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HIV IN SOUTH AFRICAN FORENSIC MEDICAL PRACTICE.

user
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Abstract: *HIV in South African forensic medical practice.*

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HIV has become a focal point for South Africa and many other parts of the world. This is owing to the dire implications the disease holds for the human race. Accurate research needs to be done in order to determine the effectiveness of current prevention, treatment and funding strategies so that future strategies can be implemented with greater success. All organisations, whether governmental or non-governmental, need to be aligned in their strategies and campaigns in order to be maximally effective.

In the South African forensic setting, it is not routine to test a decedent for their HIV status unless a needle stick injury or occupational mishap occurs. This means there is very little information on the current prevalence of HIV in the other than natural death population of South Africa. In order to address the lack of information three main areas of research have been identified as the most urgent to obtain reliable information on.

The first was a study done to document the HIV prevalence in the general Pretoria MLL population for a month. All cases admitted to the Pretoria MLL were tested for their HIV status and this was documented and analysed. The second study population was the suicide population in the Pretoria MLL. It is hypothesised that the suicide populations HIV prevalence would be higher than the general population's prevalence rate.

The third study shed light on the sub population of Sudden Unexplained and Unexpected Deaths (SUU-Death) that are admitted to the Pretoria MLL. The results of all three studies determined that the prevalence of HIV in the Pretoria MLL is higher than the current statistics for South Africa.

South African forensic medical practice has the context and potential to provide large amounts of accurate information regarding the HIV pandemic in South Africa.

Opsomming: ***MIV in Suid-Afrikaanse geregtelike geneeskunde.***

MIV is 'n brandpunt vir Suid-Afrika en baie ander dele van die wêreld as gevolg van die ernstige implikasies wat die siekte hou vir die mens. Akkurate navorsing moet gedoen word ten einde die doeltreffendheid van huidige voorkoming, behandelings en strategieë befondsings te beoordeel en om toekomstige strategieë te beplaal wat met groter sukses toegepas kan word. Alle organisasies, regerings en nie-regeringsorganisasies moet strategieëse veldtogte in plek he om maksimaal effektief te wees.
In Suid-Afrika is dit nie algemene praktyk om die oorledenes wat regsmediese nadoodse ondersoeke ondergaan, te toets vir MIV-status nie. Slegs indien ‘n besering aan diens in die vorm van byvoorbeeld ‘n naaldprik-ongeluk plaavind, word die MIV-status getoets. Dit beteken daar is tans baie min inligting oor die huidige voorkoms van MIV in die regsmediese laboratorium omgewing. Om hierdie gebrek aan inligting aan te spreek is drie hoofareas van navorsing geÏdentifiseer. Die eerste studie het gepoog om die voorkoms van MIV in oorledenes wat regsmediese ondersoeke ondergaan het in die Pretoria Regsgeneeskundige Laboratorium te dokumenteer vir een maand. Alle gevalle wat toegelaat is tot die Pretoria RGL is getoets vir MIV, gedokumenteer en ontleed. In die tweede studie is gepostuleer dat die MIV gevalle hoër sou wees in persone wat selfmoord pleeg as die algemene bevolking van die Pretoria RGL. Derdens is die MIV status beoordeel in persone wat skielik en onverwags tot sterwe gekom het. Die uitslae van al drie studies toon dat die voorkoms van MIV in die persoonbevolking van die Pretoria RGL bevolking hoër is as wat algemeen

onder die bevolking gevind word in Suid-Afrika. Die potensiaal bestaan om baie waardevolle navorsing te doen insake die aard, voorkoms en profiel van MIV onder persone wat op onnatuurlike wyse gesterf het, wat van breër nut en toepassing in die gemeenskap is.

Abbreviations:

AIDS	Acquired immune deficiency syndrome
ARV	Anti Retroviral
ASSA	Actuarial Society of South Africa
DR	Death Register
DSI	Death Scene Investigator
FPS	Forensic Pathology Service
HIV	Human Immunodeficiency Virus
HSRC	Human Sciences Research Council
ID	Identification
MLL (RGL)	Medico-Legal Laboratory / Regs Geneskindige Labratorium
OHS	Occupational Health and Safety
PM	Post Mortem
PPE	Personal Protective Equipment
PTSD	Post-Traumatic Stress Disorder
SAPS	South African Police Service
SUU- Death	Sudden Unexpected and Unexplained Death
UNAIDS	United Nations Programme on HIV/AIDS
WHO	World Health Organisation

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Chapter 1: Introduction

Human Immunodeficiency Virus (HIV) infection has become a global pandemic of unparalleled proportions. The World Health Organisation (WHO) reports that there are 33.4 million people living with the disease globally and that there were a total of 2 million deaths due to HIV in 2008.[1] The report documents the global scale of HIV and the influence that HIV infection has on the specific continental regions of the world.

The scale of infection cannot be contemplated until it is compared with other human tragedies and epidemics. The First World War claimed 15 million lives; the Second World War claimed 50 million lives.[2, 3] The two most recent influenza outbreaks, namely the Avian Influenza (H5N1) and the Swine influenza (H1N1), took 282 and 14142 lives, respectively.[4, 5] If the impact of these events on humanity is anything to go by, the HIV pandemic is going to impact on our very existence and way of life to a degree not easily imagined.

The global adult HIV prevalence rate for the 15 to 49 year old age group is 0.8% according to 2009 figures. The regional HIV statistics for the 15 to 49 year old age group are as follows:

- East Asia (0.1%);
- Western and Central Europe (0.2%);
- Middle East and North Africa (0.2%);
- South and South East Asia (0.3%);
- Oceania (0.3%);

- Central and South America (0.5%);
- North America (0.5%);
- Eastern Europe and Central Asia (0.8%);
- Caribbean (1%);
- Sub-Saharan Africa (5%).[1]

Sub-Saharan Africa has the distinction of being the epicentre of the global HIV pandemic. The region accounts for 67% of all the world's infections, 70% of new infections and 70% of global deaths due to HIV.[1] HIV has become a focal point for South Africa and the world, owing to the dire implications the disease holds for the survival of the human race. These implications stem all the way through from individual suffering and economic challenges to social collapse. It is imperative that decisive action be taken in dealing with the pandemic. For this to be possible, accurate research needs to be done in order to determine the effectiveness of current prevention, treatment and funding strategies so that future strategies can be implemented with greater success. All organisations, whether governmental or non-governmental, need to be aligned in their strategies and campaigns in order to be maximally effective.

South Africa has the privilege of having a functioning, stable government which can utilize its available resources to provide such prevention, treatment and funding strategies to the population.[6] Unfortunately, in the past, the government has side-lined world renowned academics and failed to acknowledge the extent of the pandemic in South Africa.[7] With these two opposition fronts, the statistics of HIV in South Africa will always be a

contentious political as well as scientific issue. The HIV / AIDS pandemic in South Africa stirs debate, contention and fear from cultural, social and religious paradigms. The law seeks to redress these fears by being progressive regarding laws of notifiability. The laws attempt to remove the fear and uncertainty associated with the pandemic by providing a safe environment for an HIV positive individual to function without fear of non-consensual disclosure.[8] That said, the laws, combined with stigmatism surrounding HIV infection, do make accurate data on the pandemic harder to obtain and validate as there is no onus to notify the state disease control centre or registry about a new infection. Currently, the laws are of vital importance in the protection of human rights and in stopping atrocities associated with fear and ignorance in South Africa. This does however mean there is a need for other methods of determining the prevalence of HIV in the population.

There are numerous statistical representations and methods used by institutions to determine the prevalence rate of HIV infection in South Africa. The Actuarial Society of South Africa (ASSA) calculated that the HIV national prevalence is 10.6% with the 15 to 49 year age group prevalence at 17% according to 2010 figures.[9] The data from antenatal clinics, which collectively act as a sentinel for measuring population state, estimate the prevalence in the population to be 17.6%. Further research was conducted in the South African National HIV Prevalence, Incidence, Behaviour and Communication Survey states that the prevalence estimate is 10.6% which translates into 5.2 million HIV positive South Africans.[10] Confirmatory studies need to be conducted to determine the accuracy of the

studies. For example Dorrington's study in 2007 stated that 11% of the total population was infected with HIV, which translates into 5.4 million South Africans infected with HIV in mid-2006.[11]

Unfortunately international studies don't agree with the statistics emerging from South Africa. For instance the UNAIDS report for 2009 gives South Africa's percentage prevalence as 15.6% (2002), 16.2% (2005) and 16.9% (2008). [1] These discrepancies from reliable sources are distressing as it shows that the current sampling and raw data collection is substandard for the current needs of the scientific community. These discrepancies translate into policies that are misaligned and budgets that are inappropriate for specific organisations to complete their mandated tasks successfully.

A World Health Organisation bulletin exposes the misclassification of HIV/AIDS deaths in South Africa, stating: "The bulletin documents that coverage is incomplete and that certification and coding are of questionable quality". The report also discusses the need for analytical techniques for adjusting for biases. The report concludes that 94% of all HIV / AIDS deaths in the country are being misclassified, thus adding further uncertainty to the data being produced in South Africa.[12] This study sheds doubt on the current statistics available in South Africa, further emphasizing the need for other methods of measurement and comparison in South Africa.

Currently South Africa uses two ways to identify the prevalence of HIV in South Africa. The first is a sentinel surveillance system.[13] The main sentinel population currently being monitored combines pregnant women attending antenatal care clinics and their infants' HIV status to form a representative

picture for the rest of the population. The website, "Avert," houses a detailed analysis of the surveillance systems and states that, " antenatal surveillance is internationally recognised as the most useful way of assessing HIV prevalence in countries with generalised epidemics. Pregnant women are sexually active and constitute an easily identifiable, accessible and stable population."[14] The data collected from this subpopulation is limited to a very specific sample group and not always completely representative of the entire population. This leads to over estimation of some populations (namely, women aged 15 to 19 years) and under or over estimation in male populations. None the less, this collected data is then used in conjunction with other available information in statistical models and then extrapolated to the general population. Theoretically, this method is purported to provide an accurate estimation of the current situation. The model becomes increasingly relevant and accurate as more data is assimilated into its prediction. Thus, every study population that is studied and incorporated into the data model makes the resultant predictions more accurate.

The second data collection method relies on a voluntary "household" survey which is collected by the Human Science Research Council (HSRC).[10] The survey is fraught with difficulties that are rigorously analysed and compensated for to make the study as representative as possible. This type of study gives a more representative look at the data for children and men who are not accurately represented in the antenatal population. In theory, this should provide a clearer description of the nature of the pandemic in South Africa. However, there are still specific groups that are under-

represented in this type of data collection as testing and participation is voluntary.

These methods don't intend to contradict each other. Instead they can be regarded in parallel, with each exposing different trends in separate populations within the South African context. It is imperative that the models, representations and estimations be checked and scrutinised against current, representative data. The comparison of existing data with new data creates an increased robustness in the confidence that the statistics have. An example of this being done was in a simple study of conformation by Du Plessis et al. (1999). The study was done in an autopsy population where the rate of HIV infection in the mortuary was corroborated with theoretically modelled statistics as being 11%.[15] Studies such as these give credit to statistical models of the time by corroborating their findings. This research is unfortunately severely out-dated and needs to be updated for current times. The latest statistics and models need to be validated and corroborated by as many external sources as possible to give them integrity while providing further information on the pandemic's profile and epidemiology. Such comparison will facilitate the creation of strategic interventions and tailoring of interventions to specific populations.

The Forensic Pathology Service (FPS) has a substantial burden to bear in South Africa. In 2007 there were 54216 non-natural deaths in South Africa, accounting for up to 9% of total registered deaths during that year.[13] Unfortunately the death notification system in South Africa has many flaws and loop holes, this leads experts in South Africa to view these statistics with caution. As of mid-2009, South Africa has a population of 49

320 500.[16] Arguably, this makes South Africa the peace time, non-natural death capital of the world. With some of the highest road traffic mortality rates in the world, 33.2 deaths per 100 000, and one of the highest peace time homicide rates in the world, 34.1 murders per 100 000 South African citizens.[17,18]

In the South African province of Gauteng, there were 11891 non-natural deaths totalling 10.3% of the total deaths recorded in the province in 2007.[13] Gauteng has 21.4% of the South African population living within its confines, making it the province with the highest population density and the highest number of non-natural deaths in the country.[16] The Pretoria Medico-Legal Laboratory (MLL) is a largest MLL situated Pretoria, Gauteng. Pretoria is also the capital city of South Africa. On average, the MLL deals with 2500 suspected non-natural death cases a year making it the ideal environment to conduct research due to its large capacity, the large number of cases handled and its ties to the University of Pretoria's Faculty of Health Sciences. Within the Tshwane Metropolitan district, the 3rd largest by area in the world, there are two smaller mortuaries Ga-Rankuwa and Bronkhorstspruit which make up a further 1500 cases per year.

In order to determine the risk of HIV infection in the post mortem setting a study was conducted with the aim of determining how long HIV remains viable in a refrigerated body. [19] The study determined that HIV remains viable in blood for 16.5 days post mortem, 13.8 days in a pleural liquid effusion and 15.5 days from a pericardial liquid effusion.[19] The bodies were refrigerated as per international recommended practice. Another study was conducted on the viability of HIV-1 in syringes. The study used two

quantities of blood in the syringes; 2µl and 20µl were placed in each syringe. Viable HIV virus was picked up at 42 days of storage in both samples of blood if the syringe was stored below 4°C but at the other extreme both syringes failed to yield any viable HIV for 7 days or longer at 37°C.[20] This is of concern to the forensic personnel who mistakenly cut or receive a needle stick injury while working on a HIV positive case.

The risks faced by the forensic professionals are compounded by the sheer volume of work, as well as the known and unknown risks that have to be faced on a daily basis. If the current UNAIDS statistics on South Africa are to be applied (16.9% for 2008) to the Pretoria MLL, the facility deals with approximately 423 HIV positive cases per year.[1] These are high risk autopsies which increase stress and anxiety in forensic professionals involved with them, even if no cuts or needle stick injuries occur. Such a level of constant anxiety is difficult to quantify. Forensic personnel seldom, if ever, have HIV status information prior to performing an autopsy; so every case must be treated as high risk. In the South African forensic setting it is not routine to test a decadent for their HIV status unless a needle stick or occupational mishap occurs. This intensifies the stress and fear associated with performing autopsies. If we can shed light on the virus' behaviour and provide evidence, along with relevant practical conclusions that can be implemented, anxiety, complacency and uncertainty surrounding the virus can be reduced. Such measures will contribute to the mental and physical wellbeing of the forensic professionals and will undoubtedly benefit the organisation and system as a whole by making the work environment a safer place.

The South African Occupational Health and Safety legislation mandates employers to provide and maintain a working environment that is safe and without risk to their employees. OCCUPATIONAL HEALTH AND SAFETY ACT 85 OF 1993, Chapter 8; describes the general duties of an employer: [21]

"General duties of employers to their employees

(1) Every employer shall provide and maintain, as far as is reasonably practicable, a working environment that is safe and without risk to the health of his employees.

(2) Without derogating from the generality of an employer's duties under subsection (1), the matters to which those duties refer include in particular-

(a) the provision and maintenance of systems of work, plant and machinery that, as far as is reasonably practicable, are safe and without risks to health;

(b) taking such steps as may be reasonably practicable to eliminate or mitigate any hazard or potential hazard to the safety or health of employees, before resorting to personal protective equipment;

(c) making arrangements for ensuring, as far as is reasonably practicable, the safety and absence of risks to health in connection with the production, processing, use, handling, storage or transport of articles or substances;

(d) establishing, as far as is reasonably practicable, what hazards to the health or safety of persons are attached to any work which is performed, any article or substance which is produced, processed, used, handled, stored or transported and any plant or machinery which is used in his business, and he shall, as far as is reasonably practicable, further establish what precautionary measures should be taken with respect to such work, article, substance, plant or machinery in order to protect the health and safety of persons, and he shall provide the necessary means to apply such precautionary measures;

(e) providing such information, instructions, training and supervision as may be necessary to ensure, as far as is reasonably practicable, the health and safety at work of his employees;

(f) as far as is reasonably practicable, not permitting any employee to do any work or to produce, process, use, handle, store or transport any article or substance or to operate any plant or machinery,

unless the precautionary measures contemplated in paragraphs (b) and (d), or any other precautionary measures which may be prescribed, have been taken;

(g) taking all necessary measures to ensure that the requirements of this Act are complied with by every person in his employment or on premises under his control where plant or machinery is used;

(h) enforcing such measures as may be necessary in the interest of health and safety;."[21]

The legislation is intended to make it possible for an employer to make a working environment like the MLL a safe working environment. This is done by understanding the environment and analysing all possible risks, then implementing effective, proven interventions. Finally, enforcement of the intervention strategies and re-evaluation of the strategy's efficacy on a regular basis is essential.

Chapter 12 focuses on more practical areas of intervention once it has been stated that as part of the contract the individual will have to undertake the hazardous work.[21]

"General duties of employers regarding listed work

(1) Subject to such arrangements as may be prescribed, every employer whose employees undertake listed work or are liable to be exposed to the hazards emanating from listed work, shall, after consultation with the health and safety committee established for that workplace-

(a) identify the hazards and evaluate the risks associated with such work constituting a hazard to the health of such employees, and the steps that need to be taken to comply with the provisions of this Act;

(b) as far as is reasonably practicable, prevent the exposure of such employees to the hazards concerned or, where prevention is not reasonably practicable, minimize such exposure; and

(c) having regard to the nature of the risks associated with such work and the level of exposure of such employees to the hazards, carry out an occupational hygiene programme and biological monitoring, and subject such employees to medical surveillance.

(2) Every employer contemplated in subsection (1) shall keep the health and safety representatives designated for their workplaces or sections of the workplaces, informed of the actions taken under subsection (1) in their respective workplaces or sections thereof and of the results of such actions: Provided that individual results of biological monitoring and medical surveillance relating to the work of the employee, shall only with

the written consent of such employee be made available to any person other than an inspector, the employer or the employee concerned."[21]

In the mortuary setting, the legislation seeks to address all the possible outcomes or safety issues with as little legislating as possible. It is essential that respective parties in the organisation are knowledgeable and adept at implementing intervention strategies in the environment. A first aid station, vaccinations and on site antiretroviral interventions are essential at every mortuary. In context of the current study, legislation places the onus of determining what is hazardous on the employer. Employers must then implement interventions to cope with and mitigate the possible consequences of the hazard. An active approach needs to be taken to continually be abreast of what the work environmental hazards are and this is done through thorough research. This research can take many forms as shown below but the intention is to make the working environment a safer place.

Personal protective equipment (PPE) is regulated and mandatory in the mortuary. Yet there are only recommendations as to what should be the operational standard.[22] Mortuary personnel are often provided with equipment that meet specifications but are not practical to use. For example at the Pretoria MLL, the sizes of gloves available are limited to "large" most of the time. This poses a risk for people who have to wear larger gloves in that they are at greater risk of accidently puncturing or cutting the gloves. For the people whose hands are two big it is also a risk because the gloves tear when they are donned and if not they tear when they are taken off. Another example is the wearing of eye protection and surgical masks. The

masks often cause eye protection (safety glasses) to fog up, making it difficult for personnel to see what they are doing. Often, the mask is removed, exposing airways to airborne pathogens or safety glasses are removed and individuals are exposed to splatter. After these observations were made, measures were put into place to test how well full face plastic shields will work to replace safety glasses and how well the shields will work in conjunction with the face masks. This is an example of how the legislation has helped improve the safety of the personnel in the Pretoria MLL.

If the PPE should fail there needs to be a reliable process in place that protects all personnel from possible infection with HIV. The first step in this process is immediate first aid followed by extensive irrigation of the site of exposure. This is followed by blood sample collection from the origin of the contaminant. The person is then issued with an ARV starter pack and the blood is sent off to the laboratory for testing. The incident is then reported to the internal occupational health and safety (OHS) committee. The person then has to go for an HIV test at the OHS department at Steve Biko Academic Hospital and they are issued with a month's supply of Anti-retrovirals (ARV). The completion of this course is mandatory whether the tested sample comes back as positive or negative. Follow up testing is carried out for a year after the incident. In reality, once the patient has the results of the test they discontinue the treatment if it is negative. This is because of the debilitating side effects the ARV treatment can have on an individual. The perceived risk of the test being wrong and the effects the

ARV's have are weighed and the ARV's are stopped. This highlights the importance of using reliable and accurate tests for HIV.

At the Medical Virology Research laboratory (University of Pretoria), Abbot Determine ^{TM} HIV -1/2 Rapid qualitative Immunoassay test kits are used to screen blood samples. These specific tests are used in the Medical Virology Research laboratory because of their highly sensitivity. (Annexure 1) If a result is negative, no further testing of the sample takes place. If a positive or invalid test result is documented, the result is confirmed with the confirmatory test, AxSYM HIV Ag / Ab Combo Micro-particle Enzyme Immunoassay (MEIA) from Abbot Axsym System. This test is a fourth generation HIV combined Ag / Ab assay. [23,24]Fourth generation assays reduce the window period by a further 2 days, making it one of the most accurate tests on the market today.

> HIV is an RNA virus that is "100-120nm in diameter and consists of a lipid bilayer membrane that is surrounds a dense truncated cone-shape nucleocapsid which contains the genomic RNA molecules."[24] "HIV infects and damages or destroys several types of cells, most importantly helper/inducer (CD4+) lymphocytes. In the majority of infected persons, the loss of CD4+ lymphocytes leads to a progressive reduction in both cell mediated and antibody mediated immunity. Early during infection most adults are asymptomatic, but after several years many develop symptoms representing a moderate degree of immune suppression. Eventually, most of these individuals become susceptible to the life-threatening opportunistic infections and cancers which define the acquired immunodeficiency

> syndrome." [25] Routes for transmission occur through three main methods: Through unprotected intercourse (heterosexual or homosexual); during pregnancy or at birth from the infected mother to the foetus or infant; or through infected blood contact (e.g. in drug addicts who share needles, occupational needle stick injuries or exposure to infected blood and blood transfusions). [26] Needle stick injuries have a 0.36% chance of causing HIV infection should a person be exposed in such a manner.[15,27,28] This places forensic personnel who have a needle stick injury at very high risk.

The psychological impact of HIV is not adequately understood and explained. Post-traumatic stress disorder (PTSD) has been linked to the diagnosis and living with HIV.[29] If the mental health issues of HIV positive patients are not addressed it could lead to more drastic consequences. The link between HIV and an increased risk of suicide has been extensively documented in the international literature.[30,31] The studies that have been done have had varying results, with one study determining that the rate of suicide is not higher than that of the general population.[32] In contrast the New York Medical Examiners statistics revealed that the risk of suicide for HIV positive men between the ages of 20-59 years of age was 66 times greater than for the general population and 33 times greater for men of the same demographic.[32-34] A subsequent study in found that men of the same demographic (20-59 years of age) in California were 17 times more likely to commit suicide than uninfected individuals.[33,34] in 1993 an even larger risk group (20-39 years of age) was identified with a 21 times greater incidence of suicide.[33] Although the link between HIV and suicide has been explored

in these studies, the results are conflicting. However, the fact that some studies show significant increases in suicide amongst HIV positive individuals is a worrying finding that needs to be researched in the South African context.

Another issue surrounding the mental health of individuals, and the social health of communities, is stigmatisation of HIV positive individuals. One of the main issues implicit in the formation of stigmata is that HIV contraction is controllable and preventable. It is perceived that "immoral behaviours" are associated with the contraction of HIV. Individuals often attribute these behaviours to "other" groups of people that they do not consider themselves to be a part of. [29] This disassociation with personal risk taking behaviour places the burden and responsibility for the disease's progression on another group. Logic such as this can be regarded as a social protective measure which is being implemented as an assistive, individual, protective measure. Due the large burden of stigmatism in South Africa, legislative protective measures have been implemented to deal with the ethical issues surrounding stigmata and confidentiality.

Some of the main aspects that need to be dealt with before any research commences are the ethical and confidentiality issues that arise when dealing with such a sensitive topic. In South Africa, the overarching law that governs everything is the South African Constitution (Act 108 of 1996). [35] The constitution recognises the need for confidentiality and consent in South Africa. The Health Professions Council of South Africa has seen fit to draw up guidelines for good practice in the health care sector with regard to HIV. Consent is dealt with in the document.

- It is not justifiable to test for HIV without the patient's consent, except in the circumstances set out in the National Policy on Testing for HIV, (e.g. as part of unlinked and anonymous testing for epidemiological purposes undertaken by the national, or a provincial or local health authority, or an agency authorised by any of these bodies - provided that HIV testing for epidemiological purposes is carried out in accordance with national, legal and ethical provisions regarding such testing.[26]

Furthermore The Inquests (Act 58 of 1959) addresses the circumstances that surrounds an unnatural death and when samples may be retained during the investigation:

- The Inquests Act 1959: [s3]3 Investigation of circumstances of certain deaths.[36]

    - {2}If the body of a person who has allegedly died from other than natural causes is available, it shall be examined by the district surgeon or any other medical practitioner, who may, if he deems it necessary for the purpose of ascertaining with greater certainty the cause of death, make or cause to be made an examination of any internal organ or any part or any of the contents of the body, or of any other substance or thing.

- {3} For the purposes of any examination mentioned in subsection {2}- {b}a body or any part, internal organ or any of the contents of a body so removed there from may be removed to any place.

The question arises, why is HIV not documented in the forensic setting or documented in the confidential section of death certificate by the medical professional who has a mandate too? To answer this question it is necessary to examine the nature of the virus: The HI-virus facilitates the destruction of the immune system (CD4 Cells). Destruction of the immune system increases the possibility of an infected individual contracting an opportunistic infection which leads to his/her death. At autopsy, the macro-/microscopic diagnosis of the cause of death is usually determined accurately but the underlying cause of this infection is not investigated further and HIV is not explicitly documented. A further complication is that medical professional's feel that documenting the HIV status is prejudicial to the family and that they will not be able to collect on insurance policies due to a positive HIV status.

With the possibility of obtaining additional, accurate information on the HIV pandemic it is proposed that specific targeted research be carried out. This research will answer current questions and provide a solid platform of information for policy development and serve as an alternative source of information to document the current status of the HIV pandemic. There are several ethical concerns that will need to be taken into account.

These ethical concerns all revolve around confidentiality. Multiple interventions have been put in place to safe guard the confidentiality of the individuals' identities and test results:

- Only the principal investigator and supervisor will have direct access to any primary data obtained in the study.
- No names or any personal details or identifiable details of the deceased will be made available at any time, to any irrelevant party.
- The Death Registry number and South African Police case numbers will be collected for verification of raw data but new non-representative research numbers will be given to each individual case. From the collection phase only the research numbers will be used. This will break the link between personal information and research relevant data.
- No personal identifying information will be retained concerning the deceased. (I.E: ID number, names, addresses or locations.)
- The test results will not be included in the post mortem report nor will they be made available to the medical personnel or forensic pathologist.

- The collected data forms will be kept in a locked room in the Department of Forensic Medicine, University of Pretoria.

- The confidentiality of personal data is of utmost concern and all reasonable effort will be made to break the link between personal data and research-relevant information, ensuring the rights of the deceased and their family.

The above ethical considerations' are all subject to the scrutiny and acceptance of the University of Pretoria's, Faculty of Health Sciences' Research and Ethics Committee.

Three main areas of research have been identified as the most urgent to obtain reliable information on. The first is documented in chapter 2 where a study was done to document the HIV prevalence in the general Pretoria MLL population for a month. All cases admitted to the Pretoria MLL where tested for their HIV status and this was documented and analysed. The second study population is the Suicide population in the Pretoria MLL. It is hypothesised that the suicide populations HIV prevalence would be higher than the general population's prevalence rate. The suicide study on the HIV prevalence rate is documented in chapter 3. The third study seeks to shed light on the sub population of Sudden Unexplained and Unexpected Deaths (SUU-Death) that are admitted to the Pretoria MLL. Due to the complexity and large numbers of SUU-Deaths admitted to the Pretoria MLL information regarding their HIV status would be of a significant nature. These studies are described separately in chapters 2, 3 and 4.

Chapter 2: The prevalence of HIV in cases admitted to Pretoria Medico-Legal Laboratory, South Africa

Introduction:

Infection with Human Immunodeficiency Virus (HIV) has risen to the status of a global pandemic. The World Health Organisation (WHO) reports that there are 33.4 million people living with the disease globally and that there were a total of 2 million deaths due to HIV in 2008.[1] The WHO report documents the global scale of HIV and the influence that the pandemic has on specific continental regions. Sub-Saharan Africa carries the largest burden of the disease with 22.4 million people living with HIV and 1.4 million deaths associated with the disease in 2008.[1]

South Africa is at the forefront of the pandemic and in an ideal position to facilitate the war on HIV. With a population of 49 320 500 (Mid 2009), South Africa has a stated HIV prevalence of 17% in the 15-49 year old age group and 10.6% in the entire population.[16] As of 2007 the WHO Report states that there are 5.7 million adults and children living with HIV and that there have been 350 000 HIV related deaths in South Africa.[37] This emphasises the need for research that determines the extent of the pandemic, validity of the current statistical models and the effectiveness of current intervention strategies.

Pretoria is the capital city of South Africa and has an estimated population of 1.338 million people residing within its confines (2008).[38] The Pretoria Medico-Legal Laboratory (MLL) serves the greater Pretoria region,

including the Tshwane metropolitan area and deals with approximately 2500 other than natural causes of death per year.[13] These deaths account for 10.5% of all deaths in the greater Pretoria area.[13]

There is very little data available regarding the prevalence of HIV in the South African autopsy population. The only study that addresses this issue in South Africa is one by *Du Plessis et al*, conducted in 1999. In the aforementioned study, a total of 265 peripheral blood samples were collected. The prevalence of HIV in the Pretoria MLL (1999) was found to be 11% and in the 15 to 49 year old age group it was 19%. This was in accordance with the HIV prevalence estimates of the time. Of the sero-positive cases, 30% died of natural causes.[15] Subsequently there has been little additional research done on the prevalence of HIV in the autopsy population.

The objective of this study is to fill the void of uncertainty and determine the prevalence of HIV in the autopsy population at the Pretoria MLL. The study was designed as a prospective; cross sectional study as it is not standard operating procedure for the pathologist to do a HIV test at autopsy. Data capturing was done continually for the period of one calendar month. Due to ethical and legal requirements, the study was conducted anonymously.

The null hypothesis is that the prevalence of HIV in the Pretoria MLL population should not deviate from that of the general population of South Africa. The alternate hypothesis is that there is a difference between the two

HIV prevalence statistics. A significant p-value for the chi squared analysis is 0.05.

The study shows that the prevalence of HIV in the Pretoria forensic medical population is 26.15% and the prevalence in the 15 to 49 year old age group is 30%. This is a concern, as the prevalence of HIV is 15.15% higher than in the 1999 study and significantly higher than the current statistical predictions.

Materials and Methods:

Post mortem (PM) blood samples were obtained from all cases of death due to other than natural causes admitted to the Pretoria MLL during one month. These deaths are legislated under the Inquests Act 58 of 1959, which prescribes the initiation of an investigation into the circumstances surrounding the death. The Pretoria MLL was selected due to its academic ties to the University of Pretoria and the large number of autopsies performed at the facility each year. The population under investigation was chosen because of its random nature and the accuracy of available demographic information about the samples. The study ran over one calendar month and was a prospective, cross-sectional design study. Post mortem blood was obtained where possible during the autopsy.

A forensic pathologist, who followed the standard operating procedures of the Pretoria MLL, collected the PM blood samples.

The processing of the blood samples was done by the standardized testing procedures developed and used in the Tshwane National Health Laboratory Service (NHLS), Virology Division. (Annexure 1)

- Abbot Determine ^{TM} HIV -1/2 Rapid qualitative Immunoassay test kits were used.
- Negative results on the Abbot Immunoassay will where documented as such and no further investigation took place.
- Confirmatory testing was done on a selection of positive Abbot samples to screen for false positive results. The confirmatory test was AxSYM HIV Ag / Ab Combo Micro-particle Enzyme Immunoassay (MEIA) from Abbot Axsym System.[23]

The study proposal was submitted to the University of Pretoria's Faculty of Health Sciences' Research Ethics Committee and the MSc Committee, which both gave approval for the study to be conducted.

The demographic details, case histories and procedure related information for each case were collected and stored. The confidentiality of any individual's results was of concern and every case was given a research number at autopsy. Every reasonable precaution to secure the confidentiality of the collected data was taken. This includes, but is not limited to, not providing the month in which the research took place and by severing all ties between test results and demographics collected from individualistic

identifying information (e.g. identification number, name and death register number provided by Pretoria MLL.)

All statistical analyses were done in conjunction with the department of statistics at the University of Pretoria.The null hypothesis is that the prevalence of HIV in the Pretoria MLL population should not deviate from that of the general HIV statistics in the Gauteng population. The alternate hypothesis is that there is a difference between the two HIV prevalence statistics. A significant p-value for the chi squared analysis is 0.05.The statistical program SAS was used for analysis of raw data. The use of race demographics (Black, White, Coloured and Asian) is not scientifically or anthropologically accurate or accountable but serves to inform experts on the traditionally utilised craterisations of social and ethnic groups that to this day persist in the South African context, albeit from a historical perspective.

Results:

A total of 257 other than natural deaths were admitted to the Pretoria MLL for investigation during the month. Post mortem blood was unobtainable in 19 of the 257 cases due to the nature and circumstances of death. The main reasons for the lack of sample availability were decomposition, skeletonisation and charring of the remains. These processes limit the possibility of sample collection from the cases. Thus, 238 PM samples were collected and sent to the Tshwane NHLS Virology Division for analysis. A relatively large number of invalid results, involving 43 samples, were reported from the testing phase. Positive results accounted for 51 samples

and ultimately 26% of the valid test samples. [Refer to Table 1, Figure 1 and Figure 2]

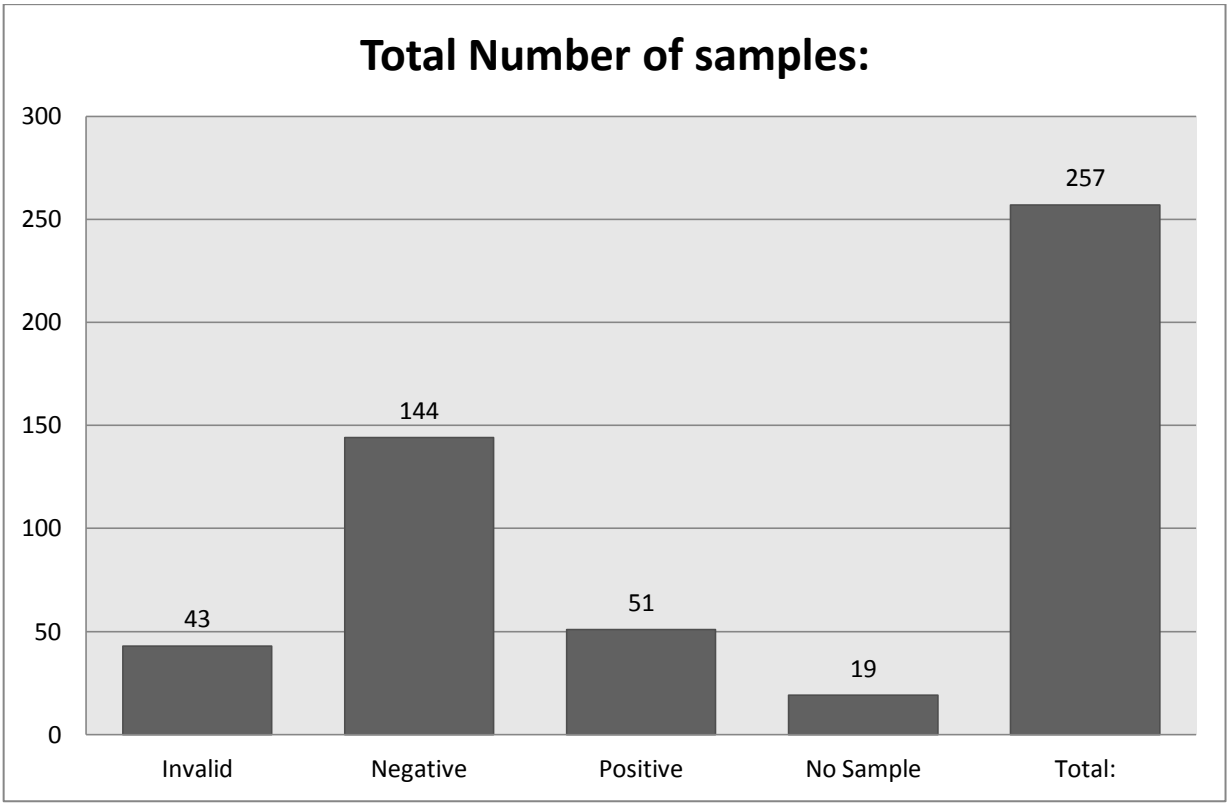

Figure 1: Sample collection break down for the general population

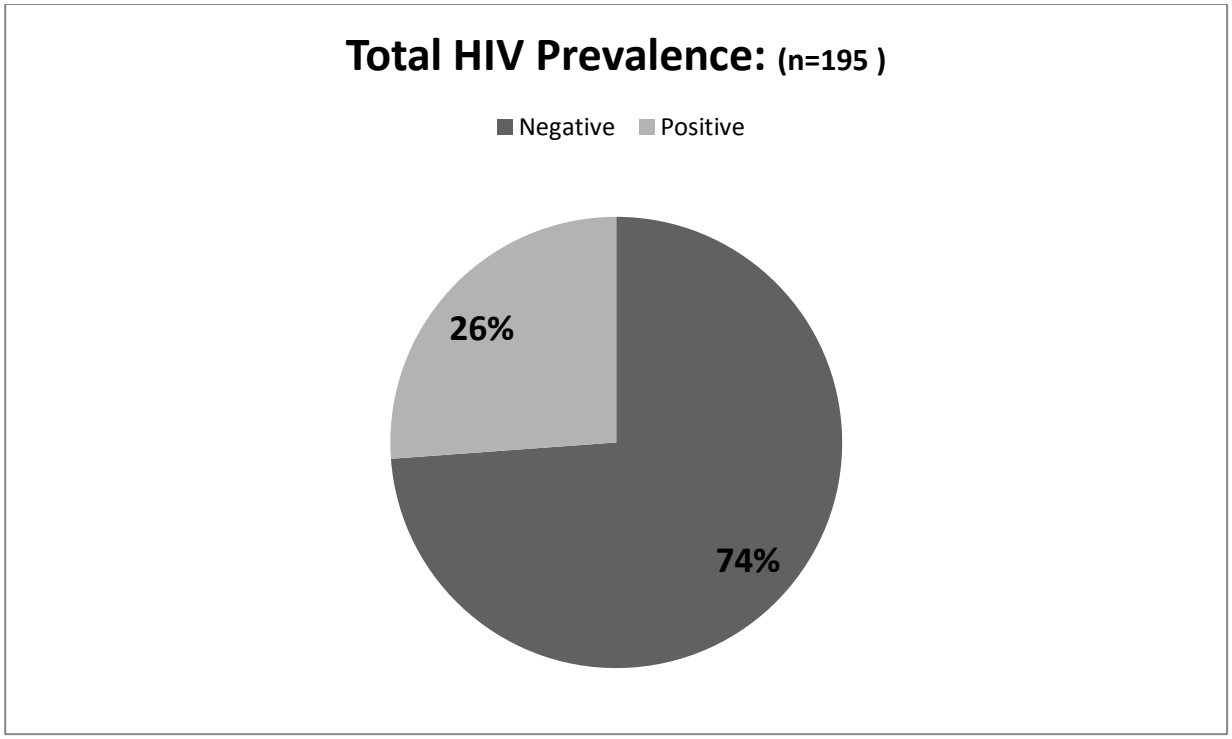

Figure 2: Valid sample HIV analysis for the general population

Table 1: Total cases analysed and viability of the samples for the general population in the Pretoria MLL

Result	**Number of cases**	**%**	**Collected samples**	**%**	**Valid test samples**	**%**
Invalid	43	17	43	18	-	
HIV Negative	144	56	144	61	144	74
HIV Positive	51	20	51	21	51	26
No Sample	19	7	-		-	
Total:	**257**	**100**	238	100	195	100

Demographics of the collected samples are listed in Table 2, Table 3 and Figures 3 to 9 below. The majority of decedents were male (78%) and only

20% of the cases were female. A surprising observation is that the prevalence of HIV in the female group is 37% as opposed to 24% in the male decedents.

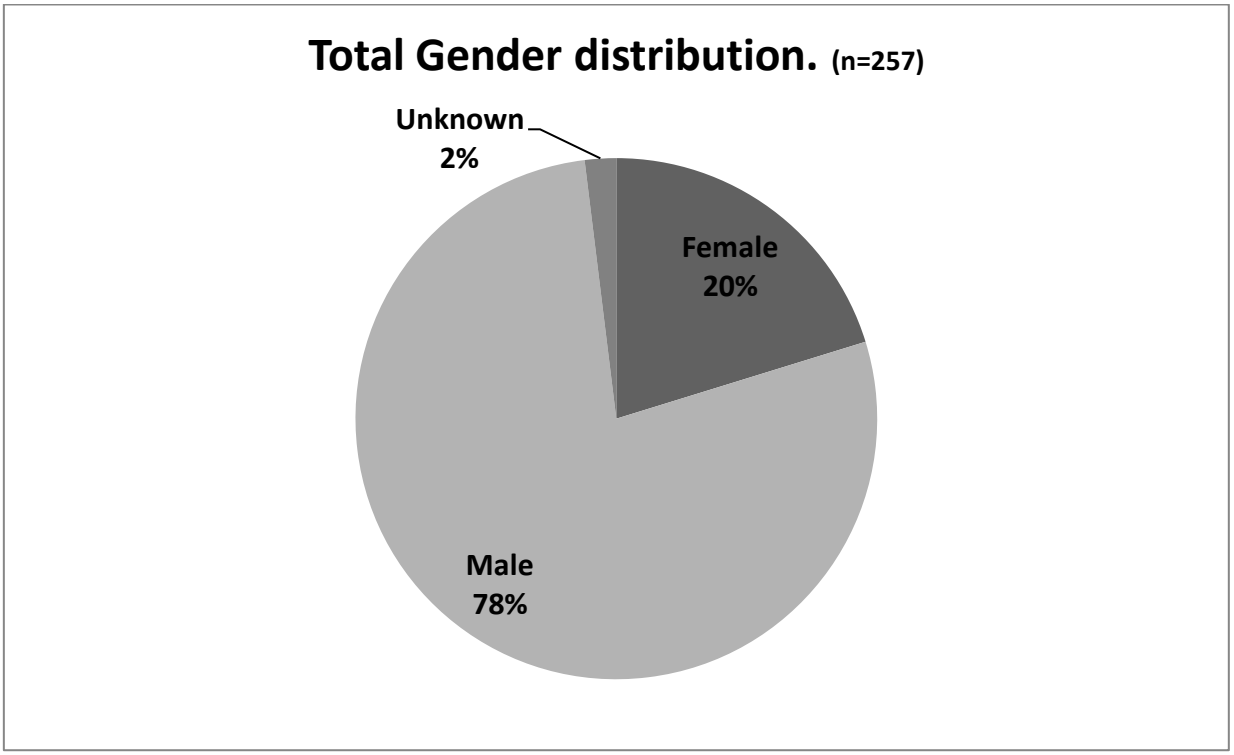

Figure 3 Gender Distribution for the general population

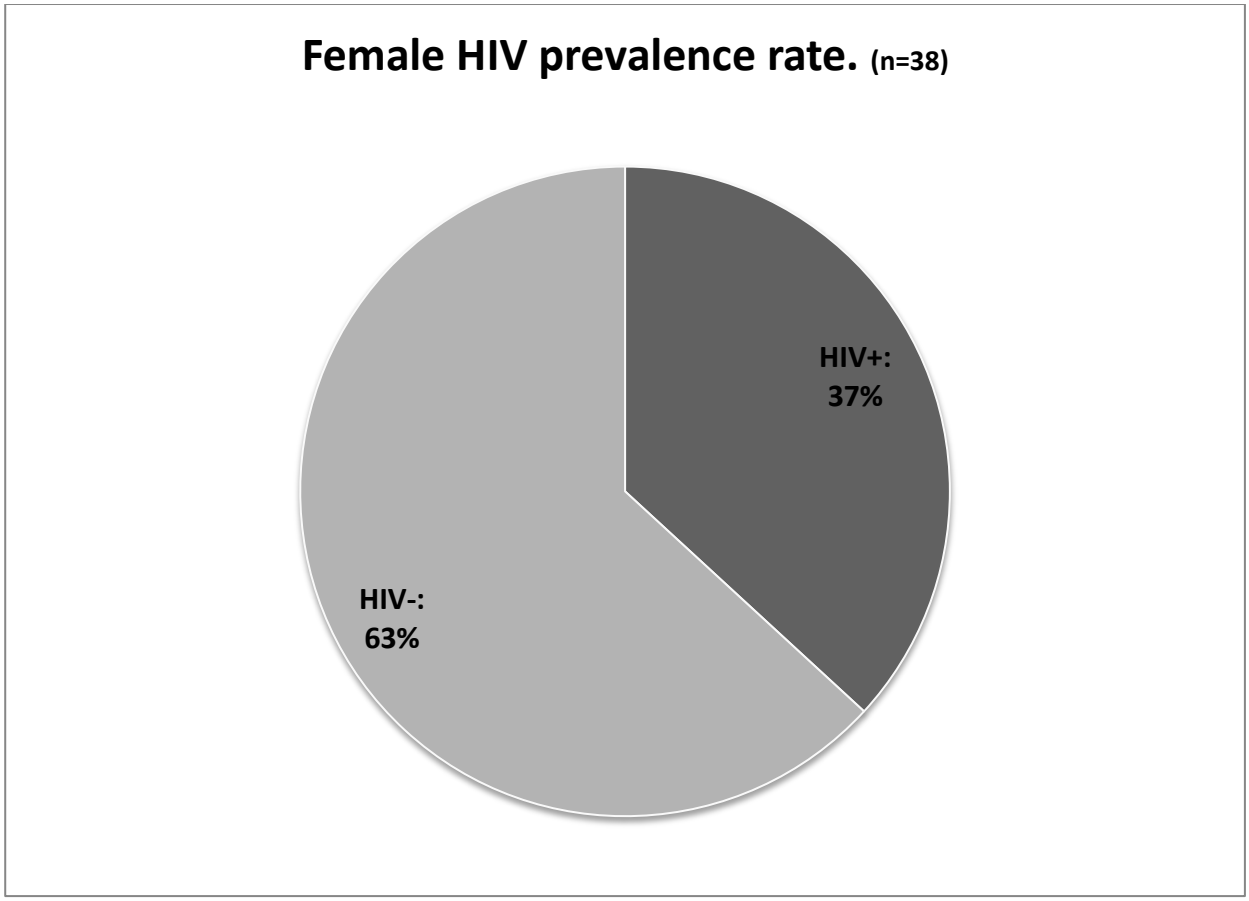

Figure 4 Female HIV prevalence rate for the general population

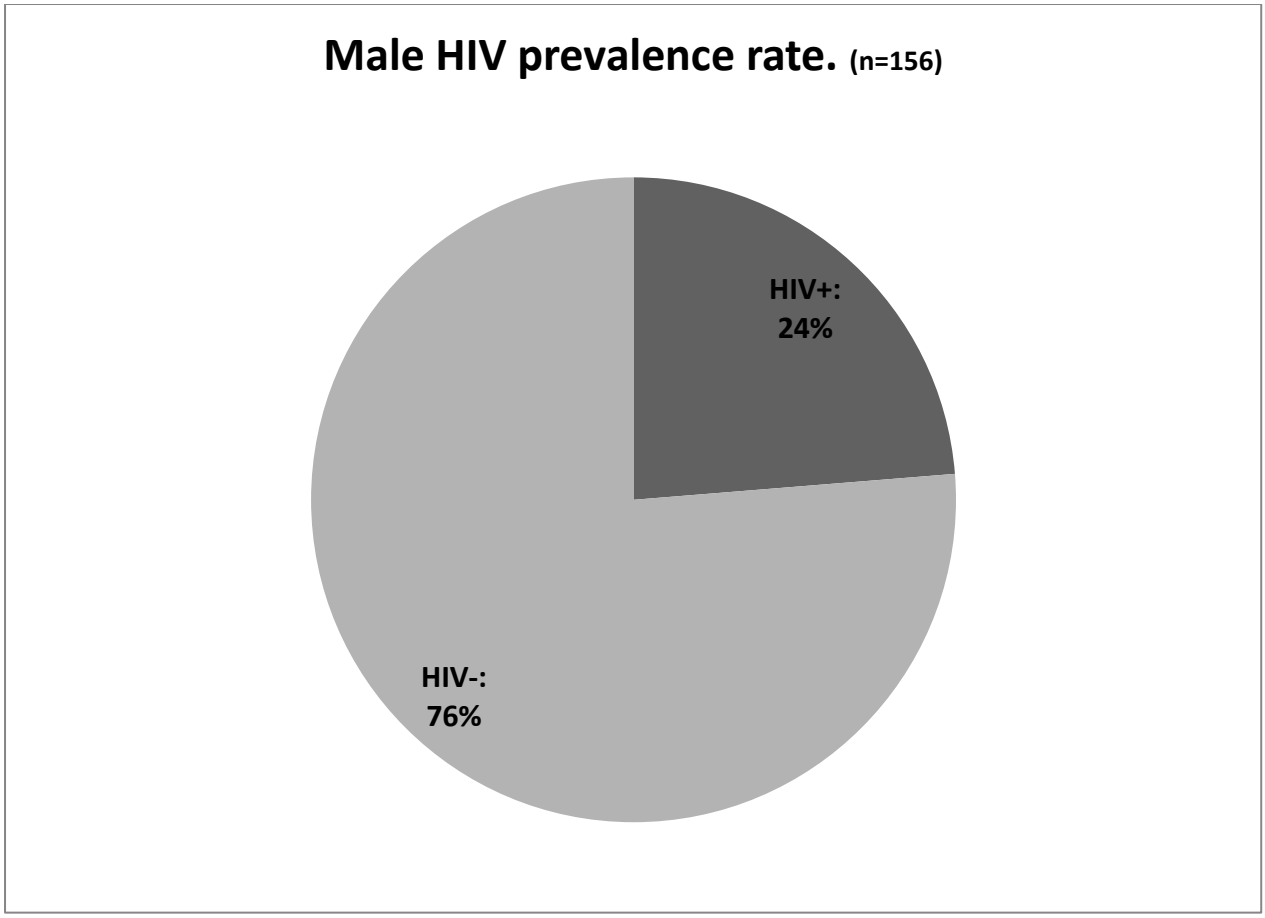

Figure 5: Male HIV prevalence rate for the general population

Table 2: Gender analysis of collected blood samples for the general population

Sex	**Number**	**%**	**HIV+**	**%**	**HIV-**	**%**	**Total number of valid samples**
Female	52	20	14	37	24	63	38
Male	200	78	37	24	119	76	156
Undeterminable	5	2	0	0	1	100	1
Total:	**257**	**100**	51		144		**195**

It was expected that the majority of decedents entering the Pretoria MLL would be Black (76%), with a larger than expected White population (18%), smaller Coloured (2%) and Asian (1%) populations. Gauteng's demographics are represented as follows for 2009, Black (79.3%), White (9.1%), Asian (2.6%) and Coloured (9%).[16]The racial distribution of HIV from valid test samples shows two significant groups in this small data set. The White population showed an 8% HIV prevalence and the Black population showed a 32% HIV prevalence.

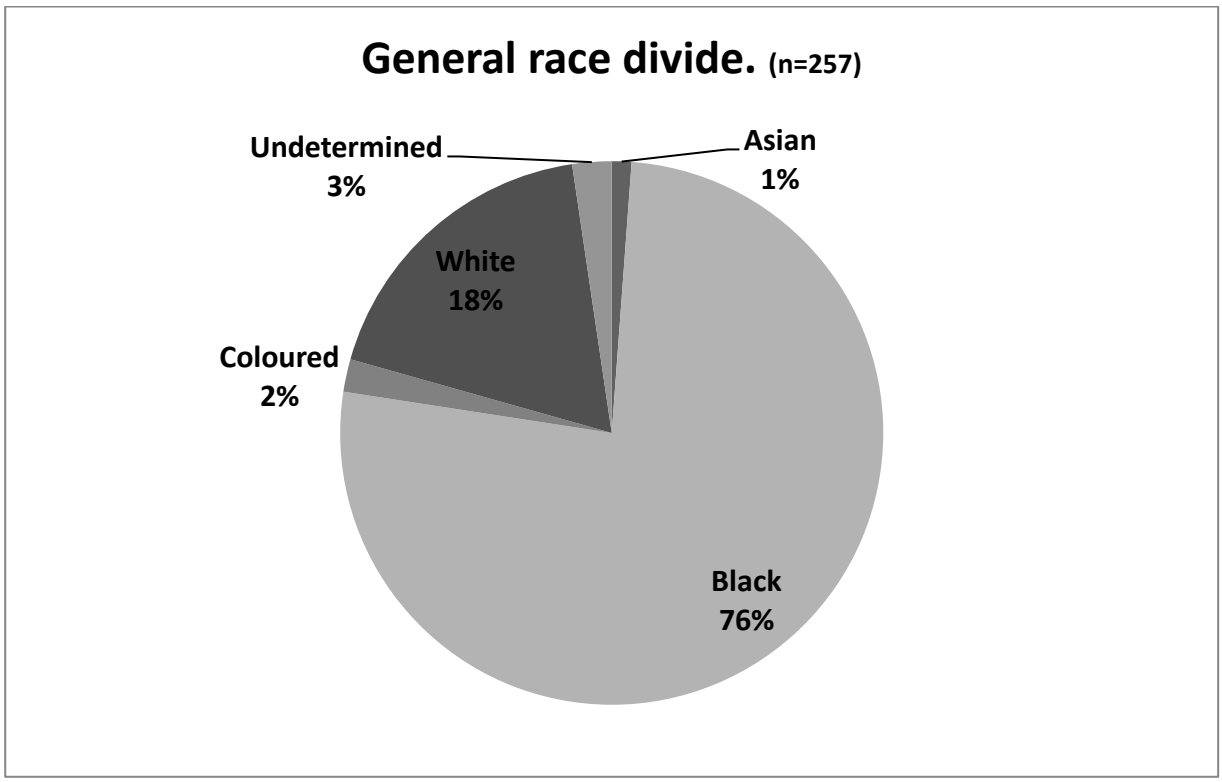

Figure 6: General race divide of the Pretoria MLL general population

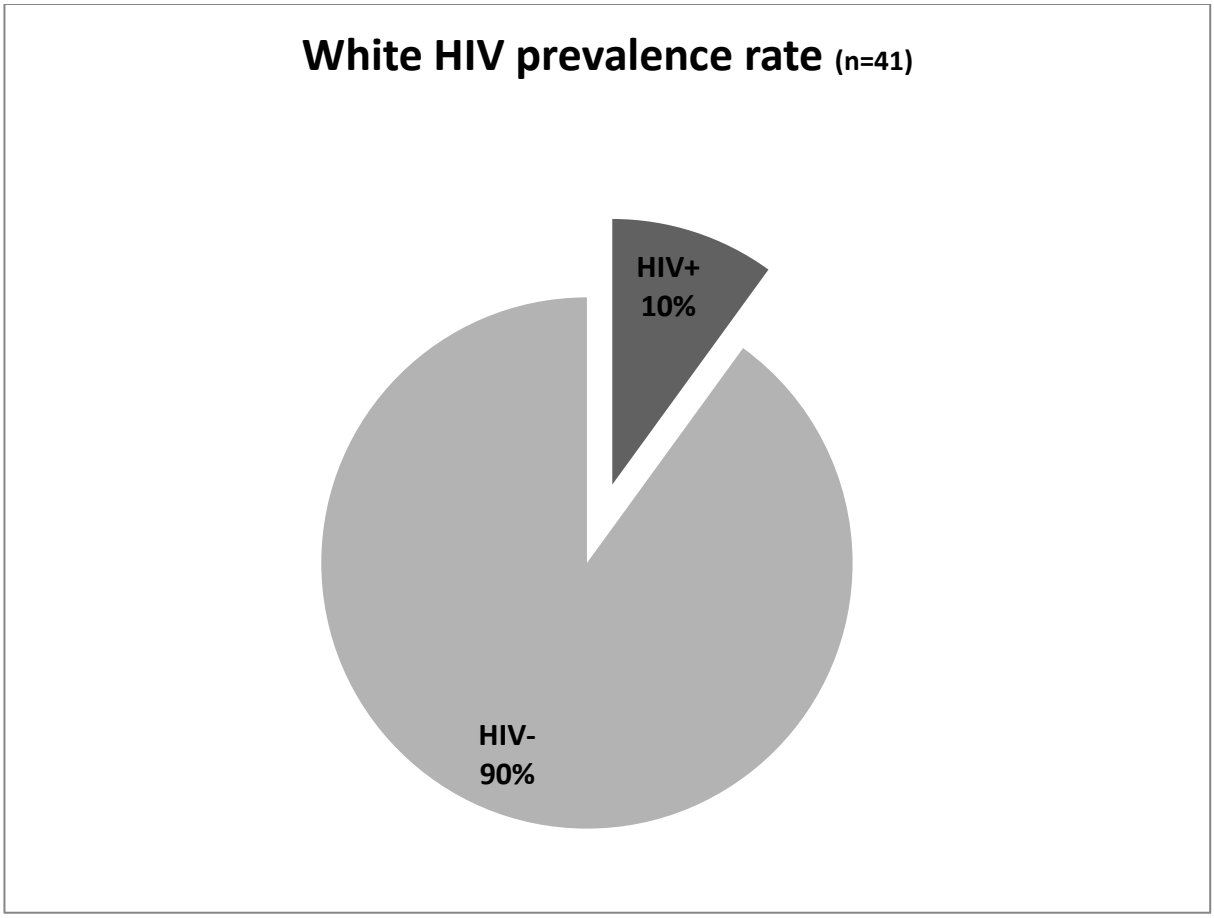

Figure 7: White HIV prevalence rate for the general population

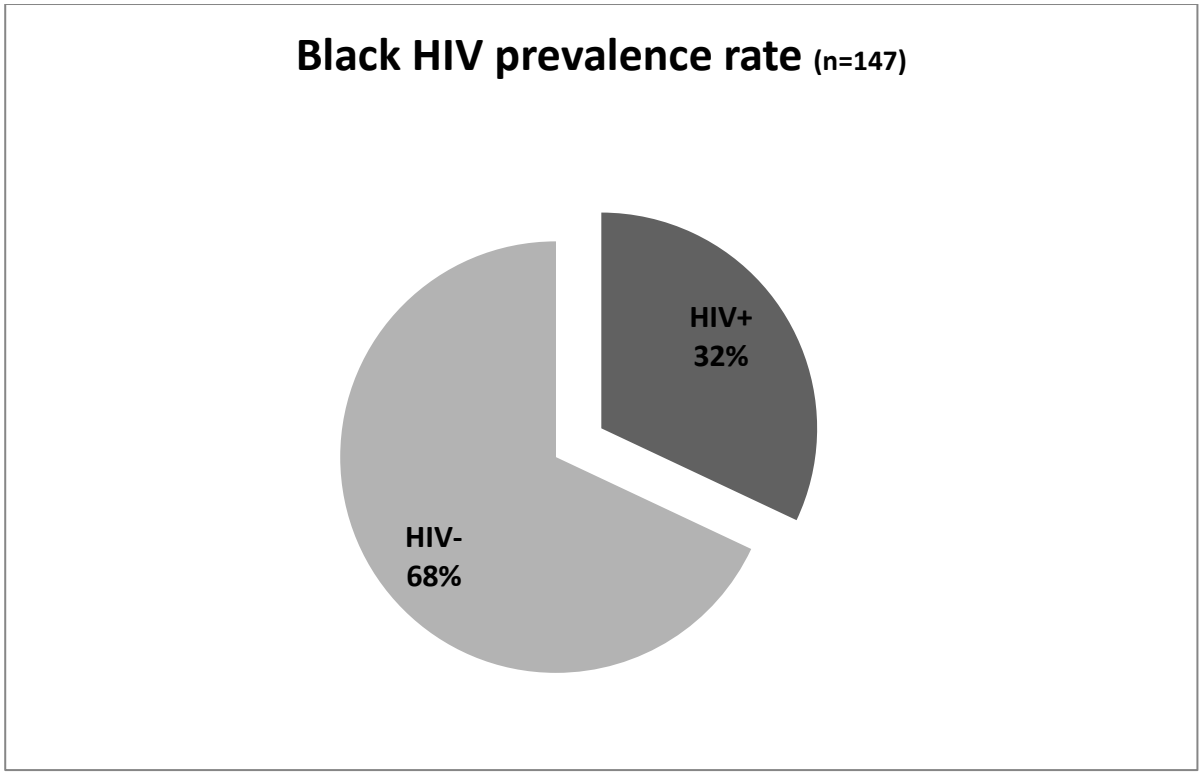

Figure 8: Black HIV prevalence rate for the general population

Table 3: Race analysis of collected blood samples for the general population

Race	**Number**	**%**	**HIV+**	**%**	**HIV-**	**%**	**Total valid samples**
Asian	3	1	0	0	3	100	3
Black	196	76	47	32	100	68	147
Coloured	5	2	0	0	3	100	3
White	47	18	4	8	37	90	41
Undeterminable	6	2	0	0	1	100	1
Total:	**257**	**100**	**51**		**144**		**195**

The ages of individual cases in this study ranged from less than 0 to 83 years. Data was grouped with the less than zero in its own category and thereafter the ages were divided up into categories with a 5 year interval between the groupings. Positive HIV immune assay results were obtained only from samples in the 0-64 year range, resulting in a 26% rate of infection across the entire Pretoria MLL population. In contrast, the prevalence for the age group 15 to 49 years of age was determined to be 30%. [Figure 9]

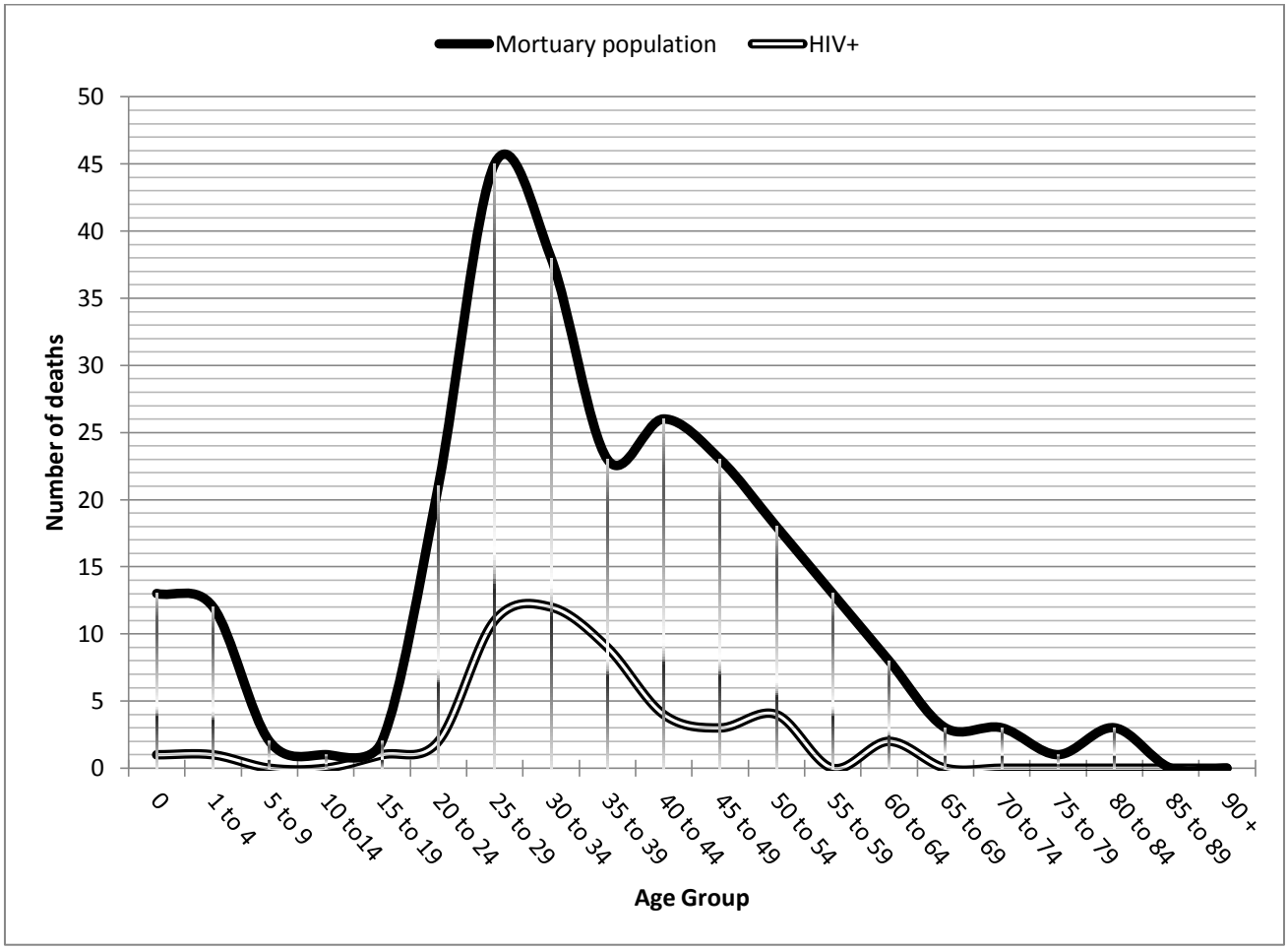

Figure 9: The age distribution of HIV infections and age range of the entire mortuary population

The cause of death in the sample population are displayed in Table 4.Cause of death is defined as "the reason or event that precipitates death".[39] The largest contributing category was transport related deaths (32 % of the total cases), followed by gunshot wounds (16%) and sudden unexplained and unexpected (SUU) deaths (14%). [Table 4; Figure 10] Another notable finding was made when the apparent manner of death was analysed and suicide accounted for 13% of the caseload with 19 cases in the month.

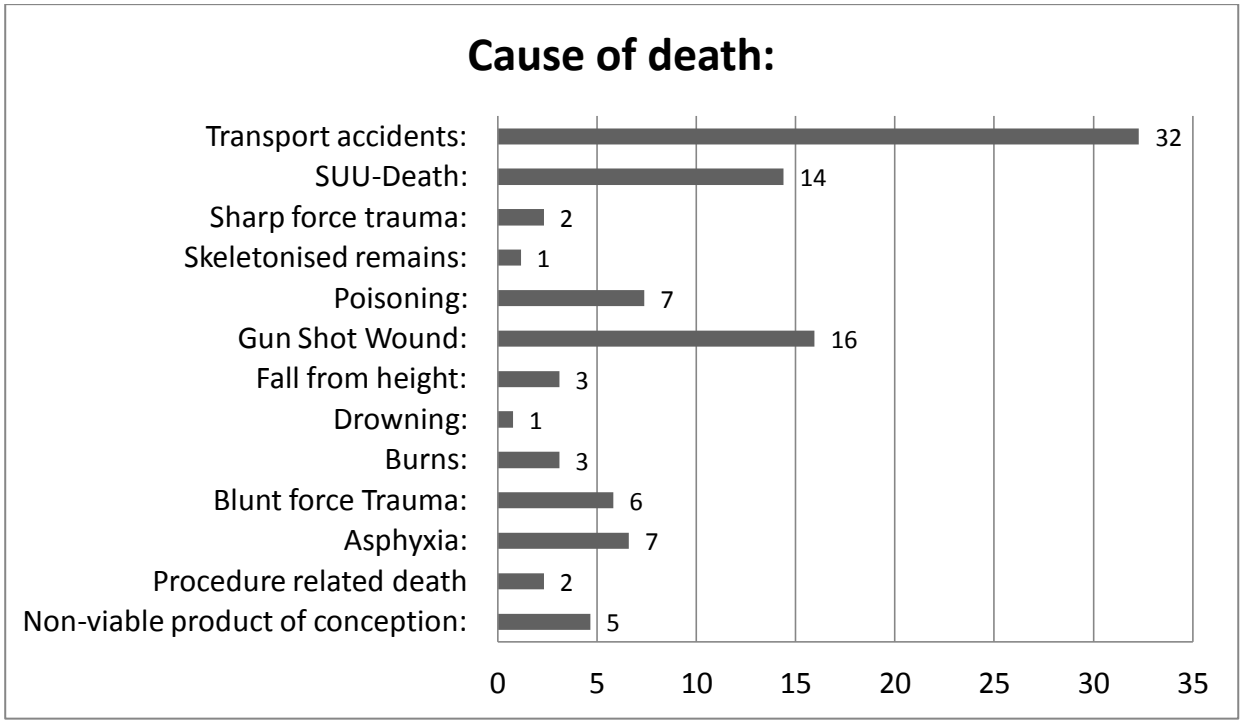

Figure 10: Cause of death for the general population

Table 4: Cause of death and HIV prevalence for the general population

External Cause of death:	Total Number	%	HIV +	%	HIV -	%	Total.(valid samples)
Non-viable product of conception:	12	5	0	0	0	0	0
Procedure related death	6	2	1	20	4	80	5
Asphyxia:	17	7	3	21	11	79	14
Blunt force Trauma:	15	6	5	42	7	58	12
Burns:	8	3	1	20	4	80	5
Drowning:	2	1	0	0	2	100	2
Fall from height:	8	3	2	40	3	60	5
Gunshot Wound:	41	16	10	30	23	70	33
Poisoning:	19	7	4	27	11	73	15
Skeletonised remains:	3	1	0	0	1	100	1
Sharp force trauma:	6	2	0	0	6	100	6
SUU-Death:	37	14	9	33	18	67	27
Transport accidents:	83	32	16	23	54	77	70
Totals:	***257***	***100***	***51***	***26***	***144***	***74***	***195***

The large number of invalids (43; 17%) warranted further analysis. Post mortem interval (PMI) was determined from the incident data and post mortem information. This was compared to the percentage of invalids obtained in specific PMI period categories (0-1 days, 2-3 days, and 4 days to

greater than 5 days). It was established that the number of invalid results increases with increasing PMI. [Figure 2]

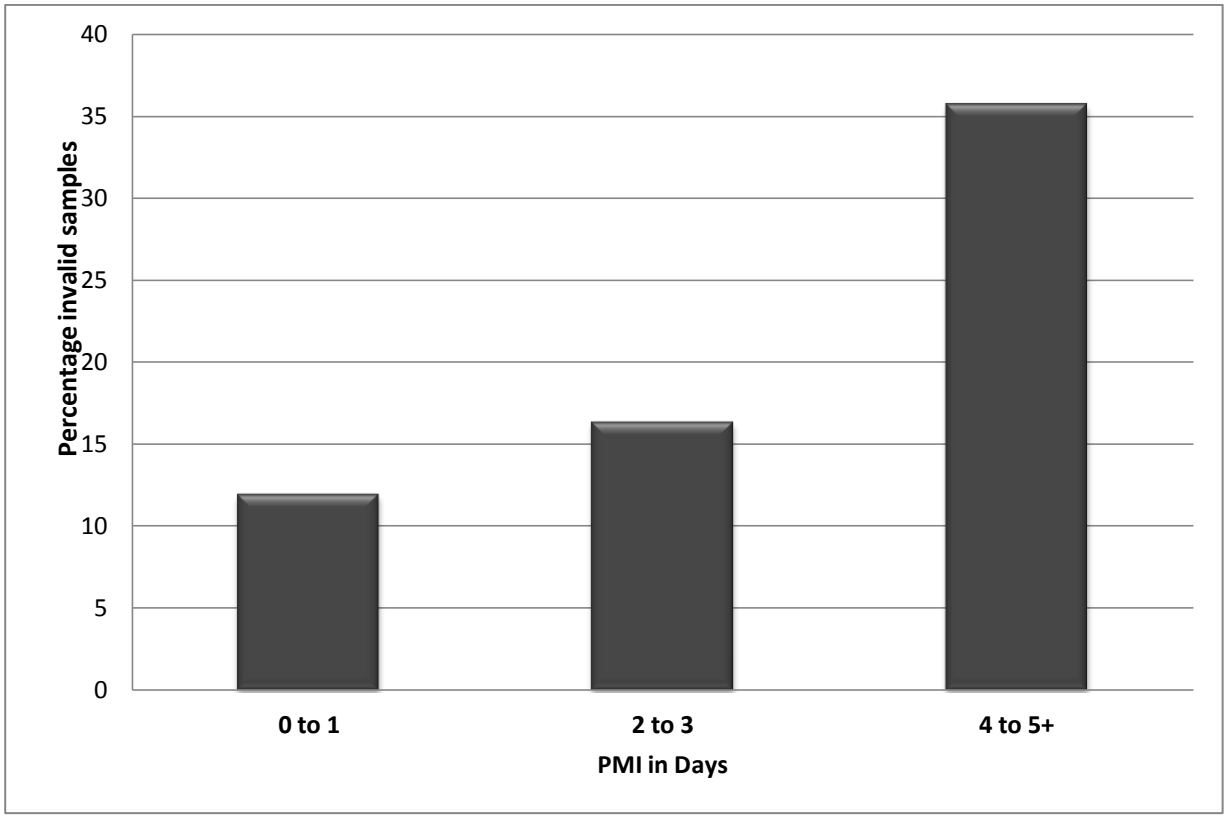

Figure 11: Graph of invalid samples (represented as a percentage of the total samples collected) compared to post mortem interval (PMI) (Chi squared test, *p*= 0.0034)

Discussion:

In 2008, the Pretoria MLL admitted 2742 cases of suspected unnatural cause of death cases. This places the Pretoria MLL as the fifth largest MLL in South Africa for 2008.[13] An earlier study done by du Plessis, at the same mortuary in 1999, documented an 11% rate of infection in the Pretoria MLL population.[15] The statistics correlated with the documented rates of infection

at the time. The current study shows a 26% HIV sero-prevalence in the whole MLL population and a HIV prevalence of 30% in the 15 to 49 year age group. These figures are not representative of the data currently published on the prevalence of HIV in South Africa. The Statistics South Africa 2009 report states that the HIV prevalence for the entire population is 10.6% and increases to 17% in the 15 to 49 year age group.[16] The 2008 WHO study reported 5.7 million cases of HIV in South Africa, which translated into a prevalence of 11.6 % in the entire population and 18.1% in the 15 to 49 year old category.[37] These discrepancies in HIV prevalence can be attributed to differences in the size of the current sample group, the demographics of Pretoria and the risk factors associated with urban living. Nonetheless, the importance of this study lies in the insight it provides into urban living, risk profiles and cultures found in a large urban city like Pretoria.

Due to the large volume of cases being admitted to the Pretoria MLL, a HIV prevalence of 26% in mortuary cases and exposure to severely traumatised bodies, the correct use of personnel protective equipment must be made a priority. These factors reflect dramatic increases in the occupational health and safety risks faced by forensic personnel and pathologists at autopsy. A further concern is the increasing unreliability of Abbot Determine ^{TM} HIV -1 / 2 Rapid qualitative Immunoassay test kits on post mortem samples as the post mortem interval increases.

This has important implications on accidental exposure protocols in the mortuary setting as it has been documented that HIV-2 can be cultured in blood for up to 16 days after death.[19] Consequently, forensic personnel,

pathologists and funeral undertakers face uncertainty if they should suffer an occupational injury involving bodily fluids. The invalid results pose an interesting conundrum and there are two apparent explanations. Firstly, post mortem haemolysis of blood samples may reduce the transportation of samples through the test's matrix. Secondly, the post mortem interval may allow an inhibiting agent to be produced. In either scenario, the efficacy of the test is reduced, resulting in a large number of invalid test results as the PMI increases. In an attempt to address the large amount of invalids it is suggested that, in follow-up studies, each test sample be spun down and that serum (rather than whole blood) be used for the testing procedure.

The demographic information used in this study was obtained during the identification phase of the forensic investigation. The gender split (Table 2) is representative of the national statistics for other than natural deaths taking place in South Africa. This is consistent with the mortality data for South Africa, where it is shown that the percentage of males dying from other than natural deaths (76%) is three times greater than for females (24%).[13] An interesting observation from this data set is the split of prevalence of HIV between gender groups. The prevalence rate for women is reported as 19.7% in 2009, yet in this study it is documented at nearly double that rate (37%).[16] The Human Science Research Council (HSRC) states, in its 2009 report, that the prevalence rate of HIV infections for males is 7.9% and for females 13.6%.[10] The increased rate of infection in females is observed in the HSRC's research study but not to the same extent as documented in this study. The discrepancy in prevalence rates can be attributed to the size of the sample group and the increased susceptibility of women to contracting

HIV through sexual intercourse and social factors. The discourse can also be explained due to the fact that there are a larger proportion of men in this study than women.[40]

The race profile documented above (Table 3) needs to be put into perspective. The Black community accounts for 79.3% of the population of South Africa; the White community, 9.1%; the Coloured community, 9.0% and the Asian community represents 2.6% of the population.[16] HIV distribution in racial groups is not well documented in the specific reports.[1,13,16] The HSRC documented the Black community's prevalence at 13.3% and the White community's prevalence at 0.6%.[10] This study documents the epidemiological change associated with race in Pretoria. Therefore, the current research is essential to documenting the changes in the profile of infection in South Africa.

The age distribution of the samples is documented above (graph 1). The only observation that is worth mentioning is that there are a number of HIV infections (6; 12%) in cases where the deceased's age is greater than 50. This highlights the changing profile of HIV as the population ages and treatment becomes more effective and available.

The accuracy and availability of information make this population ideal for use in the statistical modelling of HIV in South Africa. With sound knowledge of what the disease is doing we can monitor and adapt our treatment and intervention strategies accordingly.

Sudden unexpected death accounts for 14% of the caseload received at the Pretoria MML. In many of these cases, there is little or no medical history

present at the time of autopsy. A relatively large number of cases arriving at the Pretoria MLL are decomposed or autolysed and often only a limited amount of information can be gained from autopsies on such cases. It is hypothesised that one of the overlooked areas of inquiry in these cases is the sero-prevalence of HIV. It is not routine for the forensic pathologist in South Africa to test for the presence of HIV and the unknown impact of HIV on the case loads in South Africa needs to be investigated.

It was observed in some of the suicide notes, which have been presented at autopsy by the investigating officer, that the HIV status of the individual was a large contributory factor in their decision to end their lives. As the number of suicides (19 cases) in this study is relatively small and the prevalence of HIV is 13%, it is not possible to form statistically based conclusions. However, the evidence at autopsy suggests that there could be a link between a person's HIV status and their risk of committing suicide.

Conclusion:

Post mortem testing of other than natural deaths still holds a wealth of information that can be extracted with minimal invasion of a person's rights and in an appropriate environment, with the necessary safeguards. The data will ultimately have an invaluable role to play in the monitoring and management of HIV, as well as in formulating new intervention strategies.

Personal Protective Equipment and universal precautions need to be strictly adhered to and enforced for the safety of all forensic pathology services employees and funeral service industry employees.

The testing methods of post mortem blood samples need to be adapted to provide a study result with fewer invalids.

Finally, further research needs to be undertaken into HIV in the suicide and sudden unexplained and unexpected death sub-populations

Chapter 3: The prevalence of HIV in the suicide population of the Pretoria Medico-Legal laboratory

Introduction:

In a previous study done at the Pretoria Medico-Legal Laboratory (MLL), which looked at the prevalence of HIV in the facilities case load. It was observed that in some of the cases of suicide, where a note was written, it was stated that the persons HIV status was a significant contributory factor to them committing suicide. One of the recommendations from the said study was that further research should be done to determine if there is a significant difference in the prevalence rates of the general mortuary population and the suicide population in the Pretoria MLL.

Pretoria is the capital city of South Africa and has an estimated population of 1.338 million people residing within its confines in 2008.[38] The Pretoria MLL services the Tshwane metropolitan area and dealt with 2732 non-natural causes of death in 2007.[13] Unfortunately the validity of the statistics surrounding suicide in South Africa have been brought into question with the publication of the Statistics South Africa report on mortality and causes for death in South Africa. The report states that there were only 442

suicides in the whole of South Africa in 2008.[13] Unbelievably this would make South Africa's rate of suicide 0.896 per 100 000 citizens.[16] A number of local and international reports paint a contrasting picture. One released by the Medical Research Council states that, at 14.35%, suicide is the third leading cause of unnatural death in Pretoria South Africa, this translates into a rate of 15.7 suicides per 100 000 citizens.[41] This is on par with world average, which is 16 suicides per 100 000 people.[42] Just too muddy the water further in a study done in 2004 at the Pretoria MLL it was found that the suicide prevalence for the 1997 to 2000 period was 25 in 100 000 people.[43] These discrepancies between places and publications are discussed in an article, by Stephanie Burrows and Lucie Laflamme, where they did a city-level comparison across socio-demographic groups of suicide mortality in South Africa. They found that the suicide rate is 25 per 100 000 citizens. Further they conclude that specific city based investigations need to be conducted to explain suicide mortality in South Africa.[44]

The link between HIV and an increased risk of suicide has been extensively documented in the international literature.[30,31] The studies that have been done have had varying results, with one study determining that the rate of suicide is not higher than that of the general population.[32] In contrast the New York's Medical Examiners statistics reviled that the risk of suicide for HIV positive men between the ages of 20-59 years of age was 66 times greater than the general population and 33 times the rate for men of the same demographic.[32-34] A subsequent study found that men of the same demographic (20-59 years of age) in California were at 17 times the risk of committing suicide than uninfected individuals.[33,34] An even larger risk

group between 20 and 39 years of age was identified with a 21 times greater incidence of suicide in 1993.[33] With the current data available the link between HIV and suicide has been documented and this is a worrying trend that needs to be researched in the South African context.

This study aims to determine if the prevalence of HIV in the suicide population is different from that of the general mortuary populations' HIV prevalence. The identification of explicit links between suicide and HIV status will further the policy development, treatment and counselling strategies to the benefit of the patients in the future.[6] Thus, ensuring a better life for all parties either infected or affected by the HIV pandemic and suicide.

The statistical analysis was done in conjunction with the University of Pretoria's Statistics Department. The null hypothesis is that the prevalence of HIV in the Pretoria MLL suicide population should not deviate from that of the general Pretoria MLL population. The alternate hypothesis is that there is a difference between the two HIV prevalence statistics.

Materials and methods:

Post mortem blood samples were obtained on all cases admitted to the Pretoria MLL where the attending forensic pathologist was of the opinion that the manner of death was suicide. These deaths are legislated under the Inquests Act 58 of 1959 which prescribes the initiation of an investigation into them. The Pretoria MLL was selected due to its academic ties to the University of Pretoria and the large number of autopsies performed at the facility each year. The sample population was chosen because of its specific

nature of their deaths and the abundance of available demographic information on the samples. A total of 101 consecutive samples were collected from suicide cases and processed. This study excluded any case that blood could not be collected from the body; this could be due to the severity of decomposition or the charring of the remains.

The samples were collected, during the autopsy, by a forensic pathologist who followed the standard operating procedures for autopsy at the Pretoria MLL.

The processing of the blood samples was done by the standardized testing procedures developed and used in the NHLS Tshwane Virology Research Department.

- The sample was centrifuged and the serum was used to run the test in order to minimise the effect haemolysed blood had on the test strips. This was proposed in the preliminary study in chapter two to minimise the invalid test results.
- The screening test used was the Determine HIV-1 / 2 Ag / Ab Combo assay and the confirmatory test used was the HIV Combi, Cobas E, Elecsys and Modular (Roche).[23]

The study proposal was submitted to the University of Pretoria's Faculty of Health Sciences Research Ethics Committee and the MSc Committee, which both gave approval for the study to be conducted.

The demographic and case related Information for each individual was collected and stored. The confidentiality of the individual's results was of

concern and every case was given a research number at autopsy. Every reasonable precaution was taken to secure the confidentiality of the tested subject's identities and test results.

The statistical analysis was done in conjunction with the department of statistics at the University of Pretoria. The SAS program was used for the analysis of the raw data. The use of race demographics (Black, White, Coloured and Asian) is not scientifically or anthropologically accurate or accountable but serves to inform experts on the traditionally utilised craterisations of social and ethnic groups that to this day persist in the South African context, albeit from a historical perspective.

Results:

The study was carried out in the Pretoria MLL. Each case was selected from the general mortuary population. Table 5 demonstrates that a total of 101 samples were collected with 32 samples being invalid. The HIV prevalence of the valid samples is 23.18%. In the Pretoria MLL the demographic data is split according to gender (75.25% male; 24.75% female) and race (Black 47.52%; White 48.51%; Asian 1.98%; Coloured 1.98%). The HIV prevalence for each group is represented by gender (24% male; 21% female) and race (Black 27.27%; White 18.18%; Asian 0%; Coloured 100%) which are represented in Table 5 and Figures 12 to 17.

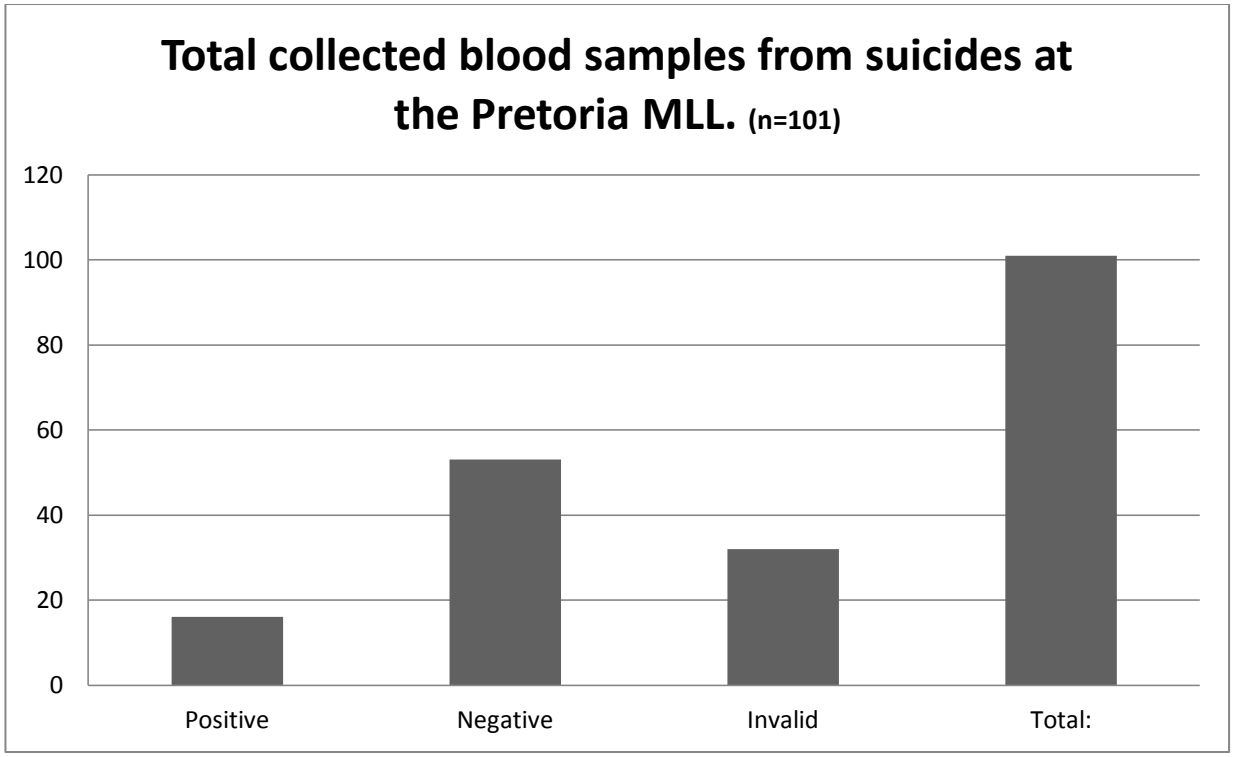

Figure 12:Total collected blood samples from suicides at the Pretoria MLL

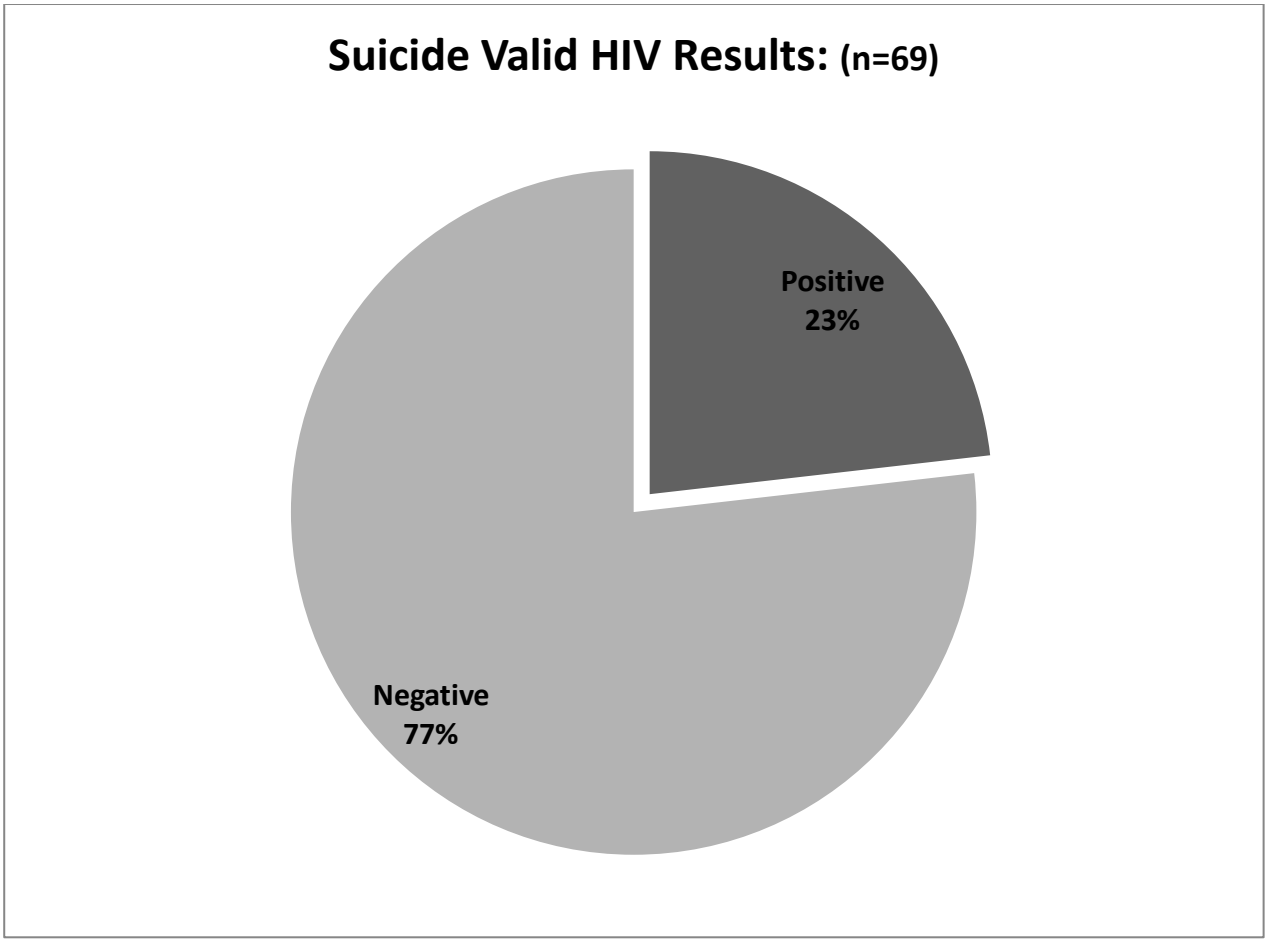

Figure 13 Prevalence of HIV in the Pretoria suicide population

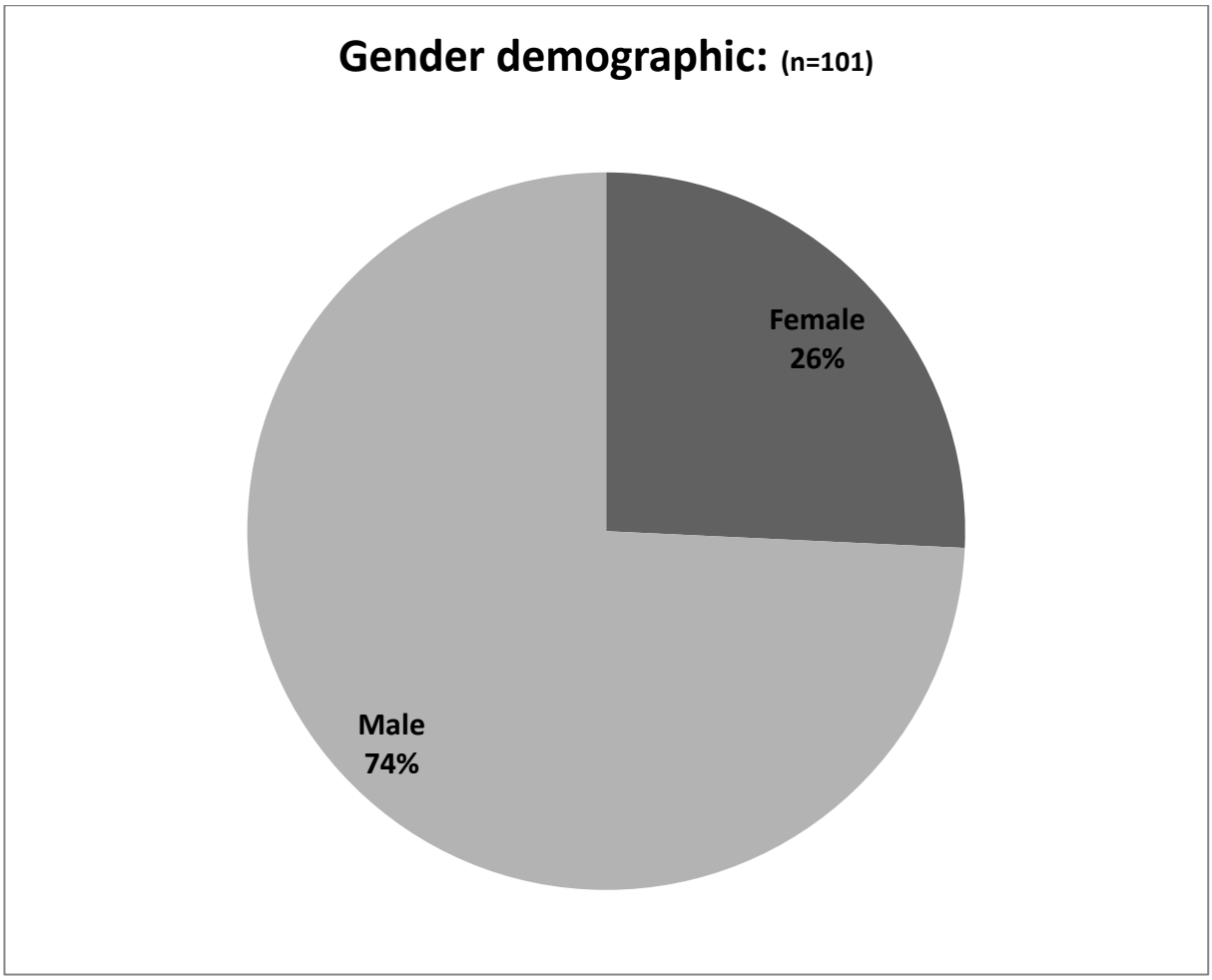

Figure 14: Gender distribution for suicide

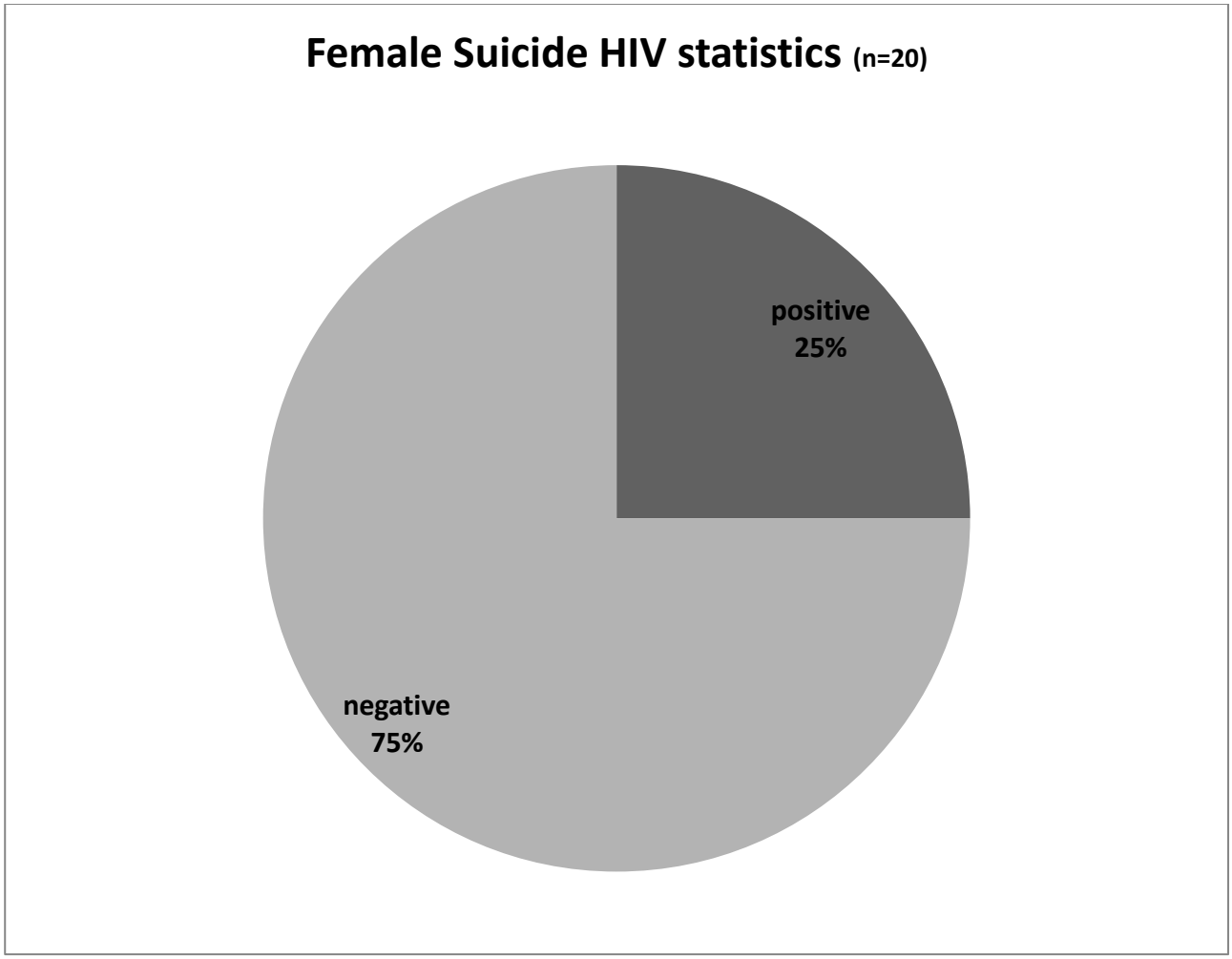

Figure 15: HIV statistics for female suicide cases

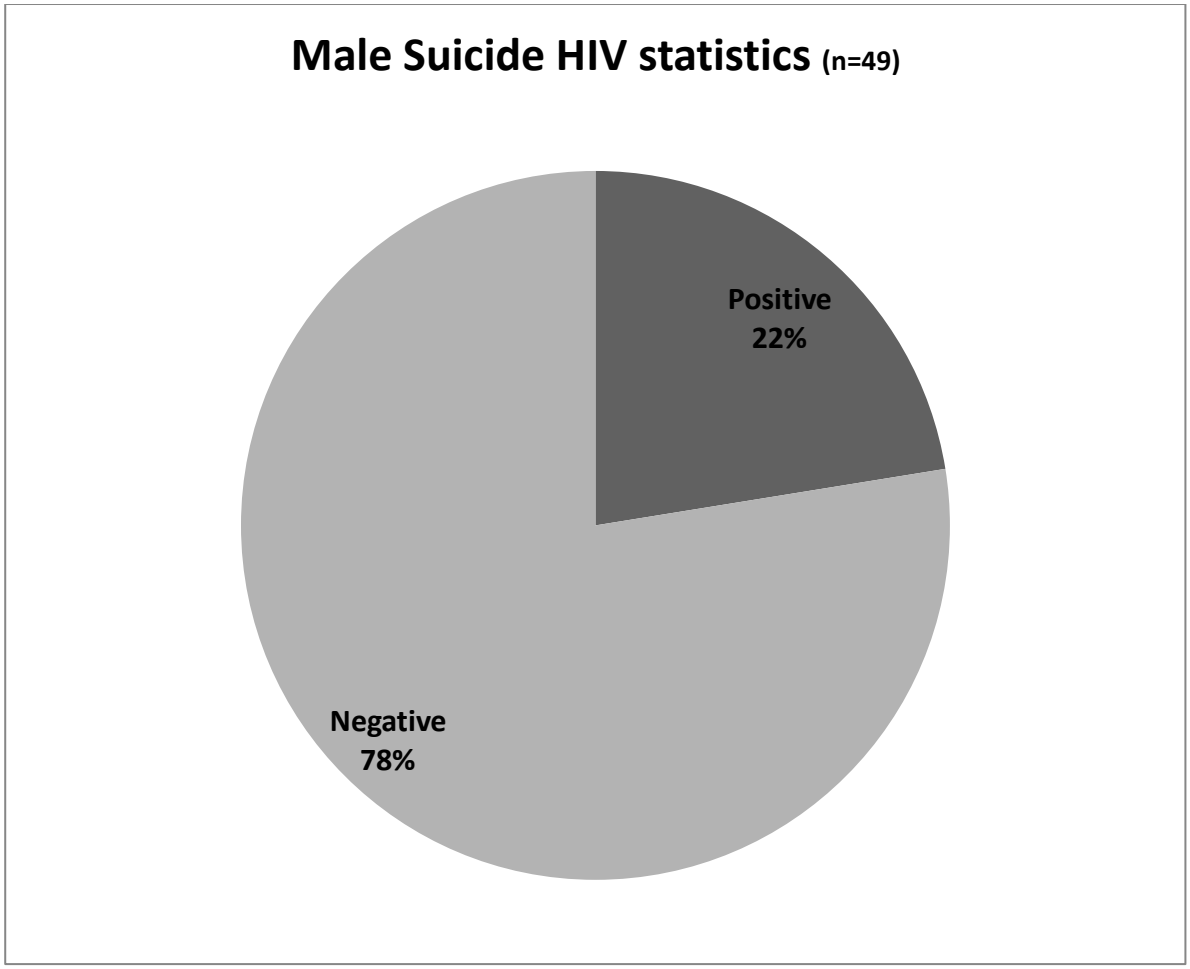

Figure 16: Statistics for male suicide cases

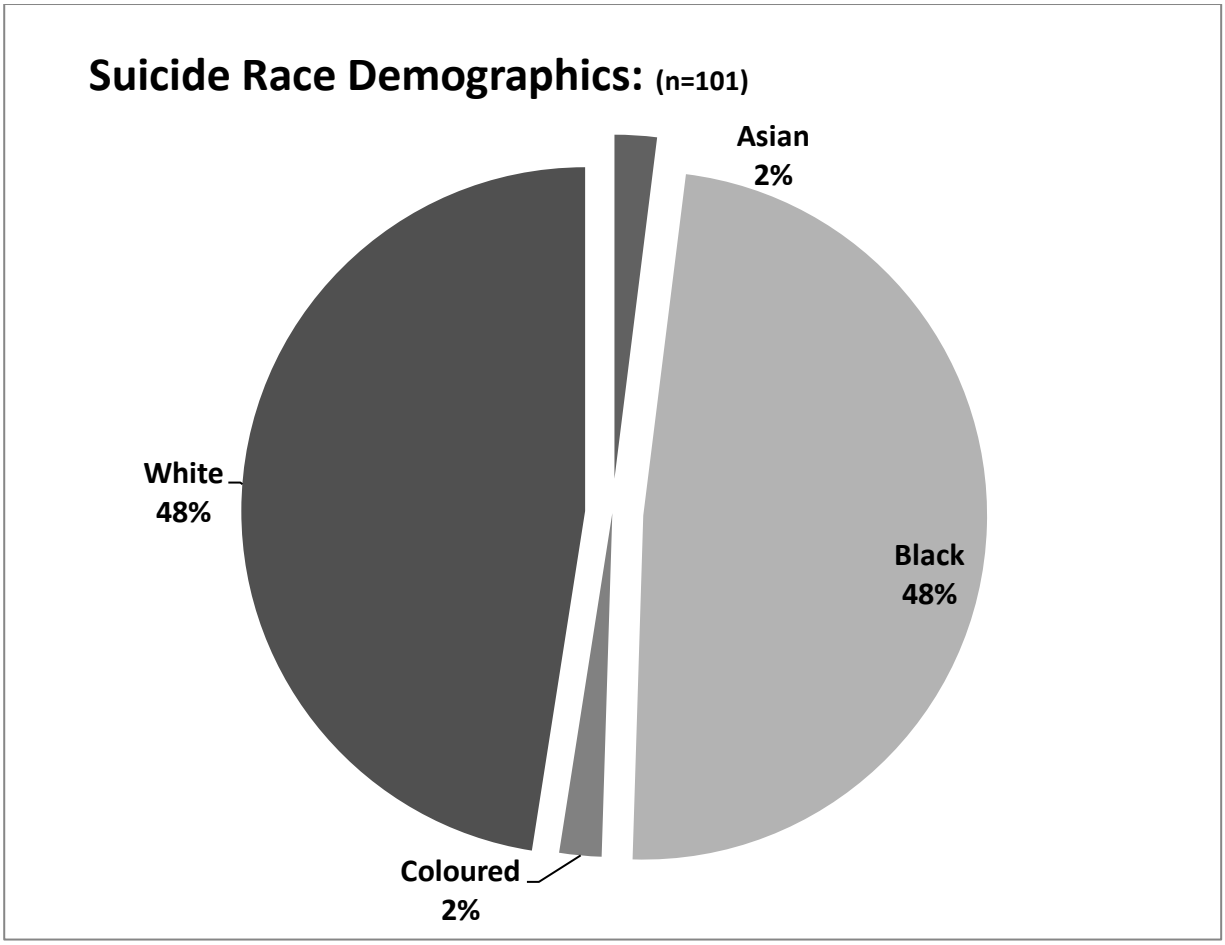

Figure 17: Suicide populations race demographics

Table 5: HIV statistics and Demographics for the suicide population in the Pretoria MLL

HIV Statistics	Totals:	Gender		Race			
		Male	Female	Black	White	Asian	Coloured
HIV Positive	16	12	4	9	6	0	1
HIV Negative	53	38	15	24	27	2	0
Invalid	32	26	6	15	16	0	1
Total: (n)	101	76	25	48	49	2	2

Table 6 and Figures 18 to 20 document the preferred methods of suicide in this study in Pretoria. Hanging (45 cases; 44.5%) was the leading method choice, followed by poisoning (23 cases; 22.8%) which includes carbon monoxide poisoning from vehicular gassings (6 cases ; 5.9%), ingestion of pills (12 cases ; 11.9 %), drug overdose (1 case ; 1%) and agricultural poisons (4 cases; 4%). The third preferred method of suicide is by firearm (gunshot wound) (18 cases; 17.8%) with 17 gunshot wounds to the head and 1 to the chest. The fourth most preferred method is to jump from a height (6 cases; 5.9%) with the fifth being vehicular impact (5 cases; 4.9%) which include jumping in front of a moving train (3 cases; 3%), jumping in front of a truck (1 case; 1%) and a car (1 case; 1%). The preferred methods of

suicide for men are hanging (52.6%), gunshot wounds (GSW; 18.4%), poisoning (15.8%), jumping from height (6.58%) and vehicular impact (5.26%). The preferred methods of suicide for women are poisoning (44%), hanging (20%), GSW (16%), burns (4%), complex suicide (4%), sharp force incision (4%), jumped from height (4%) and vehicular impact (4%).

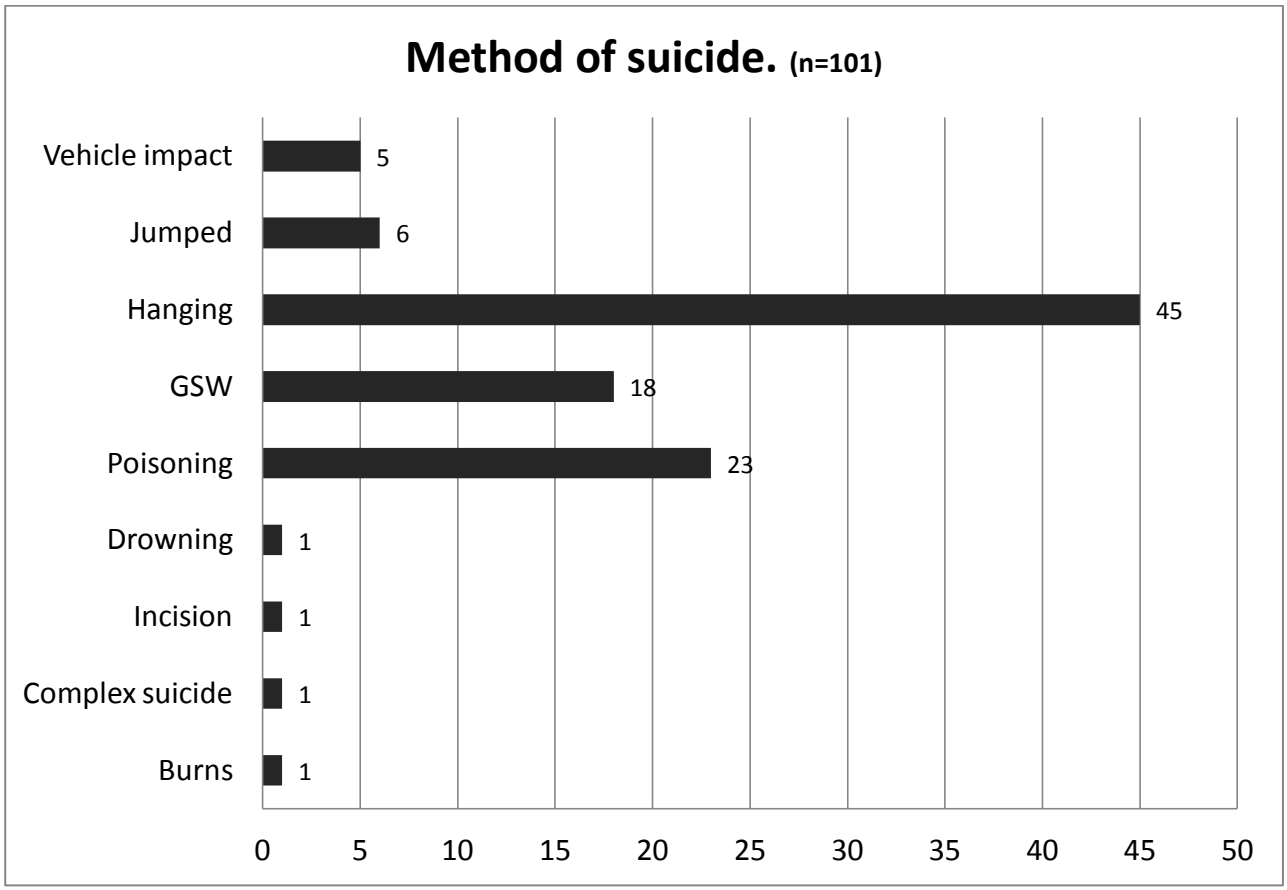

Figure 18: Method of suicide for Pretoria

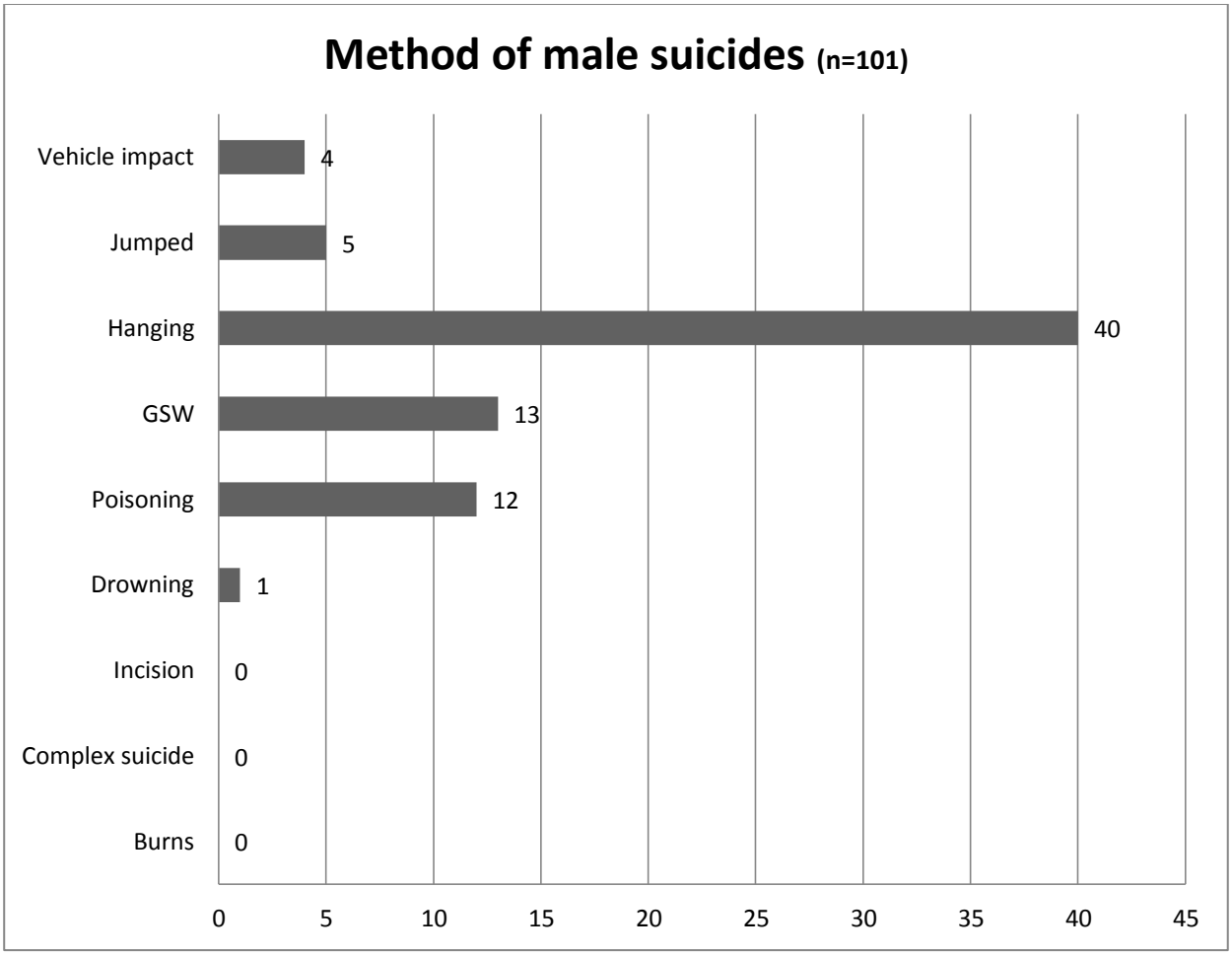

Figure 19: Method of male suicides

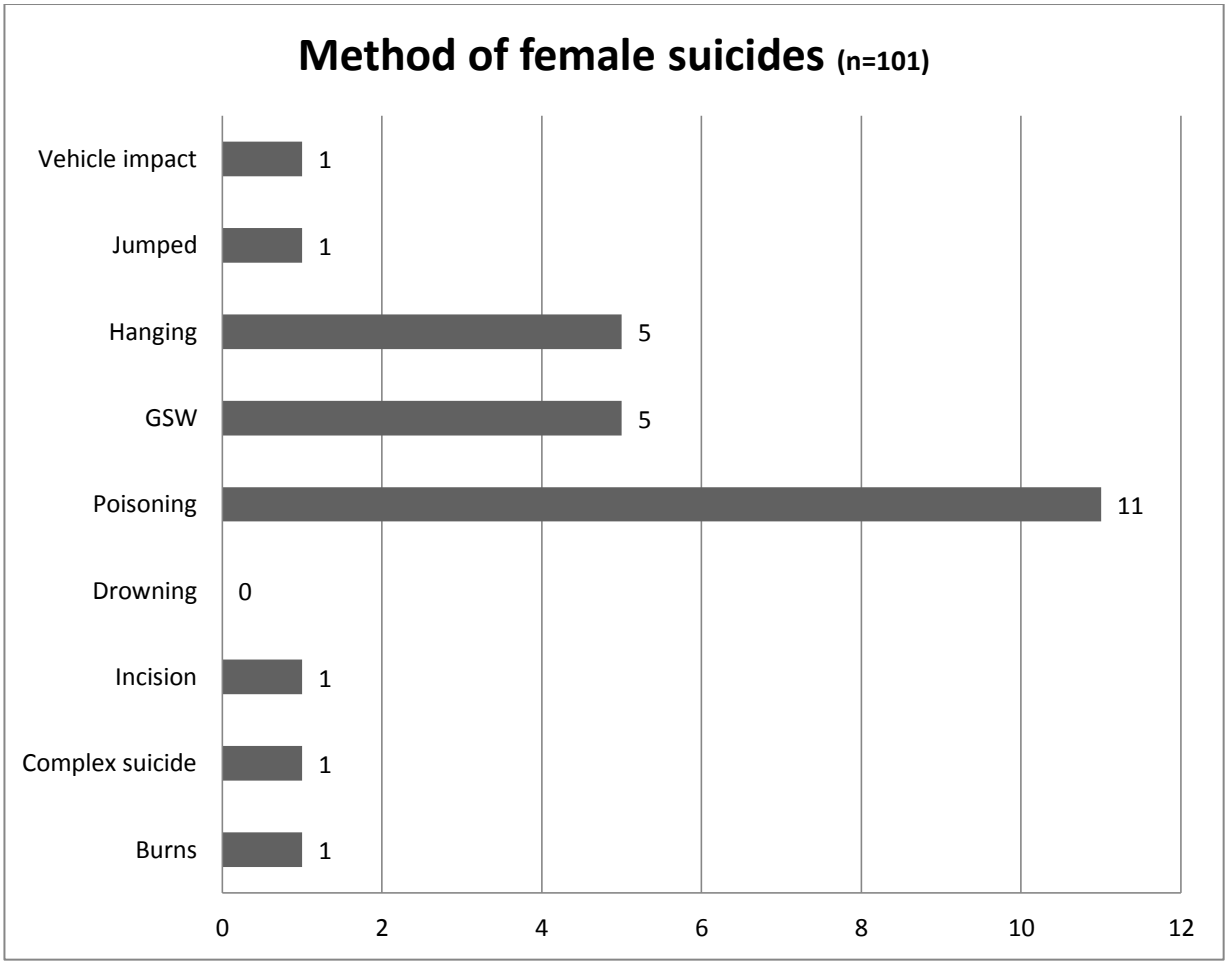

Figure 20: Method of female suicides

Table 6: Method of suicide by demographic in the Pretoria MLL

Method of suicide:	Totals	Gender		Race			
		Male	Female	Black	White	Asian	Coloured
Burns	1	0	1	1	0	0	0
Complex suicide	1	0	1	0	1	0	0
Incision	1	0	1	0	1	0	0
Drowning	1	1	0	0	0	1	0
Poisoning	23	12	11	6	17	0	0
GSW	18	14	4	5	12	1	0
Hanging	45	40	5	27	16	0	2
Jumped	6	5	1	4	2	0	0
Vehicle impact	5	4	1	5	0	0	0
total:	101	76	25	48	49	2	2

The youngest victims where 15 years of age (3 cases) in Pretoria. With an average age of suicide at 34.7 years and the average age for the HIV positive individuals who committed suicide is 38.8 years old. The average age of suicide in men is 33.4 years and 34.1 years in women. The data was disassociated further in to race demographics. Black women (30.9 years) and Black men (30 years) commit suicide earlier than White women (43

years) and White men (37.5 years), not much can be concluded in Asian men (34.5 years) and Coloured men (36.5 years) due to a lack of numbers (4 cases).

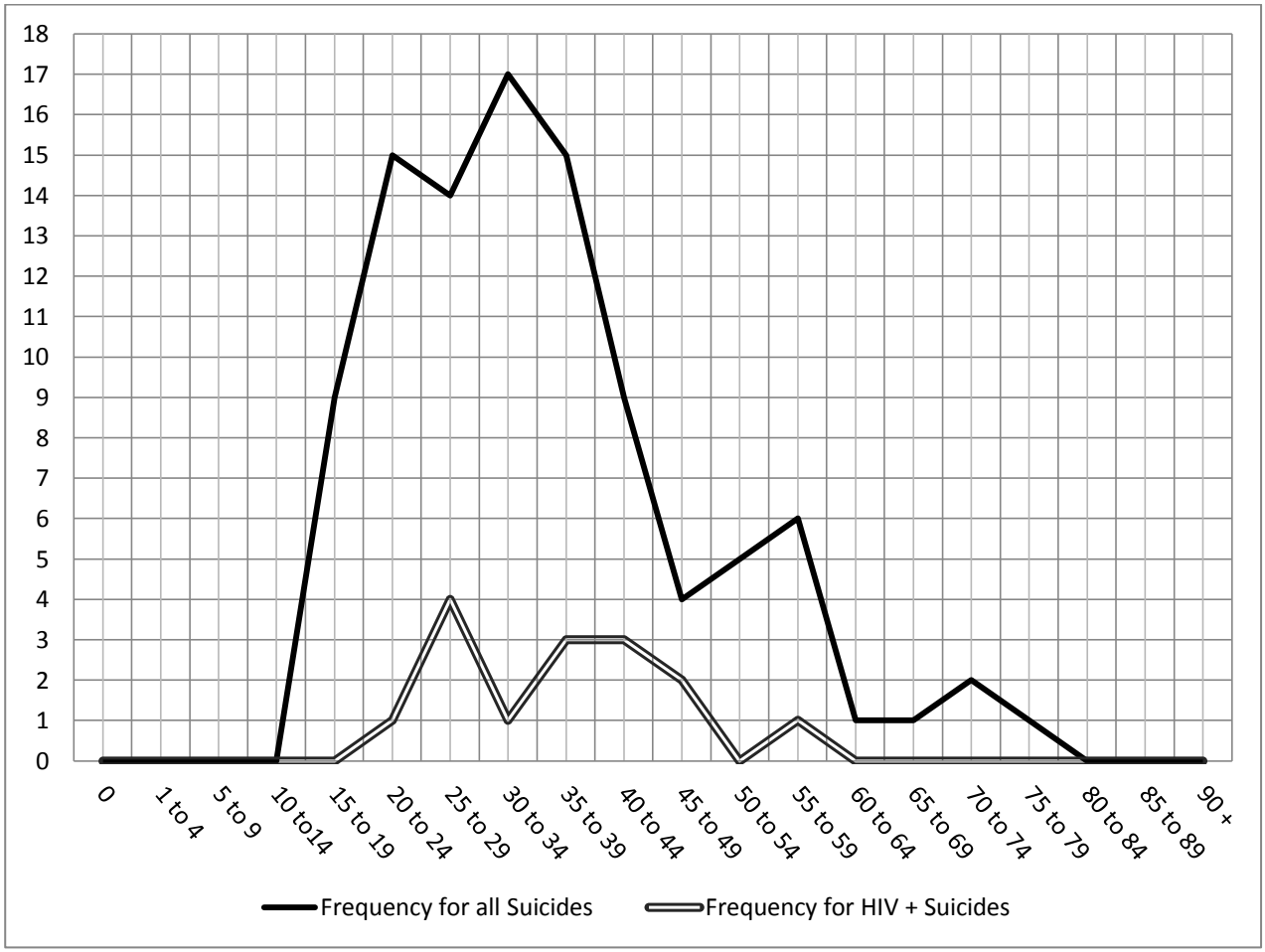

Figure 21: Age distribution for the suicide population and HIV positive suicide population in Pretoria MLL

The prevalence's of both the general populations HIV statistics and the suicide populations HIV statistics, where compared to each other in a Chi Squared analysis which yielded a statistically significant *p*-value of 0.0198. The cut off for a significant p value was a *p*-value of greater than 0.05.

Table 7: Chi squared analysis of the general mortuary populations HIV prevalence rate and the suicide populations prevalence rate

General Mortuary Population's HIV prevalence:	Suicide Population's HIV Prevalence:	Chi squared analysis: *(p-value)*
26%	23%	*p*= 0.0198

Discussion:

This study was prompted by the analysis of suicide notes in a previous unpublished study. In that study it was documented that 3 peoples HIV positive status was explicitly stated to be the initiating factor for their suicidal ideation and ultimately the suicide itself. This study was set up to determine the prevalence of HIV in the suicide population at the Pretoria MLL. Firstly, suicide is defined in the 1973 edition of the Encyclopaedia Britannica and quoted by Shneidman as "The human act of self-inflicting one's own life cessation".[45] This definition is used as is to categorise the supposed method of death in this study. In order to for the study to have any context the collected data was compared to the previous study. (Chapter 2)

The initial baseline study found that the HIV prevalence in the mortuary was 26.15%. The results in table one shows a HIV prevalence rate of 23.18%, this equates to a 2.79% difference in prevalence. On further investigation and analysis the *p*-value between the two data sets was determined to be 0.0198. The *p*-value rejects the null hypothesis, that the prevalence's in both articles should be the same making the 2.97%

difference in rates a meaningful statistical difference between the two studies. It was observed that the demographic split in the data could offer a possible explanation for the difference in prevalence between the two studies. The previous study had a different demographic split with Asians accounting for 1%, Blacks accounting for 76%, Coloureds accounting for 2% and Whites accounting for 18% of the mortuary population. The suicide demographics demonstrate the proportion of White individuals has increased to 48.51% of the selected population but the HIV prevalence in this group is 18.18% which considerably higher than in the original studies' finding (8%). A second possible explanation is that there is a greater percentage of women in the suicide population, 26%, with a lower HIV prevalence of 25%, than the general mortuary population which has a HIV prevalence of 37%. Both of the demographic and HIV statistical changes can account for the change in HIV prevalence form the original prevalence study.

An additional observation from the study was the over representation of Whites, 48.51%, in the Pretoria MLL suicide population. The impact of this data only becomes apparent when placed in context; White South Africans only make up 9.1% of South Africa's entire population and only 18% of Pretoria's MLL population.[16] It has been recommended that targeted research into specific "at risk" groups be undertaken to determine their needs and the best way to implement a suicide prevention program.[44] The continued need for pre and post counselling can also not be ignored in this setting and the direct discussion surrounding suicide and self-harm needs to take place in these counselling sessions. Information on how to seek help for

suicidal ideation needs to be freely available to South Africans but this can only be done once a needs assessment for the "at risk" populations is completed.

In any future studies involving post mortem sampling the sample size will be determined by the number of valid test results and not the total number of tests done. This will increase the accuracy and statistical significance of the study as there are a large number of invalids present in the study.

Conclusion:

There is a significantly (2.97%, $p = 0.0198$) lower HIV prevalence in the suicide population at the Pretoria MLL. Cases do exist where HIV is the initiating cause for the suicidal ideation but it is not in large enough numbers to represent a change in the HIV prevalence.

A worrying observation is the over representation of Whites in the suicide population of Pretoria. Further research needs to be done into the specific environmental, psychological and social reasons for this over representation. This should be done in order to determine the most effective means of implementing a targeted suicide prevention program in Pretoria.

<u>Chapter 4:</u> The prevalence of HIV in the Sudden, Unexplained and Unexpected Death population at the Pretoria Medico-Legal Laboratory

Introduction:

At the Pretoria Medico-Legal Laboratory (MLL) some cases are classified before autopsy as sudden, unexplained and unexpected deaths (SUU-

Deaths).This is in line with the National Health act of 2003.[22] SUU-Deaths are considered to be some of the most challenging autopsies that can be conducted by pathologists. They are classified according to the ICD-10 codes of the World Health Organisation. SUU-Death is defined as an ill-defined and unknown cause of mortality (R95-R99). [46]Simply put, it is a death under circumstances where a body is found with no prior medical history and no obvious cause of death, either traumatic or disease related. The SUU-Death definition is broad. This is done in order to secure as many of the other than natural deaths for autopsy purposes. Pretoria MLL deals with 2500 cases per year; of these, 14% to 20% are SUU-Deaths.[13] Knights forensic pathology chapter 25 states that " some of the most difficult problems in criminal and litigious cases arise not out of gross, rapidly fatal, trauma, but in deaths where concurrent natural disease or complications after trauma lead to a fatal outcome."[47]

In chapter two of the current study (above), it is stated that the rate of HIV infection in the SUU-Death group is 33%. The general mortuary population's prevalence is 26%. Due to the increase in prevalence rate, further investigation was suggested in order to get a clearer understanding and representation of the SUU-Death group in the Pretoria MLL. The further research in to the SUU-Death population could provide additional insight into the epidemiology of the virus. It will also provide useful information into the efficacy of the current intervention strategies.

Antiretroviral therapies have been associated, in a number of studies, with the increased risk of cardiovascular disease.[48,49] In a follow up study it was documented that, "Increased exposure to protease inhibitors is associated

with an increased risk of myocardial infarction, which is partly explained by dyslipidaemia."[50] In cases where a myocardial infarction is fatal, an autopsy may be performed due to the reason of SUU-Death. This is especially true if the deceased had no prior history of heart disease or was considered to be in good health. Therefore, an increased risk of cardiovascular disease and/or myocardial infarction in HIV positive individuals receiving anti-retroviral therapy, could potentially be observed as an increase in SUU-Deaths.

Tuberculosis (TB) and HIV co-infection may also be linked to SUU-Death. TB and HIV co-infected patients have a five times greater risk of dying within two years of receiving TB treatment.[51] Other studies have determined that 31% of all new TB cases in the African region can be attributed to HIV infection: "TB was the cause of 11% of all adult AIDS related deaths". In South Africa it was estimated that there were 2 million co-infected adults in 2003.[52,53]

South Africa has a jaded past with misinformation and a large issue with stigmatisation of HIV positive individuals. HIV positive individuals, in their specific communities, are being diagnosed with health and mental health problems such as Post Traumatic Stress Disorder.[7] Research into this phenomenon is being done. The role that faith-based organisations play in the process of informing and supporting people affected and infected with HIV has been under-estimated for a long time.[54] This stigmatisation and lack of support hinders and prevents the early identification of a person's HIV status, thus delaying the testing and treatment regime.

South Africa has a population of 49 320 500 people as of mid-2009 and 10.6% of the population is HIV positive.[16] The burden of disease on the state is large and costly. In order to minimise the consequences of the pandemic, every possible chance to understand and refine the information used to make important prevention/treatment decisions, needs to be taken.

This study seeks to provide relevant statistical information on the HIV prevalence rate in the SUU-Death population of the Pretoria MLL. Blood samples will be collected continually for a 100 valid HIV test results along with the relevant demographic information.

Materials and methods:

The location of the study is the Pretoria Medico-Legal Laboratory. The samples were collected, during autopsy, by a forensic pathologist who followed the standard operating procedures for autopsy at the Pretoria MLL.

The processing of blood samples was done by the standardized testing procedures developed and used in the NHLS Tshwane Virology Research Department..

- After centrifugation of whole blood, the serum was used to run the test in order to minimise the effect that haemolysed blood has on test strips.
- The screening test used was the Determine HIV-1 / 2 Ag / Ab Combo assay and the confirmatory test used was the HIV Combi, Cobas E, Elecsys and Modular (Roche).[23]

The sample number was amended to a hundred valid test samples due to the previous study determining that there is a possibility of having a high number of invalid test results in the study. Blood samples were taken from every SUU-Death case that presented at the Pretoria MLL. Cases were excluded if the age was less than 9 months of age and if it was not possible to obtain a sample due to post mortem changes (decomposition) or charring of the remains.

The study proposal was submitted to the University of Pretoria's Faculty of Health Sciences Research Ethics Committee and the MSc Committee, which both gave approval for the study to be conducted.

The demographic and case related information for each individual was collected and stored. The confidentiality of an individual's results was of concern and every case was given a research number at autopsy. Every reasonable precaution was taken to secure the confidentiality of the tested subjects' identities and test results. The original data sheets and testing documentation are kept in a secure storage facility at the Department of Forensic Medicine, University of Pretoria.

The statistical analysis was done in conjunction with the University of Pretoria's Statistics Department. The null hypothesis is that the prevalence of HIV in the Pretoria MLL SUU-Death population should not deviate from that of the general Pretoria MLL population. The alternate hypothesis is that there is a difference between the two HIV prevalence statistics. The use of race demographics (Black, White, Coloured and Asian) is not scientifically or anthropologically accurate or accountable but serves to inform experts on

the traditionally utilised craterisations of social and ethnic groups that to this day persist in the South African context, albeit from a historical perspective.

Results:

The data collection took approximately ten months to complete at the Pretoria MLL. A total of 133 samples were collected from 130 cases of SUU-Death. In 2 cases where samples were collected it was necessary to obtain more than one sample (3 samples), from different sites, as only small amounts of sample were available for collection. This was due to the traumatised state of the body.

Table 8 documents the results of the HIV screening and confirmatory testing that was done at the NHLS Tshwane Medical Virology Laboratory. A total of 33 invalid test results were documented, this translates into 25% of all samples. Of the viable samples, 43 (43%) of them were HIV positive and 57 (57%) were HIV negative.

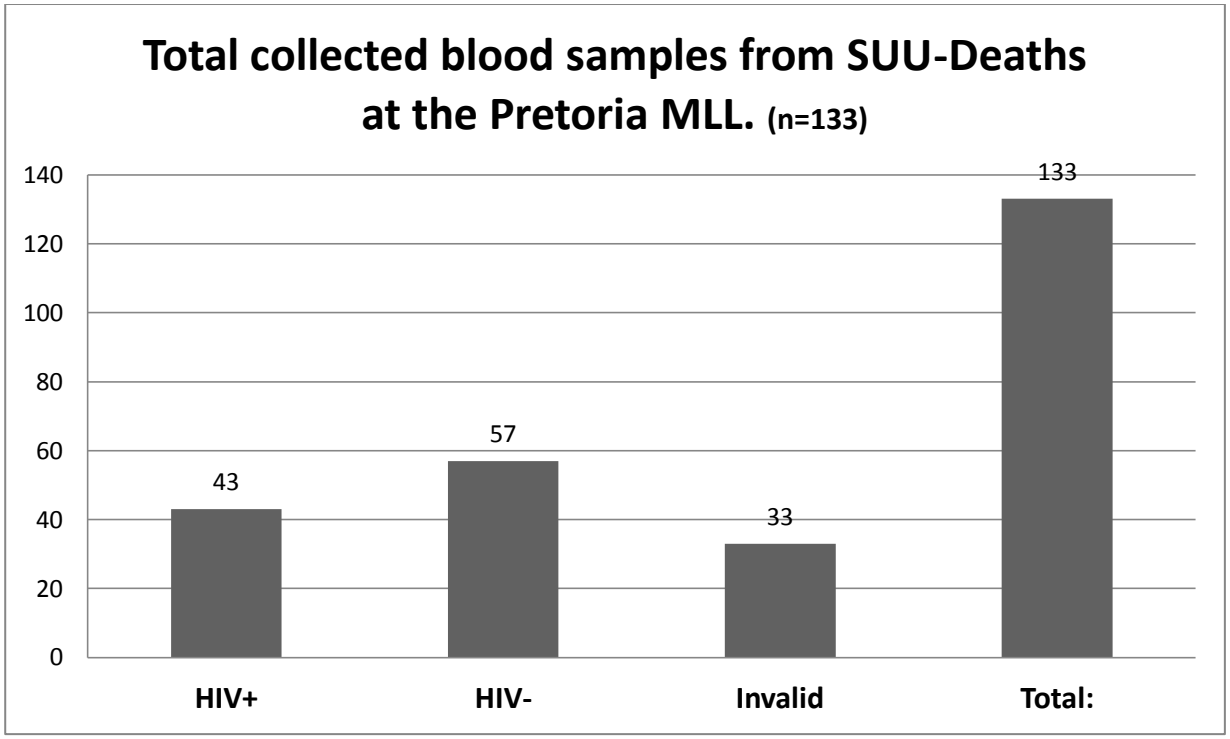

Figure 22: Total collected blood samples from the SUU-Deaths at the Pretoria MLL

Table 8: Total SUU-Death cases and HIV prevalence information in the Pretoria MLL

Result	**Number of cases**	**%**	**Number of viable samples:**	**%**
HIV Positive	**43**	**32**	**43**	**43**
HIV Negative	**57**	**43**	**57**	**57**
Invalid	**33**	**25**	**-**	**0**
Total:	**133**		**100**	

The data was segregated and analysed along demographic parameters in order to extract any additional information from the collected data. Table 9 documents the gender distribution of cases that presented at the Pretoria MLL. Twenty-two present (29 cases) of the SUU-Death sample population

were female and 78% (101 cases) were male. Of the female demographic fraction, the HIV positive cases make up 62% (13 cases) of the total valid samples. In the male demographic fraction, the HIV prevalence rate is 38% (30 cases).

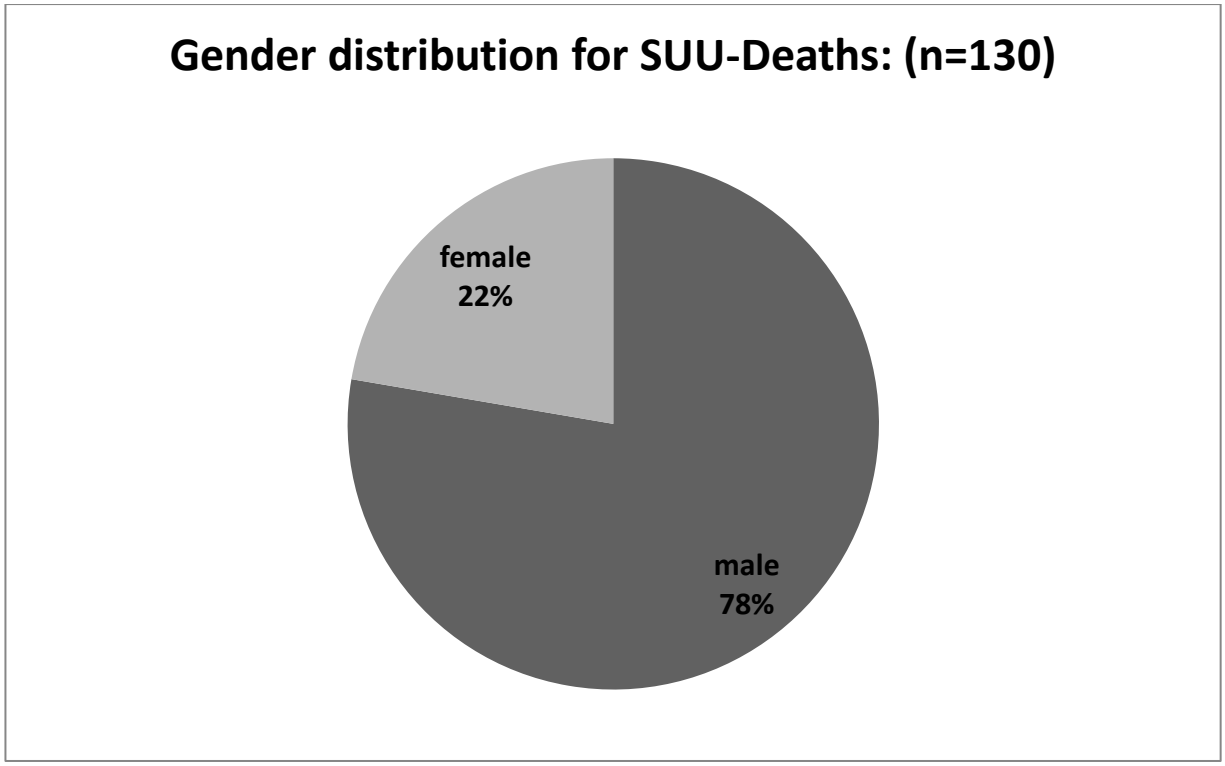

Figure 23: Gender distributions for SUU-Deaths

Table 9: Gender demographics For SUU-Death cases

Gender	**Number**	**%**	**HIV+**	**%**	**HIV-**	**%**	**Total valid samples**
Female	29	22	13	62	8	38	2
Male	101	78	30	38	49	62	79
Total:	**130**		**43**		**57**		**100**

The race demographics for the SUU-Death cases are represented in Table 10. Of the total sample population, Asian individuals make up 2% (2 cases), with none of them being documented as HIV positive. Black South Africans make up 75% (98 cases) of the cases and the prevalence of HIV is 51% (40 cases). No individuals classified as "Coloured" were documented in this study. White South Africans made up 23% (30 cases) of the SUU-Death population and the HIV prevalence is 16% (3 cases) in this group.

Table 10: Race demographics for SUU-Death cases

Race	**Number**	**%**	**HIV+**	**%**	**HIV-**	**%**	**Total valid samples**
Asian	2	2	0	0	2	100	2
Black	98	75	40	51	39	49	79
Coloured	0	0	0	0	0	0	0
White	30	23	3	16	16	84	19
Total:	**130**	**100**	**51**		**57**		**100**

The ages of individual cases in this study ranged from less than 0 years (abortions and miscarriages) to 74 years. Data was divided up into categories with a 5 year interval between the groupings. Positive HIV immune assay results were obtained only from samples in the 0-74 year range, resulting in a 43% rate of infection across the entire Pretoria MLL SUU-Death population. The average age for the general population was 43 years. The average age for females was 38.8 years and for males it was 45

years. For Black males, the average age was 45.5 years and White males were 44.9 years. For White females the average age was 46 years and Black females had an average age of 36.14 years. [Figure 24]

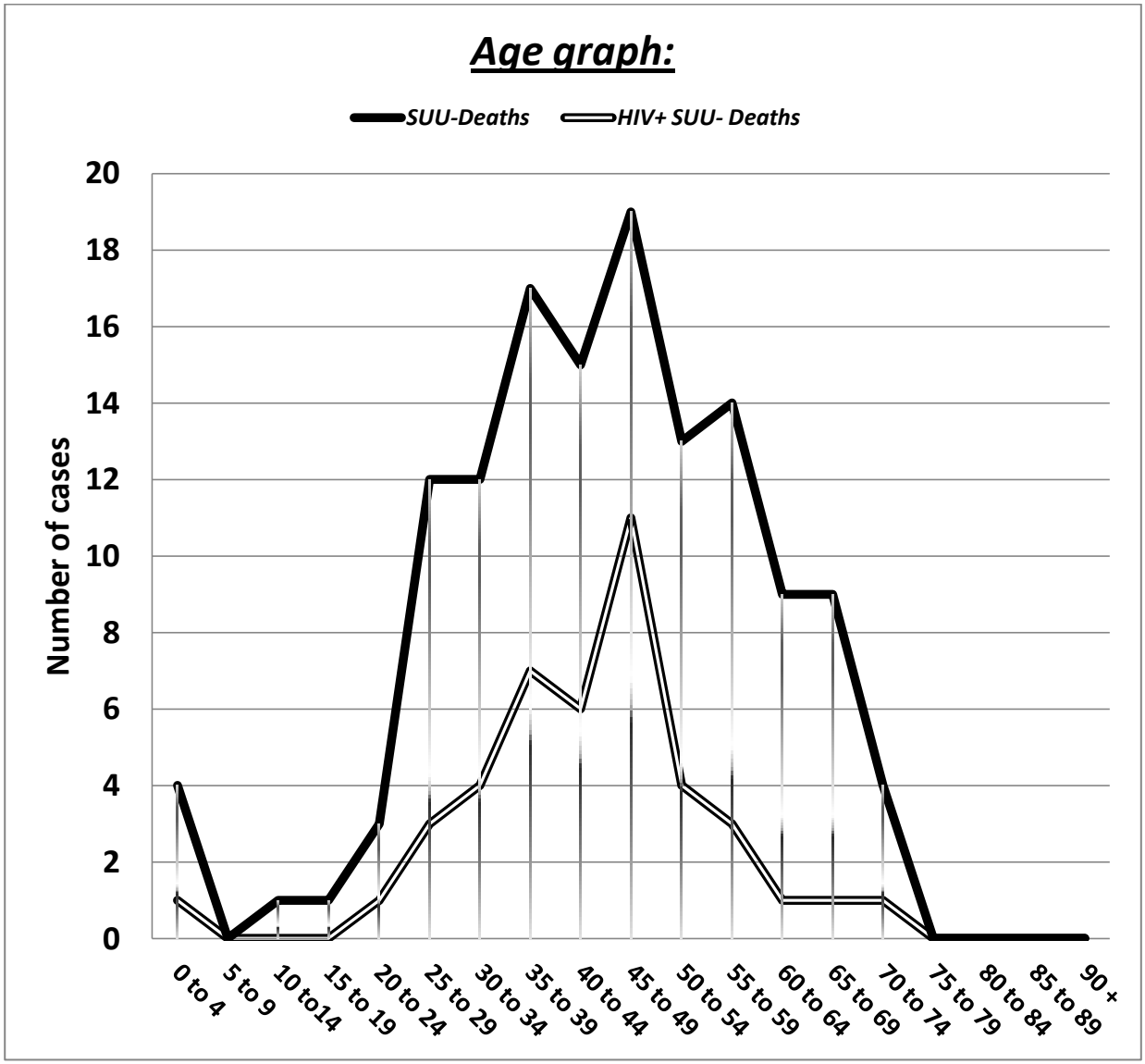

Figure 24: Age distribution of SUU-Deaths and the HIV positive results

The prevalence's of both the general populations HIV statistics and the SUU-Death populations HIV statistics, where compared to each other in a Chi Squared analysis which yielded a statistically significant *p*-value of

0.0045. The cut off for a significant p value was a *p*-value of greater than 0.05.

Table 11: Chi squared analysis of the General population HIV statistics and the SUU-Death HIV prevalence statistics

General Mortuary Population's HIV prevalence:	SUU-Death Population's HIV Prevalence:	Chi squared analysis: *(p-value)*
26%	43%	*p*= 0.0045

Discussion:

The HIV prevalence in the SUU-Death population at The Pretoria MLL is 43%. This is significantly higher than the HIV prevalence in the general population at the Pretoria MLL (26%), translating into a 17% difference in prevalence between the two groups. This was determined to be scientifically and statistically significant with a *p*-value of *0.0045*.Taken together with results from chapter 2, This study shows that there is a moderate (7%) increase in the base line HIV prevalence for the SUU-Death sample population as compared to the general mortuary sample population. In the original study there was a small sample size and it was not possible to draw any meaningful conclusions from the information available. In the follow up study, in every demographic population, there was a marked increase in the HIV prevalence. For example, White South Africans account for 18% of the general mortuary population and their HIV prevalence is 8%. However, in the SUU-Death population they account for an un-expected

23% of the population and have an HIV prevalence of 16%. Black South Africans account for 76% of the general mortuary population with an HIV prevalence of 32%. In the SUU-Death population, black South Africans account for 75% of the population but have a HIV prevalence of 51%. These increases in the separate population demographics can be explained by the very nature of HIV and its epidemiological profile. The concerning factor is that there are such large numbers of HIV positive cases being admitted to the Pretoria MLL and that HIV is not being routinely tested for in the mortuary setting. Further investigation needs to be done to determine what the post mortem findings are and if there are any correlations with mechanisms of death. TB-HIV co-infection and antiretroviral treatment interactions are two possible explanations that warrant further investigation. However, this cannot rule out the role that stigmatisation plays in the lives of people. HIV positive individuals will often not seek treatment due to fear of being ostracised in their communities or even families. [47,48,50-54]

HIV is not the ultimate cause of death in the SUU-Death cases admitted to the Pretoria MLL. Due to HIV's nature of infection it lowers the host's immune system and makes them susceptible to opportunistic infections and cancers. Therefore HIV is not routinely tested for in the South African forensic medical setting as it is seen to yield only a small contribution to the cause of death inquiry. This does unfortunately lead to inaccurate statistics on HIV in the South African forensic medical population. This study proved that there is a wealth of information available for analysis and that it can assist in many aspects in the fight against HIV.

If medical records and information leading to a clearer understanding of the cases are made available, a Death Scene Investigator (DSI) can collect the relevant records and information and present them to the forensic pathologist. This would simplify the post mortem examination for the forensic pathologist and facilitate a reduction in the number of SUU-Death cases admitted to the Pretoria MLL. A reduction in SUU-Death cases could save the Forensic Pathology Service a considerable amount of money. Pretoria MLL does approximately 2500 post mortems a year and 14% of them are SUU-Deaths. This would lessen the financial burden and psychological burden placed on the Forensic Pathology Services and personnel.

Conclusion:

Sudden, unexplained and unexpected deaths account for 14% of all cases admitted to the Pretoria MLL. They have a HIV prevalence of 43%, which is 17% higher than the general mortuary population in Pretoria (*p*-value = *0.0045)*.

Further research should be conducted into the stage of infection of the HIV positive SUU-Death cases in order to determine at what stage the individuals are most vulnerable. Screening for antiretroviral should also be undertaken to determine if the person was on treatment at the time of death.

A concerning observation is that there is a considerable lack of medical and background information pertaining to the individuals admitted as sudden, unexplained and unexpected deaths.

Chapter 5: Study Conclusion

HIV in the South African Forensic Medical setting is rife. The 26% HIV prevalence at the Pretoria Medico-Legal Laboratory is significantly higher than estimations of the general population's HIV prevalence. This increased rate of HIV prevalence in the mortuary places the personnel under immense strain and in a high risk situation. Further research needs to be conducted on a larger scale to determine the extent of the prevalence difference and the different factors at play in the different regions of South Africa. This research is a small snapshot of the potential information that could be gained in the war on HIV in South African.

With the identification of the suicide population and the SUU-Death populationat the Pretoria MLL being "at risk" populations, additional data was collected, shedding light on the complex phenomenon that is HIV/AIDS. HIV prevalence amongst suicide victims (23%) was shown to be significantly lower ($p= 0.0198$) than in the general population. This changes the current perception that, once diagnosed with HIV, a person will contemplate suicide and attempt it. This also emphasises the continued need for HIV pre- and post- test counselling. However, SUU-Deaths were shown to have a 43% HIV prevalence rate in the Pretoria MLL. In the research presented above it has been demonstrated that a wealth of information is being lost by failing to collect post mortem information about the HIV pandemic. In addition, this study shows that 48% of all suicides are happening in the White South African population at the Pretoria MLL.

Targeted intervention strategies should be implemented to understand the motivation for their suicidal ideation and attempts.

SUU-Deaths place a large burden on forensic medical practice in South Africa. The HIV prevalence rate in this population is 43%, which is a 17% ($p= 0.0045$) increase from the base line. With such a large percentage of the work being done on SUU-Deaths in the Pretoria MLL it places personnel in a high risk autopsy many times that are unnecessary. It is imperative that a greater understanding of this sub population be achieved. This further understanding will facilitate the development of effective policies and intervention strategies. These strategies will lessen the work load and the personnel's exposure to high risk autopsies.

Pretoria MLL needs to develop and then implement an official HIV policy document. This is to safe guard the personnel and the department of health from any circumstances stemming from an accidental exposure to the HI-virus. Furthermore the OHS personnel should be trained and up to date on post exposure prophylaxis and should receive first aid training. Currently the minimum requirements are met but in a high risk environment such as a mortuary these systems need to be exemplary and used when the need arises.

Another observation is that further research needs to be done into the efficacy of HIV tests on a post mortem blood sample. With the long term viability of the virus post mortem, it is essential that personnel have a reliable method of testing a post mortem sample should they come into

contact with bodily fluid. Once that is done the test needs to be made available for use by the Department of Health.

Information in the forensic medical setting can be collected with minimal concern for accidental disclosure and a breach of confidentiality. At the same time a wealth of information can be obtained and analysed to further bolster South Africa's response to the HIV pandemic. The information collected from the post mortem tests will serve as another sentinel measurement population in South Africa. It can be used to bolster the current statistical models or incorporated into its own model to determine the accuracy of the other statistics. Ultimately this will drive the formation of more effective policy.

South African forensic medical practice has the context and potential to provide large amounts of accurate information regarding the HIV pandemic.

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Annexure 1:Brief explanation of the HIV determine rapid assay

DEFINITION

HIV -1/2 is an *In Vitro* visually read immunoassay for the detection of antibodies to HIV -1 and HIV-2 in human serum, plasma or whole blood. The test is intended as an aid to detect antibodies to HIV-1/HIV-2 from infected individuals.

SUMMARY AND EXPLANATION OF THE TEST

AIDS Acquired Immunodeficiency Syndrome is characterized by the changes in the population of Lymphocytes. In an infected individual, the virus causes depletion of helper T-cells which leaves the person susceptible to opportunistic infections and some malignancies.

PRINCIPLE OF THE PROCEDURE USED

Determine HIV1/2 is an immunochromatographic test for the qualitative detection of antibodies to HIV-1and HIV-2. The sample is added to the sample pad. As the sample migrates through the conjugate pad, it reconstitutes and mixes with the selenium colloid antigen –a conjugate. The mixture continues to migrate through the solid phase to the immobilized recombinant antigen and synthetic peptides as the window patient site

If the antibodies to HIV-1 and HIV-2 are present in the sample, the antibodies bind to the antigen selenium colloid and to the antigen at the patient window forming a red line at the patient window site

To ensure the validity, a procedural control bar is incorporated in the assay device

TEST PROCEDURE

Whole blood samples

- Remove the protective foil cover from each test
- Apply 50ul of sample to the sample pad marked by arrow symbol
- Wait 1 minute and apply one drop of lysis buffer to the sample pad
- Wait a minimum of 15 minutes to 60 minutes and read the results

QUALITY CONTROL

To ensure assay validity, a procedural control is incorporated in the device pad is labelled “Control”. If the control bar does not turn red by assay completion, the test results are invalid and the sample should be retested.

INTERPRETATION OF RESULTS

- **Positive results**: Red bars appear in both the control window and patient window of the strip. The results are interpreted as positive. (Two red bars)
- **Negative results**: One red bar appearing in the control window of the strip and no red bar appearing in the patients window of strip , the results are interpreted as negative
- **Invalid results**: There is no red bar in the control and patient window even if the results appear in the patient window only the results are interpreted as invalid and the samples are repeated

DRAWBACKS

The following factors contributed to the invalid results obtained

- Samples received which are too old to process

Samples