociodemographic information and listing of household members. A single age eligible individual was randomly selected, provided with detailed study information, followed by written informed consent and or assent and enrolled. A questionnaire was administered to obtain demographic, psycho-social and behavioural information, biological samples for laboratory tests and GPS co-ordinates for the household were collected for each enrolled participant at the time of the interview. HIV prevalence, geometric mean viral load and prevalence of viraemia >1000 copies/ml were calculated and mapped per municipal ward using ArcGIS software version 10.3 (ESRI, USA). Micro-geographical cluster detection of HIV prevalence and prevalence of viraemia were performed using Kulldorff spatial scan statistic (SaTScan) at a significance level of  $p < 0.05$ .

**Results:** Based on the HIV viral load, the overall geometric mean viral load for individuals in the study area was 202 copies /ml, in men it was 735 copies/ml and in women it, was 130 copies /ml. In the south-east of the study area, two high viral load clusters were identified. The first cluster accounted for the overall population and the geometric mean viral load for this cluster was 327 copies/ml. The geometric mean viral load for individuals in the population outside of the high viral load cluster was 125 copies/ml resulting in a geometric mean viral load difference of 202 copies/ml (Log-likelihood ratio =18.95,  $p=0.001$ ). The second-high viral load cluster accounted for women and the geometric mean viral load for this cluster was 237 copies/ml. The geometric mean viral load for women outside the high viral load cluster was 79 copies/ml and the geometric mean viral load difference was 158 copies/ml (Log-likelihood ratio =18.99,  $p=0.001$ ). Both the high viral load clusters occurred to the south-east of the study area representing a peri-urban setting. A further analysis of the viral load at a threshold of >1000 copies/ml showed that viral load >1000 copies /ml exceeded 50% in 11 of the 30

municipal wards, and 10 of the 11 wards were located within urban areas. A single cluster was identified and was in the north-west of the study area and approximately over a three kilometre radius having a relative risk of 0.69 ( $p=0.02$ ). A total of 309 HIV positive individuals contributed to this cluster indicating that 69% or 213 individuals had viral load of <1000 copies/ml, whilst 31% or 96 individuals had viral load >1000 copies/ml. Based on the HIV prevalence analysis, a high-prevalence cluster with a relative risk of 1.75 ( $p=0.02$ ) was identified in the same north-west area and the HIV prevalence in this area was 71%. Overall men compared to women had higher  $\log_{10}$  mean viral load ( $\text{Log}_{10}$  mean viral load 3.21 vs 2.62;  $p<0.001$ ) and higher in the age category 15-24 years compared to 25-49 years, ( $\text{Log}_{10}$  mean viral load 3.34 vs 2.65;  $p<0.001$ ).

**Conclusions:** The findings of this study demonstrate that applying spatial analysis to the understanding of HIV epidemiology even in a hyperendemic HIV epidemic setting is a valuable tool to monitor the epidemic. Despite the unprecedented high prevalence of HIV within geographically specific areas, the promising finding of the high prevalence of viral load <1000 copies/ml underscore the importance and impact of HIV programmes that have been rolled-out in this community. Furthermore, the high HIV viral load in young women and men in this region play a significant role in sustaining the epidemic, and there is an urgent need to prioritise interventions critical to reducing the potential for HIV transmission.

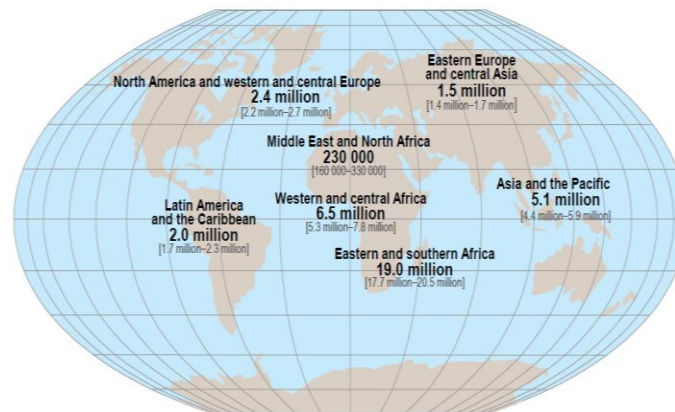
**Key words:** Spatial analysis, Geographic Information Systems, prevalence, viral load, gender differences, HIV surveillance

## **CHAPTER 1: INTRODUCTION**



## 1.1. Background

Globally, by the end of 2015, more than 36.7 million (range 34.0 million –39.8 million) individuals were living with human immunodeficiency virus (HIV). Since the discovery of HIV in 1981 and the start of the epidemic, a total of 78 million (range 69.5 million–87.6 million) people became infected and approximately 35 million (range 29.6 million–40.8 million) deaths occurred resulting from advancing HIV disease - acquired immunodeficiency syndrome (AIDS) related-sicknesses [1]. Despite a total of 18.2 million (range 16.1 million–19.0 million) people receiving antiretroviral therapy (ART) in 2015, a total of 2.1 million (range 1.8 million–2.4 million) new HIV infections and 1.1 million (range 940 000–1.3 million) AIDS-related deaths occurred in 2015 (Figure 1). The data provide an in-depth overview of the epidemic and includes the total burden of infection, with little indication of the heterogeneity of infections. Furthermore, over time the epidemics at local level have evolved, coalesced and amalgamated into diverse and interrelated local epidemics [2].



**Figure 1.1:** Global estimation of people living with HIV/AIDS in 2015. Total 36.7 million (range 34.0 million-39.8 million ) Source: Joint United Nations Programme on HIV/AIDS (UNAIDS): [3].

Sub-Saharan Africa (SSA) bears a heavy burden of HIV with over 25.8 million individuals living with the virus [1]. The western and central region has 6.5 million (range 5.3 million–7.8 million) HIV positive individuals, whilst the eastern and southern region accounts for 19.0 million (range 17.7 million–20.5 million) individuals. The region accounts for just over 50% of the total number of people living with HIV in the world. In 2015, almost 960,000 new HIV infections occurred, about 50% of the global total.

Across each country and over the last three decades the HIV epidemic has evolved into a complex mosaic of epidemics; with divergent disease burden, transmission patterns and impact on morbidity and mortality. Consequent to this, including the lack of political will, weak treatment and prevention efforts and continued high rates of new HIV infections, many countries have suffered immensely with

escalating morbidity and mortality, new HIV infections and high rates of mother to child transmission of HIV. At a country level, to better understand the HIV epidemic and curb the ongoing high rates of HIV transmission, the World Health Organisation (WHO) and the Joint United Nations Program on HIV and AIDS (UNAIDS) recommended the worldwide classification of the epidemics into several epidemic profiles to capture the dominant characteristics of the evolving epidemic at a country or regional level.

For an intensified focus, WHO/UNAIDS provided practical guidelines for intensifying HIV prevention towards universal access and encouraged countries to design and implement programmes based on the concept of “Know your epidemic and your current responses” [4]. This concept required countries to understand the key behavioural, biological and structural drivers of the epidemic that allow adapting key HIV responses and interventions. Know your epidemic characterises epidemics into low, concentrated, generalised and hyperendemic settings. Whilst these epidemic characteristics are useful when applied at a country level, their application within a country are limiting because of the recent recognition of heterogeneity of infections. Therefore, surveillance systems which include geospatial analytical methods with georeferenced data for a location-based approach to identify gaps and recognise hot-spot geographies that drive the epidemic are required.

Geospatial analytical methods are increasingly being used to better understand public health monitoring of disease and intervention programmes. These methodologies can be applied to understand diversity and location of infections or to improve targeted delivery of resources and HIV prevention interventions for public health benefit and more importantly to understand the variability of the local epidemic to identify high-risk areas. The aim of this study was to assess the spatial variability of HIV prevalence and HIV-1 RNA viral load in a geographically defined area of uMgungundlovu district, KwaZulu-Natal, South Africa. Therefore, this study has focussed on georeferenced data that had been collected in the HIV Provincial Surveillance System.

## **1.2. Overview of the dissertation**

- Chapter one (Introduction) consists of a brief background, literature review (which contributes to the supplementary published review paper attached in the appendices), study aim and objectives.
- Chapter two describes the materials and methods required to undertake the objectives of the study.
- Chapter three presents the results that were obtained from the study and

- Chapter four (discussion) integrates the findings of chapter three to discuss the findings in relation to literature and highlights conclusions and provides future recommendations.

### **1.3. Literature review**

#### ***1.3.1 The Epidemiology of HIV in sub-Saharan Africa and South Africa***

Across SSA the HIV epidemic typology is characterised as being generalised, meaning that heterosexual transmission sustains this epidemic, and countries with generalized epidemics report an HIV prevalence of about 5% in pregnant women and more than 5% in adults, with a concomitant epidemic of prenatally acquired infections. However, pockets of concentrated epidemics occur in more discrete individuals, such as sex workers (SW), men who have sex with men (MSM) and injecting drug users. Prevalence of HIV in these individuals exceeds 20% and therefore these individuals are considered to be at high risk for onward transmission.

The population prevalence in many countries in the region, such as Swaziland, Botswana, Lesotho, South Africa, Zambia and Zimbabwe are characterised as countries having a hyper-endemic generalised epidemic as the country level HIV prevalence exceeds 15% in the general population. The HIV prevalence is significantly higher in the high-risk individuals compared to the general population. Eight countries in this region, that is: Ethiopia, Kenya, Malawi, Mozambique, Uganda, Tanzania, Zambia and Zimbabwe accounted for about 50% of infections whilst about 40% were accounted for in South Africa. Almost 470 000 AIDS-related deaths occurred in this region in 2015 despite many of the country's commitments to provide and scale up ART programmes, reduce mother to child transmission of HIV, strengthening of HIV related services and management of AIDS-related illnesses. Over the last several years, through extensive commitments, many of the countries have begun showing signs of a decline in the number of new HIV infections and a substantial decline in the number of AIDS-related deaths, though the decline in the number of new HIV infections is still too slow to achieve the goals of HIV epidemic control.

Despite these declines, there were an estimated 1.4 million new infections in this region in 2015. HIV in the region is not uniformly spread across all countries since approximately 81% of infections come from only 10 out of 48 countries within SSA (Kenya, Zimbabwe, Malawi, the United Republic of Tanzania, Mozambique, South Africa, Uganda, Nigeria, Zambia and Ethiopia) [2]. HIV prevalence ranges from 0.2% in the Democratic Republic of Congo to 27% in Swaziland [5], while almost half of the infections occur in South Africa and Nigeria [2]. In this region women are disproportionately affected by HIV and account for almost 60% of adults living with HIV. The rate of new HIV infections remains high amongst young women between the age of 15 and 24 years and in 2015, almost 5000 new

HIV infections in this group of young women occurred every week, which is double the number seen in young men. Despite knowing the extent of the burden of HIV in this region and the populations affected, programmes to minimise the risk of HIV acquisition remains insufficient and many face stigma, discrimination and legal barriers that prevent them from accessing HIV services.

By the end of 2015, seven million South Africans were living with HIV with 380 000 new HIV infections and 180 000 AIDS-related deaths occurred, whilst over 3.2 million adults (age 15+ years) living with HIV were receiving ART [2, 6, 7]. To date complete and most reliable data are from the South African National HIV Prevalence, Incidence, Behaviour and Communication Surveys. In the 2012 survey the national HIV prevalence for those aged 15-49 years was reported to be 18% (95% Confidence Interval (CI) 17.5–20.3). HIV prevalence in the province of KwaZulu-Natal (KZN) was the highest at 27.9% (95% CI 25.2–30.8), while prevalence in the Western Cape was 7.8% (95% CI 5.5–10.9) [2, 3]. Similarly, the annual anonymous HIV seroprevalence surveys undertaken amongst pregnant women attending public sector primary health care clinics for the first time for their current pregnancy have shown the overall HIV prevalence to be consistently high. In 2013 the national HIV prevalence from public sector antenatal (ANC) surveillance was 29.7% (95% CI: 28.9-30.2) with KZN having the highest prevalence of 40.1% (95% CI 38.4-41.8), in contrast to 18.7% (95% CI 15.1- 23.0) in the Western Cape. HIV prevalence in this province increased from 1.2% in 1990 to 40.1% in 2013 [6, 8]. In contrast, the Northern Cape had a prevalence of <1% in 1990 and increased to 17.5% in 2013.

At the provincial level, the province of KZN consistently experiences the highest prevalence. The epidemic in the province of KZN is best described as “explosive” with a predominance in women and young people <30 years of age, and very high prevalence with no sign of saturation. Amongst the 52 health districts in the country, HIV prevalence was over 40% in five districts in KZN and one in Mpumalanga. The district of uMgungundlovu in KZN had a prevalence of 42.5% (95% CI 34.8-50.5) [4]. This heterogeneity in HIV burden at national and provincial level suggests that location-based approaches are needed to target HIV interventions in the high prevalence and incidence areas to scale up interventions to reduce the overall HIV burden. Nonetheless, such an approach requires georeferenced HIV data at local level. However, most national surveillance programmes in SSA do not incorporate such data in their routine HIV surveillance, leading to data being only available by large geographic units, thereby masking variations that exist at a micro-geographical level [9]. Geolocation of individuals is therefore necessary to understand the spatial structure of the HIV epidemic and to identify locally concentrated epidemics, which have the potential to sustain the overall burden of HIV.

Heterosexual transmission accounts for more than 90% of new infections which has resulted in young women bearing a disproportionate burden of HIV and being considered to be and described as “key populations” in this region [2]. HIV prevalence in adolescents between 15-24 years in high disease

burden communities provides a reasonable proxy for incident HIV infections because infections are likely to be relatively recent and HIV-related mortality is likely to be minimal. The burden of HIV in the South African adolescent population is unprecedentedly high, particularly in adolescent girls aged 15-19 years, who acquire HIV at least 5 to 7 years earlier than their male peers, and have a 3 to 4-fold higher incidence rate.

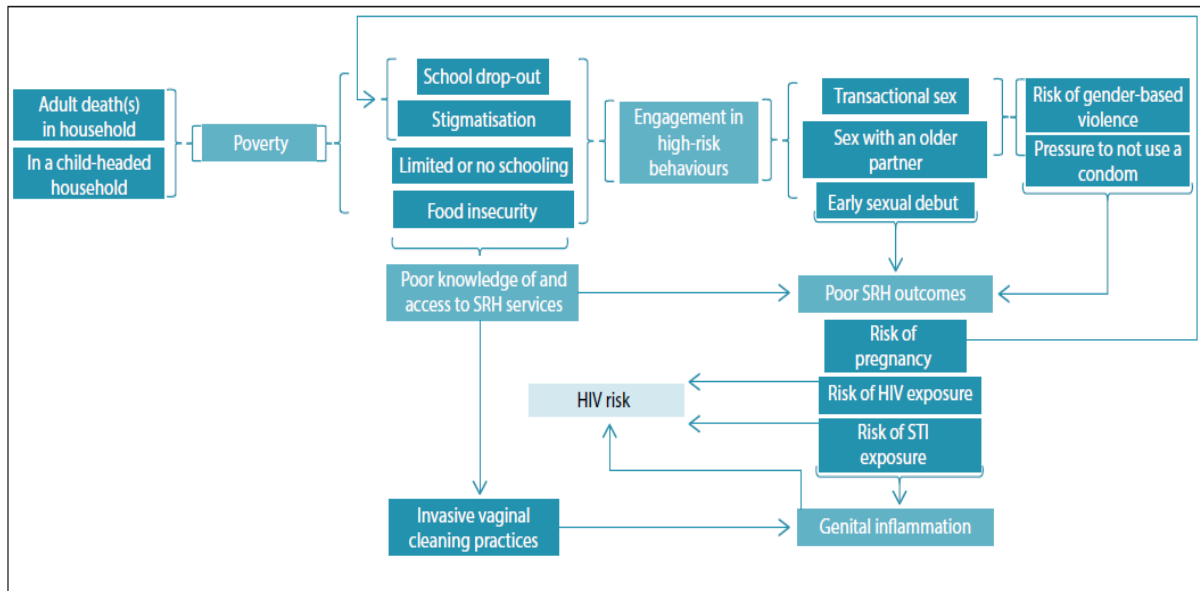
These data show that HIV is an important public health condition in this region, highlighting the importance of age and gender-specific prevalence. However, these data provide no insight into heterogeneity and diffuseness of HIV infections. Furthermore, the absence of any geographic locations where enhanced transmission might be occurring is not evident. Structurally key location are important to identify as they allow the design of programmes for implementation within target populations allowing a strategic response and to identify key specific geographic locations of infections.

### ***1.3.2 Key populations to prioritize for HIV prevention***

#### ***1.3.2.1. Adolescent girls and young women***

The World Health Organization defines an adolescent as any person between ages 10 and 19 years and who is in the transitional phase of growth and development between childhood and adulthood. Furthermore, this period could be extended to include young adult up to age 24 years. Adolescents during this period face numerous challenges in balancing life adjustments, rapid physical and emotional growth, initiation of sexual experiences, further complicated by traditional norms and conforming to the everyday life challenges. As such, many adolescents are at high-risk for adverse health conditions and health risk behaviours. Over the last 30 years, the greatest impact of the HIV epidemic globally has been on adolescents and young adults. Adolescent girls and young women (AGYW) aged 15–24 years account for only 11% of the adult population however, they are at particularly high risk of HIV infection, accounting for 20% of new HIV infections among adults globally in 2015. In SSA with higher HIV prevalence and the gender imbalance, AGYW account for 25% of new HIV infections among adults. Structural, biological and behavioural factors continue to predispose adolescents to many challenges and exacerbating their risk behaviours. AGYW acquire HIV at least 5 to 7 years earlier, have a higher prevalence and prevalence peaks at a younger age compared to their male peers. Key risk factors driving the high prevalence are: harmful gender norms and inequalities, insufficient access to education and sexual and reproductive health services, poverty, food insecurity and violence, differences in age at sexual debut between men and women, age-disparate sex, transactional sex, sexually transmitted infections (STIs), gender-based violence, limited access to health services and education, having multiple and concurrent partners and the lack of condom use is at the root of the increased HIV risk in young women and adolescent girls [10-15].

The HIV epidemic is more severe in AGYW in South Africa, with 24% of new HIV infections contributing to the total number of all new infections [16]. Preventing new HIV infections in AGYW remains a public health priority in South Africa where AGYW are recognised as a key high-risk population. In addition, more research on AGYW needs to include their sexual partners as the majority of infections are heterosexual transmissions [2]. Figure 2 shows the proposed key factors and pathways that may be associated with HIV risk in AGYW in South Africa.



**Figure 1.2:** Structural, behavioural and biological factors risk factors for HIV acquisition in young women in South Africa. Source: Dellar et al., 2015 [16].

Several studies have shown the disproportionate burden of HIV amongst AGYW. In the 2004 national survey of adolescents, AGYW were significantly more likely to be HIV positive in comparison with young men (15.5%, 95% CI 13.7–17.6 versus 4.8% 95% CI 3.9–5.9), representing a more than three-fold higher prevalence in women compared to men. Similarly, in the 2012 national survey, HIV prevalence in women aged 15-24 years was 11.4% (95% CI 9.8–13.2) compared to 2.9% (95% CI 2.1–3.9) in men of the same age [3], again a four-fold higher prevalence in women compared to men. These data highlight the importance of understanding the HIV transmission dynamics and preferentially whether these infections are from key locations in a specific geographical region or not. Whether these infections are common in urban or rural areas and the types of sexual networking patterns and whether they result from high rates of mobility.

### 1.3.2.2. Men who have sex with men (MSM)

A key risk population that is well recognised as having a higher burden of HIV are men who have sex with men (MSM). MSM were a specific group of individuals in whom AIDS-related illnesses were identified early in the 1980's [17]. Although the HIV burden has been shown to be higher in some

regions amongst MSM compared to heterosexual men, this sub-population is generally considered as a “hidden” population and not easily recognised through standard HIV surveillance methods. Despite encouraging indicators on the reduction of new HIV infections worldwide, the epidemic among MSM continues to grow and data gaps exist in understanding the epidemiology of HIV amongst MSM. There are biological, behavioural and legal factors which put MSM 24 times more at risk of HIV compared with the general population. Globally, more funding is required to support targeted HIV prevention, testing and treatment programmes for men who have sex with men. This is due to many countries criminalising homosexuality and punitive measures failing to improve health services for MSM. As is the case in most countries in SSA [2, 17]. Furthermore, the punitive approach limits prevention efforts targeted to MSM, stigmatisation and possible imprisonment for their sexual orientation as well as other biological risk factors related to anal sex.

Although South Africa has a generalised HIV epidemic, the epidemic is concentrated in the MSM population within this region, with an estimated overall prevalence of 9.2% [18]. A study conducted in three different cities of South Africa showed that MSM are disproportionately affected by HIV, and have an HIV prevalence that is higher compared to pregnant women attending public sector clinics in these three cities. HIV prevalence was 22.3% (CI 95%: 14.7-30.1) in Cape Town, 26.8% (CI 95%: 20.4-35.6) and 48.2% (CI 95%: 37.9-55.4%) in Johannesburg and Durban respectively. MSM over 25 years of age had a higher prevalence compared to those aged 18-24 years [19].

Another study by Price et al. (2012) in Kenya (Kilifi and Nairobi) and South Africa (Cape Town) reported an overall incidence rate of 6.8 per 100 person-years of follow-up (95% CI: 4.9-9.2) [20], while an HIV prevalence of 27.4% (95% CI: 17.6-40.0%) was reported amongst MSM compared to 17% (95% CI: 14-20%) reported in heterosexual men in Eastern Cape and KwaZulu-Natal by Dunkle et al. (2013) [21]. The high incidence and prevalence underscore the importance of including MSM into routine HIV surveillance systems, increasing their access to HIV services and also ending discrimination towards this sub-population in order to reduce the HIV burden [22]. It is also important to note that South Africa is the only African country that has formally recognised MSM including same-sex marriage or gay human rights [23], yet the incidence and prevalence of HIV remain high in MSM.

#### *1.3.2.3. Sex workers (SW)*

SW have been shown to have 50 fold higher HIV prevalence across countries reporting data on SW [2]. Stigma, gender-based violence, discrimination, criminalization, high number of sexual partners, inconstant condom use and lack of access to health care and support for SW remain major barriers in reducing HIV prevalence and incidence in this key population [24, 25]. In the mid-1990's, SW played a key role in “driving” the high HIV prevalence ranging from 46-69% in South Africa [26], and had

remained high until 2008. A study by Van Loggerenberg et al., (2008) reported an HIV prevalence of 59.6% in this sub-population [27].

The odds of SW acquiring HIV are 13.5 times higher compared to the general population of all women between 15-49 years of age [2]. Although it is widely believed that most SW are women, a considerable number of SW are also men and transgender women across a variety of settings [28], and understanding the epidemiology of HIV amongst SW remains important.

In 2012, an estimated 60% of SW were HIV positive in South Africa, contributing to an estimated 20% of the 350 000 new infections that occur annually in this region [29]. Six percent of infections occur amongst SW alone while 14% involve their clients or the client's intimate partners [26]. Sexual violence amongst SW and their clients remains a challenge, as it perpetuates risky behaviour associated with violence and non-condom use often leading to clients demanding for unprotected sex [26]. It is important to promote prevention methods that are women initiated and which can empower SW to protect themselves against HIV, such as pre-exposure prophylaxis and women condoms. It is also important to identify and target areas that are high HIV burden areas for SW as they are likely to operate in specific geographic locations for example across major transport routes [30].

#### *1.3.2.4. People who inject drugs (PWID)*

PWID play a significant role in HIV transmission, with 13 % of the total number of people injecting drugs being HIV positive (approximately 1.7 million of the total 12.7 million drug injecting individuals) globally in 2013 [31]. When compared to the adult population HIV prevalence, PWID have 28 times higher prevalence. Approximately 30% of incident infections that occur outside SSA are amongst PWID. Some of the major challenges facing PWID are criminalization and stigma which in turn lead to less health care seeking behaviours. The shortage of needles (90 needles available per person in a year instead of 200) leads to sharing of contaminated needles increases HIV transmission amongst PWID and onward transmission to their sexual partners [2]. Needle and Syringe Programs (NSP) have only been made available in 90 out of 158 countries that have reported injecting drug use. While only five (Senegal, Kenya, Tanzania, Mauritius and South Africa) of the NSP providing countries are in SSA despite a drastic increase in HIV prevalence (ranging from 5.5% to 42.9% in 2010 and 3.9 to 73.1% in 2016) in PWID in this region [32, 33].

Very little literature has been published on PWID over the last decade in South Africa, and due to SW being a clandestine population only two studies recruited a sample of about 50 PWID participants [34, 35]. A more recent study by Scheibe et al. (2016) enrolled 450 PWID from five different cities, namely, Cape Town, Durban, Centurion, Pretoria and Johannesburg. An overall HIV prevalence of 14% was reported, with 13% and 18% HIV prevalence amongst men and women respectively. Factors that were



associated with PWIDs being HIV positive include being from Gauteng province, belonging to a racial group other than white, ever being involved in sex work and being 25 years or older [32].

More studies are required to understand the epidemic within this population since these individuals potentially overlap with other key populations such as MSM and SW or may be considered a “bridging” population such that these high-risk individuals may have partners from the general population and transmit infections. Therefore, investments are needed to prioritize towards programmes that address specific interventions for HIV prevention in PWID such as: NSPs, Medically Assisted Therapy (MAT), HIV Counselling and Testing (HCT) and ART provision.

#### *1.3.2.5. Transgender people*

There are approximately 15 million transgender people globally [36]. Transgender people have been socially excluded and marginalized due to their gender identity and also neglected in HIV prevention programmes while they bear a heavy burden of HIV infection. The odds ratio of getting HIV infected compared to the general sexually active adult population is 48.8 (95% CI: 21.2-76.3) in transgender people [2, 37]. The global HIV prevalence in this population has been shown to be an estimated 19.1% ranging from 8% to 68%, and 27.3% for those transgender individuals who also engage in sex work [37-39]. Limited opportunities for employment and exclusion in society are major reasons for transgender to engage in sex work.

High risk of HIV acquisition facing the transgender population may be due to stigma, violence, absence of gender identity recognition leading to discrimination and exclusion to education, employment and access to healthcare. In 2014, despite this population being vulnerable to HIV acquisition, 61% of strategic plans for different countries to address AIDS has not included transgender people [2]. Globally, there is a huge gap in HIV research on transgender people especially in SSA and South Africa, and this needs to be addressed in order to prevent the spread of HIV.

Several studies have shown that HIV infection is higher amongst transgender people, MSM, SW, AGYW, and PWID [28, 40-42]. These key populations have been shown to perpetuate the ongoing transmission of HIV, meaning both them and their sexual partners remain at high-risk for HIV acquisition. Therefore, there is a greater need to identify geographic locations where these high-risk populations are located such that HIV prevention and treatment programmes are directed to these individuals.

Identifying and locating key populations strengthens HIV surveillance efforts to better inform health policy makers regarding the allocation of resources to locations where they are most needed, especially in geographic areas where these populations are located. Geographic information systems (GIS) and

spatial analysis could be useful tools to enable identification of these hard-to-reach populations at a micro-geographical level, and in settings where they may play a role in HIV transmission even within generalised epidemics.

### ***1.3.3. HIV Surveillance and monitoring of the HIV epidemic***

Since the discovery of HIV in the late 1980's and with the exponential increase in infections over the last 30 years, it has been critical to monitor and understand the epidemiology of HIV through surveillance activities. Enhancing surveillance through collection of behavioural data has been a useful way to track the epidemic in different regions and across populations [43]. Therefore, improvements in HIV surveillance methods provides a nuanced understanding of the epidemic and allows planning and evaluation of health care programmes at a global, national and local level.

HIV surveillance has evolved over time, beginning from the first generation surveillance that looked exclusively at AIDS cases, mortality reports and other sentinel HIV prevalence studies [44]. This approach had gaps in understanding the progression of the epidemic and behaviours that were associated with the spread of the epidemic [43]. These limitations led to the development of the second-generation approach which incorporated data from tuberculosis (TB) clinics, STI clinics, sentinel, behavioural, socio-demographic and household surveillance data in order to increase the explanatory power and understanding of the trends of the epidemic [45-47]. This type of surveillance systems has been important even though they reported on overall, sex and age aggregated HIV prevalence only, and estimated incidence based on prevalence data. Knowing incidence is important as it is a sensitive measure of whether any disease is continuously spreading to uninfected individuals, whereas prevalence reports on the total burden of infection, limiting our understanding and knowing when individuals acquired HIV.

Although there has been progress in ending the AIDS epidemic over the past 15 years through scale-up of HCT, antiretroviral therapy (ART), medical male circumcision (MMC), pre-exposure prophylaxis (PrEP) and condom roll-out, there are still 17.1 million people who do not know their status and 22 million that do not have access to life saving ART, including 1.8 million children [48].

Key to ending the HIV epidemic globally and in SSA is the Joint United Nations Programme (UNAIDS) 90-90-90 target that aspires to get 90% of people living with HIV knowing their status, 90% of those that know their status being on treatment and 90% of those on treatment being virally suppressed [49]. This fast-track target aims to reach less than 500 000 new HIV infections and AIDS-related deaths and to eliminate HIV-related discrimination by 2020. These targets have been shown to be possible even in resource-limited countries such as Botswana where an average of 70.2% of individuals had viral

suppression (only 2.8% short of the UNAIDS overall target of 73%) [50]. With the UNAIDS targets in place, it is crucial to track the epidemic in real time and to monitor the effectiveness of prevention services (in a “non-trial” setting) for different populations in order to direct prevention strategies where they are most needed and where they could have the greatest impact in order to achieve these targets. However, this requires robust HIV surveillance methods that can allow understanding of the epidemic at a granular level and in real time.

Although prevalence data have limitations in terms of informing prevention strategies, an individual’s risk of HIV acquisition can be strongly associated with living in a high prevalence area [51]. Application of geographic information systems (GIS) and spatial analysis to study patterns of HIV infections and viral load could help enhance the understanding about which geographical areas to prioritise for HIV prevention, or are being missed by interventions and health care services such as access to ART, condoms and circumcision. Therefore, application of these tools could also guide implementation of public health interventions at a localised level.

#### ***1.3.4. Application of GIS and spatial analysis in health care***

GIS has been used extensively as a mapping tool as it provides a geographic representation of the phenomenon under study [52]. However, GIS could be applied in analysing spatial data in different forms which include: visualization (mapping), exploration (analysis of “uncommon” spatial trends or patterns) and model building to predict future events or current aetiology of diseases [53, 54]. Spatial analysis and GIS have been used to study spatial patterns of diseases in order to understand disease patterns, including environmental factors, biological, physical and socioeconomic factors associated with disease transmission with reference to space and time [55]. GIS has the ability to integrate and overlay spatial data, support quantitative analysis of disease aetiology and improve the allocation of healthcare resources, through its ability to handle, visualise and analyse large datasets [56]. GIS and spatial analysis graphically inform health care providers as to where resources are lacking by allowing identification of geographic locations where particular diseases occur since many diseases could be associated with location [57]. Utilization of GIS with spatial analytical methods could also provide information on population distribution, clustering of mortality cases and utilization of healthcare services, which is crucial to health care planning [53, 58, 59].

The practice of GIS to address health problems dates back to as far as 1854 when Dr Snow mapped cases of cholera outbreak proving that certain water pumps were associated with neighbouring cholera cases, and that removal of handles of those pumps resulted in a rapid decrease of incident cholera cases [60]. Since then, the use of spatial analytical methods in the field of health has grown rapidly in various areas of healthcare planning and research [58]. This includes the use of GIS to map infectious diseases

such as malaria and schistosomiasis, where GIS showed that there were other environmental factors which affected the distribution of these two diseases such as topography and slope. These factors affected the utilisation of land use, soil type and other factors that affect vegetation cover. This had an effect on snail breeding sites and hence, the prevalence of schistosomiasis. In addition, factors that were affected by elevation, such as humidity and temperature had an effect on malaria cases [61]. This study explored factors that could be neglected by routine public health approaches to diseases and showed that favourable conditions for snail breeding being associated with higher prevalence of schistosomiasis.

The study by Munch et al. (2003) used spatial analysis and exploratory mapping to demonstrate clustering of TB cases in areas of overcrowding, specifically in taverns rather than across enumeration area [62]. Furthermore, spatial analysis and GIS have been applied to other infectious diseases such as *Chlamydia trachomatis*, syphilis, gonorrhoea and also to non-infectious diseases such as cancer and to measure appropriate admission to surgical care [63-67].

Disease control and prevention strategies that involve utilization of GIS and spatial analysis could improve public health interventions since these methods can allow a more focused approach and a better understanding of the local epidemiology of diseases [9, 68]. Application of GIS and spatial epidemiology over the years has also enabled mapping of HIV infections and cluster detection through spatial statistics and analyses.

#### *1.3.4.1. The use of GIS and spatial analysis in HIV prevention*

GIS and spatial techniques have also been applied in the context of HIV epidemiology where access to healthcare resources was assessed and shown to have a positive effect on health care [69]. Several studies have assessed: distance to a healthcare facility and how it affects the use of the facility by the community [53, 70], geographical patterns of HIV sero-discordancy in countries with high burden of HIV [71], evaluation of ART coverage in relation to reduction in community viral load [72] and understanding where clusters and micro-geographical differences in HIV prevalence exist [51].

GIS technology has enabled utilising the strategic allocation of new health care facilities through evaluating physical access to the healthcare facility by those in need of care based on supply and demand for care [52, 54, 70, 73]. For example, low-income populations have been shown to be more likely to use the nearest health care facilities regardless of the standard of care [53]. This underscores the importance of appropriate “location-allocation” of health care facilities for low-income individuals in order to improve access to health care [52, 53]. Geospatial approaches can also be applied in implementing recent HIV prevention strategies such as treatment-as-prevention for high-risk

populations [74]. For example, GIS and spatial analysis could potentially examine the coverage of ART, medical male circumcision and determine the impact of these on HIV incidence.

Targeted interventions such as HCT, MMC and PrEP for high-risk groups have already been implemented in various countries including those in SSA [75] and GIS and spatial techniques have useful tools for efficient roll-out of these public health interventions [74]. Table 1.1 is a summary of research studies from peer-reviewed primary research articles that have applied different GIS and spatial mapping methods in the context of HIV in different countries in SSA to better understand the spatial distribution of HIV including identifying areas with an exceptionally high burden of HIV, also previously known as “hot-spots”. The search engines used were PubMed, Science Direct, EBSCOhost and Google Scholar. The keywords that were used in the literature search in these search engines were HIV, spatial analysis, geospatial analysis and sub-Saharan Africa.

**Table 1.1:** Summary of published studies that applied geographic information systems and spatial analysis of HIV in countries across sub-Saharan Africa.

Authors	Study setting	Design	Sample size	Study population	GIS or spatial method used	Key findings	Key conclusion
Kleinschmidt et al. 2007 [76]	South Africa	Cross-sectional study	N = 11 904	General population (young men and women 15 – 24 years old)	Bayesian and linear spatial models	<p>The mean HIV prevalence was 13.6% for women and 4.8% for men.</p> <p>There were differences in HIV prevalence within provinces. Inland rural areas of Western Cape had the lowest prevalence while KZN, Mpumalanga and Free State had the highest HIV prevalence.</p> <p>Residing in an urban area, being unemployed and ethnicity were associated with HIV prevalence</p>	Comprehensive maps of HIV prevalence can be successfully utilized to guide and focus interventions to regions where they are predominantly needed.
Tanser et al. 2009 [51]	South Africa	Population-based cohort	N = 12 221	General population (men 15 - 54 years old and women 15 – 49 years old)	Kulldorf spatial scan statistics and two-dimensional Gaussian kernel	<p>The mean HIV prevalence was 21.7% (95% CI: 20.1% – 22.5%)</p> <p>There were three overlapping HIV high-risk clusters identified next to the national road, while other three low risk clusters were located elsewhere in the study area.</p> <p>HIV prevalence varied geographically at a local level and there was localized clustering of HIV infections.</p>	<p>There is an existence of localized HIV sub-epidemics of different intensity that exist within geographically defined areas.</p> <p>There is a need to target communities at higher risk of infection despite overall high prevalence in rural South African settings.</p>
Wand et al. 2010 [77]	South Africa	Randomized clinical trial	N = 5753	Sexually active women 16 – 49 years old	Kulldorf spatial scan statistics	<p>Three high prevalence hot-spots were identified which had an HIV prevalence that ranged from 39% – 56%.</p> <p>There were more married women outside the hot-spot compared to inside.</p> <p>There were more women who reported</p>	<p>Risk of HIV infection is associated with defined geographic space.</p> <p>Localised monitoring of the HIV epidemic is essential especially in areas where the epidemic is rampant such as KwaZulu-Natal.</p>

						having sex more than three times a week and having multiple sexual partners inside the “hot-spot” compared to outside.	
Cuadros and Abu-Raddad (2014)	Tanzania, Malawi, Kenya, Zimbabwe	Cross-sectional study	Not specified	General population (women aged 15 - 64 years)	Kulldorff spatial scan statistics	The study suggests that the declines in the national HIV prevalence in some of the SSA countries may not be representative of downward trends in prevalence in areas of high HIV prevalence, as much as the result of sharp declines in prevalence in areas of already low HIV prevalence.	The national declines in prevalence appear to be driven by sharp declines in prevalence in areas of already lower HIV prevalence.
Lakew et al. (2015) [78]	Ethiopia	Cross-sectional study	N = 30 625	General population (men and women aged 15 - 59 years old)	Kulldorff spatial scan statistics and geographic information system	<p>The overall HIV prevalence was 1.5%, while women and men had 1.9% (95% CI: 1.7% – 2.12%) and 1.1% (95% CI: 0.84% – 1.18%).</p> <p>HIV prevalence ranged from 10 - 21% in geographic clusters of central, eastern and western regions of the study area.</p> <p>Odds of being positive increased with age from 25 - 49 years and were higher in individuals with multiple sexual partners compared to those with a single partner.</p>	HIV prevalence was higher in women than men and amongst urban communities compared to rural, and there was clustering of HIV infections in western, eastern and central administrative areas of Ethiopia.
Okango et al. 2015 [79]	Kenya	Cross-sectional study	N = 4864	General population (women aged 15 - 64 years)	Bayesian semi-parametric spatial joint modelling	<p>The odds of being HIV positive were higher for women who resided in urban areas compared to rural. Furthermore, divorced women were 1.78 times likely to be HIV positive compared to married women living with a one partner.</p> <p>Spatial variation occurred in HSV-2 and HIV with higher prevalence for both</p>	There was positive spatial correlation between HIV and HSV-2 [correlation = 0.068 (95% CI: 0.39 - 0.87)]. Age, HIV and HSV-2 status had a nonlinear association.

						diseases closer to Lake Victoria region.	
Manda et al. 2015 [80]	Malawi	Cross-sectional study	N = 13 633	General population and pregnant women (men and women aged 15 - 49 years)	Bayesian multivariate joint spatial modelling and inverse probability weighting	Overall prevalence was 11.05%.  Where poverty and population density were high, there was also a comparatively higher prevalence.  There was a high HIV attributable risk to ANC women in central-eastern and northern parts of the country.	Inverse probability weighting can be applied to correct for non-response to HIV-testing, in situations where the number tested at a subnational level is minimal.
Gonzalez et al. 2015 [81]	Mozambique	Cross-sectional study	N = 1511	General population (men and women aged 18 - 47 years old)	Kulldorff Spatial Scan statistics	The overall incidence was 3.6 per 100-person years at risk [95% CI: 1.56 - 7.88] and one high-risk cluster was identified.  This cluster had an HIV prevalence of 79% in 2010 and 52.3% in 2012.	There is a need to combine HIV incidence estimation with geospatial analysis of micro-geographical patterns of infection in order to monitor and allow effective prevention strategies.
Wand et al. 2015 [82]	South Africa	Cohort study	N = 3462	Sexually active women aged 18 - 49 years	Geo-additive modelling	The HIV incidence was 6.8 per 100 person-years.  There were spatial differences within a small geographic area in behavioural factors which clustered in different specific regions.	There is an existence of spatial variation in measured and unmeasured risky behaviours within a small geographic space.
Niragire et al. 2015 [83]	Rwanda	Cross-sectional study	N = 6592	General population (women aged 15 - 49 years)	Bayesian geo-additive logistic regression model	The overall HIV prevalence was 3.8%.  STIs, high income, age of first sex, being < 19 years old and residing in woman-headed households was associated with high risk of HIV infection.  Living in a rural area and high knowledge and perception of HIV was protective.	There are distinct geographic patterns of HIV risk which are not statistically significant, however these may still suggest that interventions should be targeted to certain districts.
Barankarina et al. 2016 [84]	Burundi	Cross-sectional study	N = 4512	General population (men and women)	Kulldorff Spatial Scan statistics	Overall prevalence was 1.4%.  High risk cluster had 3.9% HIV prevalence	The study showed where and which populations are in greater need of resources in Burundi.



				aged 15 - 49)		while the low risk cluster had no case observed.	
Wabiri et al. 2016 [85]	South Africa	Cross-sectional study	N = 23 369	General population (men and women aged 15 - 49 years)	Geographical weighted regression model	The relationship between local HIV prevalence and key social determinants of HIV had a non-stationary relationship.	Detection of main social “drivers” for HIV in different locations can direct intervention programmes to areas of specific need.
Chang et al. 2016 [86]	Uganda	Open population-based cohort	N = 17 119	General population (15 - 49 years old men and women)	Hierarchical Bayesian modelling	HIV prevalence ranged from 9% to 43% across the study area.  HIV prevalence was higher in fishing communities compared to trading and agrarian communities.	There is high HIV prevalence heterogeneity which underscores the significance of local surveillance and focused HIV response.
Carrel et al. 2016 [87]	Democratic Republic of Congo	Cross-sectional study	N = 9275 (in 2007) and 18 257 (2013)	General population (Men and women 15 - 49)	Bayesian kriging	HIV prevalence was higher in 2013 compared to 2007, while residing farther from the cities was no longer protective against HIV as it was in 2007.	HIV prevention efforts need to be from urban to rural areas as the epidemic has started to spread from urban to rural settings.  There are spatial differences in HIV infection in woman of reproductive age.
Dobra et al. 2017 [88]	South Africa	Population-based cohort	N = 17 743	General population (men and women ≥ 15 years of age)	Interval – censored Cox proportional hazard models	Men and women had an incidence rate of 2.16 (95% CI: 1.98 – 2.34) and 3.27 (95% CI: 3.11 – 3.44) per 100 person-years respectively.  The risk of acquiring HIV increased by 50 % for spending 44% and 90% of the time outside the rural study area for both men and women respectively.	The relationship between distance migrated and HIV acquisition is non-linear.  Short distance migration also has an additional contribution to risk of HIV acquisition.

#### *1.3.4.2. Limitations and challenges of GIS application in health care*

The challenges facing the use of GIS and spatial analysis include the unavailability of georeferenced data, lack of trained and qualified staff and cost of commercial software programs (although some programmes are freely accessible such as Spatial Scan Statistics Software (SaTScan™) [89] and Quantum GIS (Swiss QGIS user group) [90]. Privacy and confidentiality issues related to identifying individuals and or communities' disease status are a challenge as there could potentially be stigmatisation, discrimination and/or marginalisation if the geographic location of individuals could be identified [52, 84].

Stigma has been shown to increase the risk of HIV acquisition and result in poor uptake of HIV testing and concomitant high-risk behaviours, especially among high-risk groups such as MSM [91, 92]. These behaviours include false reporting of self-reported HIV status (which underestimate prevalence thus leads to being neglected for intervention programmes), escalated unprotected anal sex and poor adherence to ART [91]. However, identifying such high-risk groups is important as they may share some risky behaviours [93]. For example, being a PWID could be related to being involved in transactional sex work in some areas [94].

Since stigma and discrimination remains a challenge especially for high-risk populations, it is important to protect GIS data obtained for personal privacy. The application and use of GIS data should be disclosed clearly to the participant and consent for data to be used must be provided by the participant. Confidentiality of participants should be simultaneously maintained through non-disclosure of personal information [95]. However, there is still a need to implement methods to improve the level of privacy when using GIS data. Furthermore, the use of georeferenced data must be limited to service providers and those involved in healthcare planning and it should be highly controlled and safeguarded to prevent misuse to criminalize or stigmatise vulnerable groups [96]. Knowing where, how and who is infected with HIV is crucial in HIV response [97], and GIS and spatial analysis can be applied to answer such important questions in HIV prevention.

#### *1.3.5. Targeting “Hot-spots” and key geographies for HIV transmission*

HIV control and prevention strategies that target key populations and “hot-spot” (areas of high incidence and/or prevalence) geographies for transmission have the potential to reduce acquisition of new HIV infections when compared to a general approach since there is high heterogeneity in HIV prevalence and incidence within different regions [68, 86, 98]. Targeted interventions for HIV are critical to SSA countries with limited resources, especially in South Africa (KwaZulu-Natal province) since it carries a high burden of HIV [77]. There is also a greater chance that such a focused approach

will have better outcomes with regards to prevention where there is geographical clustering of HIV incidence cases or clustering within sub-groups [99].

Biomedical interventions including pre-exposure prophylaxis, early initiation of ART, condom roll-out and medical male circumcision have been used to reduce HIV acquisition [98, 100]. These interventions have been used in generalised epidemic settings to reduce HIV infections. However, disparities observed in the HIV epidemic at different population levels have shown that individuals are not at similar risk of HIV infection even in generalised epidemics [101]. A review by Tanser et al. (2014) also suggested that targeted interventions directed to the most vulnerable groups such as MSM, SW and PWID have been neglected while such approaches can be effective in reducing HIV in the general population as part of an overall strategy [75].

The incidence of HIV still remains high in some local communities of Southern Africa [102, 103]. This suggests that prevention programmes, risk and health-promoting behaviours are not equally distributed across the country, but are concentrated more in some communities than others [104]. Therefore, as a result of geographical heterogeneity in the HIV epidemic [75, 105], there is an increasing need to strengthen sub-national responses and the understanding of HIV spread within different local communities in the country. Otherwise, a “one size fits all strategy” will not work in the presence of numerous localized epidemics that differ epidemiologically and of significant socio-geographical variations [51, 106]. These variations emphasize the significance of a location-based approach in order to acquire knowledge about where the highest density of HIV infections are occurring and where those individuals are geographically located. This approach can allow scale-up of treatment and prevention strategies in populations where the overall impact could be greatest. Application of spatial techniques and GIS is relevant in enhancing the understanding of the HIV epidemic globally and at a micro-geographical level. When applied correctly, these tools can allow identification of locations that are disproportionately affected by HIV.

#### **1.4. Rationale**

For the past decade, the Centre for AIDS Programme of Research in South Africa (CAPRISA) has been conducting research in the Vulindlela sub-district of uMgungundlovu municipality to monitor the HIV epidemic, implications for research and programmatic priority setting.

uMgungundlovu is one of the leading districts with regards to HIV infections in KwaZulu-Natal and in South Africa [6]. The results from the annual antenatal HIV surveillance conducted by CAPRISA in seven PHC clinics in Vulindlela community in the uMgungundlovu district revealed that the overall

HIV prevalence was extremely high at 35.3% (95% CI: 32.3% – 38.3%) from 2001 - 2003; 39.0% (95% CI: 36.8% – 41.1%) in the period 2004-2008 and 39.3% (CI: 37.2% – 41.4%) from 2009-2013 [107]. At least 30% of pregnant women attending these clinics were below 20 years old and the HIV prevalence was consistently high in this age group and was an estimated 22.5% (95% CI: 17.5% - 27.5%) between 2001 - 2003; 20.7% (95% CI 17.5-23.8) in the period 2004-2008 and 17.2% (95% CI: 14.3% - 20.2%) from 2009-2013 [107].

These data suggest that HIV infection in young women is more likely to be recent since, in this setting, HIV acquisition is almost synonymous with sexual debut which is estimated at around age 16 years. The burden of teenage pregnancies in the community was an average of 30% of the surveyed pregnancies sampled per year from 2001 to 2013 [107]. Another study by Abdool Karim (2011) [102], showed that the incidence rate was 6.4/100 person-years (95% CI: 2.6% - 13.2%) in women 14-30 years in this rural community Vulindlela and as high as 4.7 /100 person years [95% CI: 1.5% - 10.9%) in young women <18 years [108]. These data provide empiric evidence for the high HIV burden, however, the heterogeneity of HIV infections and the spatial distribution of prevalence including the HIV-1 RNA viral load has not been undertaken. Thus, this study was designed to address the research gap that exists in understanding the micro-geographical areas that have the highest or that contribute most of HIV infection in this community.

## **1.5. Aims and Objectives**

### **Research Question**

- Do HIV positive individuals and those with high viral loads cluster in space?

### **Aim**

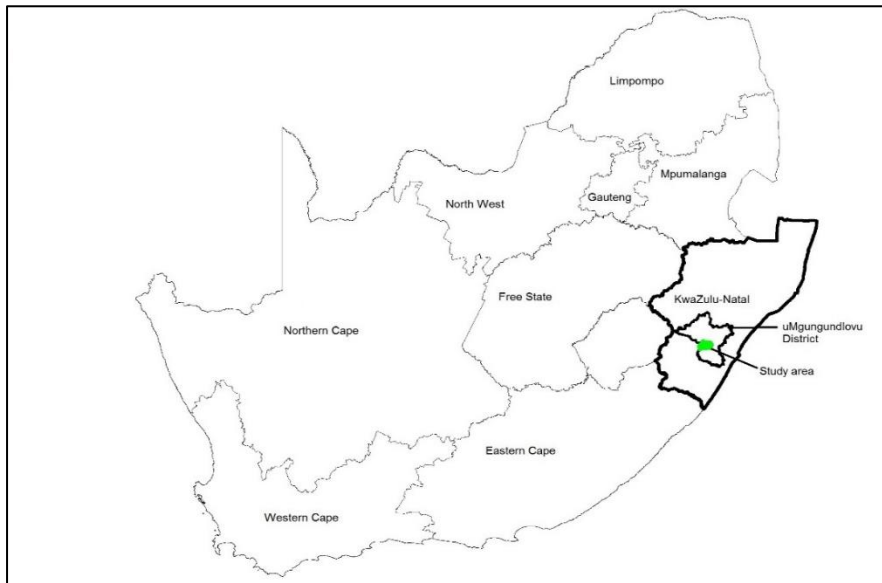
- To assess the spatial variability of HIV prevalence and HIV-1 RNA viral load at the sub-district level of uMgungundlovu municipality, KwaZulu-Natal, South Africa.

### **Objectives**

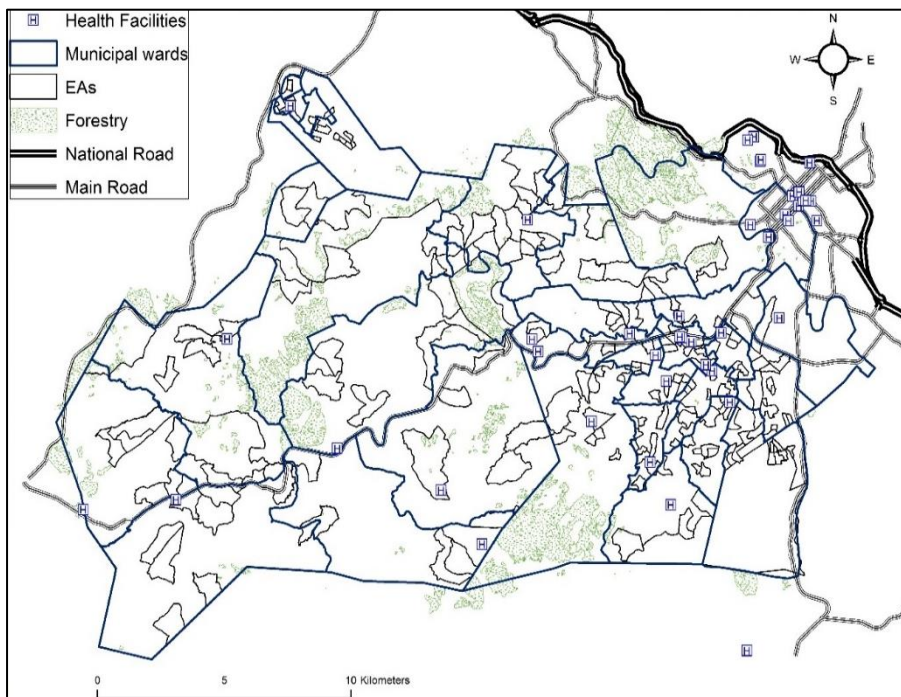
- To determine specific locations within the uMgungundlovu sub-districts that are high burden areas for HIV prevalence.
- To spatially analyse and map the viral load distribution within the uMgungundlovu sub-district.

## **CHAPTER 2: MATERIALS AND METHODS**

## 2.1. Study Area and population



**Figure 2.1:** Location of the study area within the uMgungundlovu district, province of KwaZulu-Natal, South Africa.



**Figure 2.2:** Location of the two sub-districts of Vulindlela (Western part) and Greater Edendale (Eastern part).

The study area comprised of Vulindlela (rural) and Greater Edendale (peri-urban) in the uMgungundlovu District, KZN, South Africa. The governance of this area is under the traditional leadership and is represented in the local municipality. The Vulindlela area incorporates habitation in traditional settlements, farmlands or forestry within a rural setting whilst the Greater Edendale area is the main economic hub. In Vulindlela, employment opportunities exist through the farmlands or through the forestry projects, whilst in the Greater Edendale area, a large number of mid-level factories provide some employment opportunities. However, the employment opportunities are limited resulting in high levels of unemployment which is around 35% and high levels of poverty. Access to health care is through the 17-primary health care clinic in the area and several donors funded non-governmental organisations that provide additional health and social related support to the community. The closest hospital is the Edendale Hospital which provides additional secondary healthcare support. The HIV Incidence Provincial Surveillance System (HIPSS) is an ongoing longitudinal study, established as surveillance platform in a geographically defined region in KZN, South Africa; to monitor HIV related measures in association with the scale-up of prevention efforts in a “real world”, non-trial setting. Community-based partnerships evaluate and support the research programmes in the area; recognising that the district-level antenatal HIV prevalence of 42.5% in 2013 has been the highest in South Africa [6].

## **2.2. Study design**

HIPSS was a multi-stage random sampling of enumeration areas (EAs), households and individuals from the household-based representative sample of men and women (15-49 years) in the Vulindlela and Greater Edendale sub-districts of KZN, South Africa. The study protocol has been described in detail [109]. HIPSS baseline survey was undertaken from June 2014 to June 2015 and enrolled 9812 participants. Participant’s households were geo-located, and participants responded to interviewer-administered questionnaires and had peripheral blood samples collected. For this analysis, the EAs were further divided into municipal wards as service delivery, health care and community services are at the ward level.

## **2.3. Laboratory testing**

HIV antibody testing was done using 4th generation HIV enzyme immunoassay assay Vironostika HIV Uniform II Antigen/Antibody microELISA system (BioMérieux, Marcy l’Étoile, France) and Elecsys HIV 1/2 combi PT assay (Roche Diagnostics, Penzberg, Germany). All HIV antibody positive samples were confirmed with the Western Blot – HIV-1 kit (Bio-Rad Laboratories, Redmond, WA, USA). Viral

load testing was done using the COBAS AmpliPrep/COBAS TaqMan HIV-1 version 2.0 assay (Roche Diagnostics, Penzberg, Germany) as per manufactures' instructions.

## **2.4. Measurements**

HIV infections were defined as both HIV ELISA and Western blot positive. Prevalence of viraemia was reported as viral load of >1000 copies/ml in HIV positive individuals. Geometric mean viral load was categorised into five strata: <1000 copies/ml, 1001-2000 copies/ml, 2001-4000 copies/ml, 4001-8000 copies/ml and >8000 copies/ml.

## **2.5. Spatial analysis**

### ***2.5.1. HIV prevalence and viral load maps***

The analysis has not been weighted in this study, since, it focuses on identifying specific geographic locations where spatial clustering of HIV prevalence and HIV-1 RNA viral loads may occur. To maintain household and individual confidentiality, the collected Global Positioning System (GPS) coordinates were randomly moved between 0 to 500 meters away from the exact household coordinates. To determine HIV prevalence per municipal ward, the GPS coordinates of the geo-located households were grouped by municipal wards and HIV prevalence was calculated for each ward using ArcGIS software version 10.3 (ESRI, USA) to create a choropleth map showing HIV prevalence. All viral loads were used to determine the prevalence of viraemia at viral load >1000 copies/ml and to calculate the geometric mean viral load (copies/ml) over a three kilometre radius using a standard Gaussian kernel interpolation method to produce a continuous map of viral distribution across the study area [110].

### ***2.5.2. Kulldorff's spatial scan statistic for cluster detection of HIV infections***

The Kulldorff's spatial scan statistic (SaTScan) is a statistical method that has been designed to detect a local excess of diseases and to test whether such an excess is reasonably due to chance [111]. This method was applied to this study to detect clusters of HIV infection, prevalence of viraemia >1000 copies/ml and high geometric mean viral load. SaTScan has the capability to detect both the location of clusters and their statistical significance [112], and adjust for differences in population density in order to prevent clustering due to variations in spatial distribution of the study population [113].

SaTScan analysis was done by scanning a circular window across the study area and the circle centre of this window was allowed to gradually move over each selected household – generating an infinite



distinct number of circles that contain different sets of neighbouring households at different locations while noting the number of observed and expected cases (HIV infections) inside each circle using a two-dimensional Bernoulli model.

Each circle was a possible candidate cluster. For each distinct circle generated, SaTScan performed a likelihood ratio test statistic (described further in SaTScan User Guide) [113] to determine if the number of observed cases (HIV infections) inside the circle were higher than expected compared to those outside the circle. This was done under the null hypothesis which assumed that the number of HIV infected individuals in each circle have spatial randomness. Subsequently, leading to constant prevalence across the study population (many random replications of the dataset were generated under this null hypothesis – 999 times). These sets of clusters detected were then ranked in accordance with the likelihood ratio test statistic. The circle with the highest likelihood ratio among all possible locations was considered as the most likely cluster (least likely to have occurred by chance) and the  $p$ -value was obtained using Monte Carlo hypothesis testing [113] by comparing the rank of the test statistic from the real data set with that from the simulated random data sets.

The null hypothesis was rejected if  $p$  was less than 0.05. The geographic extent of each circle was allowed to increase from zero to a maximum radius of 3 km and also to include a maximum percentage of 50% of the total population [113]. With these specifications, a reported cluster could only comprise of 50% - at most, of the total population at risk. A similar method (two-dimensional Bernoulli model) was used to determine clusters for viral load less or greater than a 1000 copies/ml.

### ***2.5.3. Detection of viral load clusters***

SaTScan Normal model [114] was used to detect spatial clustering of the geometric mean viral load. The null hypothesis for this model stated that all cases come from a similar distribution, while the alternative hypothesis stated that there is a single cluster location where cases have a larger or smaller mean compared to those outside the cluster. The circular scanning window was moved over different point locations of HIV positive individuals (households), while permuting observed continuous observations [geometric mean viral load (copies/ml)], the radius of the circle kept changing continuously from zero to the maximum upper limit which was set at <50% of all the cases. For each possible cluster, a log likelihood ratio was calculated and the test statistic was demarcated as the cluster with the maximum likelihood compared to other clusters. The  $p$  value permutations were based on the Monte Carlo hypothesis testing (described elsewhere) [113] at  $p < 0.05$ .

#### ***2.5.4. Cartographic display of high HIV prevalence and viral load clusters detected by Kulldorff's spatial scan statistic***

ArcGIS (version 10.3) [110] was used to cartographically superimpose and visualize the spatial scan statistic results on HIV prevalence and viral load maps in order to denote the geographical location of viral load >1000 copies/ml, geometric mean and HIV prevalence clusters.

### **2.6. Statistical Analysis**

A One-way ANOVA statistical test was performed using IBM SPSS statistics version 24.0 (IBM Corporation, Armonk, NY) to compare the mean  $\log_{10}$  viral load across all different age categories at  $p < 0.05$ . This was followed by a univariate (Two-way ANOVA) analysis if the  $p < 0.05$  for the One-way ANOVA to compare the mean  $\log_{10}$  viral load within each age group by gender and between different age groups. A chi-square test was used to test the association of demographic, behavioural and biological characteristics of being inside or outside a localised cluster of high HIV prevalence ( $p < 0.05$ ).

## **CHAPTER 3: RESULTS**

### **3.1 HIV prevalence and localised cluster of high numbers of HIV infections**

The overall HIV prevalence was 36.3% (95% CI 34.8-37.8). The prevalence in women was 44.1% (95% CI 42.3-45.9) and 28.0% (95% CI 25.9-30.1) in men ( $p < 0.0001$ ) [115]. Of the 9812 enrolled participants, there were 9,788 participants with household GPS coordinates, whilst 24 participants had missing household coordinates. HIV prevalence mapped by municipal ward ranged from 30.1% (95% CI: 27.1-33.2) to 50.1% (95% CI: 44.9-55.4) (Figure 3.1). At a micro-geographical level, one localised cluster of high HIV prevalence was identified in the north-west of the study area by the Kulldorff spatial scan statistic where HIV prevalence was 71% [Relative-risk (RR) = 1.75;  $p = 0.02$ ]. This cluster was in a semi-rural community and in close proximity to a primary health care facility.

### **3.2 Prevalence of viraemia >1000 copies/ml in municipal wards**

Figure 3.2 shows the prevalence of viraemia >1000 copies/ml overall and disaggregated by sex. A single cluster for low levels of viraemia >1000 copies/ml was present in the same location as the high HIV prevalence cluster approximately over a three kilometre radius having a relative risk of 0.69 ( $p = 0.02$ ) (Figure 3.2a). A total of 309 HIV positive individuals contributed to this cluster indicating that 69% (213 individuals) had a viral load of <1000 copies/ml, whilst 31% (96 individuals) had a viral load >1000 copies/ml. Prevalence of viraemia >1000 copies/ml exceeded 50% in 11 of the 30 municipal wards within the study area and 10 of the 11 wards were located within urban areas while one was in a semi-rural area. Disaggregated by sex, the prevalence of viraemia >1000 copies/ml was higher in men across a wider geographical area compared to viraemia in women (Figure 3.2b and Figure 3.2c).

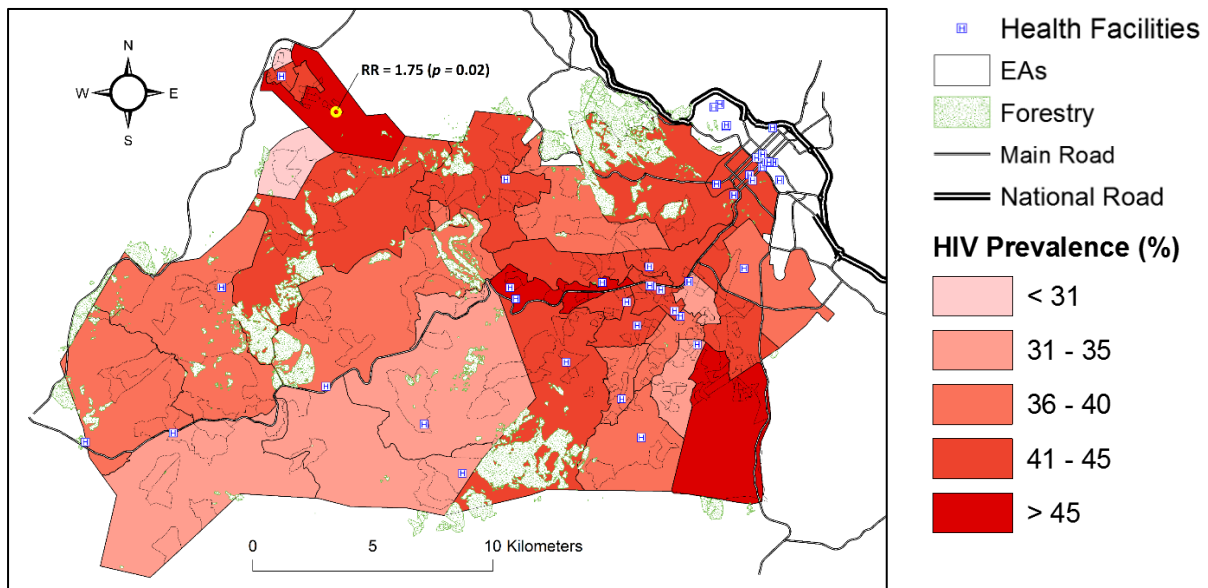
### **3.3 Geometric mean viral load maps and clusters**

The geometric mean viral load is shown in Figure 3.3, overall and disaggregated by sex. The overall geometric mean viral load for individuals in the study area was 202 copies/ml, in men it was 735 copies/ml and in women, it was 130 copies/ml. In the south-east of the study area, two high viral load clusters were identified. The first cluster accounted for the overall population and the geometric mean viral load for this cluster was 327 copies/ml. The geometric mean viral load for individuals in the population outside of the high viral load cluster was 125 copies/ml resulting in a geometric mean viral load difference of 202 copies/ml ([log likelihood ratio ( $LLR$ )=18.95;  $p = 0.001$ ) (Figure 3.3a). The second-high viral load cluster accounted for women and the geometric mean viral load for this cluster was 237 copies/ml. The geometric mean viral load for women outside the high viral load cluster was 79 copies/ml and the geometric mean viral load difference was 158 copies/ml ( $LLR = 18.99$ ;  $p = 0.001$ ) (Figure 3.3b). Both the high viral load clusters occurred to the south-east of the study area representing a peri-urban setting. Furthermore, the geometric mean viral load in different geographical areas across

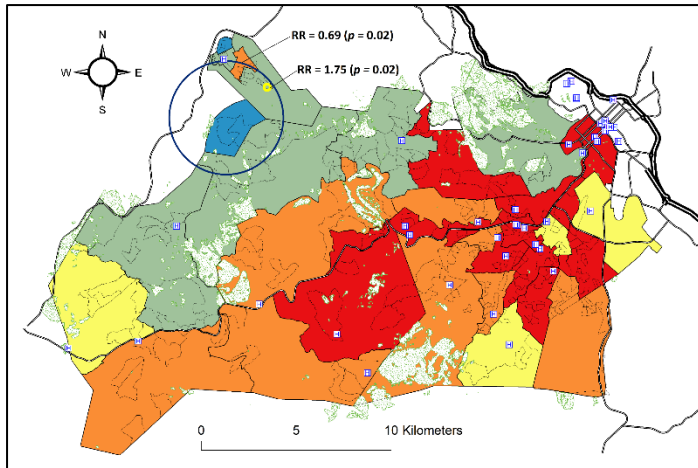
the study population was higher in men compared to women (Figure 3.3b and 3.3c), however, there was no significant spatial clustering of geometric mean viral load amongst men (Figure 3.3c).

### 3.4 Univariate analysis of $\log_{10}$ mean viral load amongst men and women

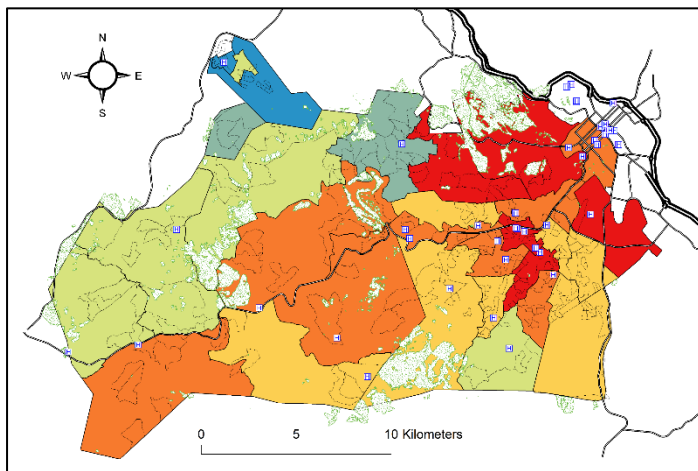
Based on the  $\log_{10}$  HIV mean viral load, there was a statistically significant difference in the overall  $\log_{10}$  mean viral load between men ( $\log_{10}$  mean viral load=3.211) and women ( $\log_{10}$  mean viral load=2.623) [F (1, 3953) = 97.834,  $p<0.001$ ] and in different age groups [F (6, 3948) = 32.334,  $p<0.001$ ], as well as in the interaction between gender and age on viral load [F (6, 3941) = 5.367,  $p<0.001$ ]. Comparison of the  $\log_{10}$  mean viral load in young men and women 15-24 years ( $\log_{10}$  mean viral load=3.342) to older men and women 25-49 years ( $\log_{10}$  mean viral load=2.654) showed that there was a difference in the  $\log_{10}$  mean viral load between the two groups [F (1, 3953) = 101.175,  $p<0.001$ ].



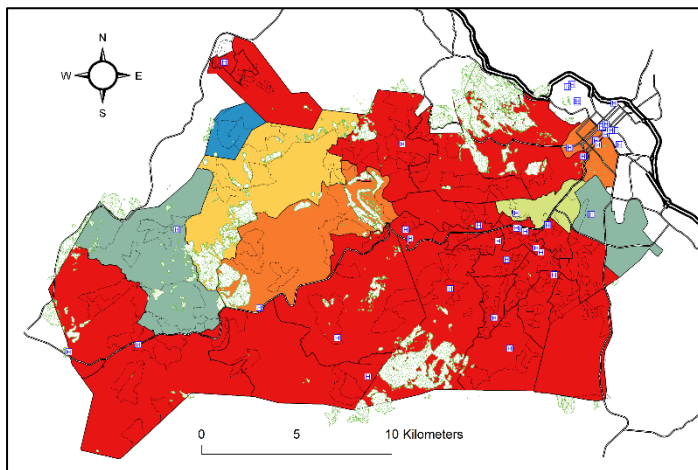
**Figure 3.1:** HIV prevalence map by municipal ward for men and women aged 15-49 years (N=9788), with a localised cluster of high numbers of HIV infected individuals [Prevalence=71%; RR=1.75;  $p=0.02$ ], yellow circle superimposed on the north-west of the study area, independently obtained by SaTScan Bernoulli model].



**Figure 3.2A:** Prevalence of viraemia >1000 copies/ml for men and women aged 15-49 years (n=3954) by municipal ward, with a localised cluster (blue circle) of low numbers of individuals with prevalent viraemia and high numbers of HIV infected individuals (yellow circle), both obtained by SaTScan Bernoulli model.

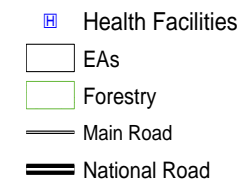


**Figure 3.2B:** Prevalence of viraemia >1000 copies/ml in women aged 15-49 years (n=2945) by municipal ward.

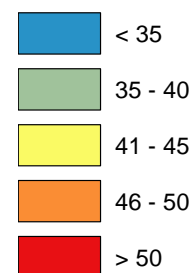


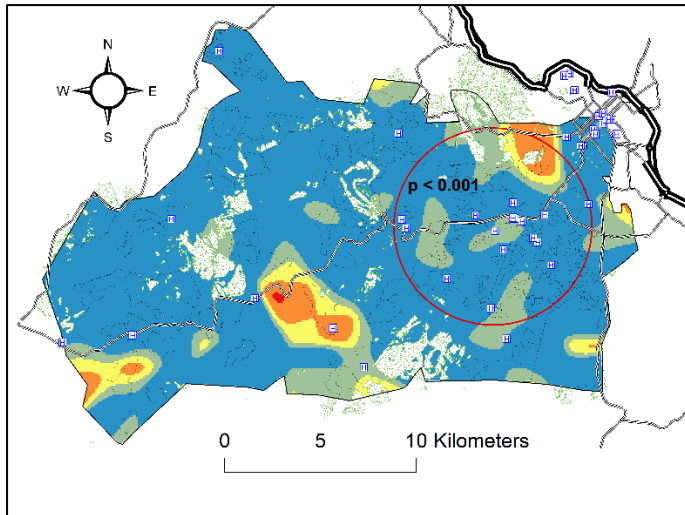
**Figure 3.2C:** Prevalence of viraemia >1000 copies /ml for men aged 15-49 years (n=1009) by municipal ward

**Figure 3.2 A-C:** Prevalence of viraemia at >1000 copies/ml by municipal ward. The blue shaded area showing the lowest prevalence of viraemia (<35%) and the red showing highest prevalence of viraemia (> 50%).

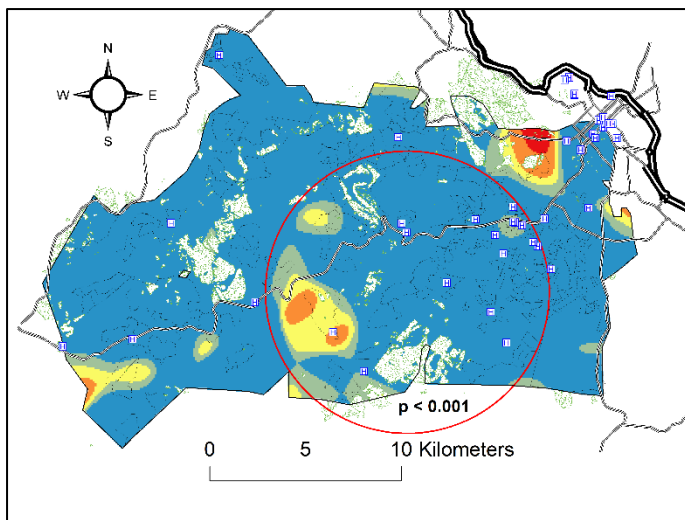


**Prevalence of viraemia >1000 copies/ml (%)**

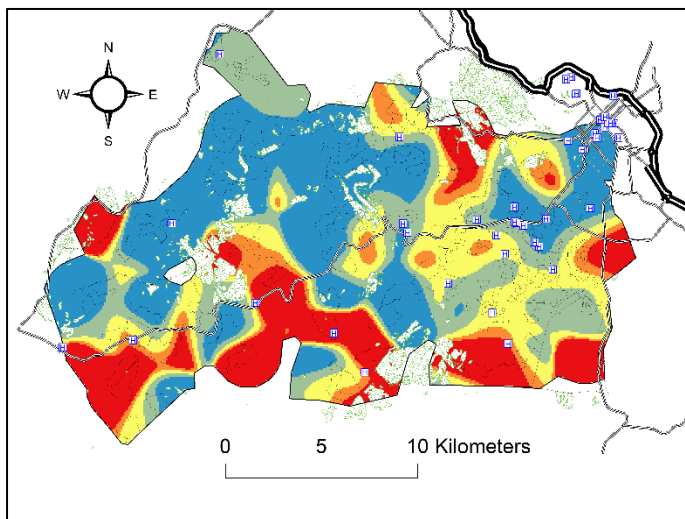




**Figure 3.3A:** Geometric mean viral load in men and women 15-49 years (n=3954) determined by 3 km standard Gaussian kernel showing the localised cluster for high geometric mean viral load obtained by SaTScan (Normal model)



**Figure 3.3B:** Geometric mean viral load in women 15-49 years (n=2945), determined by standard Gaussian kernel over a 3 km radius showing the localised cluster for high geometric mean viral load obtained by SaTScan (Normal model),



**Figure 3.3C:** Geometric mean viral load in men 15-49 years (n=1009), determined by standard Gaussian kernel over a 3-km radius (No significant cluster).

**Figure 3.3 A-C:** Geometric mean viral load determined by 3 km standard Gaussian kernel.

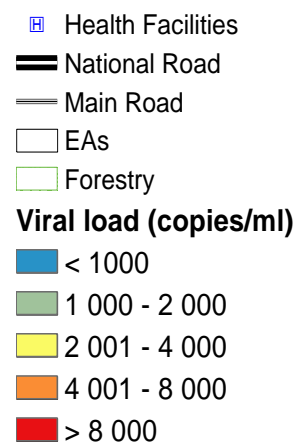


Table 3.1 shows the differences in the log<sub>10</sub> mean viral load between men and women in similar age group. There were no statistically significant differences in the log<sub>10</sub> mean viral load between men and women in the age groups 15-19 years and 45-49 years old. However, there were significant differences in the log<sub>10</sub> mean viral load of the age groups between 20 to 44 years old.

**Table 3.1:** Univariate analysis of log<sub>10</sub> mean viral load in men and women by age groups.

Age group	Log <sub>10</sub> mean viral load in women	Mean difference in Log <sub>10</sub> viral load between men and women (95% CI)	<i>P</i> -value *	(N= 3954)
15-19	3.471	-0.293 (-0.876 – 0.291)	0.326	166
20-24	3.214	0.641 (0.277 – 1.005)	<b>0.001</b>	520
25-29	2.799	1.104 (0.834 – 1.374)	<b>0.001</b>	747
30-34	2.542	0.831 (0.582 – 1.080)	<b>0.001</b>	775
35-39	2.318	0.762 (0.507 – 1.017)	<b>0.001</b>	721
40-44	.3161	0.343 (0.068 – 0.619)	<b>0.015</b>	606
45-49	2.185	0.310 (0.075 – 0.651)	0.075	419

\*. The mean difference is significant at  $p < 0.05$  level.

Demographic, behavioural and biological factors that were associated with being in an HIV localised cluster compared to being outside of the cluster are shown in Table 3. Completion of high school education, marital status (being married), total household monthly income, condom use, herpes simplex virus type 2 (HSV-2) infection and reported antiretroviral (ART) status were significantly associated with being inside the cluster for HIV infection. There were no statistically significant differences between populations which resided inside the cluster compared to those that were outside the cluster in terms of gender, age at first sex, age of partner at first sex, number of lifetime sex partners, percentage circumcised and being diagnosed with a sexually transmitted infection (STI) (*Neisseria gonorrhoea*, *Chlamydia trachomatis*, *Trichomonas vaginalis* and syphilis).



**Table 2.2:** Characteristics of individuals inside the high HIV prevalence (71%) cluster compared to individuals with HIV infections outside of this cluster.

<b>Variable</b>	<b>Inside Cluster (n/N)</b>	<b>%</b>	<b>Outside cluster (n/N)</b>	<b>%</b>	<b>p-value</b>
<i>HIV cases</i>	50/70	71.4	3904/9742	40	<b>0.001</b>
<i>Sex</i>					0.672
<b>Men</b>	27/70	38.6	3520/9742	36.1	
<b>Women</b>	43/70	61.4	6222/9742	63.9	
<i>Median age (IQR)</i>	32 (23-39)		28 (21-38)		0.184
<i>Relationship status</i>					<b>0.004</b>
<b>Married</b>	13/70	18.6	849/9742	8.7	
<b>Unmarried</b>	57/70	81.4	8893/9742	91.3	
<i>Level of education</i>					<b>0.003</b>
<b>Completed secondary school</b>	20/70	28.6	4541/9742	46.6	
<b>Incomplete secondary school</b>	50/70	71.4	5201/9742	53.4	
<i>Total household monthly income (South African rand)</i>					<b>0.008</b>
<b>None - R500</b>	10/69	14.5	2187/8860	24.7	
<b>R501 - R2500</b>	28/69	40.6	4128/8860	46.6	
<b>&gt; R2500</b>	31/69	44.9	2545/8860	28.7	
<i>Sexual history</i>					
<b>Ever had sex</b>	61/70	87.1	8241/9742	84.6	0.555
<b>Median age at first sex (IQR)</b>	17 (16-19)		18 (16-19)		0.206
<b>Median age of partner at first sex (IQR)</b>	19.5 (16-22)		20 (17-22)		0.451
<b>Number of life-time sex partners (range)</b>	3 (1-5)		3 (1-4)		0.621
<i>Condom use in the last 12 months</i>					<b>0.022</b>
<b>Always</b>	15/49	30.6	1572/6657	23.6	
<b>Never</b>	3/49	6.1	1498/6657	22.5	
<b>Sometimes</b>	31/49	63.3	3587/6657	53.9	
<i>Medically circumcised</i>					0.538
<b>Yes</b>	8/27	29.7	1244/3522	35.3	
<b>No</b>	19/27	70.3	2278/3522	64.8	
<i>Reported being on ART</i>	29/50	58.0	1563/3919	39.88	<b>0.009</b>
<i>Prevalent sexually transmitted infections</i>					
<b>Herpes simplex virus type 2 (antibodies)</b>	54/70	77.1	6075/9716	62.5	<b>0.012</b>
<b>Syphilis (antibodies)</b>	2/70	2.9	291/9742	29.9	0.949
<b><i>Neisseria gonorrhoea</i>*</b>	2/70	2.9	293/9708	30.2	0.938
<b><i>Chlamydia trachomatis</i>*</b>	3/70	4.3	740/9708	76.2	0.294
<b><i>Trichomonas vaginalis</i>*</b>	11/70	15.7	1146/9707	11.8	0.313

\* Aetiological diagnosis using multiplex PCR

**CHAPTER 4: DISCUSSION**

Chapter one provided an overview of the HIV burden globally and locally, demonstrating the rationale for the application of GIS and spatial analysis to direct prevention intervention strategies where they are most needed. In this study, GIS was applied and was useful in identifying the selected household, and to analyse household-level data to map HIV prevalence and geometric mean viral load and prevalence of viraemia >1000 copies/ml at a community level. The spatial analyses identified localised clusters of high prevalence of HIV infection including areas with high viral load within this small geographic area of a larger community.

#### **4.1 Localised high HIV prevalence and viral load >1000 copies/ml clusters**

This study has identified clusters of excessively high HIV prevalence within an already hyper-endemic generalised epidemic setting. The HIV prevalence across the district is reported to be 42.5% among pregnant women, however, this study showed that within this well demarcated geographical area in the uMgungundlovu district, the prevalence was unevenly distributed across the area. Areas of unprecedented high prevalence and the localised HIV prevalence cluster of 71% emphasises the intense burden of HIV in this community.

Apart from identifying high prevalence clusters, we identified a high prevalence of viral load >1000 copies/ml which was widespread across urban and rural areas of uMgungundlovu district. In addition, we identified one localized cluster of high HIV prevalence and one cluster for low prevalence of viraemia >1000 copies/ml which were both located north-west of the study area in a semi-rural community. This is an important finding as it suggests that these community members are benefitting from the ART programme and in parallel are likely to be adherent as evidenced by community viral suppression of the low prevalence of viral load >1000 copies/ml amongst these individuals.

#### **4.2 Geometric mean viral load, age and sex differences in viral load**

The two clusters of high geometric mean viral load identified in the urban community located south-east of the study area highlight a potential hotspot for viral transmission. Although there were no areas with spatial clustering of the geometric mean viral load in men, there were numerous geographical areas across the study area where the geometric mean viral load exceeds 8000 copies/ml. This corresponded with high prevalence of viraemia >1000 copies/ml across the study area in men compared to viraemia and geometric mean in women, suggesting a high transmission potential and lack of viral suppression in men.

This study showed that age and gender played an important role in determining viral load. Mapping of viral load spatially demonstrated that a higher proportion of men 15-49 years had viral load >1000 copies/ml compared to their women counterparts. The prevalence of viraemia was higher in most geographical locations of the study area in men compared to women. The viral load (copies/ml) was higher in men compared to women as they reached ages 20-29 years old until the maximum age of 49 years. There were insignificant differences in  $\log_{10}$  mean viral load between men and women aged 15-19 and 45-49 years old, possibly due to a smaller sample size of individuals in those age groups in this study.

One of the potential factors that result in women having lower viral load compared to men is that women in this community access public sector facilities for contraception and pregnancy and [116], therefore, are likely to have HIV Counselling and Testing (HCT) and be linked to care during these visits. Whereas, men are less likely to seek health care sooner and more frequently [117], which could result in them not accessing ART and being virally suppressed.

### **4.3. Relevance of the study findings**

This study indicated that spatial heterogeneity of HIV infection exists in a mature epidemic context and allowed identification of geographical areas to prioritise for HIV prevention in uMgungundlovu district. These findings are important as they support the UNAIDS strategy of focusing on location and population to have the greatest impact on the HIV epidemic [116]. Identification of viral load clusters was crucial as a decrease in community viral load could lead to a reduction in incident infections [118-120]. Therefore, identifying areas and populations with high viral load could guide policymakers on identifying populations that are being missed by ART and where viral transmission is high.

There were no significant differences among the individuals in the HIV cluster compared to those outside the cluster in terms of median age, gender distribution, age of sexual debut, age of partner at sexual debut, number of lifetime partners and the percentage of individuals who reported to be circumcised. Of all STIs tested, only HSV-2 was significantly higher in the HIV cluster. Our findings are consistent with other published studies which indicate that HSV-2 enhances the risk of HIV acquisition [79, 121]. Our study also showed that marriage, condom use in the last 12 months and total household monthly income were higher in the HIV cluster, while the percentage of persons who completed high school was lower compared to the population outside the cluster. Our findings highlight the heterogeneity of HIV even at the sub-district level [84, 112, 121-124]. The key finding of this study is the clear sex differences in the prevalence of viraemia in defined geographical locations [121], and

therefore emphasizes the need for ongoing interventions to be targeted to men in this community to reduce the transmission potential.

The findings of this study are consistent with other studies in showing that HIV heterogeneity exists at a local level [51, 77, 86, 125, 126]. Whilst studies have been undertaken in Rakai (Uganda), uMkhanyakude district in northern KZN (South Africa) and in Durban (South Africa) - the findings from this study are the first to be generated from the two sub-districts of uMgungundlovu district. These findings are critical as they could allow prioritising supplementary HIV prevention interventions such as:

- HCT to link older men and younger women for immediate services for ART.
- HCT to link young men to MMC and ART for those in need. Furthermore, to maximise the coverage and density of prevention efforts in geographically targeted high burden areas for the greatest impact in these sub-districts which could impact on the HIV epidemic trajectory.

Although reporting on incidence is more sensitive and informative measure on the state of the epidemic, measuring incidence at a population level still remains a challenge, due to the constraints of cost and longitudinal follow-up of HIV uninfected individuals [127]. Thus, measuring community viral load has been proposed to be a reliable proxy to assess transmission probability and incidence. The magnitude and spatial mapping of the community viral load measures and assesses the impact of prevention programmes due to a correlation between prevalence of viremia and incidence [128]. Therefore, it was important to spatially map viral load data in settings as in this study since it could potentially show areas of high viral load leading to high incidence rates.

Although an individuals' risk of acquiring HIV is increased in high HIV burden settings, it is important that in the context of scale-up of combination prevention interventions that the impact of ART on community viral load is assessed reliably. Thus, spatially mapping rising prevalence resulting from better survival following ART and community viral load provides a nuanced understanding of the HIV epidemic [51].

#### **4.4. Limitations**

This study has several strength and limitations. The strengths of this study were the design and sampling strategy of the robust random sampling and selection of a single individual from the household which prevented inherent clustering that could potentially occur at the household level. Furthermore, the

sampling strategy provided extensive coverage of the study area and permitted identifying “locations” to prioritise for HIV interventions. This is the first large survey from this community that has collected extensive data on men providing a comprehensive analysis of the overall, age-specific HIV prevalence and prevalence of viraemia. A potential limitation of this study was that there were geographical gaps in the data collected because of not sampling all enumeration areas for more geographic coverage, therefore other areas of the study were estimated based on the nearest areas by interpolation. Another potential limitation was that SaTScan guide recommends cluster size setting which is a window size of up to a maximum of 50% of the population at risk for the detection of clusters. However, this setting may not be appropriate for every type of study, hence this area remains unclear considering that the detection window size for cluster detection remains unstandardized for this method. Furthermore, self-reported data such as condom use, number of partners, sexual debut and other sexual behavioural data are likely to be biased because of potential recall and social desirability bias.

#### **4.5. Conclusions**

In our study, we applied geographic information systems and spatial techniques to identify localised clusters of high numbers of HIV infection and clusters of high viral loads that exist in micro-geographical areas of a larger community. Our findings show high spatial variability of HIV prevalence and viral load across this typical rural South African area. The results also demonstrate that applying spatial analysis to the understanding of HIV epidemiology even in a hyperendemic HIV epidemic setting is a valuable tool to monitor the epidemic. Despite the unprecedented high prevalence of HIV within a geographically specific area, the promising finding of the high prevalence of viral load <1000 copies/ml underscore the importance and impact of HIV programmes that have been rolled-out in this community. Furthermore, the high HIV viral load in young women and men in this region play a significant role in sustaining the epidemic, and there is an urgent need to prioritise interventions critical to reducing the potential for HIV transmission.

#### **4.6. Recommendations**

Whilst prevalence provides information on the extent and burden of HIV disease, it is also important to know and understand the contribution of ART on survival and its impact on prevalence which is likely to increase [129]. To date over 3.3 million South Africans, predominantly in KZN are on ART and the provincial government has continued to scaled-up HIV prevention and treatment programmes at ward level and these will require advanced monitoring and evaluation of their impact on incident infections. Parallel to measuring the spatial distribution of HIV prevalence it will be critical to measure coverage and fidelity of ART programmes which has the potential to substantially lower the viral load in the population and in turn lower the number of new infections to the point of achieving and sustaining epidemic control. It will be critical to have an in-depth understanding of the longitudinal distribution

of HIV infections and prevalence of viraemia at the micro-geographical level for ongoing transmission potential.

Traditional HIV surveillance focused on HIV prevalence and AIDS-deaths. However, as survival of individuals improves with ART [26, 27] it is becoming imperative to add newer tools to determine the effectiveness of treatment as prevention [28] and prevalence of viraemia as a correlate of transmission potential and incidence to understand the underlying dynamics of transmission within communities and different geographical areas. Using tools such as geospatial mapping of prevalence of viraemia and the sex differences could meaningfully influence and strengthen services at macro and local levels [30, 31]. Thus, comprehensive surveillance must include tools to allow understanding the magnitude and characteristics of the epidemic and delivery of and uptake of services.

Surveillance of HIV infections and viral load measures including geospatial mapping of these are additional tools that can identify locations and populations for prioritising interventions. Viral load measures are central to the continuum of care for individuals on ART and more importantly for potential transmissions. Given high viral load in men, strategies to link men to health care need to be urgently developed.

#### **4.7. Future studies**

- With the rapid scale-up of ART, it is important to test whether proximity to the health facility, area of employment and time to health care facility could impact on the long-term HIV continuum of care in this community. It is possible these factors may contribute to poor adherence to ART leading to high viral loads and contributing to sustaining the epidemic or the potential for the emergence of ART-resistant HIV epidemic. Therefore, ongoing mapping of prevalence and community viral load in the context of facilitators and barriers of continuum of care is crucial for monitoring and achieving long-term epidemic control. Innovative mapping methods provide protection of individual's confidentiality but could potentially induce bias [130].
- There is also a need to assess biological, behavioural, psychosocial and structural factors that are associated with being in high viral load clusters and factors that are associated with high viral loads in men, 20 years and older.

- There is an important opportunity to utilise laboratory-based HIV assays to identify new infections and to evaluate whether these infections geographically cluster in particular locations.



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## **Appendices**

### **Appendix1: Review article**

The review article attached below, titled: “Strengthening HIV surveillance: measurements to track the epidemic in real time” is complementary and was adopted from the literature review of this dissertation and it gives a broad understanding about why the research conducted in this dissertation is relevant to current and future HIV surveillance.

## Strengthening HIV surveillance: measurements to track the epidemic in real time

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Surveillance for HIV as a public health initiative requires timely, detailed and robust data to systematically understand burden of infection, transmission patterns, direct prevention efforts, guide funding, identify new infections and predict future trends in the epidemic. The methods for HIV surveillance have evolved to reliably track the epidemic and identify new infections in real time.

Initially HIV surveillance relied primarily on the reporting of AIDS cases followed by measuring antibodies to HIV to determine prevalence in key populations. With the roll-out of antiretroviral therapy (ART) resulting in better survival and the corresponding increase in HIV prevalence, the landscape of surveillance shifted further to track HIV prevalence and incidence within the context of programmes. Recent developments in laboratory assays that potentially measure and differentiate recent versus established HIV infection offer a cost-effective method for the rapid estimation of HIV incidence. These tests continue to be validated and are increasingly useful in informing the status of the epidemic in real time.

Surveillance of heterogeneity of infections contributing to sub-epidemics requires methods to identify affected populations, density, key geographical locations and phylogenetically linked or clustered infections. Such methods could provide a nuanced understanding of the epidemic and prioritise prevention efforts to those most vulnerable. This paper brings together recent developments and challenges facing HIV surveillance, together with the application of newer assays and methods to fast-track the HIV prevention and treatment response.

**Keywords:** geospatial locations, HIV assays, incidence, phylogenetics, prevalence, surveillance

### Introduction

To advance the ambitious goal of ending the AIDS epidemic, the post-2015 Joint United Nations Programme on HIV/AIDS (UNAIDS) Fast-Track aims to achieve ambitious targets over the next five years to include the 90-90-90 treatment strategy and zero discrimination by the year 2020 (UNAIDS, 2014a). These strategic milestones work within the new paradigm of realising an end to the HIV epidemic by 2030. These targets include reducing new HIV infections from the current 2 million (1.9–2.2 million) in adults and children to less than 500 000; to reduce the number of people dying from AIDS-related causes to less than 500 000 and to eliminate HIV-related discrimination. Reaching these targets could avert 28 million new HIV infections including 5.9 million infections in children and 21 million AIDS-related deaths by 2030 (UNAIDS, 2014b, 2015c).

Achieving these Fast-Track milestones necessitates timely, detailed and robust HIV surveillance; and the efficient use of these activities could facilitate better understanding of transmission patterns, direct prevention efforts, guide funding, evaluate the impact of HIV related services, predict future HIV burden of infection and identify new trends (Parkin et al., 2016; Puren & Takuva, 2011; UNAIDS/WHO Working Group on Global HIV/AIDS and STI surveillance, 2011; Wilson & Halperin, 2008).

Surveillance relies on diverse and multiple data sources to obtain a well-defined picture of the epidemic and trends over time, and is crucial in monitoring the disease globally. Whilst surveillance data provides extensive knowledge on the prevalence of HIV, information on prevalence alone is not sufficient to understand the rate of new infections (incidence), including where and within which networks viral transmissions might be occurring. HIV incidence remains a key indicator for monitoring the impact of HIV-related programmes, especially in countries which bear a disproportionate burden of HIV/AIDS (WHO, 2004). As the field of HIV surveillance evolves, additional tools and techniques are in development for the rapid, concurrent systematic measurement of incidence in real time to assess the achievements of the Fast-Track targets (Mastro, 2013; Rosenberg, Pilcher, Busch, & Cohen, 2015; UNAIDS, 2010).

Laboratory tests for recent infection (TRIs) are increasingly being used to identify and differentiate new and established infections, though these require additional biological marker information such as HIV-1 RNA viral load and exposure to antiretroviral therapy (ART). These improved surveillance tools for incidence measurements are important applications for more accurate testing of recently acquired infections; to identify concentrated localised sub-epidemics and sexual networks of individuals who are less likely to test for HIV or be linked to care and could therefore contribute to



sustaining the epidemic (Tanser, de Oliveira, Maheu-Giroux, & Barnighausen, 2014; UNAIDS, 2015b).

Using a combination of tests to improve surveillance would further assess the impact of scaled up HIV services for diagnoses, treatment and prevention interventions and ultimately move countries closer to the post 2015 Fast-Track targets.

This paper aims to describe and critique methods used for the measurement of existing and new HIV-1 infections complemented by the use of phylogenetics and geospatial epidemiology, including the complex convergence of these tools for surveillance.

### Sources of data for HIV surveillance

HIV antibody tests have been the mainstay of sentinel surveillance of pregnant women attending public sector primary health care clinics for antenatal care. These data sources have been supplemented by national population based household surveys and surveillance of HIV in high risk individuals attending specialist clinics for sexually transmitted infections (STIs) and tuberculosis (TB) (Calleja et al., 2005; Mahy, Garcia-Calleja, & Marsh, 2012). Traditionally these data sources provided a reliable understanding of the total number of existing HIV infections (prevalence) in contrast to understanding where new HIV infections are occurring (incidence). As HIV is linked in time, place and population group, it is critical to know where prevalent and incident infections are occurring and the modes of transmission. Furthermore, aggregating the data by age and gender is useful to identify key populations, and assist in understanding trends and dynamics of transmission (Puren & Takuva, 2011; Sun et al., 2007).

Whilst these traditional surveys generate important reliable data on national estimates, as epidemics mature with increasing coverage of ART, prevalence of HIV infection becomes a less reliable marker of the evolving epidemic since survival improves, prevalence increases and masks new infections; especially in key populations such as young adolescents, men who have sex with men (MSM), sex workers (SW) and people who inject drugs (PWID) (UNAIDS, 2014b).

### Methods of measuring HIV incidence

Accurate and timely measurements of HIV incidence are critical elements of HIV surveillance (Moyo et al., 2015) especially in the context of HIV intervention efforts. However, none are without limitations. Table 1 provides a summary of methods to estimate HIV incidence, reviewing the benefits and limitations of each method.

#### **Cohort estimation**

Measurement of HIV incidence through prospective cohorts is the gold standard method as well defined groups of HIV-negative individuals are followed and tested and retested for new infection over time (Puren & Takuva, 2011; UNAIDS/WHO Working Group on Global HIV/AIDS and STI surveillance, 2011). However, this method has intrinsic disadvantages, as it is influenced by biases that could lower or exaggerate incidence measurement, especially true for high HIV endemic and resource-limited regions (Mastro

et al., 2010; Puren & Takuva, 2011). As the high cost of establishing cohorts and potential loss to follow-up are key challenges of cohort studies, there is a drive for robust HIV surveillance strategies that directly measure incidence in real time that are accurate, cost-effective and less resource-demanding. Such tests would facilitate measurement of true incidence dynamics of the HIV epidemic and assist with better planning, tracking and evaluation of HIV prevention and treatment strategies in a timely manner (Diaz, De Cock, Brown, Ghys, & Boerma, 2005; Mahy et al., 2012; Pervilhac, Stover, Pisani, Brown, & Mayorga, 2005).

#### **Mathematical modelling**

HIV incidence and trends have been estimated using input parameters such as prevalence, mortality data and ART use within mathematical models. Models have been valuable for informing HIV trends, but have limitations due to the ready availability of reliable prevalence data, prevention and treatment programme coverage, fidelity of interventions and geographical region (Stover, Brown, & Marston, 2012) as well as the bias of inaccurate assumptions within the model. Models include the Asian Epidemic Model (AEM) and the Estimation and Projection Project (EPP/Spectrum) which are annually updated and continue to adapt for correcting biases and inclusion of additional information sources (Brown et al., 2014; UNAIDS, 2010). Within EPP/Spectrum, the model generates estimated predictions on, amongst others, HIV prevalence, treatment requirements and mortality rates, and calculates incidence from prevalence data while considering the number of people on ART (Stover et al., 2012; UNAIDS, 2010). Data from incidence assays could be incorporated into the EPP model to potentially narrow parameters and improve estimates (Bao, Ye, & Hallett, 2014). Estimates from models assist countries with their health service planning, policy development and budgeting. Furthermore, comprehensive behavioural data collection and information regarding population size and STIs in key populations assist with determining HIV infection patterns and correspondingly the effectiveness of HIV programmes and initiatives (Gouws, White, Stover, & Brown, 2006). The need for such thorough data may therefore limit the programmatic utility in resource-limited settings and specifically in countries already carrying the highest HIV burden. Modelling is less financially and labour intensive than population based surveys and therefore commonly used to estimate incidence. However, these models can still have a time delay, and remain dependant on the quality and accuracy of available empirical data sources used for estimation (Brookmeyer, 2010).

#### **Inferring HIV incidence from prevalence among pregnant women aged 15–24 years**

Prevalence of HIV infection among pregnant 15 to 24-year-olds attending antenatal clinics could be used to approximate trends in incidence with an assumption that prevalence is due to recent sexual debut mirroring recent infection in this age group (Zaba, Boerma, & White, 2000). While this is not a direct measure it has been used as a proxy, especially in countries with generalised epidemics (Ghys, Kufa, & George, 2006). However, over time as ART use increases and changes in behaviour occur (i.e., with a shift towards delayed sexual debut), this method is likely to become less reliable.

**Table 1:** Summary of methods to measure HIV incidence

Research method	Principle	Data source	Advantages	Disadvantages	Going forward
<b>Prospective Cohort Studies</b>					
	<ul style="list-style-type: none"> <li>• Directly observed measure of incidence — Follow HIV-uninfected people over time and test for HIV at fixed intervals</li> </ul>	<ul style="list-style-type: none"> <li>• Observational studies</li> <li>• HIV prevention preparedness studies.</li> <li>• Clinical trials</li> </ul>	<ul style="list-style-type: none"> <li>• Considered "gold standard"</li> <li>• Direct measure of HIV sero-conversion</li> </ul>	<ul style="list-style-type: none"> <li>• Large sample size required</li> <li>• Costly</li> <li>• Logistically challenging</li> <li>• Loss to follow-up, particularly those at high risk</li> <li>• Intrinsic bias (participant behaviour change)</li> <li>• Infrequently conducted</li> </ul>	<ul style="list-style-type: none"> <li>• Improve retention of study participants</li> </ul>
<b>Biological Assays Testing for acute/new and recent/early infections</b>					
Antigen based tests for acute/new HIV infection	<ul style="list-style-type: none"> <li>• Uses "biomarkers" such as HIV-1 RNA or P24 antigen that develop during the recent/acute stage of infection prior to sero-conversion</li> </ul>	<ul style="list-style-type: none"> <li>• Large-scale cross-sectional surveys</li> </ul>	<ul style="list-style-type: none"> <li>• Single sample required</li> <li>• Follow-up not required</li> <li>• Can distinguish between acute HIV infection in absence of HIV antibodies and post sero-conversion.</li> <li>• Acute HIV infection stage used for incidence estimation</li> </ul>	<ul style="list-style-type: none"> <li>• Inadequate sensitivity and specificity</li> <li>• Short duration of detection period (~28 days)</li> <li>• Misclassification of acute with post sero-conversion infections</li> <li>• Requires confirmatory testing</li> <li>• No "gold standard" test</li> </ul>	<ul style="list-style-type: none"> <li>• Development of new fourth generation HIV antibody assays decreases the "window-period" of acute HIV infection in absence of HIV antibodies</li> </ul>
Antibody avidity based tests for recent/early HIV infection	<ul style="list-style-type: none"> <li>• Several tests available which use antibody avidity maturation following sero-conversion to determine recent/early HIV infections</li> </ul>	<ul style="list-style-type: none"> <li>• Large-scale cross-sectional surveys</li> </ul>	<ul style="list-style-type: none"> <li>• Follow-up not required</li> <li>• Detection period (~130 days)</li> <li>• Can distinguish between early and established HIV infection</li> <li>• Early HIV infection used for incidence estimation</li> </ul>	<ul style="list-style-type: none"> <li>• Requires confirmatory testing</li> <li>• Requires additional information on ART exposure and HIV-1 RNA viral load</li> <li>• No "gold standard" test</li> </ul>	<ul style="list-style-type: none"> <li>• Requires monitoring and estimation of incidence as HIV treatment is moving towards test and treat strategy — more recently infected individuals may be on ART, and this could affect accuracy of incidence measurement</li> </ul>
<b>Combination of HIV testing algorithms</b>					
	<ul style="list-style-type: none"> <li>• Combination and sequence of assay tests for detecting "recency" of HIV infection with clinical data</li> </ul>	<ul style="list-style-type: none"> <li>• Cross-sectional surveys</li> </ul>	<ul style="list-style-type: none"> <li>• Multiple tests selected to streamline for improving accuracy</li> <li>• Addition of clinical data to reduce correct for misclassifications for interpreting results</li> </ul>	<ul style="list-style-type: none"> <li>• Expensive</li> <li>• Still requires correction for misclassification despite addition of clinical data</li> </ul>	<ul style="list-style-type: none"> <li>• Data obtained with this approach is crucial to compare with other incidence estimates obtained by other methods such as cohorts and mathematical modelling</li> <li>• Field validation using population-based surveys is required</li> <li>• With increase in ART roll-out, surveys that include individuals on ART are required</li> </ul>
<b>Mathematical modelling</b>					
	<ul style="list-style-type: none"> <li>• Several models based on parameters of HIV prevalence data, modes of transmission, assumptions about survival after infection and mortality, risk behaviour, population size, STI prevalence, incubation information and ART coverage</li> </ul>	<ul style="list-style-type: none"> <li>• Cross-sectional surveys providing HIV prevalence trend data</li> <li>• Sentinel HIV prevalence data routinely collected</li> </ul>	<ul style="list-style-type: none"> <li>• Cost-effective</li> <li>• Moderate-high levels of confidence</li> <li>• Relatively simple</li> </ul>	<ul style="list-style-type: none"> <li>• Depends on accuracy of prevalence and measurements of population size, ART coverage, mortality</li> <li>• Can have high uncertainty range</li> <li>• Time between studies may affect precision of results</li> <li>• Key populations may not be representatively sampled</li> </ul>	<ul style="list-style-type: none"> <li>• Cross-sectional surveys need to have greater inclusion of adolescents and key populations</li> <li>• Less reliance on young people as proxy for sexual debut and recent infection</li> </ul>



### **Laboratory tests to estimate HIV incidence**

The comprehensive use of laboratory tests to estimate HIV incidence is growing. These tests measure immunologic biomarkers in acutely and recently HIV-infected persons from cross-sectional samples (Kassanjee et al., 2011; Mastro et al., 2010; McDougal et al., 2005). The major advantage of these tests is that only one sample is required to classify an infection as acute (new) or established (long term) (UNAIDS/WHO Working Group on Global HIV/AIDS and STI surveillance, 2011). Before implementation of a testing programme for incident infections for surveillance, epidemiological factors complement and guide the application of test-specific prerequisites such as calibration, validation, quality assurance and performance characteristics that may influence interpretation. When properly and judiciously applied, the capacity of these tests to enhance surveillance would provide precise and timely analysis of the dynamics of the epidemic and assess the effectiveness of public health interventions.

**Markers of acute (new) HIV infection:** HIV-1 RNA and p24 antigen are the first set of biomarkers that are readily detectable before sero-conversion (Daar, Pilcher, & Hecht, 2008). The nucleic acid amplification test (NAAT) for the detection of HIV-1 RNA and p24 antigen assays are both highly sensitive and specific, with HIV-1 RNA having an added advantage of being detected much earlier than p24 antigen (McDougal et al., 2005; Quinn et al., 2000). HIV-1 RNA testing was developed to monitor patients and has more recently been adopted as a way to identify acute (new) HIV infection, i.e. identifying acutely infected individuals by pooling and testing HIV seronegative samples. Several surveillance programmes used NAAT of pooled HIV seronegative specimens to estimate HIV incidence (McDougal et al., 2005; Quinn et al., 2000). A further advance in diagnosing acute HIV infection for estimating HIV incidence has been the development of fourth generation HIV-1 assays which detect p24 antigen and HIV antibody simultaneously (Daar et al., 2008). However, the detection levels of these assays differ as key viral markers evolve rapidly in acute HIV infection with a “window-period” of approximately 28 days (Cohen, Gay, Busch, & Hecht, 2010). Assays which identify acute (new) HIV infection are useful for estimating HIV incidence, but require large sample sizes (McDougal et al., 2005).

**Markers of recent (early) HIV infection:** To determine recent (early) infection and estimate HIV incidence, the assay using gp41 immunodominant sequences from HIV subtypes B, E and D in the capture enzyme immunoassay (BED assay) (UNAIDS/WHO Working Group on Global HIV/AIDS and STI surveillance, 2011) had been used extensively. However, the test misclassified early HIV infection even after many years of infection, resulting in an overestimate of true population incidence. Misclassification was particularly high among individuals on ART and those with very low CD4 cell counts, therefore data on ART and CD4 cell counts at the individual level are collected to exclude such persons from the incidence analysis. Compared to the BED assay, the newer TRIs have overcome many of the misclassification issues and are less likely to be affected by advanced HIV disease or low CD4 cell counts. Whilst assay misclassification rates have

been shown to vary across countries (Busch et al., 2010; Longosz, Serwadda, et al., 2014), by using adjustment factors, assay-derived estimates can be calibrated to correct for misclassification or incorporated into the mathematical formula to improve incidence estimates (Stover et al., 2012; UNAIDS/WHO Working Group on Global HIV/AIDS and STI surveillance, 2011).

The newer TRIs for recent (early) HIV infection are based on antibody avidity, that is, the maturity of the HIV antibody response which increases over time following sero-conversion (Duong et al., 2012). Antibody avidity is more robust than antibody titre because it is a functional property of maturing antibodies. Antibodies of low avidity are usually indicative of recent (early) infection and could be used to determine HIV incidence (Duong et al., 2012; Parekh et al., 2002).

Several new assays are currently being evaluated to determine their accuracy in distinguishing recent (early) from established (long term) HIV infection and translate this to measuring HIV incidence on a population level (UNAIDS/WHO Working Group on Global HIV/AIDS and STI surveillance, 2011). These include the rIDR-M-Avidity Index Assay (rIDR-M AI EIA) developed by the US Centers for Disease Control and Prevention Global AIDS Program (CDC GAP) Serology/Incidence laboratory. This test is an avidity index assay using a recombinant protein (rIDR-M) which incorporates three sequences derived from the immune-dominant region (IDR) of gp41; representing divergent HIV-1 subtypes A through E (group M) (Wei et al., 2010). This assay uses a pH 3.0 buffer to dissociate low avidity antibodies which are characteristic of recent infection. The greater the proportion of high avidity antibodies remaining bound increases the avidity index, therefore indicating established (long term) infection (Suligoi et al., 2002). The *Bio-Rad Avidity Enzyme Immune Assay (EIA)* is a modification of the genetic system (GS) HIV-1/HIV-2 Plus O EIA (Redmond, MA) (Hauser et al., 2014). This assay uses 0.1M diethylamine (DEA) to dissociate low avidity antibodies characteristic of recent infection. The greater the proportion of high avidity antibodies remaining bound increases the avidity index, therefore indicating established infection. The *Limiting Antigen (LAg) Avidity EIA* developed by the CDC GAP Serology/Incidence laboratory is an avidity-based assay that the same recombinant multi-subtype protein (rIDR-M), but at a limited coating concentration, such that it is even easier to dissociate low avidity antibodies. Advantages of the LAg are that the test requires only a single well as opposed to two wells, therefore allowing for an increased number of specimens to be tested, is also easier to perform and is able to dissociate low avidity antibodies more readily. Furthermore, the LAg Avidity EIA has been evaluated, commercially available avidity-based HIV-1 incidence assay and used in several national population based surveys in Kenya (Kimanga et al., 2014), South Africa (Simbayi et al., 2014) and Swaziland (Swaziland Ministry of Health, 2012).

The two main performance parameters of TRIs are the mean duration of recent infection (MDRI) — the average time spent “recently” infected (usually a period of 4–12 months); and the false-recent rate (FRR) — the misclassification of recent with non-recent infection (Kassanjee, McWalter,



Barnighausen, & Welte, 2012; Kim et al., 2011; Welte, McWalter, Laeyendecker, & Hallett, 2010). Therefore an accurate estimate of MDRI is required for a more precise incidence estimation (Hanson et al., 2016). TRIs could fail to correctly identify recent infections due to the following variances: unique viral progression and immune system responses, dissimilar HIV subtypes between different individuals and populations (Busch et al., 2010; Mastro, 2013; Mastro et al., 2010; Puren & Takuva, 2011; Longosz, Mehta, et al., 2014), elite controllers (i.e., people who sustain low viral RNA and low antibody responses to HIV) (Puren & Takuva, 2011; UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance, 2013), ART status (those having low HIV progression due to drug-induced suppression therefore have decreased antibody concentration) and those with AIDS having a low CD4 cell count and low HIV antibody concentration (Mastro et al., 2010). These factors can lead to varying MDRI (Parekh et al., 2011) and FRR (Kassanjee, 2011; Kassanjee et al., 2011; WHO/UNAIDS, 2013), depending on the assay used. Such misclassification can lead to biased incidence estimates and therefore is not yet recommended that TRIs be used for individualised testing and diagnosis of recent HIV infection or as stand-alone tests for incidence measurement, but can be applied at a population level in a form of an algorithm (UNAIDS/WHO Working Group on Global HIV/AIDS and STI surveillance, 2011).

#### **Combination of HIV testing algorithms**

To address limitations of individual incidence assays and improve accuracy, TRIs are often applied through Recent Infection Testing Algorithm (RITA) (WHO, 2009) and Multiple-Assay Algorithm (MAA) (Laeyendecker et al., 2013). These algorithms involve utilising two or more incidence assays that detect different components of the recent/early HIV infection together with other clinical indicators such as CD4 cell count, ART use and HIV-1 RNA viral load (Busch et al., 2010; Longosz, Serwadda, et al., 2014). While improving accuracy of test results this method can be impeded by cost, especially in resource-limited regions since CD4 cell count requires the whole blood sample, immediate testing and not dried blood spots which are usually used in national surveys (Busch et al., 2010; Mastro et al., 2010). Samples with detectable ART and with viral load of <1 000 copies/ml have been excluded from analysis to increase the accuracy of TRIs (Mastro et al., 2010) as ART use may lead to misclassification (Laeyendecker et al., 2009). However, even with the exclusion of those on ART or with AIDS, misclassification still occurs (Longosz, Mehta, et al., 2014). TRIs continue to undergo extensive validation for use in RITA for HIV (Consortium for the Evaluation and Performance of HIV Incidence Assays (CEPHIA), 2015; Hanson et al., 2016).

#### **Methods to enhance understanding of viral transmissions**

Essential to surveillance and targeted response is knowing and understanding the biological and behavioural risk factors contributing to HIV viral spread. The novel areas of phylogenetics and using locations to prioritise target populations are methods to further understand epidemic incidence dynamics.

#### **Concept of HIV-1 phylogenetics**

Phylogenetics is a molecular epidemiological strategy that explores viral evolution, diversity transmissions, and characterises epidemics on the basis of the genetic interrelatedness of HIV-1 viral sequences. Phylogenetic analysis has been used to determine the emergence of HIV globally from non-human primate reservoirs, estimate the age of Simian Immunodeficiency Virus (SIV) and map the spread of HIV between continents early in the epidemic (Courgnaud et al., 2002). In the context of HIV-1, this technique takes advantage of the high error rate and rapid pace of HIV replication, which leads to a predictable rate of evolution over long periods of time, or a "molecular clock". Phylogenetic trees can be generated and used to determine the most recent common ancestor in a given set of sequences, revealing linkages that define their transmission history (Grabowski & Redd, 2014). Sexual networks contributing to HIV acquisition are poorly understood in many regions for various reasons. Among these include mis- and under-reporting of sexual relationships, social desirability bias, the transient nature of risky relationships, migrancy and various cultural factors. Furthermore, traditional methods to map sexual networks are labour intensive and require extensive engagement with participants and verification of relationships. Therefore HIV-1 sequence data could be used as a tool to gain information on networks that are otherwise difficult to obtain.

Phylogenetic linkages have empirically been assessed in HIV-1 clinical trials as in the HPTN 052 (Cohen et al., 2011) and Partners in Prevention HSV/HIV Transmission trials (Campbell et al., 2011). The analysis of HIV-1 *pol* sequences from HIV-1 sero-conversions showed that at least a quarter of these occurred within stable partnerships, were unlinked and demonstrated the magnitude of sexual networks that potentially sustain epidemics. Recently, phylogenetic methods have been applied to generally understand HIV transmission, especially among MSM to detect transmission clusters leading to outbreaks (Brenner, Roger, Otis, & Wainberg, 2013; Middelkoop et al., 2014; Ratmann et al., 2016). Fewer studies have been carried out in Africa. However, based on the phylogenetic analyses of the *gag* and *env* genes, a major study conducted in the Rakai district of Uganda provided some indication that viral transmissions occurred beyond household partnerships and were common from outside the communities (Grabowski et al., 2014). Applying phylogenetics to understand "linked" or "clusters of linked" HIV transmission in the areas most affected by the pandemic could assist with optimal direction for targeted interventions to interrupt viral transmission (Castro-Nallar, Pérez-Losada, Burton, & Crandall, 2012). A recent phylogenetic study showed that a higher escalation in cluster size was linked with recently infected persons, which may indicate individuals with new infections are a driving force of the epidemic (Ragonnet-Cronin et al., 2010). Estimating recent HIV infection by diversity has also been performed in many studies (Andersson et al., 2013; Cousins et al., 2011; Ragonnet-Cronin et al., 2012; Xia et al., 2014; Yang et al., 2012). These methods are based on the premise that HIV diversity increases as the duration of infection increases (Andersson et al., 2013). Therefore HIV diversity is a possible biomarker for classifying HIV infection as recent or non-recent (Cousins et al., 2011; Yang et al., 2012).



The high resolution melting (HRM) assay is one of the methods that has shown great potential for determining HIV incidence using viral diversity as a biomarker (Cousins et al., 2011). This assay has also shown that AIDS is not correlated with misclassification as in serologic incidence assays (Cousins et al., 2011). The markers obtained from this assay are less related to markers of serological methods and therefore have the potential to be used in an HIV algorithm together with serological tests to further reduce misclassification (Cousins et al., 2011). This approach has been used with the MAA (James et al., 2013). However, ongoing evaluations are needed to set parameters and standardise this assay for its use across various populations. It should be noted that HRM has its own limitations, that is, not entirely showing the sequence differences in a DNA fragment (Tong & Giffard, 2012). Intra-patient pattern-based viral genetic diversity methods have also been used to measure recent (early) infection (Yang et al., 2012). This method is also based on diversity patterns from recent (early) and established infected individuals and is unaffected by AIDS, HIV-1 RNA viral load and ART use. The MDRI for this method is 200 to 350 days to potentially detect recent infections (Yang et al., 2012) in combination with serological tests. As surveillance requires high throughput of specimens, these methods may be difficult to establish in resource-limited settings.

Phylogenetic analysis of HIV-1 sequences is therefore useful to identify linked sequences in dyads and potentially characterise the epidemiologic relationships of these in clusters. HIV transmission clusters within high-risk groups (Poon et al., 2015) and including demographic, geographic and behavioural characteristics of individuals in clusters could provide crucial information of evolving HIV strains and transmission dynamics ("phylogenetics") for prevention initiatives (Castro-Nallar et al., 2012).

#### **Concept of locations to prioritising populations**

Scaling up HIV testing to identify newly infected individuals is key to achieving the Fast-Track targets, especially within hyper-endemic regions consisting of concentrated localised sub-epidemics which tend to cluster and sustain the epidemic (Chen et al., 2015; Tanser et al., 2014; UNAIDS, 2015b).

Analysing the nature of the epidemic at a local level, using location-based approaches to deliver high impact and locally relevant programmes in populations with the primary need (high burden) to have the greatest impact, underscore the Fast-Track approach (UNAIDS, 2014a). Thus, surveillance systems must include spatial analytical methods to identify gaps in HIV prevention programmes and hot-spot geographies that are currently driving the epidemic (UNAIDS, 2013; UNAIDS/WHO Working Group on Global HIV/AIDS and STI Surveillance, 2013). The location-based approach also includes Fast-Track cities within different countries, as they are known to contribute significantly to the epidemic, with 156 cities within 30 countries contributing 89% of all new infections (UNAIDS, 2015a). The HIV epidemic in sub-Saharan Africa tends to be higher in urban than in rural communities and it is estimated that by 2030, a total of 60% of the global population will live in cities including areas in sub-Saharan Africa and Asia which are currently less urbanised (UNAIDS, 2015a).

ART use together with HIV prevention strategies targeting key populations and hot-spot geographies (areas of high incidence and/or prevalence) have the greatest potential to reduce HIV acquisition, as compared to a general approach, due to existence of high heterogeneity of HIV infections within different regions (Anderson et al., 2014; Jones et al., 2014; Wand & Ramjee, 2010). Geographically targeted prevention interventions have proven successful where geographical clustering of HIV incident cases occur within sub-groups (Aral & Cates, 2013) and commercial sex work (Halperin, de Moya, Perez-Then, Pappas, & Garcia Calleja, 2009). "Knowing your epidemic" is key to meeting the UNAIDS targets, and this requires knowing the profile of the local epidemic in order to choose the right combination of interventions that have the greatest, longest-lasting impact and are cost effective (Wilson & Halperin, 2008).

The future of HIV surveillance is tailored towards a more location-specific approach and incorporating RITAs, CD4 cell count and HIV-1 RNA viral load testing of the participants (UNAIDS, 2013; WHO, 2015). Understanding the epidemiology and location is crucial, however, most national surveillance studies do not include these data due to lack of resources. Such an approach requires georeferenced HIV data at a local level with data only available by large geographic units (UNAIDS, 2015b; Wilson & Halperin, 2008).

The major limitations to full-scale implementation of a location-based approach are the lack of georeferenced data, trained and qualified staff and the cost of commercial software programs. The issues of privacy and confidentiality related to disease status are significant challenges when addressing HIV because of stigma (Mbonu, van den Borne, & De Vries, 2009), meaning the target of zero discrimination is equally important to the 90-90-90 treatment target. However, different geographic masking methods can be used to protect privacy of HIV infected individuals and communities.

#### **Conclusions**

Estimating HIV incidence rapidly and accurately offers great benefits to population-based HIV surveillance, particularly in the era of post-2015 Fast-Track targets. Surveillance continues to evolve and countries cannot use a "one-size fits all" approach. This paper is not an exhaustive list of all methods, but it is intended to stress the importance of incidence measurements and bring together some of the more recent developments and ongoing challenges.

New biomarkers and methods that are cost-effective, commercially available, with good quality assurance, simple to use, accurate and less affected by factors such as ART use and advancing state of infection remain essential. An ideal incidence test would simultaneously diagnose HIV and give an accurate estimation of how recently the infection occurred. Such an assay could allow robust surveillance studies to accurately measure incidence and therefore more meaningfully inform public health programmes. HIV epidemic control is achieved when the numbers of new infections are below the numbers of AIDS-related deaths. However, the global drive is moving beyond simple epidemic control to advocate for drastic reduction and an



eventual end of the HIV epidemic. This reality will only be achieved through concentrated and targeted investments and interventions adjusted to the local specific geographies where the people most affected by HIV are rapidly identified and linked to treatment in an effective care cascade. Fast-tracking the response to HIV involves monitoring the epidemic through effective, timely and targeted surveillance systems. This is especially true for resource-poor regions which continue to face a disproportionate share of the global burden of HIV/AIDS.

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## **Appendix 2: Ethical Approval**

13 May 2014

Prof Ayesha BM Kharsany  
CAPRISA  
2<sup>nd</sup> Floor  
KRITH Building  
Medical School  
[Kharsany@ukzn.ac.za](mailto:Kharsany@ukzn.ac.za)

**PROTOCOL: HIV Incidence Provincial Surveillance System (HIPSS): A longitudinal study to monitor HIV incidence trends in the uMgungundlovu District, KwaZulu-Natal, South Africa. REF: BF269/13.**

The Biomedical Research Ethics Committee (BREC) has considered the abovementioned application.

The study was provisionally approved by a quorate meeting of BREC on 14 August 2013 pending appropriate responses to queries raised. Your responses dated 06 May 2014 to queries raised on 09 December 2013 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 13 May 2014.

This approval is valid for one year from 13 May 2014. 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 (2004), South African National Good Clinical Practice Guidelines (2005) (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).

Professor D Wassenaar (Chair)  
Biomedical Research Ethics Committee  
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Founding Campuses: ■ Edgewood ■ Howard College ■ Medical School ■ Pietermaritzburg ■ Westville



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18 April 2016

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**PROTOCOL: HIV Incidence Provincial Surveillance System (HIPSS): A longitudinal study to monitor HIV incidence trends in the uMgungundlovu District, KwaZulu-Natal, South Africa. REF: BF269/13.**

We wish to advise you that your letter dated 08 March 2016 requesting approval of Amendments for the above study has been noted and approved by the Biomedical Research Ethics Committee at a meeting held on 12 April 2016.

The following have been noted and approved:

- Inclusion of two additional secondary study objectives which require analyses of data that has already been collected as part of study procedures (objectives 9 and 10).

Please note that the meeting was not quorate and this approval will be ratified at the next BREC meeting to be held on 10 May 2016.

Yours sincerely

Mrs A Marimuthu  
Senior Administrator: Biomedical Research Ethics





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18 April 2016

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**PROTOCOL: HIV Incidence Provincial Surveillance System (HIPSS): A longitudinal study to monitor HIV incidence trends in the uMgungundlovu District, KwaZulu-Natal, South Africa. REF: BF269/13.**

#### RECERTIFICATION APPLICATION APPROVAL NOTICE

Approved: 13 May 2016  
Expiration of Ethical Approval: 12 May 2017

I wish to advise you that your application for Recertification received on 08 March 2016 for the above protocol has been noted and approved by the Biomedical Research Ethics Committee (BREC) at a meeting that took place on 12 April 2016 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.

Please note that the meeting was not quorate and this approval will be ratified at the next BREC meeting to be held on 10 May 2016.

Yours sincerely

Mrs A Marimuthu  
Senior Administrator: Biomedical Research Ethics



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07 November 2016

Mr UE Buthelezi (210503421)  
Discipline of Medical Microbiology  
School of Laboratory Medicine and Medical Sciences  
[210503421@stu.ukzn.ac.za](mailto:210503421@stu.ukzn.ac.za)

**Study Title:** Spatial analysis of HIV Infections in high burden sub-districts in KwaZulu-Natal, South Africa.

**Degree:** MMedSc

**BREC REF NO:** BE312/16 (sub-study of BF269/13)

### EXPEDITED APPLICATION

A sub-committee of the Biomedical Research Ethics Committee has considered and noted your application received on 19 May 2016.

The study was provisionally approved pending appropriate responses to queries raised. Your response received on 20 September 2016 to BREC correspondence dated 11 August 2016 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 07 November 2016.

This approval is valid for one year from 07 November 2016. 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 13 December 2016.

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

Yours sincerely

Professor J Tsoka-Gwegweni  
Chair: Biomedical Research Ethics Committee

cc: supervisor: [ksansa@ukzn.ac.za](mailto:ksansa@ukzn.ac.za)

cc: postgraduate administrator: [dj.dhrainin@ukzn.ac.za](mailto:dj.dhrainin@ukzn.ac.za)

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