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Assisted Reproduction Services: Accessible screening and semen profiling of HIV-positive males

By

Melissa Stander

(23112396)

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Supervisor:

Prof C. Huyser
PhD Reproductive Biology
Department of Obstetrics and Gynaecology
Reproductive Biology Laboratory
University of Pretoria
Steve Biko Academic Hospital

Co-Supervisor:

Dr K. Richter
MBChB, MMed (Path) Virology
Department of Medical Virology
University of Pretoria
National Health Laboratory Services
Tshwane Academic Division

DEDICATION

To my father, Maurice Graham Stander:
Thank you for all your support throughout my life, for always being proud of me and for teaching me to fight the odds and never give up

"If 'plan A' fails, the alphabet has 25 more letters!"
Anonymous

DECLARATION BY CANDIDATE

'I hereby declare that the dissertation submitted for the degree MSc Reproductive Biology, at the Faculty of Health Sciences, University of Pretoria, is my own original work and have not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references.'

MELISSA STANDER

Name in block letter

Signature

03/02/2014

Date

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Faculty of Health Sciences Research Ethics Committee

23/08/2010

Number	:	**S64/2010**
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Investigator	:	M Stander, Department of Obstetrics and Gynaecology, University of Pretoria (SUPERVISORS: DR C HUYSER / DR K RICHTER)
Sponsor	:	**None**
Study Degree:		**MSc. (Reproductive Biology)**

This Student Protocol was approved by the Faculty of Health Sciences Research Ethics Committee, University of Pretoria on 23/08/2010. The approval is valid for a period of 3 years.

Prof M J Bester	BSc (Chemistry and Biochemistry); BSc (Hons)(Biochemistry); MSc(Biochemistry); PhD (Medical Biochemistry)
Prof R Delport	(female)BA et Scien, B Curationis (Hons) (Intensive care Nursing), M Sc (Physiology), PhD (Medicine), M Ed Computer Assisted Education
Prof V.O.L. Karusseit	MBChB; MFGP (SA); MMed (Chir); FCS (SA)
Prof J A Ker	MBChB; MMed(Int); MD – Vice-Dean (ex officio)
Dr M L Likibi	MBChB; Med.Adviser (Gauteng Dept.of Health)
Dr MP Mathebula	Deputy CEO: Steve Biko Academic Hospital
Prof T S Marcus	(Female) BSc (LSE), PhD (University of Lodz, Poland)
Prof A Nienaber	(Female) BA (Hons) (Wits); LLB (Pretoria); LLM (Pretoria); LLD (Pretoria); PhD; Diploma in Datametrics (UNISA)
Prof L M Ntlhe	MBChB(Natal); FCS(SA)
Mrs M C Nzeku	(Female) BSc(NUL); MSc Biochem(UCL,UK)
Snr Sr J. Phatoli	(Female) BCur (Et.Al); BTech Oncology
Dr R Reynders	MBChB (Pret), FCPaed (CMSA) MRCPCH (Lon) Cert Med. Onc (CMSA)
Dr T Rossouw	(Female) MBChB.(cum laude); M.Phil (Applied Ethics) (cum laude), MPH (Biostatistics and Epidemiology (cum laude), D.Phil
Mr Y Sikweyiya	MPH (Umea University Umea, Sweden); Master Level Fellowship (Research Ethics) (Pretoria and UKZN); Post Grad. Diploma in Health Promotion (Unitra); BSc in Health Promotion (Unitra)
Dr L Schoeman	(Female) BPharm (NWU); BAHons (Psychology)(UP); PhD (UKZN); International Diploma in Research Ethics (UCT)
Dr R Sommers	**Vice-Chair** (Female) - MBChB; MMed (Int); MPharMed.
Prof T J P Swart	BChD, MSc (Odont), MChD (Oral Path), PGCHE
Prof G van Biljon	(female)FCP (Paed)SA
Prof C W van Staden	**Chairperson** - MBChB; MMed (Psych); MD; FCPsych; FTCL; UPLM; Dept of Psychiatry

Student Ethics Sub-Committee

Prof R S K Apatu	MBChB (Legon,UG); PhD (Cantab); PGDip International Research Ethics (UCT)
Dr A M Bergh	(female) BA (RAU); BA (Hons) (Linguistics) (Stell); BA (Hons) (German) (UNISA); BEd (Pretoria); PhD (Pretoria); SED (Stell)
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Prof D Millard	(female) B.Iur (Pretoria); LLB (Pretoria); LLM (Pretoria); AIPSA Diploma in Insolvency Law (Pretoria); LLD (UJ)
Dr S A S Olorunju	BSc (Hons). Stats (Ahmadu Bello University –Nigeria); MSc (Applied Statistics (UKC United Kingdom); PhD (Ahmadu Bello University – Nigeria)
Dr L Schoeman	CHAIRPERSON: (female) BPharm (North West); BAHons (Psychology)(Pretoria); PhD (KwaZulu-Natal); International Diploma in Research Ethics (UCT)
Dr R Sommers	**Vice-Chair** (Female) MBChB; M.Med (Int); MPhar.Med

DR L SCHOEMAN; BPharm, BA Hons (Psy), PhD; Dip. International Research Ethics
CHAIRPERSON of the Faculty of Health Sciences Student Research Ethics Committee, University of Pretoria

DR R SOMMERS; MBChB; M.Med (Int); MPhar.Med.
VICE-CHAIR of the Faculty of Health Sciences Research Ethics Committee, University of Pretoria

☎ 012 354 1677 🖷 0866516047 deepeka.behari@up.ac.za http://www.healthethics-up.co.za
✉ P O Box 667, Pretoria, 0001 31 Bophelo Road, HW Snyman South Building, Level 2, Room 2.33, Gezina, Pretoria

SUMMARY

Introduction

International guidelines endorse the screening of patients for human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) and *Chlamydia trachomatis* before assisted reproductive techniques (ART). At present no such guidelines exists in South Africa. At the Reproductive and Endocrine Unit (referred to as "the Unit") of Steve Biko Academic Hospital, all patients with unknown HIV status are counselled and a blood sample is collected during the initial visit for automated laboratory based HIV screening. These HIV results are not available before semen samples are processed. Furthermore, patients are not screened for HBV, HCV and *Chlamydia trachomatis*. Couples attending the Unit are of a low to middle socio-economic status and experience financial constraints. Moreover, automated laboratory based assays are expensive to perform. Rapid testing is a cost effective and practical method from screening patients, with a 20–30 minute result turnover time. Until screening at the Unit is improved, the possible identification of semen characteristics that could indicate HIV infection would be a useful tool.

Materials and Methods

The following rapid point-of-care assays were evaluated: Determine® HIV-1/2 combo test (n=100), Determine® HBsAg test (n=100), DIAQUICK HCV kit (n=74), and the DIAQUICK *Chlamydia trachomatis* kit (n=30). For profiling, parameters from a basic semen analysis of HIV-positive males (n=60) were compared with HIV-negative males (n=60). Information pertaining to CD4 count, antiretroviral treatment and plasma viral load of HIV-positive males were analysed.

Results

From all patients included in the study, 8% tested positive for HIV. The risk of a female being HIV-positive was 3.73 times higher than for males. In the pilot study to explore rapid testing for HBV and HCV, 1% and 1.4% of patients tested

positive respectively. When testing for *Chlamydia trachomatis* 31.3% of females, but no males tested positive. Comparing semen profiles, no significant differences were found between samples from HIV positive and negative males or between HIV positive males categorised by CD4 cell count ($p>0.05$). For the HIV-positive group with a detectable plasma HIV viral load (>40 copies/ml), a significant difference was observed in the semen viscosity ($p=0.0460$). Significant differences were noted in the sperm motility (immotile sperm $p=0.0456$, progressive sperm $p=0.0192$) of patients receiving antiretroviral (ARV) therapy.

Discussion and Conclusion

The use of rapid testing is an acceptable and feasible option for improving current screening protocols at the Unit. The absence of definite alterations in the semen characteristics of HIV-positive men further motivates the need for a simpler, point-of-care screening protocol. The prevalence of HBV was lower than that reported in the general population of South Africa and further investigation is needed. Although the sample size was small, HCV prevalence was similar to that of the general population. One third of females tested positive for *Chlamydia trachomatis*. The methodology used was possibly not appropriate for males. This study highlighted the need for guidelines that address the specialised needs of ART clinics in resource-limited and developing countries with a high HIV prevalence.

Key words: HIV, Public service sector, Accessible ART, Rapid screening, Semen profiling, Semen parameters, Blood-borne virus, Hepatitis B virus, Hepatitis C virus, *Chlamydia trachomatis*

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LIST OF ABBREVIATIONS

Ab – Antibody
Ag – Antigen
AIDS – Acquired immunodeficiency syndrome
ART – Assisted reproductive techniques
ARV – Antiretroviral
ASRM – American Society for Reproductive Medicine
BBVs – Blood-borne viruses
CI – Confidence interval
DNA – Deoxyribonucleic acid
ELISA – Enzyme-linked immunosorbent assay
ESHRE – European Society of Human Reproduction and Embryology
HAART – Highly active antiretroviral therapy
HBcAb – Hepatitis B core antibodies
HBeAb – Hepatitis e-antibodies
HBsAg – Hepatitis B surface antigen
HBV – Hepatitis B virus
HCV – Hepatitis C virus
HIV – Human immunodeficiency virus
HIV-1 – Human immunodeficiency virus type 1
ICSI – Intracytoplasmic sperm
IUI – Intra uterine insemination
IVF – *In vitro* fertilisation
LLD – lower limit of detection
ml – millilitres
NASBA – Nucleic acid sequence based amplification
NHLS – National Health Laboratory Service
NICD – National Institute of Communicable Diseases
P – Probability value
PCR – Polymerase chain reaction
R – Rand

RNA – Ribonucleic acid
SBAH – Steve Biko Academic Hospital
SD – Standard deviation
SOP – Standard operative procedure
STI – Sexually transmitted infection
WHO – World Health Organisation
€ – Euros

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Section A: *Study Overview*

Overview

1. Motivation for study

Infertility is a worldwide problem with millions of couples in need of assisted reproductive techniques (ART). The majority of these couples reside in developing countries which encounter an absence of facilities at all levels of health care, particularly infertility diagnosis and treatment.[1] While between 8 and 12% of couples suffer from infertility worldwide, the rates in Sub-Saharan Africa rise to 30% and more.[2] The main causes of infertility are sexually transmitted infectious diseases, cultural practices and substandard health care.[2]

Human immunodeficiency virus type 1 (HIV-1) is a growing epidemic throughout Sub-Saharan Africa. South Africa has one of the highest HIV infection rates in the world.[3] An estimated 15.9% of adults in their reproductive years (15 – 49 years of age) and 17.4% of women in this age group are HIV positive.[3] According to the 2013 mid-year population estimates of Statistics South Africa, approximately 10% of the population is infected with HIV.[3] Even though South Africa is a developing country, antiretroviral (ARV) therapy has become more readily available, ARV drugs have improved and patients are treated at a higher CD4 threshold (CD4 count ≤350 cells/ml).[4] Thus the quality and life expectancy for individuals infected with HIV has improved. The desire for safer means of conception by couples with HIV has been driven by a perception change, as HIV is no longer regarded as a terminal disease, but rather as a chronic illness. With the right treatment and lifestyle changes, HIV is a manageable condition and infected individuals are able to live a near normal life. Following the improved prognosis, couples are requesting ART to achieve conception and reduce the risk of HIV-1 transmission to a partner and unborn child.[5-7]

Patients entering an ART programme at the Reproductive and Endocrine Unit of Steve Biko Academic Hospital (SBAH) are required to undergo screening for

blood-borne viruses (BBVs) such as HIV. Initial screening of patients for HIV is performed using automated fourth generation combo Enzyme-linked immunosorbent assay (ELISA) tests – e.g. Architect HIV Ag/Ab Combo assay (Abbot). Patients with positive HIV ELISA blood results are further tested through sophisticated quantitative analysis using molecular techniques such as polymerase chain reaction (PCR) and nucleic acid sequence based amplification (NASBA). These methods are not just financially taxing for patients, but result turnover time is measured in days. Results of BBV analysis are therefore not always available on the commencement of basic semen analysis putting both staff and other patients at risk through possible contamination of the work environment. There is a need for the timely identification of potentially infected patients and samples and a more cost effective avenue for the determination of patient HIV status.

Using less expensive, rapid, point-of-care assays as opposed to sophisticated, costly analysis, may be a solution to the lack of resources faced by developing countries. All procedures should be modified at different levels: simplification of diagnostic procedures, simplifying ART and lessening of complication rates.[2] Rapid HIV antibody tests aid the effort to increase access to HIV testing and counselling services in remote, rural and poor parts of the world.[8] Rapid testing for HIV infection is a viable first line detection method in lower socio-economic countries. The advantages and disadvantages of point-of-care rapid testing should be weighed against each other and must be well understood by clinics that implement these assays. Rapid HIV tests may have a lower sensitivity and a longer window period when compared to fourth generation automated HIV ELISA assays. There is the risk of not diagnosing patients in the early acute HIV seroconversion phase. Fourth generation assays are able to detect HIV p24 antigen (Ag) a few days before the detection of third generation test can detect HIV antibodies (Ab) (see Figure A).[9] The Determine® HIV-1/2 Ag/Ab Combo Test (manufactured by Alere Healthcare; Massachusetts, USA) is the first rapid assay commercially available that has the capacity to detect the p24 Ag

(present during early stage infection) and antibodies against HIV 1 and 2, which will shorten the window period.

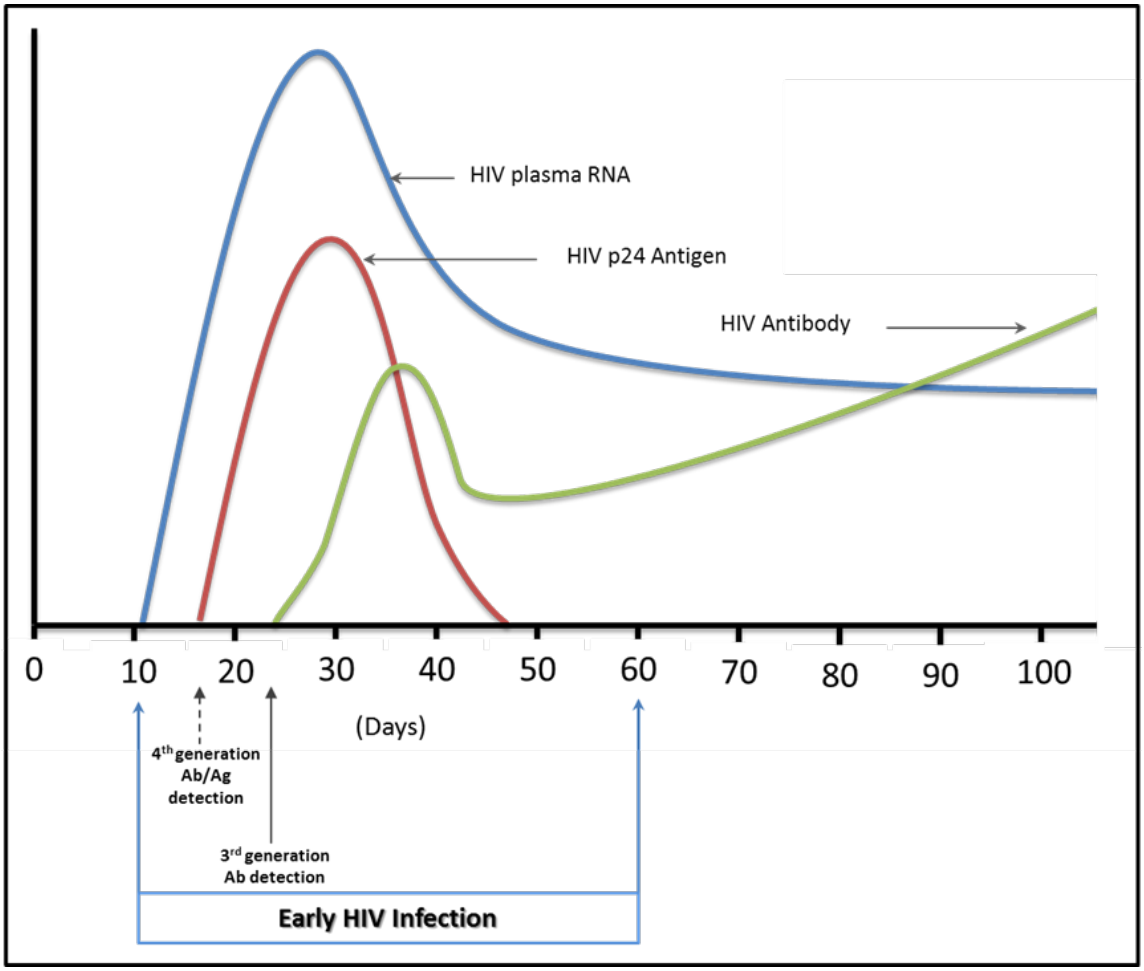

Figure A: Window period of detection of HIV markers (adapted from Patel *et al.,* 2012).[9]

Studies have demonstrated that HIV-1 is found in the semen of infected men[5-7] even when not detectable in the blood.[6] Infectious HIV-1 is found in the seminal plasma as well as in the seminal leukocytes.[6] The effect of HIV-1 on semen parameters such as ejaculate volume, viscosity, sperm motility, concentration, total count, and morphology as well as non-spermatic cells has been addressed in several studies.[5-7,10] To our knowledge, the semen parameters of South African HIV-positive men have yet to be addressed.

Recently there has been a global rise in the request for ART in patients infected with BBV infections such as HIV, HBV or HCV.[11] Sexually transmitted diseases have always preoccupied staff performing ART.[12] Cross contamination between patients during ART is a concern. Nosocomial infection of patient samples has been described for HBV, HCV and HIV in the last 20 years.[12] The article by Englert *et al* (2004) discusses the occurrence of HIV infection after artificial insemination with donor sperm.[12] These incidents demonstrate that sperm, independent of any sexual contact, can transmit the virus.[12] To ensure safety, samples from such patients should be handled within a separate high security laboratory or laboratory area, technically adapted to ensure minimal cross-contamination risk to uninfected gametes and embryos.[12]

Furthermore, co-infection of patients with BBV and other pathogens can negatively affect the prognosis and treatment of these patients. For this reason, couples seeking ART should have a full and comprehensive work up, including the screening for HIV, HBV, HCV and other pathogens such as *Chlamydia trachomatis*. The screening of patients for *Chlamydia trachomatis* is recommended practice internationally and the infection is easily treatable with antibiotics. *Chlamydia trachomatis* is not currently screened for in the population group at the Unit. However, *Chlamydia trachomatis* is an important public health concern as infection is asymptomatic in many cases among men and women.[13,14] There are frequent and severe complications with *Chlamydia trachomatis* including epididymitis, proctitis, tubal factor infertility, ectopic pregnancy and pelvic inflammatory disease.[14]

Most people's life plans include reproduction and consequent family building.[15] This desire is extended through to HIV positive couples. If the desire to have a child is considered as a fundamental health need, it is society's obligation to ensure equity.[15] There is extensive concern about equity in access to reproductive health care as a result of the high cost involved.[15] This study will focus on the affordable BBV screening of couples attending an ART programme, and the establishment of a typical semen profile or pattern of HIV-seropositive males attending the Unit at SBAH.

2. Research questions

1. Can routine screening for HIV-1, HBV, HCV and *Chlamydia trachomatis* of male patients be streamlined to fit into an affordable ART programme through means of rapid point-of-care testing?
2. What is the prevalence of HIV-1/2, HBV, HCV and *Chlamydia trachomatis* in a patient population attending an assisted reproduction programme at SBAH?
3. Are semen parameters from HIV-positive males different from that of HIV-negative males seeking reproductive assistance at SBAH?

3. Hypothesis

1. H_0: The screening and handling of HIV-positive patients should differ from that of HIV-negative patients:
    I. A combination of macroscopic and microscopic semen characteristics can be associated with the HIV status of the patient.
    II. Semen parameters are influenced by blood CD4 count, HIV-1 viral load and antiretroviral drug use.
    III. Rapid point-of-care testing is a cost effective and efficient manner to determine the HIV-1/2, HBV, HCV and *Chlamydia trachomatis* status of patients seeking ART assistance.
2. H_a: The screening and handling of HIV-positive patients should not differ from that of HIV-negative patients.

4. Objectives

1. To investigate the prevalence of HIV, HBV, HCV and *Chlamydia trachomatis* and cost effective screening using rapid point-of-care assays for patients in an assisted reproduction programme
2. To establish an easily recognisable profile for semen characteristics of HIV-positive males attending a semen decontamination programme
    I. To determine whether CD4 count, HIV-1 viral load and antiretroviral drugs have an influence on semen parameters

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Section B: *Research study*

Chapter 1: Literature Review

1.1 The epidemic of HIV in South Africa

Human immunodeficiency virus type 1 (HIV-1) was discovered in the early 1980's.[1,2] HIV is a retrovirus that forms double-stranded deoxyribonucleic acid (DNA) from single-stranded ribonucleic acid (RNA). There are two types of known HIV in humans: HIV type-1 (HIV-1) and HIV type-2 (HIV-2), further classified into different groups and subtypes. The origin of the global HIV pandemic was traced back to a cross-species transmission of simian immunodeficiency virus to humans in West-Central Africa approximately 100 years ago, resulting in HIV-1 group M.[3] Even though the percentage of people infected with HIV globally has started to stabilise since 2000, there is still a trend of slow increase.[2]

An estimated 10% of the South African population is HIV positive.[4] The most prominent type of HIV found in South Africa is HIV-1, sub-type C. Without treatment progression of HIV-1 disease ultimately leads to acquired immunodeficiency syndrome (AIDS). The number of people living with HIV-1 in South Africa has increased from 4 million in 2002 to 5.26 million in 2013.[4] An estimated 15.9% of adults in their reproductive years (15 – 49 years of age) and 17.4% of women in this age group are HIV positive.[4] The estimated life expectancy for males and females in 2013 was 57.7 and 61.4 years respectively.[4] The lifespan of HIV positive persons has increased due to better access to ARV drugs, improved ARVs and the introduction of highly active antiretroviral treatment (HAART) in the mid '90s,[1] as well as earlier treatment of patients at a higher CD4 threshold. The percentage of AIDS deaths in South Africa has decreased from 40.4% to 31.9% in the last 12 years.[4]

1.2 Viral transmission and the impact on reproduction

The transmission of HIV can take place through sexual, vertical, blood and undefined means.[1] Sexual transmission accounts for 75% of cases around the world.[1] While homosexual intercourse is the main manner of infection in developed countries, heterosexual transmission is the foremost means in developing countries.[1] HIV-1 is sexually transmitted by the presence of HIV virions in the blood which promotes the shedding of HIV-1 into genital secretions.[5] The levels of HIV-1 virions vary at different stages of the disease. The amount of HIV-1 virus in the blood plays a role in predicting the decline of CD4+ cells and advancement of the disease into AIDS.[5] Co-infection with other sexually transmitted pathogens in the form of bacterial (such as *Chlamydia trachomatis*) and viral infections (such as HBV and HCV), unfavourably affects the prognosis of HIV and have an impact on ARV therapy.

The ARV availability has greatly improved along with the enhanced treatment of opportunistic infections. This has resulted in a rise in life expectancy and enhanced quality of life for adults living with HIV.[6] Historically, HIV positive couples were faced with the challenge of not having their own children, but now with improved ARV drugs and reproductive assistance, they have the opportunity to overcome this challenge. The desire for safer means of conception by couples with HIV has been driven by a perception change, as HIV is no longer regarded as a terminal disease, but rather as a chronic illness. With the right treatment and lifestyle changes, HIV can be managed and infected individuals are able to live a near ordinary life. Couples living with HIV-1 are requesting ART to achieve conception, while decreasing the risk of HIV-1 transmission to an uninfected partner or unborn child.[7-9] This demand for reproductive assistance for safer conception is a growing facet which extends to the general population too. With a decline in fertility rates (2.71 children per women in 2002 to 2.34 children in 2013)[4] the demand for ART has grown throughout South Africa and across cultures.

When a serodiscordant couple wishes to conceive a child naturally through unprotected intercourse, the uninfected female partner and potential unborn child are at risk of infection. HIV-positive couples can conceive safely through medically assisted reproduction.[7-10] The use of ART can reduce the risk of viral transmission.[7-9] The foundation for ART is based on the premise that the HIV virus does not attach to or enter the sperm cell. For the HIV virus to enter the host cell, the CD4 receptor and CCR5 and CXCR4 co-receptors are required, which are absent in sperm cells.[11] Studies have shown that HIV is present in the semen as free virus and as cell-associated virus in the non-sperm cells of seminal plasma, particularly in CD4 positive lymphocytes and macrophages (which are the main targets for infection), and can negatively alter semen parameters.[11-14]

The aim of ART in concordant and serodiscordant couples is to reduce the risk of super-infection and viral transmission to the uninfected partner and / or unborn child. In couples where the male is positive, the risk of transmission to the female partner can be reduced, however not eradicated by using condoms with timed unprotected intercourse during ovulation. This practice is however, unsafe and not encouraged if alternatives are available. Studies have demonstrated that HIV-1 is found in the semen of infected men[7-9] even when not detectable in the blood.[8] In serodiscordant couples where the female is HIV positive, transmission to her partner can be prevented by intra uterine insemination (IUI) with washed sperm from her partner or donor sperm. Sperm washing in HIV positive males is aimed at the removal of infected seminal plasma and non-sperm cells from the viral-free sperm. Many previous studies have verified the effectiveness of sperm washing techniques.[15-19]

1.3 ART in RSA

1.3.1 Current practice

SBAH is a public sector hospital located in Pretoria, Gauteng, South Africa. The hospital houses the Reproductive and Endocrine Unit which caters to patients seeking reproductive assistance. The Unit is a tertiary unit and offers advanced as well as basic diagnostic and therapeutic infertility treatment.[20] Patients attending the ART Unit are predominantly of a lower to middle socio-economic status.[20] The cost of a single ART cycle at the Unit of SBAH can come to as much as R28 000 (€2066.04[†]).[20] The majority of patients at the Unit have limited financial resources (an average monthly income per house hold of R16 700 ± R11 500 [€1232.24 ± €848.55]).[20] Even though screening for microbial pathogens and BBVs such as HIV, HCV and HBV is an expected norm in developed countries,[21] this type of screening can be very costly. Screening for BBVs and transmittable pathogens is often an unrealistic financial constraint on patients making use of an ART unit in the public sector of a developing country.

1.3.2 Policies, existing screening and testing guidelines

The Ethics Committee of the American Society for Reproductive Medicine (ASRM) states that it is unethical to withhold fertility treatment from individuals with chronic viral infections.[22] It is recommended by international guidelines that all patients undergoing fertility treatment should be screened for BBVs (such as HIV, HBV and HCV).[21-15] The European Society of Human Reproduction and Embryology (ESHRE) state in the 2008 revised guidelines for good practice in IVF laboratories that:[23]

- "Screening patients and gamete donors for HIV, HBV, HCV and other sexually transmissible diseases before processing or cryopreservation should be routinely adopted according to Commission Directive 2006/17/EC Annex III."[23]

[†] Euro exchange rate as on 28 October 2013 (R1 = €0.07)

- "As patients' admittance to IVF treatment cycles is regulated by physicians, and although the laboratory staff should treat each sample as potentially infectious, the laboratory staff must be informed about the risk of handling infected biological material, whenever the information is available."[23]

An effective means of recognising patients with BBVs is necessary in an assisted reproduction programme.[21] There are regrettably, no specific guidelines available for ART clinics in South Africa. The Reproductive and Endocrine Unit at SBAH screens patients for HIV. However, no screening is performed for HBV and HCV unless there is reason to suspect the patient is at risk of carrying these infections.

Patients entering the ART programme at SBAH are routinely tested for HIV-1 at their initial visit. A repeat HIV test is performed a year after initial screening. If the patient is considered to be a "high risk" for contracting HIV (e.g. have multiple sexual partners, have a STI, use intravenous drugs or have sexual relations with a known HIV positive partner), a repeat test is performed at 6 months (if possible). HIV ELISA testing is done, if results are positive further quantitative testing is performed using techniques such as polymerase chain reaction (PCR) or nucleic acid sequence based amplification (NASBA). These techniques require specialised equipment and laboratory staff, take time to perform and results are not available on the day of initial testing due to logistical delays such as specimen transportation. Consequently HIV-positive samples are not always identified before entering the ART laboratory for diagnostic sperm evaluation, placing both staff and other patients at risk. Annual testing is also an ineffective measure to safeguard the embryologist or provide a trustworthy guarantee of the patient's HIV status. Simpler and quicker testing methods – such as rapid testing – for applicable BBVs may address this problem. HIV testing and counselling is currently recognised as a priority in national HIV programmes, because such policies form a starting point to HIV/AIDS prevention, care, treatment and support mediations.[26] Rapid HIV tests may aid this in many settings, especially in services that will benefit from

knowledge of HIV status.[26] For example, in services associated to the deterrence of mother-to-child transmission including assisted reproduction services.[26] Furthermore automated analysis can be more than 10 times the cost of performing a rapid test.

When an HIV-positive female is pregnant HIV monitoring and the use of antiretroviral treatment is essential to ensure the health of the mother and unborn child.[27] The Unit at SBAH uses a multidisciplinary approach for the handling of HIV positive patients. After the completion of assisted reproduction, the further care, monitoring and management of pregnant HIV-positive patients is referred to an obstetrician (according to the standard operative procedures (SOP) of the Reproductive and Endocrine Unit at SBAH). Female patients must however be on HAART (According to unit SOP). The ART Unit at SBAH is dedicated to the assisting of HIV positive discordant and concordant couples. The unit has a dedicated, separate ART laboratory and equipment for the treatment of HIV positive couples. The use of a separate laboratory and equipment for HIV positive and HIV negative patients ensure safety from cross contamination and a level of higher vigilance and security when staff works with infected samples and is a recommended practice of international guidelines.[25]

1.3.3 Sperm washing and semen decontamination

Sperm washing is a technique used to isolate the motile sperm segment from the seminal fluid. Even though the HIV virus may be able to attach to sperm cells,[28] one can effectively separate non-infected motile sperm form viral infected seminal fluid and its infected non-sperm cells. This separation is achieved through sperm washing and sperm decontamination techniques, that include the use of density gradient centrifugation combined with swim-up methods and decontamination devices, achieving a purified sperm sample.[29] Studies have shown that a positive HIV RNA rate of between 4% and 8% have been detected in processed sperm samples after sperm washing and before swim-up.[19,30] After density gradient centrifugation, the top layers containing

infected cells and seminal plasma are removed by aspiration of the layers from the sperm pellet. During aspiration of the top layers, the sperm pellet can be re-contaminated by the seminal plasma as it runs down the inner surface of the tube by means of surface tension.[17] Therefore, it is common practice to incorporate the swim-up method (spontaneous migration of highly motile sperm) in sperm washing techniques. However, the method of sperm washing for HIV positive semen samples has been improved by the development of a polypropylene novel tube insert (ProInsert™; Nidacon International, Mölndal, Sweden). Re-contamination of the sperm pellet can be prevented by the use of this sperm decontamination device (ProInsert™) without an extra swim-up method.[17]

1.3.4 Service provision to HIV serodiscordant and seroconcordant couples

Numerous treatment options are available for HIV-positive couples wishing to conceive. Treatment choice will depend on whether the couple is HIV serodiscordant or seroconcordant. The options available to HIV couples (see Table 1.1) include adoption, self-insemination of husband or donor sperm, timed unprotected intercourse and sperm washing in conjunction with IUI, *in vitro* fertilisation (IVF), or intracytoplasmic sperm injection (ICSI).[25]

A specialised semen decontamination programme (unique to the ART laboratory at SBAH) has been refined over the past 12 years. The decontamination programme combines a unique sperm washing method with extensive semen (pre and post washing) viral validation to provide a comprehensive risk reduction to serodiscordant and seroconcordant couples. The semen decontamination programme at the Reproductive and Endocrine Unit is aimed at the elimination of HIV-1 from semen. From the decontamination programme semen samples from 80 HIV-1 positive males that had a detectable viral load in either their semen or plasma were extracted and evaluated (see figure 1.2). From this data it is clear that the plasma viral load does not directly correlate with the semen viral load of patients enrolled in the programme.

Approximately 26% of patients with an undetectable viral load in their blood had a detectable seminal viral load. Viral loads in blood and semen can be lowered by the use of HAART. Semen washing techniques can thus be used to prepare samples with undetectable RNA viral loads for IUI, IVF or ICSI.[31] Several published studies have shown little to no risk of transmission between mother and child after sperm washing.[32-35]

Table 1.1: Treatment options for HIV-positive couples wishing to conceive (adapted from Agboghorom et al., 2012).[25]

HIV status	Treatment option
Male positive serodiscordant couple	• Timed unprotected intercourse (infected partner should be on ARV therapy) • IUI • IVF • ICSI } with washed/donor sperm • Adoption
Female positive serodiscordant couple	• Timed unprotected intercourse (infected partner should be on ARV therapy) • Self-insemination with partners semen • Adoption
Seroconcordant couple	• Any of the above

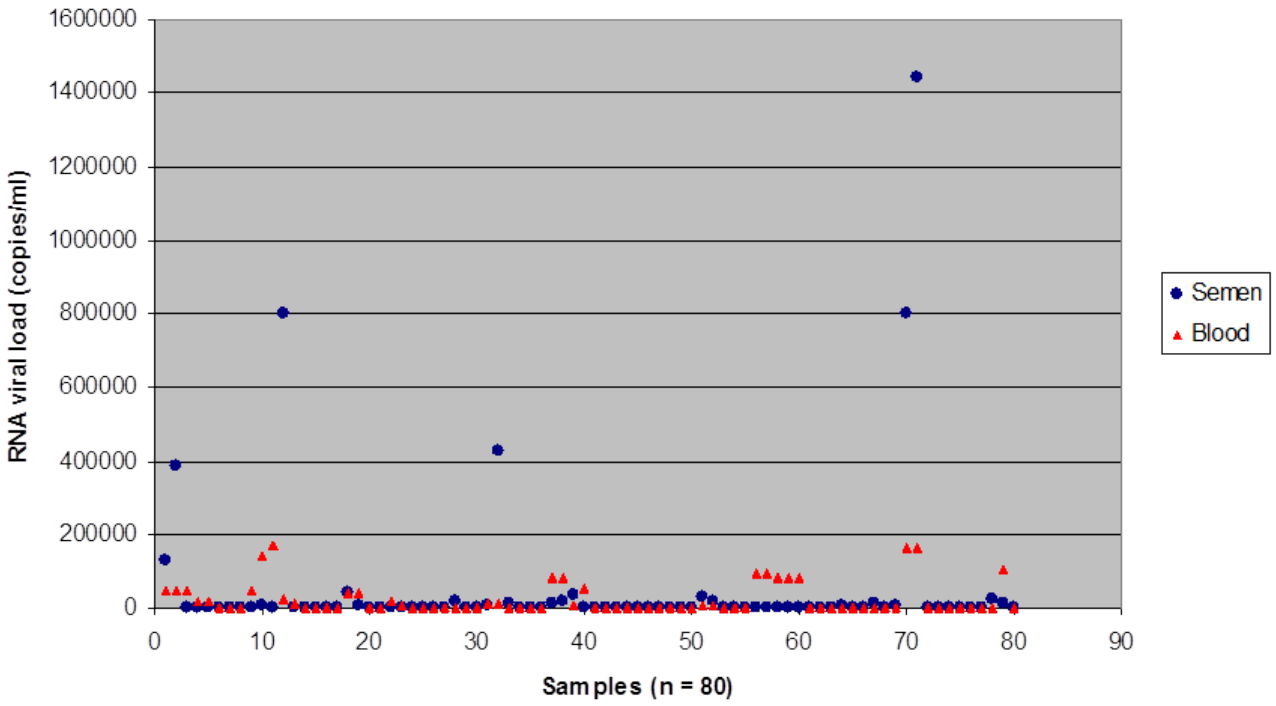

Figure 1.1: Blood and semen viral loads of HIV-1 positive patient samples (n = 80), only sample with a positive detectible viral load in the blood or semen are shown in the graph (unpublished data with permission from Prof C. Huyser, Head Reproductive Biology Laboratory, SBAH).

The aim of sperm washing in HIV positive males is to remove cell-free and cell-associated pathogens from sperm fractions, while trying to achieve an optimal sperm quality.[29,36] This can be achieved through various sperm washing techniques. Even though each step of a sperm wash is highly effective in reducing the viral content, the final sperm pellet should still be tested for HIV contamination. Viral validation can be done by modified PCR or NASBA[31,33,36,37] on both the neat semen sample and the washed sperm pellet. At the Reproductive and Endocrine Unit, studies were initiated to test the effectiveness of a decontamination method involving the ProInsert™ combined with density gradient centrifugation. The studies focused on elimination of amongst others, HIV-1 from *in vitro* spiked semen samples.[17,38] The decontamination procedure

was proven effective in the removal of the bacteria and all HIV-1 pro-viral RNA and DNA from all neat semen.[29] The decontamination procedure involves processing semen samples using density gradient centrifugation, making use of the ProInsert™ without an additional swim-up step. An elongated micropipette is used to aspirate the sperm pellet through the ProInsert™ after density gradient centrifugation to prevent contamination of the purified sperm population.[15] The aspirated sperm pellet should be clear of viral infection and can be used for ART to reduce the risk of transmission to uninfected parties. Institutions offering ART to HIV seropositive couples should provide counselling on risk reduction measures, and reproduction options.[39]

1.4 The impact of HIV on fertility

1.4.1 Course of disease and consequence on fertility potential

Subfertility in HIV-positive women may be caused by the reproductive mechanisms undergoing biological changes. A negative impact on reproductive potential may be brought about by factors related to HIV/AIDS such as general illness, co-infection, weight loss, stress, drug abuse and wasting.[37] The extent of wasting and immunosuppression seen in AIDS may be revealed by the number of ovulatory cycles and the frequency of sexual intercourse.[37] Wasting and immunosuppression are associated with the degree of HIV/AIDS clinical status, and noticeably have an adverse effect on fertility.[37] The HIV epidemic has further manipulated fertility in women by means of proximate determinates of fertility.[40,41] Fertility is directly influenced by biological and behavioural factors which constitute the proximate determinates.[41] Fertility determined through social, economic and cultural variables include factors such as onset of permanent sterility, marriage, postpartum infecundability, natural fecundability or use and effectiveness of contraception, frequency of sexual intercourse, spontaneous intrauterine mortality, and deliberate abortion.[40,41]

In a study performed in Sub-Saharan Africa, Ntozi reports that HIV/AIDS may result in a decline in fertility due to sexual relations being put on hold in a marriage or reduced frequency, while premarital sexual relations are also decreased.[40] Lower pregnancy rates and higher levels of abortion are experienced in HIV-positive women, and induced sterility as well as foetal mortality have increased.[40] The impact of fertility is not only determined by the HIV status and the use of ARVs, but social, cultural, economic, and gender related factors also have an influence.[41] However, ARV therapy does play a role in directing the proximate determinants towards increased infertility.[41] Since lower morbidity and mortality rates are achieved by ARV therapy, ARVs have the potential to counteract the decreased fertility trends seen in HIV infected women.[41]

1.4.2 Impact of HIV on semen parameters

In HIV-serodiscordant couples in which the male is HIV positive and the female is HIV negative, unprotected intercourse carries an average transmission risk of 0.1% to the uninfected partner.[39,42] HIV-positive males in HIV serodiscordant or seroconcordant relationships who wish to conceive should be counselled on practising protected intercourse. Safer conception options include IUI with either donor or washed sperm. In HIV seroconcordant couples HIV super-infection with an altered or resistant strain of HIV is of concern. Recurrent co-infections have the ability to heighten viral replication and CD4 cell decline, fast-tracking the deterioration in immune function and increase in HIV transmission.[43] Similar to many other ART units, the HIV status of patients undergoing diagnostic evaluation at the Reproductive and Endocrine Unit of SBAH is not always available upon the commencement of basic semen analysis. Although all semen samples are handled as if they are potentially infected (universal precautions) there is a need for the detection of infected samples entering the laboratory.[29] The Unit is an academic training unit; therefore samples are often handled by staff or students undergoing supervised training. The handling of

possibly infected samples by such inexperienced hands remains a risk even when following universal precautions.

Infectious HIV-1 is found in the seminal plasma in the form of free virions while HIV-1 DNA is cell bound (i.e. seminal leukocytes).[8,44] The effect of HIV-1 on semen parameters such as ejaculate volume, viscosity, non-spermatic cells and sperm motility, concentration, and morphology has been addressed in several studies,[7-9,45] however a consensus has yet to be established on whether or not these differences are significant. Controversy exists concerning the effects of the HIV-1 virus on semen parameters. Certain studies have reported insignificant or no differences in sperm parameters of HIV-infected semen samples when compared to uninfected controls.[9,46] While other studies have demonstrated significant reductions in ejaculate volume, total sperm count, sperm concentration, motility and normal sperm morphology when compared to uninfected controls[6,7,12,447] (see Table 1.2).

A lowered CD4 cell count (<350 cells/ml) and detectable HIV plasma viral load are indicative of a decreasing HIV immune status in patients, these factors can also lead to changes in semen parameters.[12] A noteworthy positive link was observed between CD4 cell count and sperm motility, concentration, and post preparation concentration and a significantly negative link with normal sperm morphology was reported in a study done by Nicopoullos et al. (2004). However, in the same study, no significant associations were observed between viral load and sperm parameters.[12] Contrastingly, a negative link between plasma RNA viral load and sperm motility was observed in a study done by Bujan et al. (2007). Research has also that certain ARV drugs can negatively alter semen parameters predominantly sperm motility.[7,47,48] Mitochondrial toxicity is induced by ARVs, which can negatively affect sperm mitochondria, inhibiting energy production by the sperm and therefore reducing motility.[49] Studies have compared the semen parameters of HIV positive men using HAART compared to HIV infected men without HAART.[9,47,50] Some studies showed no significant difference in semen parameters such as ejaculate

volume, sperm concentration and motility[9] while others showed a decrease in ejaculate volume and sperm motility.[47]

Even though many studies have been published on the effects of HIV-1 on semen parameters, to our knowledge no data has been published from a South African perspective. During sperm diagnostic procedures the presence of HIV infection in semen samples is often unknown. The ability to recognise a pattern or certain semen characteristics that may serve as a warning for further infection or pathogen investigation needs to be identified. Coupled with this, there is an obligation for more effective screening of patients to ensure the status of all samples is known before entering the laboratory for semen evaluation.

Table 1.2: Summary of published literature: semen parameters of HIV-positive vs HIV-negative males.

Semen parameters	Nicopoullos et al. (2004)		Garrido et al. (2005)		Bujan et al. (2007)		Lorusso et al. (2010)		Kelh et al. (2011)	
	HIV- (n=234)	*HIV+ (n=105)*	HIV- (n=234)	*HIV+ (n=105)*	HIV- (n=234)	*HIV+ (n=105)*	HIV- (n=234)	*HIV+ (n=105)*	HIV- (n=234)	*HIV+ (n=105)*
Volume (ml)	**3.3**	***2.9****	3.4	*3.6*	**3.9**	***3.3****	3.0	*2.5*	**3.0**	***2.2****
Total sperm count (10^6 / ml)	-	-	-	-	**353.8**	***330.9****	-	-	-	-
Concentration (10^6 / ml)	**70.1**	***54.8****	82.3	*82.9*	-	-	27.1	*24.7*	-	-
Motility (%)	**66.1**	***52.7****	48.3	*39.9*	**43.6**	***39.2****	57.3	*48*	**57**	***51****
Abnormal sperm morphology (%)	**72.0**	***76.7****	-	-	**71.4**	***73****	67.5	*68*	**43**	***50****

*p-value < 0.05

1.5 Other infections in ART

The success rate of an ART programme is dependent on a variety of factors, such as stimulation protocol, age of the women, quality of embryos[51] and contamination and transmission of infections.[52] The screening of *Chlamydia trachomatis* has been recommended for ART programmes[52] while the EU directives recommend the screening of male and female partners for HBV, HCV and HIV before ART.[53] Although HIV testing is routinely (but not repeatedly) performed at the Reproductive and Endocrine Unit of SBAH, Hepatitis and *Chlamydia trachomatis* are not screened for amongst patients entering the ART programme. The prevalence of these infections amongst ART patients at the Unit is therefore unknown. Rapid testing (as mentioned before) can however, be extended to incorporate these and other infections providing a more holistic view of a patient's reproductive health status. The primary aim of rapid testing is to make sure that infected individuals receive the correct treatment and management in order to lessen the risk of transmission to their partner and unborn child.[39]

With approximately 350 million HBV carriers globally, it is one of the most common infectious diseases.[54] In South Africa the occurrence of mono-infection with HBV has been estimated between 1% in urban areas to roughly 10% in rural areas.[55] The precise prevalence of HBV in the HIV-infected population is not well established and HBV screening is not regular practice in public service HIV clinics.[55] However, the rate of HBV infection (defined by surface antigen positivity) in HIV-infected individuals in urban South Africa was five times the rate of people who were HIV-negative.[55] HBV is a double-stranded DNA virus that multiplies with an RNA intermediate step using the reverse transcriptase enzyme. This enzyme is also used by HIV, making the treatment of HBV and HIV co-infection complicated.[56] Co-infection with HBV and HIV can impact antiretroviral action and prognosis of both diseases. The chance of mother-to-child transmission where the mother has chronic HBV infection is 2 – 15% when

she is only HBsAg-positive and 80 – 90% if she is HBsAg-positive and HBeAg- or HBV DNA-positive.[51,52]

HCV is one of the foremost causes of chronic liver disease globally. The World Health Organisation (WHO) estimates that 3% (170 million) of people in the world are chronically infected with HCV.[55] Sub-Saharan Africa is of specific interest since the region has the highest HCV prevalence rate (5.3%), and a simultaneous HIV epidemic.[57] The estimated overall prevalence of HCV in Sub-Saharan Africa is 3.0%.[57] Central Africa has the highest estimated prevalence of HCV (6.0%), while western Africa has an estimated prevalence of 2.4% and the lowest estimated prevalence is reported in southern and eastern Africa with a prevalence of 1.6%.[57] HCV is a blood-borne RNA virus predominantly transmitted through parenteral exposure such as blood products, shared needles and needle stick injuries.[54] Sexual and vertical transmissions are plausible secondary modes as HCV has been detected in semen, vaginal secretions, saliva, urine and breast milk.[54] There is a mother-to-child transmission rate of less than 1% if the mother has antibodies against HCV and is HCV RNA-negative, however, the transmission rate increases to 11% if the mother has HCV-Ab and is HCV RNA-positive.[51,52] When coupled with HIV infection the transmission rate of HCV ranges from 0 – 36% (average of 16%).[51,52]

The most common STI in Europe and America is *Chlamydia trachomatis*.[52] The incidence of *Chlamydia trachomatis* infection globally is approximately 92 million cases annually, of which 16 million occur in Sub-Saharan Africa.[58] *Chlamydia trachomatis* is an important public health concern as infection is asymptomatic in many incidences between men and women.[58,59] Electron microscopy has shown that *Chlamydia trachomatis* elementary bodies cling to spermatozoa, giving a possible explanation for infection to the upper regions of the female reproductive tract.[60] *Chlamydia trachomatis* is a prevalent STI with frequent and severe complications including epididymitis, proctitis, tubal factor infertility, ectopic pregnancy and pelvic inflammatory disease.[61] The

complication of *Chlamydia trachomatis* can result in poor pregnancy rates among infected women.

Besides the three infections mentioned in this section, other infections are present in the reproductive tracts and in semen. For an overview of sexually transmitted diseases in Africa refer to the article “Current status of reproductive behaviour in Africa” by Bambra (1999).[62] Infections found within the semen may alter the characteristics in various ways. The Reproductive and Endocrine Unit routinely submit semen samples for microscopy, culture and sensitivity, and microbiological testing for the presence of *Mycoplasma hominis* and *Ureaplasma urealyticum*. Screening for infections is costly though and therefore the degree of screening is limited in the public service sector.

1.6 Conclusion

Patients seeking assisted reproduction are often faced with more problems than just childlessness. The influence of other factors such as infection can further lower the chances of conception for these couples. The aim of the Reproductive and Endocrine Unit is to provide patients in the public sector with the best possible treatment for their specific situation. In order to provide the optimal service to all patients a comprehensive screening programme needs to be in place. However, the majority of patients seeking assistances at the Unit are not always financially able to perform costly screening tests and definitely not on a repeated basis (for infections such as HIV). Therefore, simple, quicker and more cost effective screening methods have been reviewed in this research study for the identification of HIV. Rapid testing was implemented in the Unit to see whether or not the incorporation is a viable screening option in an ART setting. Because screening results for HIV are not available to staff at the Unit on commencement of semen analysis, an attempt was made at the semen profiling of the HIV positive population at the Unit. The identification of infected semen samples by means of altered semen parameters is a useful and cost effective means to identify samples for possible further pathogen infection.

Furthermore, rapid testing was extended (in an exploratory capacity) to determine the prevalence of HBV, HCV and *Chlamydia trachomatis* to see if these infections are present in the population group attending the Reproductive and Endocrine Unit.

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Chapter 2: HIV rapid screening

2.1 Background

South African assisted reproduction clinics are not governed by a formal set of guidelines for the infection screening of patients before assisted reproduction. The automated HIV combo ELISA test platform is currently used to screen patients at the Reproductive and Endocrine Unit at SBAH. Assays relying on dedicated and expensive equipment, trained personnel and physical resources such as uninterrupted electricity are often not suited to resource-limited settings.[1] The Unit at SBAH screens patients for HIV on their initial visit. Repeat testing is not often performed unless the patient is a "high-risk" patient engaging in unsafe sexual and lifestyle practises. Patients not deemed to be a "high-risk" are tested annually at most and are not always tested again in the case of a repeat ART cycle. Furthermore, there is often a lag period (of up to a year) between the initial visit to the Unit and the commencement of an ART cycle. The lag period is often a result of financial constraints as patient need to save up money[2] to pay for costly ART procedures.[3]

The current screening protocol of the Reproductive and Endocrine Unit (annual testing) is an ineffective means to safeguard the health care workers and patients (especially partners of serodiscordant couples) or provide a reliable HIV status of the patient. Rapid testing in conjunction with automated analysis could provide a simpler point-of-care method for identification of applicable BBVs within a maximum of 20 – 30 minutes.[4] Rapid testing is also more cost effective as automated analysis can be more than 10 times the cost of performing a single rapid test. Same day identification of patient HIV statuses will ensure that samples are handled in the correct time and space (i.e. dedicated laboratory or patient batching). Patients in the public service sector are of a low to middle socio-economic status[3] and financial constraints contribute to the lack of follow up and patient drop out, preventing HIV-positive patients from receiving their status results and the necessary treatment.

2.2 Materials and Methods

Rapid testing for HIV was performed using the Determine® HIV-1/2 Ag/Ab Combo 100 Test kit with accessories from Alere Healthcare SA (Alere Healthcare; Massachusetts, USA). Confirmatory rapid testing was performed on the BIO-RAD Multispot HIV-1/HIV-2 rapid test from Bio-Rad Laboratories, Inc. (California, USA). All rapid testing was performed by the principal investigator according to the manufacturers specifications (see figure 2.1 and 2.2). HIV counselling was provided to participating patients by the nursing staff of the Reproductive and Endocrine Unit at SBAH. All participants signed a written informed consent for rapid testing to be performed.

The Determine® kit consisted of:

1. *Determine® HIV-1/2 Ag/Ab Combo test cards, ten cards (10 tests per card, i.e. 100 tests) coated with HIV-1/2 recombinant antigen and synthetic peptides, anti-p24 antibodies and avidin.*
2. *Chase Buffer, 1 Bottle (2.5 ml) (Cat No. 7D2243) prepared in phosphate buffer. Preservatives: Antimicrobial Agents.*
3. *EDTA Capillary Tubes (Cat No. 7D2222), 100 capillary tubes.*

The BIO-RAD Multispot kit consisted of:

1. *Multispot HIV-1/HIV-2 cartridge (50 cartridges, i.e. 50 tests): foil sealed base container with specimen pre-filter, membrane with 1 procedural control spot and 3 test spots.*
2. *Positive control (1 dropper bottle, 1ml): heat-inactivated human serum/plasma containing anti-HIV-1 and anti-HIV-2 immunoglobulin, nonreactive for HBsAg and antibody to HCV, 0.1% sodium azide, 0.5% ProClin™ 300.*
3. *Negative control (1 dropper bottle, 1ml): human serum, nonreactive for HBsAg and antibody to HIV and HCV, 0.1% sodium azide, 0.5% ProClin™ 300.*

4. *Specimen diluent (1 dropper bottle, 25ml): 0.1% ProClin™ 300, 0.125% ProClin™ 300.*
5. *Conjugate (1 dropper bottle, 9.5ml): anti-human IgG (H+L) (goat) alkaline phosphatase conjugated solution, ProClin™ 150.*
6. *Wash solution (2 x 85ml dropper bottles): TRIS, urea, propylene glycol, nitroblue tetrazolium, 0.1% ProClin™ 150.*
7. *Development reagent (1 dropper bottle, 8.5ml): 3-indoxyl phosphate.*
8. *Stop solution (1 dropper bottle, 55ml): 0.1 N H_2SO_4 (sulphuric acid).*
9. *Disposable transfer pipettes (60 pipettes): polyethylene transfer pipettes.*
10. *Eyedropper (1 dropper): polyethylene eyedropper and cap with rubber bulb.*

The Determine® HIV-1/2 Ag/Ab Combo 100 Test and BIO-RAD Multispot HIV-1/HIV-2 Rapid test kits were stored according to the manufacturers guidelines. The Lot numbers and expiry dates were recorded of all tests for each sample.

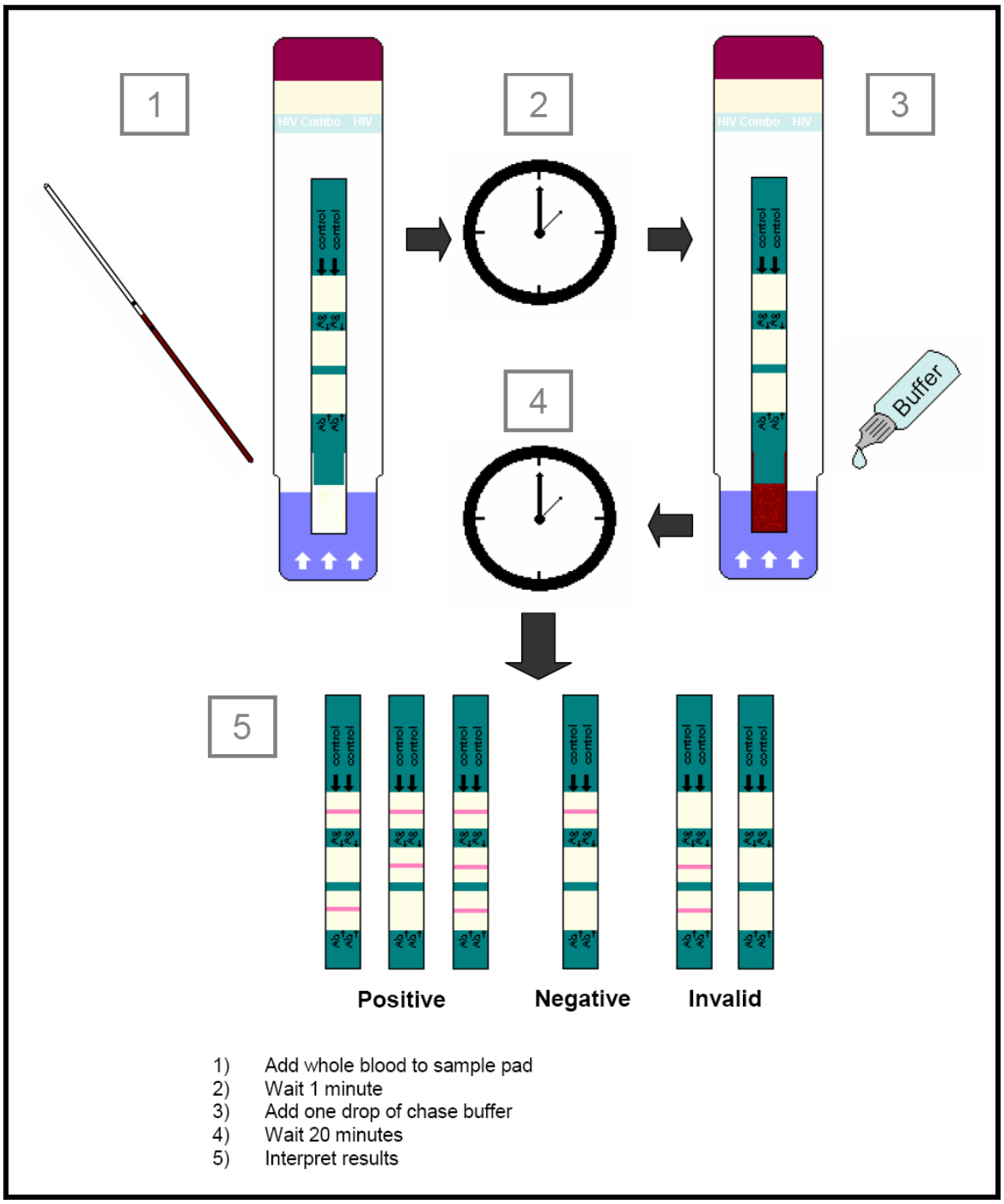

Figure 2.1: Determine® HIV-1/2 Ag/Ab Combo test procedure: illustrating steps 1 – 5 and possible results interpretation.

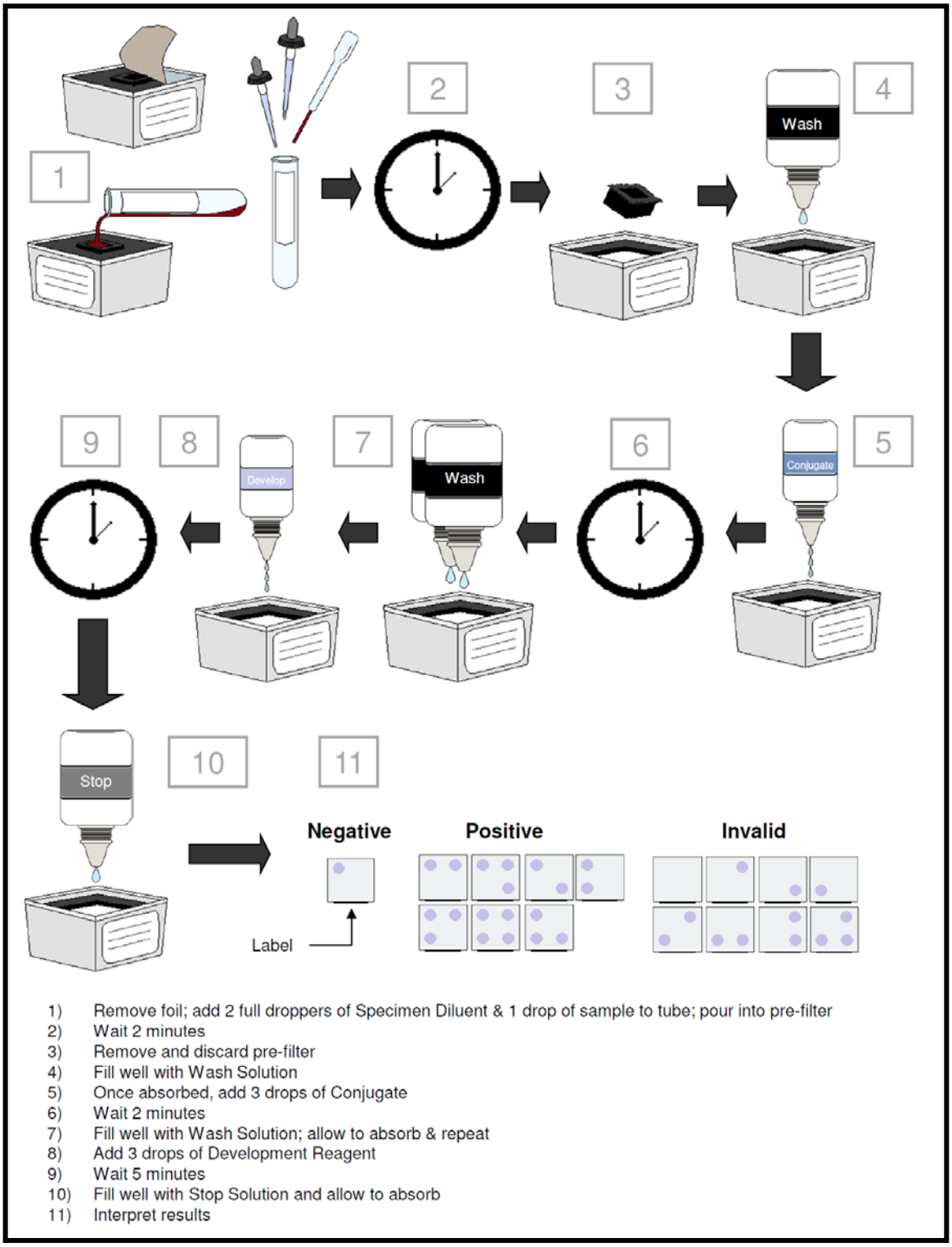

Figure 2.2: Illustration demonstrating the BIO-RAD Multispot HIV-1/HIV-2 rapid test procedure and result interpretation.

2.2.1 Participants

Patients attending the Reproductive and Endocrine Unit of SBAH for the first time, unaware of their HIV status (n = 100), were randomly requested to participate in HIV screening. The Determine® HIV-1/2 Ag/Ab Combo Test was used. Patients participating in this study fell in the age range of 23 – 55 years, with a mean age of 35 (female age range 24 – 39, male age range 23 – 55).

2.2.2 Data Gathering

Sampling was performed between November 2010 and March 2012. Blood samples were drawn from consenting male and female patients, by a qualified Nurse, into BD Vacutainer® Blood Collection Tubes, spray-coated K_2EDTA (plastic, 4 ml, Lavender Hemogard Closure), according to the standard protocol for patients attending the Infertility Unit. Of the blood samples (collected in the BD Vacutainer® K_2EDTA tubes):

i. One BD Vacutainer® blood collection tube sample was sent for HIV testing at either the National Health Laboratory Services (NHLS) or Lancet Laboratories for analysis on the automated combo ELISA test platform – i.e. Architect HIV Ag/Ab Combo assay (Abbot).
ii. One BD Vacutainer® blood collection tube sample was utilised for HIV screening on the Determine® HIV-1/2 Ag/Ab Combo Test performed at the Reproductive Biology Laboratory (RBL), SBAH.

2.2.3 Quality Control

Before the Determine® HIV-1/2 Ag/Ab Combo Test was used in the study an internal quality control was performed by testing 10 blood samples from known HIV-positive patients (that formed part of the HIV semen decontamination programme at RBL). A control bar is present in each Determine® HIV-1/2 Ag/Ab Combo Test, if the pink control bar does not appear the test is considered invalid. The control bar servers as an individual, internal quality control for the

rapid test. All results obtained from the Determine® HIV-1/2 Ag/Ab Combo (initial rapid test) were confirmed through the automated combo ELISA test (Unit's standard protocol).

Rapid test confirmation was performed as follows (see figure 2.3):

- Negative initial Determine® HIV-1/2 Ag/Ab Combo Test result:
  - Verification was obtained through the automated combo ELISA test performed on the same day.

- Positive initial Determine® HIV-1/2 Ag/Ab Combo Test result:
  - A repeat Determine® HIV-1/2 Ag/Ab Combo Test was performed.
  - Where the repeat Determine® HIV-1/2 Ag/Ab Combo Test also returned a positive result, a confirmatory rapid test was performed using the BIO-RAD Multispot HIV-1/HIV-2 rapid test (secondary conformation).
  - Determine® HIV-1/2 Ag/Ab Combo and BIO-RAD Multispot HIV-1/HIV-2 rapid test results were further confirmed by the automated combo ELISA test (performed on the same day).
  - Where the repeat Determine® HIV-1/2 Ag/Ab Combo or BIO-RAD Multispot HIV-1/HIV-2 rapid test results were negative, results were verified against the automated combo ELISA test from the same day.

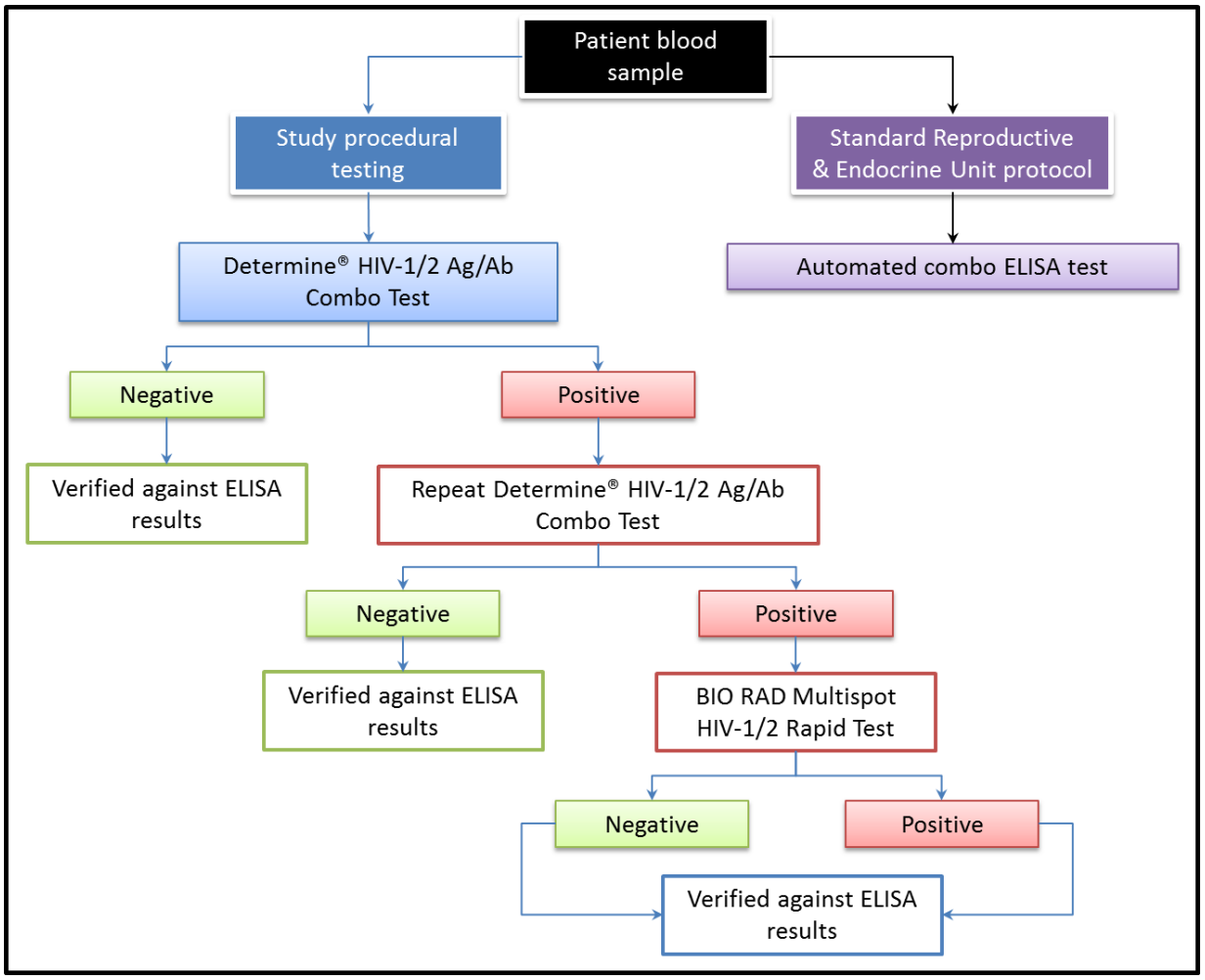

Figure 2.3: Verification procedure for positive and negative patient HIV blood results.

2.2.4 Test Appraisal

The ease of use of the Determine® HIV-1/2 Ag/Ab Combo 100 Test kit with accessories was appraised. A rapid test appraisal form was developed for the study based on the WHO, HIV Assays: Operational Characteristics and National Institute of Communicable Diseases (NICD), Laboratory Evaluation of HIV Rapid Assay.[5,6] The scoring system used in the rapid test appraisal form was based on the scoring system used by the WHO, HIV Assays: Operational Characteristics and National Institute of Communicable Diseases (NICD), Laboratory Evaluation of HIV Rapid Assay.[5,6] Test suitability was assessed

through the rapid test appraisal form. The rapid test appraisal was completed by the primary investigator of this study. A second rapid test appraisal was completed by a junior embryology staff member at the Unit. The second appraisal was done to verify the ease of use of the rapid test in inexperienced hands. The junior staff member was not formally trained in the use of the Determine® HIV-1/2 Ag/Ab Combo 100 Test kit before the appraisal was completed.

2.2.5 Cost Analysis

A cost analysis was performed to determine the feasibility of using the Determine® HIV-1/2 Ag/Ab Combo 100 Test kit with accessories as a repetitive screening system for patients during their work-up and treatment at the Reproductive and Endocrine Unit (see figure 2.4 for the current vs. study screening protocol). Quotations from Alere Healthcare, Lancet Pathology Laboratory and NHLS were compared for the year 2013.

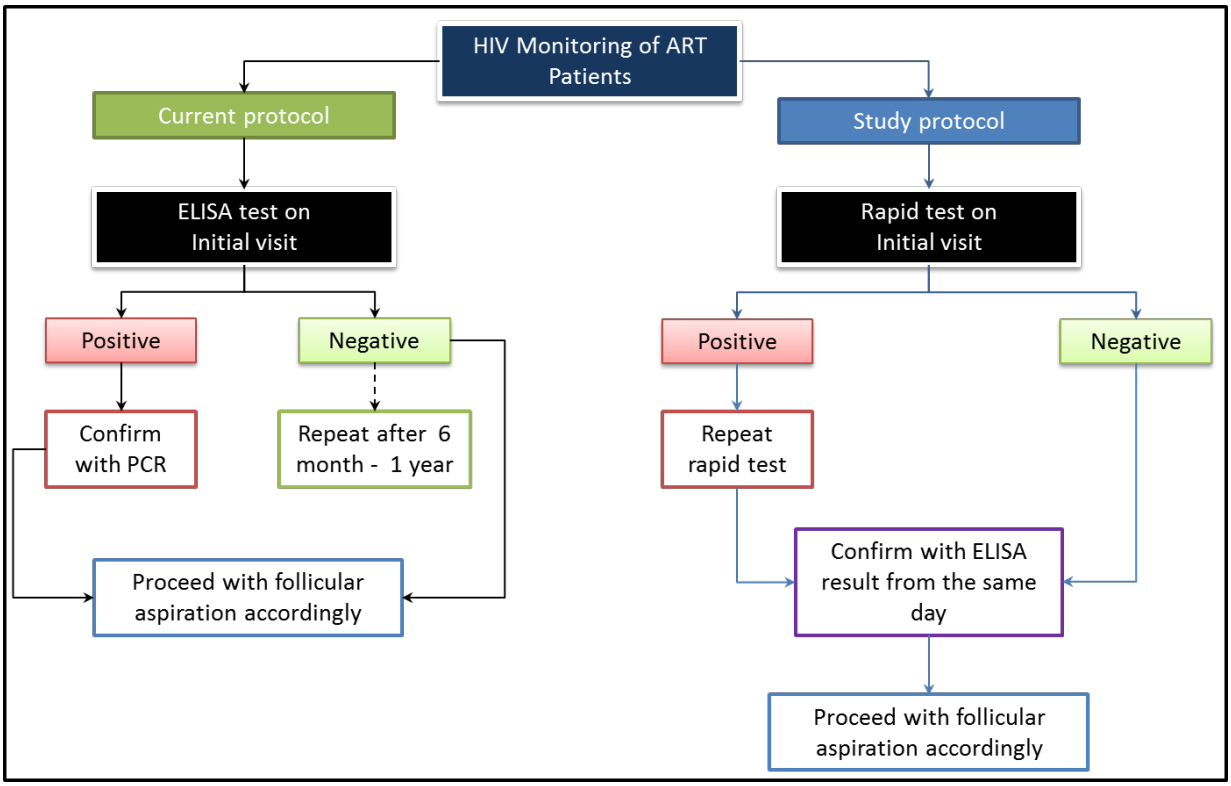

Figure 2.4: HIV screening of patients: current vs. study protocol.

2.3 Statistical Analysis

In a sample of 100 patients, rapid tests were used to test for HIV. Data was summarised using percentage positive along with 95% confidence interval. The outcome for HIV was also cross-tabulated against country of origin and proportion positive was reported. The categories of gender were summarised for HIV status using the odds ratio, determined from logistic regression.

2.4 Results

2.4.1 Data Analysis

The population group was comprised of predominantly South Africans (n = 89), however patients from Cameroon (n = 1), Congo (n = 3), Malawi (n = 1), Nigeria (n = 3) and Zimbabwe (n = 3) were also represented in the sampling cohort. The distribution of patients amongst the fore mentioned countries is shown in Table 2.1. The mean age of the sample cohort was 35 (with a mean age for female patients of 32 and 37 for male patients).

Not all male and female partners in a couple gave consent for rapid testing therefore the sample cohort was made-up of 40 couples (male and female partner), 13 individual male partners and 7 individual female partners. A total of 47 female and 53 male patients were represented in the sample cohort giving a total of 100 samples. Of the samples tested using the rapid screening method, 8 out of 100 (8%) were positive for HIV when run on the Determine® HIV-1/2 Ag/Ab Combo rapid test. All positive samples came from serodiscordant couples with 25% of positive patients being male and 62.5% being female (see Table 2.2). In the study population the risk of a female being HIV positive was 3.73 fold that of males.

Negative Determine® HIV-1/2 Ag/Ab Combo rapid test results all correlated with the automated combo ELISA results from the same day. For the verification of positive results, no discrepancies were found between the initial, repeat Determine® HIV-1/2 Ag/Ab Combo rapid tests, BIO-RAD Multispot HIV-1/HIV-2 Rapid Test and automated combo ELISA test results from the same day.

Table 2.1: Distribution of HIV in sample cohort through country of origin.

Country of Origin	Total	HIV-positive	HIV-negative	% HIV-positive
Cameroon	1	0	1	0%
Congo	3	0	3	0%
Malawi	1	0	1	0%
Nigeria	3	0	3	0%
South Africa	89	7	82	7.9%
Zimbabwe	3	1	2	33.3%
Total	**100**	**8**	**92**	**8%**

Table 2.2: Percentage of positive results in the sampling population.

Sample Group	Total	HIV-positive	% HIV-positive
Total study population	100	8	8%
Males	53	2	25%
Females	47	5	62.5%

2.4.2 Test Appraisal

The Determine® HIV-1/2 Ag/Ab Combo 100 Test kit with accessories was appraised according to the rapid test appraisal form. The technical appraisal and suitability – as assessed by the rapid test appraisal forms – is summarised in Tables 2.3 and 2.4. The test appraisal was scored with regards to: number of steps, clarity of instructions, reagent packaging and labelling and ease of performance (Table 2.3), while test suitability was scored for: sensitivity, specificity, incubation requirements, shelf-life, storage, ease of performance, test duration and reading visibility (Table 2.4).

Table 2.3: Summary of test appraisal for Determine® HIV-1/2 Ag/Ab Combo rapid test.

Test appraisal	Weight*	Score	
		Principal investigator	Staff member
Number of steps			
1 – 2	*6*	**6**	**6**
3 – 5	*3*		
> 5	*1*		
Clarity of instructions			
Good	*2*	**2**	**2**
Improvable	*1*		
Reagent packaging & labelling			
Good	*2*	**2**	**2**
Improvable	*1*		
Ease of performance			
Very easy	*5*	**5**	**5**
Easy	*3*		
Less easy	*1*		
Total	**15 (max)**	**15**	**15**

*Weight is according to WHO and NICD[5,6]

Table 2.4: Summary of test suitability for Determine® HIV-1/2 Ag/Ab Combo rapid test.

Test suitability	Weight*	Score	
		Principal investigator	Staff member
Sensitivity			
100%	*5*	**5**	**5**
98 – 100%	*3*		
< 98%	*0*		
Specificity			
> 98%	*5*	**5**	**5**
95 – 98%	*3*		
< 95%	*0*		
Incubation			
Room temperature	*3*	**3**	**3**
Other	*1*		
Shelf-life			
> 1 year	*3*	**2**	**2**
≥ 6 months ≤ 1 year	*2*		
< 6 months	*1*		
Storage			
Room temperature (opened)	*5*	**5**	**5**
Room temperature (unopened)	*2*		
2 – 8°C	*1*		
Ease of performance			
Very easy	*5*	**5**	**5**
Easy	*3*		
Less easy	*1*		
Test duration			
< 10 minutes	*3*	**2**	**2**
10 – 30 minutes	*2*		
> 30 minutes	*1*		
Reading Visibility			
Inter-reader variability ≤ 3%	*5*	**5**	**5**
Inter-reader variability > 30 %	*3*		
Total	**34 (max)**	**32**	**32**
Suitability for use			
Less suitable	*< 19*	**Very suitable**	**Very suitable**
Suitable	*19 ≤ x ≥ 26*		
Very suitable	*> 26*		

*Weight is according to WHO and NICD[5,6]

2.4.3 Cost Analysis

The feasibility of using the Determine® HIV-1/2 Ag/Ab Combo 100 Test kit with accessories was evaluated through a cost analysis comparing quotations from Alere Health, Lancet and NHLS laboratories for:

1. Determine® HIV-1/2 Ag/Ab Combo 100 Test kit with accessories (Alere Heath).
2. Automated combo ELISA test (Architect HIV Ag/Ab Combo assay-Abbott) (Lancet Laboratories and NHLS).
3. PCR test (Lancet Laboratories and NHLS).

All quotations obtained were for the year 2013. Table 2.5 below shows the cost of HIV testing on different platforms and at different laboratories. The costs for HIV screening and the proposed use of rapid testing for continual screening are displayed in Table 2.6.

Table 2.5: HIV screening cost for NHLS and Lancet Laboratories (year 2013).

Test	NHLS	Lancet
Automated ELISA Combo	R133.47 (€9.85[#])	R179 (€13.21)
PCR – RNA Viral load	R798.93 (€58.95)	R967 (€71.35)
CD4 Cell Count	R199.98 (€14.76)	R184 (€13.58)
Total	**R1132.38** (€83.55)	**R1330** (€98.14)

[#] Euro exchange rate as on 28 October 2013 (R1 = €0.07)

Table 2.6: Continual screening costs using the proposed Determine® HIV-1/2 Ag/Ab Combo rapid test (year 2013).

Visit	Test	Cost	
		NHLS	Lancet
Initial	**ELISA**	R133.47 (€9.85)	R179 (€13.21)
		Alere Healthcare	
Initial	**Rapid test**	R12.05 (€0.89)	R12.05 (€0.89)
Day 2 of patient cycle	**Rapid test**	R12.05 (€0.89)	R12.05 (€0.89)
Last follicular sonar	**Rapid test**	R12.05 (€0.89)	R12.05 (€0.89)
Total		**R169.62** (€12.52)	**R215.15** (€15.88)

2.5 Discussion

According to the current protocol all patients seeking assisted reproduction treatment at the Unit of SBAH are tested for HIV at their initial visit. Patients that are deemed “high risk” for contracting HIV, are (where possible) retested for HIV every 6 months while receiving ART. Couples are classified as “high risk” if one or both of the patients have multiple sexual partners, have a STI, use intravenous drugs or have a sexual relationship with a known HIV positive partner (personal communication, Dr K. Sing, Medical Officer, Reproductive and Endocrine Unit, SBAH).

Access to ARV treatment in low-and-middle-income countries has increased more than ten times from 2004 to 2009,[7,8] resulting in the global decrease in AIDS-related deaths to 19%.[7,8] With better antiretroviral drugs and improved treatment strategies HIV is no longer regarded as a terminal disease, but rather as a chronic illness. Through ART, couples can conceive while decreasing the risk of HIV transmission to a partner and unborn child.[9-11]

From rapid test results, 8% of patients who were not aware of their HIV status before their initial visit at the Unit tested HIV positive. Women were 3.73 times more likely of being HIV-positive than males, with 62.5% of females and only 25% of males testing positive. This higher prevalence among women is also seen in the overall population in Sub-Saharan Africa. According to the UNAIDS Global AIDS Report (2010) more women than men are living with HIV in Sub-Saharan Africa, and young women aged 15–24 years are up to eight times more likely than men to be HIV positive.[7]

The prevalence of HIV in developing countries such as South Africa is considerably higher than developed countries such as Europe and America. In 2009 the HIV prevalence among 15 – 49 year olds was 5% in Sub-Saharan Africa, compared to 0.5% in the Americas, and 0.2% in Western and Central Europe.[8] There is a need for more accessible and regular HIV screening in a high HIV epidemic county such as South Africa. This regular screening will identify new incidence cases of HIV. The research study at the Reproductive and Endocrine Unit of SBAH showed that all HIV positive patients (identified through the rapid testing) formed part of a serodiscordant couple. Patients served by the South African public health service generally have very limited resources and regular screening can become expensive. However, regular screening is an essential need, lacking in the current protocol at the Reproductive and Endocrine Unit.

According to current protocol all patients are tested with an automated combo HIV ELISA test at their first visit. These tests provide accurate and reliable results. However, regular screening by means of automated HIV ELISA testing is more expensive than rapid testing. To compromise between added financial strain and the need for regular screening, rapid tests can be incorporated. The use of rapid tests to increase HIV screening has been endorsed by the WHO for resource-poor settings.[12,13]

The Determine® HIV-1/2 Ag/Ab Combo test is the first fourth generation HIV assay with the ability to detect HIV-1/2 antibodies and the p24 antigen.[13,14] The

window period is reduced to an average of 2 weeks by the detection of the p24 antigen (Ag).[13] However, the p24 Ag sensitivity of the Determine® HIV-1/2 Ag/Ab Combo test was found to be low (10%) in a study conducted by the NHLS.[13] Even though the window period is not considerably shorted by the addition of the p24 antigen to the Determine® HIV-1/2 Ag/Ab Combo test according the NHLS study,[13] all HIV positive patients in the study population at the Reproductive and Endocrine Unit were detected. This could indicate that patients tested at the Unit were potentially not in the early stages of HIV infection. The Determine® HIV-1/2 Ag/Ab Combo test was found, in this study, to be an easy, accurate and affordable test (R12.05 [€0.89] per test). A Test suitability score of 32 (out of 34) and test appraisal of 15 (out of 15), sets the Determine® HIV-1/2 Ag/Ab Combo test as a very suitable test for use at the Unit.

Repeated screening for new HIV infections is therefore possible at a minimal additional cost to the patient (current cost per patients is R133.47 – R179). The additional HIV testing with the Determine® rapid test is affordable at R12.05 per test. When following the proposed HIV screening protocol in Figure 2.5, patients at the Unit can be continually screened at predetermined intervals (R169.62 [€12.52] – R215.15 [€15.88] per cycle) which will help overcome the problem of lack of sensitivity during the window period. In the proposed protocol (Figure 2.5) a minimal additional cost, R36.15 (€2.67) per patient, per cycle, will allow for continual HIV screening.

In conclusion, it is important to discern the HIV status of patients to provide each patient with the best possible treatment and care available. Protection of both partners and the unborn child are of great importance. The use of rapid testing will allow for the identification of HIV positive patients during their initial consultation at the Unit and ensure HIV positive patients (especially serodiscordant couples) are identified earlier and managed appropriately and reduce the loss to follow-up of these patients. Patient drop-out and resultant loss of follow-up is especially prevalent in a resource-limited setting such as the

public service sector in a developing country like South Africa. Early detection by the clinician will also allow for the screening and prevention of seroconversion by uninfected partners of serodiscordant couples. Furthermore, patients often have to wait to enter into an ART cycle for various reasons (financial constraints, waiting to perform other tests or procedures such as office laparoscopy, bacterial infection requiring treatment and repeat semen analysis thereafter, weight loss etc.). Therefore the time it takes for a patient to enter an ART cycle can vary from one month to more than a year. This lag period between the initial visit to the Unit and entering an ART cycle strengthens the need for more continual HIV screening.

The screening strategy proposed by this research can improve identification of HIV-positive patients. Continuous screening for HIV seroconversion can be performed at a minimal addition cost, without adding excessively to the already large financial strain experienced by patients seeking assisted reproductive services.

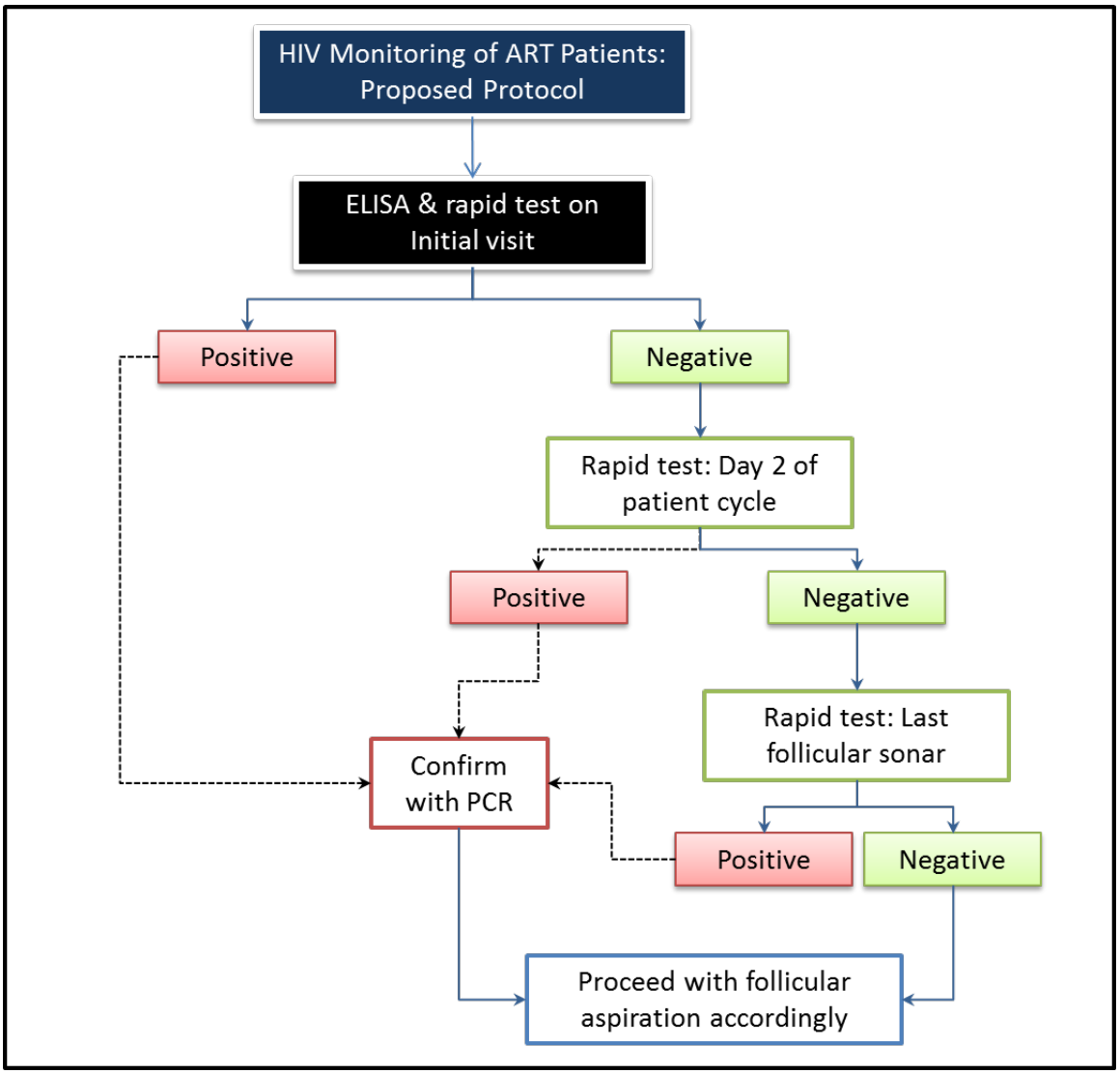

Figure 2.5: Proposed strategy for continual HIV screening of ART patients.

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Chapter 3: Chlamydia trachomatis, HBV & HCV exploratory screening: Prevalence amongst patients using rapid tests

3.1 Background

International guidelines relating to assisted reproduction recommend the screening of patients for BBVs such as HBV and HCV.[1] It is also suggested that patients be screened for pathogens such as *Chlamydia trachomatis* before an assisted reproduction attempt is made.[1] The reproductive and Endocrine Unit of SBAH do not routinely screen patients for HBV, HCV or *Chlamydia trachomatis*. As a result, infected patients go untreated. Co-infection of HIV-positive patients with other BBVs and pathogens could have a negative impact on the general and reproductive health and treatment of patients and their partners. Furthermore, co-infection with HBV and HCV has a potential impact on disease progression, drug interactions and side effects.[2]

The prevalence of HBV, HCV and *Chlamydia trachomatis* in the population group at the Reproductive and Endocrine Unit is currently unknown. Rapid testing may be a means of exploring the prevalence of these infections amongst the population group at the Unit. The feasibility and appropriateness of using rapid tests in the current setting and the acceptability of patients and operators to the concept of rapid testing will be explored. Results obtained from this pilot study will aid in determining the importance of testing patients at the Unit for these infections and assess the need for possible future studies.

3.2 Materials and Methods

Rapid testing for *Chlamydia trachomatis*, HCV and HBV were performed using the DIAQUICK *Chlamydia trachomatis* Cassette (for detection in female cervical

swab, male urethral swab or male urine sample) (Dialab; Hesse, Germany), DIAQUICK HCV Ab Cassette (for serum and plasma samples) (Dialab; Hesse, Germany) and Determine® HBsAg Whole Blood Assay 100 Test kit with accessories (Alere Healthcare; California, USA). The study was intended to be an exploratory study to determine if *Chlamydia trachomatis*, HCV and HBV is present amongst the patient population at the Reproductive and Endocrine Unit of SBAH.

Couples were randomly selected on their initial visit to participate. Individual consent was obtained from male and female partners. All rapid test kits were purchased from Alere Healthcare SA. Rapid tests for *Chlamydia trachomatis*, HCV and HBV was stored (at room temperature) and performed in accordance with the manufacturer's specifications for each rapid test kits (See figures 3.1 – 3.3). All rapid test Lot numbers and expiry dates were recorded for each sample.

3.2.1 *Chlamydia trachomatis*

The DIAQUICK *Chlamydia trachomatis* kit consists of:

1. *DIAQUICK Chlamydia trachomatis Cassette, 20 individually packed test cassettes (Cat No. Z98226B) coated with antigen-specific monoclonal antibodies on the test line.*
2. *Extraction Reagent A: 1 plastic dropper bottle (7ml) containing 0.2M sodium hydroxide (Detection code B0100448-01).*
3. *Extraction Reagent B: 1 plastic dropper bottle (7ml) containing 0.2M hydrochloric acid (Detection code B0100449-01).*
4. *Extraction tubes and dropper caps, 20 per kit.*
5. *Plastic holder for extraction tubes.*
6. *Sterilized swabs with Dacron tips (Cat No. CE0482), 20 per kit, for cervical sample collection.*

3.2.1.1 Participants

For the Chlamydia Rapid Testing section of the study, 30 patients with a mean age of 33 years, ranging from 22 – 46 years(females: 31 with range 22 – 39; males: 36 with range 29 – 46) attending the Reproductive and Endocrine Unit were randomly asked to participate. Rapid test screening was performed using the DIAQUICK *Chlamydia trachomatis* Cassette test kit. The nature of the study (aimed to be exploratory) and budget constraints where the main reasons for the low sample size (n = 30) in this study.

3.2.1.2 Data Gathering

Sampling was performed between July and October 2012 from couples who gave written informed consent. Cervical swabs were taken from female patients by the Unit's clinical staff (according to the manufacturer's guidelines). Male patients were requested by the embryologist to collect a urine sample in a sterile, 50 ml, BD Falcon Polypropylene test tube. Urine samples were centrifuged for 10 min at 10,000 rpm in an Ependorf centrifuge. The pellet was used for *Chlamydia trachomatis* extraction and testing.

3.2.1.3 Quality Control

Chlamydia trachomatis is not a regularly performed test at the Infertility Unit of SBAH. Therefore, alternative assay result to compare Rapid Test results with, were not available. Each DIAQUICK *Chlamydia trachomatis* Cassette has an internal quality control. A control bar is present in each Cassette, if the pink control bar does not appear the test is considered invalid (See figure 3.1, step 5). Confirmation of negative and positive results through a second or repeat rapid test on the DIAQUICK *Chlamydia trachomatis* Cassette was not possible, as each cervical swab or urine sample provided only enough extracted fluid for a single cassette run. In order to repeat a sample a second swab or urine sample would be needed. Positive DIAQUICK *Chlamydia trachomatis* rapid test results were reported to the Reproductive and Endocrine Unit's clinical staff, a follow-up consultation was scheduled (where possible) and an antibiotic

regiment, to include *Chlamydia trachomatis*, was prescribed to both female and male partners.

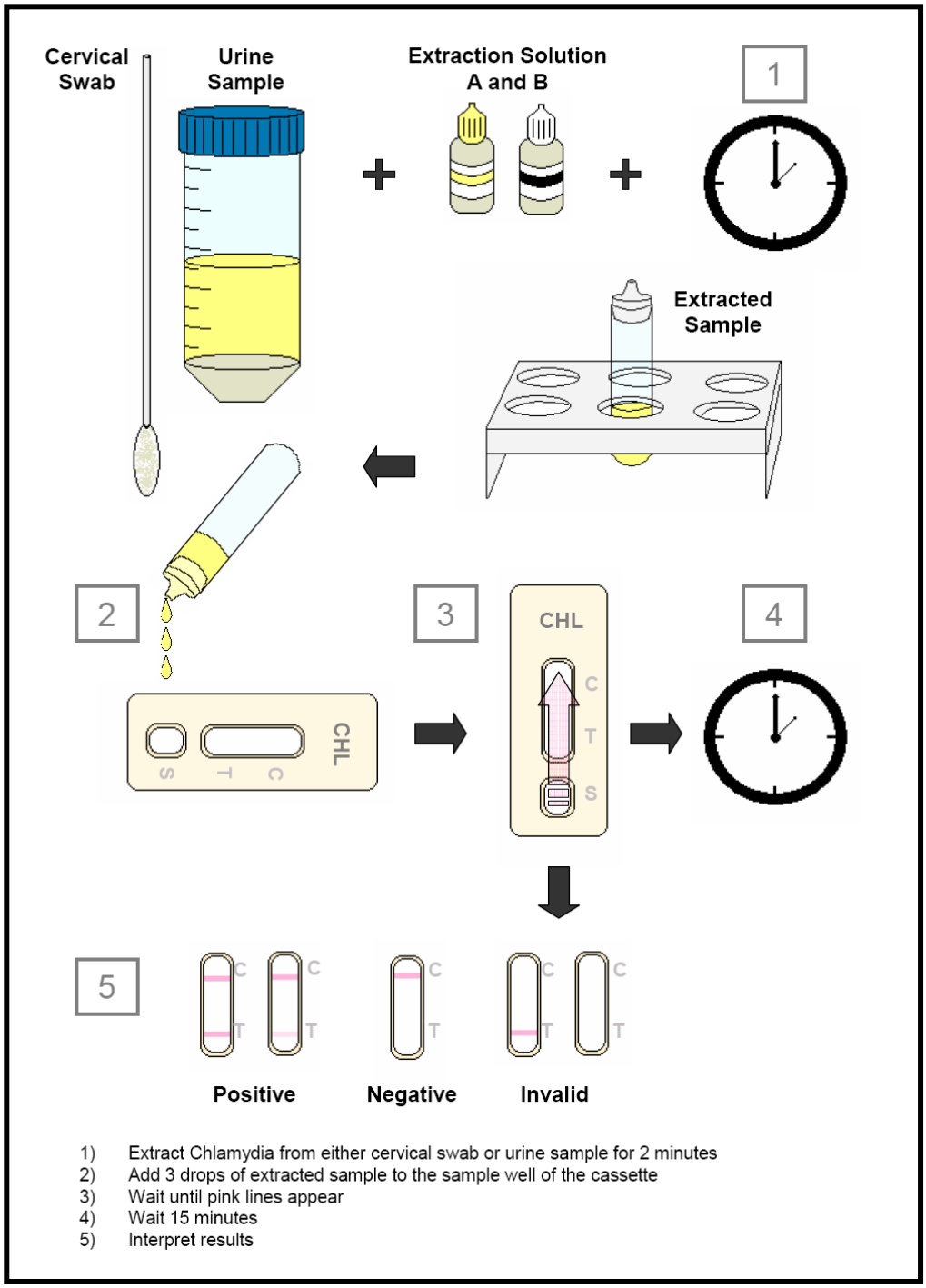

Figure 3.1: DIAQUICK Chlamydia Cassette test procedure: the illustration demonstrates the use and possible results that may be obtained for the rapid testing of Chlamydia.
(C = control bar, T = test line, S = sample well)

3.2.2 Hepatitis C Virus

The DIAQUICK HCV kit consists of:

1. *DIAQUICK HCV Cassette, 30 individually packed test cassettes (Ref No. W02100B) coated with recombinant HCV antigen on the test line.*
2. *Buffer, 2 vials sufficient for 30 tests.*
3. *Disposable plastic pipettes, 30.*

3.2.2.1 Participants

Patients attending the Reproductive and Endocrine Unit of SBAH for the first time were asked at random to participate in HCV screening using the DIAQUICK HCV Cassette kit. Patients participating in this study had a mean age of 35, ranging between 23 and 55 years.

Sampling for HCV was performed during the same period and collect procedure as samples for HIV (n = 100) and HBV (n = 100) testing. Due to insufficient stock and the need to re-order rapid tests (initially only 1 kit containing 30 tests was ordered), there was a lag period where no HCV sampling was performed and no rapid HCV tests run. As a result only 74 patient samples were tested for HCV using the DIAQUICK HCV Cassette kit.

3.2.2.2 Data Gathering

Sampling was performed on consenting male and female partners between November 2010 to Jan 2011 and August 2011 to January 2012. Blood samples were drawn by a qualified nurse, into spray-coated K_2EDTA (plastic, 4 ml, Lavender Hemogard Closure), BD Vacutainer® Blood Collection Tubes, according to the standard protocol at the Unit of SBAH. Blood samples were centrifuged (Ependorf Centrifuge) at 1000 rpm for 10 minutes to separate the serum and plasma from blood. Testing was performed on the plasma. The plasma was removed from the centrifuged sample using the disposable plastic pipette provided in the rapid test kit.

3.2.2.3 Quality Control

A control bar is present in each DIAQUICK HCV Cassette, test validity is determined through the appearance of the pink control bar (See figure 3.2, step 6). The control bar servers as an individual, internal quality control for each rapid test. Positive cassette results were not verified by further automated analysis due to financial constraints and patients not available for follow-up.

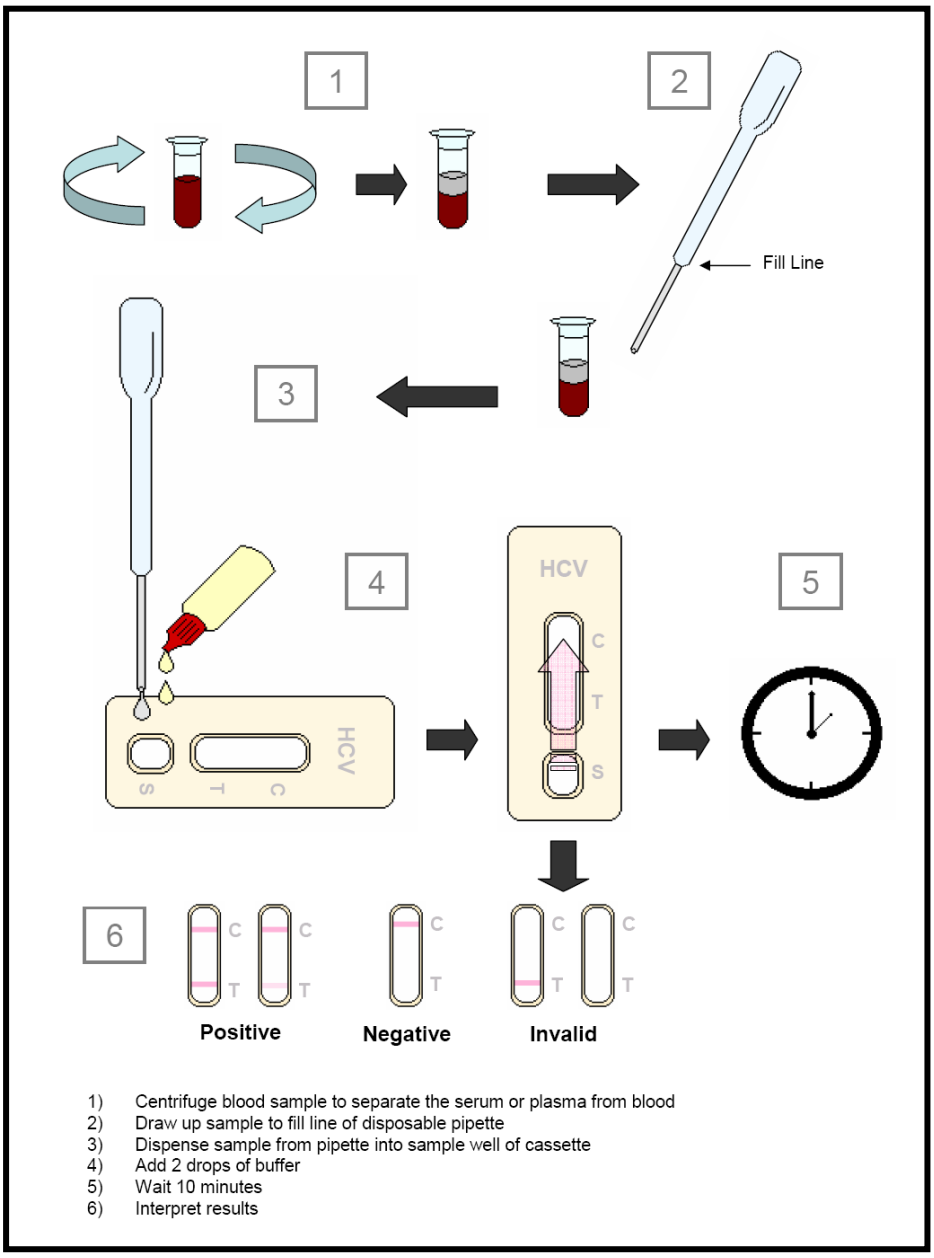

Figure 3.2: Illustration demonstrating the use and possible results for the DIAQUICK HCV Cassette test.
(C = control bar, T = test line, S = sample well)

3.2.3 Hepatitis B Virus

The Determine® kit consisted of:

1. *Determine® HBsAg Test Cards, ten cards (10 tests per card, ie 100 tests) coated with Anti-HBs (mouse monoclonal) antibodies.*
2. *Chase Buffer, 1 Bottle (2.5 ml) (cat no. 7D2243) prepared in phosphate buffer. Preservatives: Antimicrobial Agents.*
3. *EDTA Capillary Tubes (cat no. 7D2222), 100 capillary tubes.*

3.2.3.1 Participants

Randomly selected patients (n = 100), attending the Reproductive and Endocrine Unit of SBAH for the first time, were asked to participate in HBV screening using the Determine® HBsAg Whole Blood Assay Test . Samples for HBV rapid testing were collected and performed during the same period as for HIV and HCV sampling.

3.2.3.2 Data Gathering

From November 2010 to March 2012, blood samples were drawn from consenting male and female partners. All blood sampling was performed by a qualified nurse according to the standard protocol for patients attending the Reproductive and Endocrine Unit of SBAH. Blood samples were collected in BD Vacutainer® Blood Collection Tubes, spray-coated K_2EDTA (plastic, 4 ml, Lavender Hemogard Closure).

3.2.3.3 Quality Control

An individual, internal quality control is built into each Determine® HBsAg Whole Blood Assay Test. Validity of each test is verified through the appearance of a pink control bar (see figure 3.3, step 5). Patients attending the Reproductive and Endocrine Unit are not regularly tested for HBV. All positive Determine® HBsAg test results were compared with results obtained from automated HBsAg, HBsAb and HBcAb analysis (performed on the remaining sample).

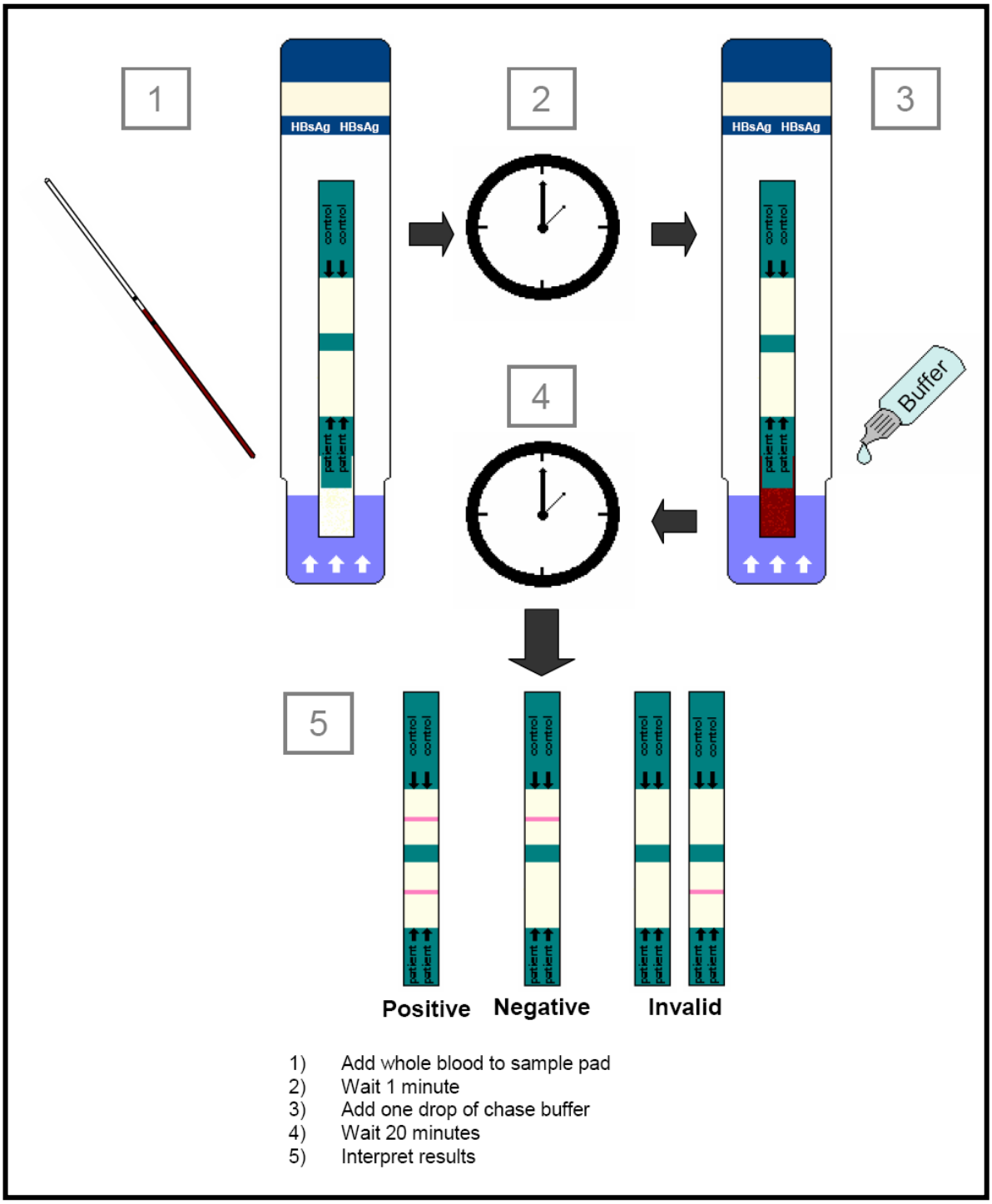

Figure 3.3: Determine® HBsAg test procedure: the illustration demonstrates the use and possible results that may be obtained for the rapid testing of HBV.

3.2.4 Test Appraisal – *Chlamydia trachomatis*, HCV & HBV

A test appraisal for the ease of use of the three rapid tests (DIAQUICK *Chlamydia trachomatis* Cassette, DIAQUICK HCV Cassette and Determine® HBsAg Whole Blood Assay) was performed. A rapid test appraisal form was developed for the study based on the WHO, HIV Assays: Operational Characteristics and NICD, Laboratory Evaluation of HIV Rapid Assay.[3,4]

The rapid test appraisal form incorporated the technical appraisal and suitability score of each rapid test. The rapid test appraisal was completed by the primary investigator of this study.

3.2.5 Cost Analysis – *Chlamydia trachomatis*, HCV & HBV

A cost analysis was performed to determine the feasibility of using the DIAQUICK *Chlamydia trachomatis* Cassette, DIAQUICK HCV Cassette and Determine® HBsAg Whole Blood Assay as a screening system for patients during their work-up and treatment at the Infertility Unit. Quotations from Alere Healthcare for the different rapid tests were compared for the year 2013.

3.3 Statistical Analysis

In a sample of 100 patients rapid tests were used to test for HBV (n = 100) and HCV (n = 74) and data was summarised using percentage positive along with 95% confidence interval. The outcomes for HBV and HCV were also cross-tabulated against country of origin and proportion positive was reported. The proportion of positive *Chlamydia trachomatis* among 30 patients, 14 couples and 2 single females was determined by taking account of the dependence that may be present within a couple, with couples considered as clusters and the two single females were separate clusters each of size one. Since none of the males were positive the proportion of positive *Chlamydia trachomatis* was determined for females only. These proportions were then expressed as percentage and were reported along with 95% confidence intervals.

3.4 Results

Due to the lack of information on the prevalence of *Chlamydia trachomatis*, HBV and HCV amongst the patients of the Reproductive and Endocrine Unit at SBAH, an exploratory study was performed (a minimum of 30 patients tested). The results of the study aim to guide the Unit in the need for possible deeper research.

3.4.1 *Chlamydia trachomatis*

The population group (n = 30) was comprised of patients from Cameroon (n = 2), Congo (n = 2), South African (n = 24) and Somalia (n = 2) (see Table 3.1). The total number of positive samples was 5 out of 30 (16.7%). The sample cohort contained 53.3% (n = 16) females and 46.7% (n = 14) males of which 31.3% of female samples and 0% of male samples tested positive (see Table 3.2). All positive results were obtained from the cervical swab samples (from females) while none of the urine samples (from males) returned a positive result. Samples were only obtained from consenting patients, as a result the sample cohort was comprised of 14 couples (male and female partners) and 2 individual females (male partners did not consent to testing).

Positive test results could not be confirmed due to insufficient sample volume after *Chlamydia trachomatis* extraction from the cervical swabs or urine pellet. Because of financial constraints and appointment logistics, confirmatory PCR testing was not performed. Patients that tested positive on the DIAQUICK *Chlamydia trachomatis* Cassette were managed by the Units clinical staff. An antibiotic regiment targeting *Chlamydia trachomatis* was prescribed to positive female patients as well as their male partners (in couples that were not lost to follow-up). The male partners were also treated due to the suspected inaccuracy of the DIAQUICK *Chlamydia trachomatis* Cassette on the urine samples received from male patients.

Table 3.1: Distribution of *Chlamydia trachomatis* sample cohort through country of origin.

Country of Origin	Total	Positive	Negative	Percentage Positive
Cameroon	2	1	1	50%
Congo	2	1	1	50%
South Africa	24	3	21	12.5%
Somalia	2	0	2	0%
Total	**30**	**5**	**25**	**16.7%**

Table 3.2: Percentage of positive results for *Chlamydia trachomatis* in the sampling population.

Sample Group	Total	HIV-positive	Percentage HIV-positive
Total study population	30	5	16.7%
Males	14	0	0%
Females	16	5	31.3%

3.4.2 Hepatitis C Virus

Countries represented in the study cohort were Cameroon 1% (1/74), Congo 4% (3/74), Malawi 1% (1/74), Nigeria 4% (3/74), South Africa 87% (64/74) and Zimbabwe 3% (2/74). The distribution of patients through country of origin is shown in Table 3.3.

The sample group contained 40 couples (both male and female partners gave consent for testing), 13 individual males (female partner did not consent to testing) and 7 individual females (where male partner did not consent). In the study population, 1 sample (1.4%) tested positive for HCV. Females comprised 45.9% (n = 34) and males comprised 54.1% (n = 40) of the study population,

with 2.9% of females and 0% of males testing positive for HCV (see Table 3.4). The sample that tested positive on the DIAQUICK HCV Cassette was repeated on the DIAQUICK HCV Cassette and returned a positive result on the second rapid test as well. However, the positive result could not be confirmed through further blood pathology tests due to financial constraints and a problem with the patients not returning for follow-up. Consent was therefore, not obtained for further confirmatory HCV testing by an external laboratory.

Table 3.3: Distribution of HCV sample cohort through country of origin.

Country of Origin	Total	Positive	Negative	Percentage Positive
Cameroon	1	0	1	0%
Congo	3	0	3	0%
Malawi	1	0	1	0%
Nigeria	3	0	3	0%
South Africa	64	1	63	1.6%
Zimbabwe	2	0	2	0%
Total	**74**	**1**	**73**	**1.4%**

Table 3.4: Percentage of positive results for HCV in the sampling population.

Sample Group	Total	HCV-positive	Percentage HCV-positive
Total study population	74	1	1.4%
Males	40	0	0%
Females	34	1	2.9%

3.4.3 Hepatitis B Virus

Patients tested (n = 100) came from Cameroon (n = 1), Congo (n = 3), Malawi (n = 1), Nigeria (n = 3), South Africa (n = 89) and Zimbabwe (n = 3) (see Table 3.5). The study group was made up of 47 females and 53 males. Represented in the study group were 40 couples, 13 individual males and 7 individual females. Not all male and female partners in a couple gave consent for rapid testing.

Of the 100 samples, 1 tested positive for HBV with rapid testing, obtained from a female patient. For male patients 0% tested positive, while 2.1% of females tested positive (see Table 3.6). The positive sample was send to the Medical Virology laboratory at NHLS for further testing using the Abbott Architect automated assays for hepatitis B markers. Hepatitis B surface antigen (HBsAg), total hepatitis B core antibodies (HBcAb) and hepatitis B e-antibodies (HBeAb) tested positive.

Table 3.5: Distribution of HBV sample cohort through country of origin.

Country of Origin	Total	Positive	Negative	Percentage Positive
Cameroon	1	1	0	100%
Congo	3	0	3	0%
Malawi	1	0	1	0%
Nigeria	3	0	3	0%
South Africa	89	0	89	0%
Zimbabwe	3	0	3	0%
Total	**100**	**1**	**99**	**1.0%**

Table 3.6: Percentage of positive results for HBV in the sampling population.

Sample Group	Total	HBV-positive	Percentage HBV-positive
Total study population	100	1	1.0%
Males	53	0	0%
Females	47	1	2.1%

3.4.4 Test Appraisal – *Chlamydia trachomatis*, HCV & HBV

Table 3.7 and 3.8 below summarises the test appraisal and suitability – as assessed by the rapid test appraisal forms – for the DIAQUICK *Chlamydia trachomatis* Cassette, DIAQUICK HCV Cassette and Determine® HBsAg Whole Blood Assay. The following aspects were assessed by the appraisal test: number of steps, clarity of instructions, reagent packaging and labelling and ease of performance (Table 3.7), while the test suitability was scored with regards to: sensitivity, specificity, incubation requirements, shelf-life, storage, ease of performance, test duration and reading visibility (Table 3.8).

Table 3.7: Summary of test appraisal for rapid tests (DIAQUICK *Chlamydia trachomatis* Cassette, DIAQUICK HCV Cassette and Determine® HBsAg Whole Blood Assay).

Test appraisal	Weight*	Score		
		Chlamydia	HCV	HBV
Number of steps				
1 – 2	*6*	**3**	**3**	**6**
3 – 5	*3*			
> 5	*1*			
Clarity of instructions				
Good	*2*	**2**	**2**	**2**
Improvable	*1*			
Reagent packaging & labelling				
Good	*2*	**2**	**2**	**2**
Improvable	*1*			
Ease of performance				
Very easy	*5*	**1**	**3**	**5**
Easy	*3*			
Less easy	*1*			
Total	**15 (max)**	**8**	**10**	**15**

*Weight is according to WHO and NICD[3,4]

Table 3.8: Summary of test suitability for rapid tests (DIAQUICK *Chlamydia trachomatis* Cassette, DIAQUICK HCV Cassette and Determine® HBsAg Whole Blood Assay).

Test suitability	Weight*	Score		
		Chlamydia	HCV	HBV
Sensitivity				
100%	*5*	0	0	3
98 – 100%	*3*			
< 98%	*0*			
Specificity				
> 98%	*5*	0	5	5
95 – 98%	*3*			
< 95%	*0*			
Incubation				
Room temperature	*3*	3	3	3
Other	*1*			
Shelf-life				
> 1 year	*3*	2	2	2
≥ 6 months ≤ 1 year	*2*			
< 6 months	*1*			
Storage				
Room temperature (opened)	*5*	5	5	5
Room temperature (unopened)	*2*			
2 – 8°C	*1*			
Ease of performance				
Very easy	*5*	1	3	5
Easy	*3*			
Less easy	*1*			
Test duration				
< 10 minutes	*3*	1	2	2
10 – 30 minutes	*2*			
> 30 minutes	*1*			
Reading Visibility				
Inter-reader variability ≤ 3%	*5*	5	5	5
Inter-reader variability > 30 %	*3*			
Total	**34 (max)**	**17**	**25**	**30**
Suitability for use				
Less suitable	*< 19*	**Less suitable**	**Suitable**	**Very suitable**
Suitable	*19 ≤ x ≥ 26*			
Very suitable	*> 26*			

*Weight is according to WHO and NICD[3,4]

3.4.5 Cost Analysis – *Chlamydia trachomatis*, HCV & HBV

All rapid test kits were obtained from Alere Health Care SA to try and simplify, centralise and manage ordering and delivery of rapid tests. Of the rapid tests used the cost of a single *Chlamydia trachomatis*, HCV and HBV rapid test is shown in Table 3.9 below. The cost of a single *Chlamydia trachomatis* test is almost twice that of an HCV test and about two and a half times that of an HBV rapid test. The cost factor had a large influence on the number of rapid kits ordered per pathogen.

Table 3.9: Cost comparison of a single rapid test for *Chlamydia trachomatis*, HCV and HBV (DIAQUICK *Chlamydia trachomatis* Cassette, DIAQUICK HCV Cassette and Determine® HBsAg Whole Blood Assay, respectively).

		Cost	
Rapid Test	**Units per kit**	**Rapid test kit**	**Single rapid test**
Chlamydia trachomatis	20	R 533.95 (€39.40)	R 26.70 (€1.97)
Hepatitis C Virus	30	R 487.60 (€35.98)	R 16.25 (€1.20)
Hepatitis B Virus	100	R 1 121.20 (€82.73)	R 11.21 (€0.83)

3.5 Discussion

The rapid testing of *Chlamydia trachomatis*, HCV and HBV was performed as an exploratory study to determine the prevalence of these pathogens in the patient population of the Reproductive and Endocrine Unit at SBAH, and to determine the need for possible further study. A minimum of 30 samples were tested for *Chlamydia trachomatis*, HCV and HBV. The nature of the study, cost of rapid tests and method of sample collection had a strong influence on the number of samples obtained for testing of *Chlamydia trachomatis*, HCV and HBV.

The DIAQICK *Chlamydia trachomatis* rapid test cassette was used for the testing of either cervical swab or urine samples. Cervical swabs needed to be obtained from the female partners by a trained professional (Unit clinician) while urine samples were requested from males partners by the consulting embryologist. The nature of sample collection and the fact that female and male patient samples were collected differently made the collection of samples a difficult task. Sample collection for the DIAQUICK HCV cassette and Determine® HBsAg rapid test was less complicated as blood samples are routinely collected by a trained nurse at the Unit. Blood samples were drawn from consenting patients as per the standard protocol of the Unit. An extra tube of blood was draw for the testing of HIV (discussed in Chapter 2), HCV and HBV. It was therefore possible to run the HIV, HBV and HCV rapid testing on the same cohort of patients.

Due to the cost and nature of sample collection the minimum of 30 samples was tested for *Chlamydia trachomatis*. For HBV 100 patients were tested while for HCV only 74 patients were tested. The discrepancy in the number of HCV samples tested is due to the slightly higher cost of the HCV than HBV rapid test kits (originally only one HCV kit was purchased). After initiation of the rapid testing it was decided that the minimum number of 30 samples for HBV and HCV could be increased as blood samples were available from the testing of HIV (n = 100). As a result new rapid test kits for HCV had to be ordered and delivered before testing of HCV could continue.

A test appraisal of the DIAQUICK *Chlamydia trachomatis* cassette, DIAQUICK HCV cassette and Determine® HBsAg rapid test was performed. From the appraisal of the rapid tests the ease of use and suitability of each rapid test was assessed. As show in the result of this study the DIAQUICK *Chlamydia trachomatis* cassette scored the lowest in both appraisal and suitability due to the test scoring low on sensitivity and specificity. The *Chlamydia trachomatis* rapid test was difficult to perform as it required extraction of samples from either a cervical swab or urine sample (urine samples required centrifugation).

Extracted sample was limited and therefore only a single rapid test could be run, for a repeat test a second swab or urine sample would be required.

The DIAQUICK HCV cassette and Determine® HBsAg rapid test scored higher and were found to be “suitable” and “very suitable”, respectively. Sample collection for the DIAQUICK HCV cassette requires a few millilitres of blood that should be centrifuged (according to the guidelines of the manufacturer) to separate the serum or plasma from the blood. The plasma was then used to run the rapid test. A suitable volume of plasma was available to repeat the rapid test if needed. The ample amount of sample available is an advantage for repeat testing. However the fact that a centrifuge is required does complicate the test and limit the use in the absence of laboratory equipment. If centrifugation is not possible the whole blood sample could be left to separate naturally over a period of time and the rapid test could then still be performed, however that is not the recommended procedure according to the guidelines of the manufacturer.

The Determine® HBsAg rapid test scored the highest out of the three rapid tests in this study. Due to the logistics of the study, blood samples were drawn from consenting patients by a qualified nurse into a BD Vacutainer® Blood Collection Tube, spray-coated with K_2EDTA. However, a simple finger prick can be performed and whole blood collected into a capillary tube, supplied as an optional accessory of the kit. Finger prick for whole blood collection is not a complicated procedure and can be performed by any individual that has been shown how to perform the technique correctly. This method of collection allows for easy resampling if a repeat test is required. The Determine® HBsAg rapid test was, furthermore, easy to perform and had a fair sensitivity and good specificity. As a result, information from the study indicates that for the Reproductive and Endocrine Unit the Determine® HBsAg rapid test is very suitable, while the DIAQUICK HCV cassette is suitable and the DIAQUICK *Chlamydia trachomatis* cassette is not suitable. The time required to perform the rapid tests for HBV (on the Determine® HBsAg) and HCV (on the DIAQUICK

HCV cassette) was between 10 and 30 minutes. However, the rapid testing of *Chlamydia trachomatis* took longer than 30 minutes to perform, due to the centrifugation and extraction steps, further reducing the suitability of the test.

Even though the *Chlamydia trachomatis* rapid test was not found to be a suitable test in the appraisal, the rapid test results showed a high prevalence of *Chlamydia trachomatis* infection amongst the females tested in this study. The total number of positive samples was 5 out of 30 (16.7% of the study cohort) and 5 out of 16 females (31.3%). All positive samples were obtained from the female partner of the tested couples. Results obtained for the male partners were not accurate owing to the non-specificity of time of urine sample collection. In a study conducted by the Andrology Unit of SBAH, the prevalence of *Chlamydia trachomatis* was found to be 25.2% among infertile male patients when their urine samples were tested by enzyme immunoassay (EIA) and direct immunofluorescent antibody (DFA).[5] The collection of samples from males can be (according to the manufacturer's guidelines) done via a urethral swab or a urine sample. In the current study conducted at the Reproductive and Endocrine Unit, the urine sample was used as performing a urethral swab at the Unit was not practical in the current Unit protocol.

The manufacturer's guidelines require a urine sample collected from the first urine of the day, believed to hold the highest concentration of *Chlamydia trachomatis*. Because of the scheduled time of couple's appointments, the distance couples travel to the Unit and the fact that couples were not previously see by the Unit (initial visit and therefore could not be informed of the requirements), the urine samples were most likely not the first urine of the morning. The levels of *Chlamydia trachomatis* in the collected urine samples could then have been low and therefore difficult to detect with the DIAQUICK *Chlamydia trachomatis* cassette. This may be a reason for the male partners testing negative on the DIAQUICK *Chlamydia trachomatis* cassette while their female counter parts tested positive. A study conducted at the Termination of Pregnancy Clinic of Kalafong Hospital in Pretoria, Gauteng, showed a

Chlamydia trachomatis infection prevalence of 25.8% amongst pregnant women and women who experienced spontaneous abortion.[6] This is in agreement with the 31.3% (5/16) prevalence found among female partners in the current study.

In South Africa there is approximately a 1% prevalence of HBV in urban areas.[7] The global prevalence of HCV is 3%.[7] In the current study the DIAQUICK HCV cassette as well as the Determine® HBsAg rapid tests only yielded one positive sample each (1/74 and 1/100, respectively). The HCV prevalence was 1.4% according to the results obtained from the DIAQUICK HCV Cassette. The positive HCV result was, unfortunately not confirmed by automated analysis due to financial constraints and the patient not returning for a follow-up visit. For HBV the prevalence was found to be 1% in the study cohort on the Determine® HBsAg rapid test. The positive rapid test result was sent for further Hepatitis B analysis at NHLS, by testing the remaining portion of the blood sample collected for rapid testing. The results from the NHLS tests showed positive for: HBcAb (total), HBsAg and HBeAb. Furthermore the NHLS results showed negative for HBcAb IgM, hepatitis B immunity and hepatitis B e-antigen. The results indicated a possible acute or chronic infection.

In conclusion, it was shown in the current study that there is a lack in the screening of patients for pathogens such as *Chlamydia trachomatis*, HCV and HBV. The prevalence for HCV and HBV were low amongst the study cohort. However, the prevalence of *Chlamydia trachomatis* was high and warrants further investigation into the actual prevalence, as the sample size was small (n = 30) and DIAQUICK *Chlamydia trachomatis* cassette was suspected to be inaccurate for the analysis of the male urine samples as collected in the current study.

Chlamydia trachomatis is a prevalent STI with frequent and severe complications including epididymitis, proctitis, tubal factor infertility, ectopic pregnancy and pelvic inflammatory disease.[6,8] An alternative *Chlamydia trachomatis* rapid test with higher sensitivity and specificity that is more cost

effective, easier to perform and using a sample type that is more convenient to collect, should be investigated as a screening tool for the Unit. Eley *et al* (2010) describes the importance of testing for *Chlamydia trachomatis* among men of infertile couples and also discusses different methods for the detection in clinical specimens.[9] *Chlamydia trachomatis* is easily treated with antibiotics. The identification of patients infected with *Chlamydia trachomatis* will allow for treatment and could possibly improve the chance of pregnancy during ART.

3.6 References

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Chapter 4: Semen Profiling of HIV positive males

4.1 Background

Not unlike many other assisted reproduction units, the HIV status of patients undergoing diagnostic evaluation at the Reproductive and Endocrine Unit of SBAH is not always available upon the initiation of a semen evaluation. It is well reported in literature that HIV-1 virions may be present in the seminal fluid and non-sperm cells of HIV-positive male samples. However, the effect that HIV-1 infection has on semen characteristics is much debated. A South African view point has yet to be published. The Reproductive and Endocrine Unit of SBAH has, for the past 12 years, been conducting a semen decontamination programme aimed at the elimination of HIV-1 from semen. From research conducted in the semen decontamination programme it is clear that the plasma viral loads and semen viral loads do not always correspond with each other. The possibility exists that HIV-1 virions may be present in the semen of an HIV-positive male while his plasma viral load is undetectable.

The need for HIV screening in an ART programme is not always recognised by some clinicians in private and public practices. Healthcare worker are often handling HIV-positive samples without prior knowledge.[1] Although all bodily fluids, including semen samples, entering an IVF laboratory are handled as if they are potentially hazardous (universal precautions applied) there is a need for the detection of infected samples as they enter the laboratory. Until the Unit's HIV screening protocols are improved, the identification of semen characteristics that may serve as a warning sign for further infection and pathogen investigation could be a useful tool. The Unit at SBAH is an academic training unit and samples are often handled by inexperienced staff or students undergoing supervised training. The handling of samples by students and staff under training, even under supervision, is a great risk area. Sample status

identification is needed to not only safe guard staff but also to identify the correct laboratory area in which a sample should be handled. The treatment of HIV-positive patients should be executed in laboratories with devoted areas, in which suitable safety measures are followed.[2,3] At the Unit of SBAH, samples from HIV-negative patients are handled in a separate area of the diagnostic laboratory as HIV-positive samples. While samples from patients of unknown HIV status are handled in the HIV-positive area of the laboratory. For therapeutic procedures the Reproductive and Endocrine Unit has dedicated, separate ART laboratories and equipment for the treatment of HIV-positive and HIV-negative couples.

4.2 Materials and Methods

Patient information was gathered with the aim of profiling semen characteristics of the patient population attending the HIV semen decontamination programme at Steve Biko Academic Hospital. Semen decontamination is an on-going programme at the unit. The standard operating procedures (SOPs) has been refined over the years to the current standard protocol by which patient samples are processed for ART. The semen samples of the first 60 patients for which the most recent standard protocol was used, were considered for analysis in the study. Samples were collected between March 2010 and May 2012.

After the semen evaluation of the HIV-positive patients in the decontamination programme, parameters of neat semen samples were entered into a database. Semen parameters of the HIV-positive study population were compared to that of HIV-negative males who had a successful assisted reproductive attempt as a control group and the data was analysed for statistically significant differences. Furthermore, information pertaining to the health status of HIV-positive patients (i.e. CD4 count, plasma viral load and anti-retroviral treatment) was also captured to compare the health and semen quality parameters of HIV-seropositive patients.

4.2.1 Participants

Semen parameters from the HIV-positive study population and the HIV-negative control group were entered into the study database for analysis.

i. Study population:
   Semen samples of 60 HIV-positive male patients partaking in the semen decontamination programme at SBAH were collected and a basic semen evaluation performed on their first visit. The mean age of the study population was 39 years (range from 26 to 54 years of age).

ii. Control group:
   Retrospective data of male patients (n = 60) who were HIV-negative (according to current screening protocol of the unit) and had a successful ART cycle (βHCG positive 10 to 14 days after embryo transfer, 47 IVF and 13 ICSI cycles) were used. Semen parameters as recorded on the day of the successful assisted reproduction cycle were entered into the study database for comparison with the HIV-positive population. Patients in this group ranged from 24 to 47 years of age with a mean age of 37.

4.2.2 Data Gathering

Male patients participating in the semen decontamination programme at SBAH were asked to provide a semen sample after 2 to 5 days abstinence by means of masturbation into a sterile sample collection jar (according to the SOP of the Reproductive Biology Laboratory). The patient's information sticker was placed on the sample jar before collection, with the patient as witness. Once the sample was collected patients were instructed to seal the sample jar in a provided plastic sealable bag and then place into a polystyrene cup for safe keeping in the lab during liquefaction. The semen sample was left to liquefy in a Quincy Lab Warming oven at 37°C for 30 minutes. The times the semen sample was produced and evaluated were recorded. Once the semen sample was liquefied, a basic semen analysis was performed according to the guidelines set out by the WHO laboratory manual for the examination and processing of

human semen,[4] and the SOP of the decontamination programme of the unit (see Figure 4.2 for summary of evaluation procedure).

The following semen parameters were recorded:

i. Volume
ii. Viscosity
iii. pH
iv. Morphology
v. Non-spermatic cells
vi. Concentration
vii. Motility

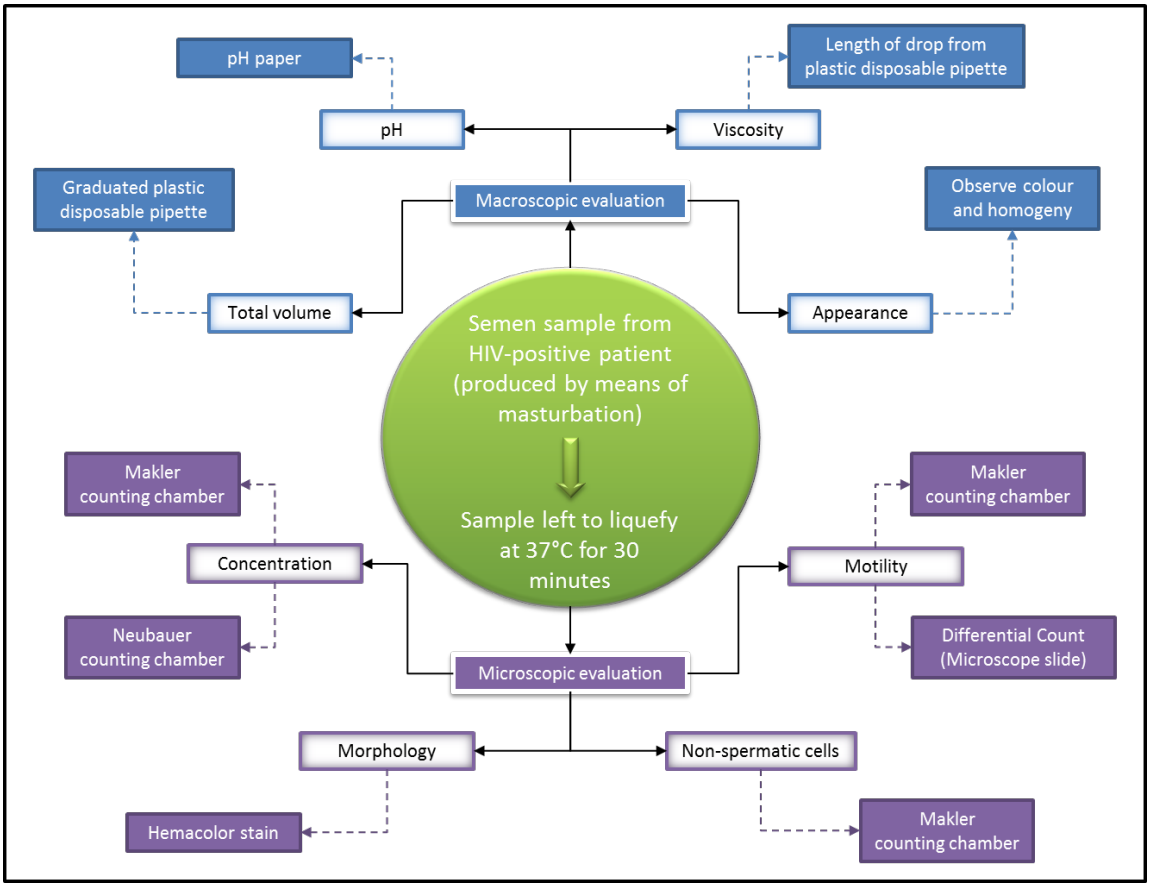

Figure 4.1: Summarised diagram of parameters assessed in a basic semen analysis at the Unit.

Semen volume was measured by weight and reported in millilitres. Viscosity was measured by dropping semen from a wide bore plastic disposable pipette and thread length measured. A thread length greater than 2cm was considered viscose, samples were classified as either viscose or not and the percentage viscose was compared. The pH was determined using pH paper. Sperm morphology was evaluated from a Hemacolor stained slide smear and percentage normal sperm determined according to the Tygerberg Strict Criteria.[5] Non-sperm cells were calculated from the Makler counting chamber, samples were classified as either elevated (>1 million per millilitre) or not and the percentage elevated were compared. Sperm concentration was determined with the Neubauer and Makler counting chambers, the Neubauer concentration was reported for comparison. The sperm motility was established through a differential count and on the Makler counting chamber. Sperm motility was reported in three parameters: immotile, non-progressive and progressive sperm. The sperm motility from the differential slide counts were reported and compared.

4.2.3 Quality Control

Sperm morphology results were verified through assessment of the slide by a second embryologist. To determine sperm concentration, two different counting chambers were used (i.e. Neubauer and Makler counting chambers). The concentration determined from each of the counting chambers was expected to be similar. If too large a difference (more than 20%) was observed, the concentration assessment was repeated. Similarly two methods of sperm motility assessment were used, a differential count on a microscope slide as well as by means of the Makler counting chamber. If the motility counts were not similar, the motility assessments were repeated.

4.2.4 CD4 count, HIV-1 viral load and antiretroviral drug treatment

Patient information was obtained during consultation at the initial semen decontamination visit. ARV usage was noted and blood pathology results (CD4 cell count and RNA viral load) were obtained from patients and were no older than 1 month from the date of the semen evaluation. Commercial validation assays were used to determine HIV-1 RNA viral load (COBAS® Ampliprep/COBAS® TaqMan® HIV-1 version 2, Roche Diagnostics, Indianapolis, USA). The linear range of the commercial assay is 20 – $10x10^6$ RNA copies/ml. Parameters were then statistically analysed to validate if health had an impact on semen parameters of HIV-positive patients as follows:

i. CD4 cell counts lower than 350 cells/ml vs. CD4 counts ≥350 cells/ml[20]
ii. Undetectable (LLD) plasma RNA viral load vs. detectable plasma viral load (LLD <40 copies/ml)
iii. ARV treatment vs. no ARV treatment

The duration of ARV treatment and type of drugs were not taken into consideration for this study.

4.3 Statistical Analysis

The study and control group were compared for the semen parameters volume, viscosity, pH, morphology, non-spermatic cells, concentration and motility. The categories of HIV (positive / negative), CD4 count (<350 cells/ml; ≥350 cells/ml), RNA viral load (positive / undetectable) and ARV treatment (yes / no) were compared with respect to continuous semen parameters (i.e. volume, pH, morphology, concentration and motility) using the Welch t-test for unequal variance. Reported are mean and standard deviation by category along with the p-value and the 95% confidence interval for the difference between means of the categories for the respective semen parameters. Categories were compared regarding the binary semen parameters (i.e. viscosity and non-spermatic cells) using Fisher's exact test. The percentage patients in each category group with positive / elevated values are reported as a percentage along with the p-value.

4.4 Results

4.4.1 Semen parameters of HIV-negative vs. HIV-positive samples

Descriptive statistics (mean values) of the semen parameters for the study group (HIV-seropositive males) compared to the control group (HIV-negative males) are summarized in table 4.1: i) semen volume: 2.7 ml vs. 2.3 ml; ii) viscose semen samples: 21.6% vs. 30%; iii) pH: 7.4 vs. 7.3; iv) sperm morphology: 6% vs. 6.8%; v) elevated non-spermatic cells: 23.3% vs. 15.3%; vi) sperm concentration: 53.1 x 10^6/ml vs. 40.4 x 10^6/ml; vii) sperm motility: immotile: 43.4% vs. 44.2%; viii) non-progressive: 8.5% vs. 9.2% and ix) progressive: 47.7% vs. 46%. The results showed no statistical difference between the groups ($p > 0.05$) for all the compared semen parameters for this study.

4.4.2 CD4 count, RNA viral load and ARV treatment

In the study group of HIV-positive patients (n = 60), semen parameters were compared in 3 categories:

1) CD4 cell count: <350 cells/ml vs. ≥350 cells/ml;
2) Plasma RNA viral load: detectable vs. undetectable (LLD) and
3) ARV treatment: yes vs. no.

4.4.2.1 CD4 cell count

There were 41 (68%) patients with a CD4 count lower than 350 cells/ml while 19 (32%) patients had a count higher than or equal to 350 cells/ml. The mean values of the semen parameters for the CD4 cell count category (<350 cells/ml vs. ≥350 cells/ml) were as follows (see Table 4.2): i) semen volume: 2.8 ml vs. 2.8 ml; ii) viscose semen sample: 33.3% vs. 31%; iii) pH: 7.4 vs. 7.4; iv) morphology: 5.4% vs. 5.9%; v) elevated non-spermatic cells (>1x10^6): 17.4% vs. 21.4%; vi) sperm concentration: 42.4 x 10^6/ml vs. 58.0 x 10^6/ml; sperm

motility vii) immotile: 40.8% vs. 44.6%; viii) non-progressive: 7.3% vs. 9%; and ix) progressive: 50.8% vs. 46.2%. There was no statistical difference in the semen parameters between the <350 and ≥350 cells/ml CD4 groups ($p > 0.05$).

4.4.2.2 Plasma RNA viral load

Of the 60 HIV positive patients, 27 (45%) presented with a detectable plasma viral load. The semen parameters were compared between the detectable vs. the undetectable viral load categories and the means of the different parameters were as follows (see Table 4.3): i) semen volume: 2.6 ml vs. 3 ml; ii) viscose semen samples: 44.4% vs. 18.2%; iii) pH: 7.4 vs. 7.5; iv) sperm morphology: 5.3% vs. 6.1%; v) elevated non-spermatic cells: 18.5% vs. 27.3%; vi) sperm concentration: 45.5 x 10^6/ml vs. 59.3 x 10^6/ml; vii) sperm motility: immotile: 42.1% vs. 44.4%; viii) non-progressive: 7% vs. 9.6%; ix) progressive sperm: 50.1% vs. 45.8%.

The only statistical difference observed between the plasma RNA viral load categories was for semen viscosity. The percentage of viscose samples in the detectable viral load category was 44.4% while the undetectable category was 18.2% ($p = 0.0460$). Most of the viscose samples in the detectable RNA viral load category where not on ARV treatment (8 out of 12). All the viscose samples in the undetectable category were on ARV treatment.

4.4.2.3 ARV treatment

There were 21 (35%) patients not on ARV treatment and 39 (65%) patients on treatment in the HIV-positive study group. For the comparison of semen parameters between the ARV categories (treatment vs. no treatment – see Table 4.4) the mean values were as follows: i) semen volume: 2.8 ml vs. 2.7 ml; ii) viscose semen samples: 38.1% vs. 25.6%; iii) pH: 7.4 vs. 7.4; iv) sperm morphology: 5.5% vs. 6.2%; v) elevated non-spermatic cells: 25.6% vs. 19.1%; vi) sperm concentration: 53.5 x 10^6/ml vs. 52.4 x 10^6/ml; vii) sperm motility:

immotile: 45.8% vs. 38.8%; viii) non-progressive: 9.3% vs. 6.8%; and ix) progressive: 44.6% vs. 53.4%.

The majority of the semen parameters were not statistically different between the group receiving ARV treatment compared to those not receiving treatment. However, the percentage of immotile sperm for the group using ARV treatment was 45.8% vs. 38.8% for the group not receiving treatment, with a p-value of 0.0456. Furthermore the group receiving ARV treatment had a significantly lower percentage of progressively motile sperm than the group not on ARVs (44.6% vs. 53.4%) with a p-value of 0.0192.

Table 4.1: Descriptive semen parameters of HIV-positive study group compared to HIV-negative control group, reported by mean and standard deviation (SD) along with the p-value and the 95% confidence interval (CI).

	HIV-positive (n = 60)		HIV-negative (n = 60)			
Semen Parameter	**Mean**	**SD**	**Mean**	**SD**	**p-value**	**95% CI**
Volume (ml)	2.7	1.27	2.3	0.93	0.0548	[-0.80 ; 0.01]
Viscosity (% viscose)	21.6	-	30	-	0.4040	-
pH	7.4	0.22	7.3	0.33	0.0943	[-0.19 ; 0.02]
Morphology (%)	6	2.96	6.8	3.06	0.1683	[-0.35 ; 2.00]
Non-spermatic cells (% >1x10^6)	23.3	-	15.3	-	0.3540	-
Concentration (x10^6/ml)	53.1	47.95	40.7	34.12	0.1073	[-27.4 ; 2.73]
Sperm motility: Immotile (%)	43.4	14.78	44.2	20.02	0.8051	[-5.49 ; 7.06]
Sperm motility: Non-progressive (%)	8.5	5.71	9.2	7.57	0.5237	[-1.64 ; 3.21]
Sperm motility: Progressive (%)	47.7	15.03	46	20.39	0.6007	[-8.20 ; 4.76]

Table 4.2: Semen parameters of HIV-positive study group (n = 60) compared with respect to CD4 cell count, reported by mean and standard deviation (SD), p-value and the 95% confidence interval (CI).

	CD4 < 350 cells/ml (n = 41)		CD4 ≥ 350 cells/ml (n = 19)			
Semen Parameter	**Mean**	**SD**	**Mean**	**SD**	**p-value**	**95% CI**
Volume (ml)	2.8	1.00	2.8	1.32	0.8738	[-0.57 ; 0.67]
Viscosity (% viscose)	31	-	33.3	-	1.0000	-
pH	7.4	0.18	7.4	0.30	0.5698	[-0.11 ; 0.19]
Morphology (%)	5.9	2.66	5.4	2.63	0.4943	[-0.98 ; 1.99]
Non-spermatic cells (% >1x10^6)	17.4	-	21.4	-	0.707	-
Concentration (x10^6/ml)	58.0	54.1	42.4	54.1	0.1529	[-6.0 ; 37.2]
Sperm motility: Immotile (%)	44.6	13.3	40.8	15.9	0.3756	[-4.78 ; 12.3]
Sperm motility: Non-progressive (%)	9	5.73	7.3	5.65	0.2769	[-1.45 ; 4.93]
Sperm motility: Progressive (%)	46.2	15.4	50.8	14.2	0.2622	[-12.8 ; 3.57]

Table 4.3: The descriptive semen parameters of HIV-positive study group (n = 60) compared by RNA viral load. Results reported by mean, standard deviation (SD), p-value and the 95% confidence interval (CI).

	RNA – Detectable (n = 27)		RNA – Undetectable (n = 33)			
Semen Parameter	**Mean**	**SD**	**Mean**	**SD**	**p-value**	**95% CI**
Volume (ml)	2.6	1.23	3.0	1.21	0.2716	[-0.28 ; 0.99]
Viscosity (% viscose)	**44.4**	**-**	**18.2**	**-**	**0.0460**	-
pH	7.4	0.28	7.5	0.15	0.2271	[-0.05 ; 0.20]
Morphology (%)	5.3	2.39	6.1	2.81	0.2453	[-0.56 ; 2.13]
Non-spermatic cells (% >1x10^6)	18.5	-	27.3	-	0.5440	-
Concentration (x10^6/ml)	45.5	31.5	59.3	57.8	0.2448	[-9.75 ; 37.4]
Sperm motility: Immotile (%)	42.1	13.0	44.4	15.2	0.5451	[-5.06 ; 9.50]
Sperm motility: Non-progressive (%)	7	4.14	9.6	6.56	0.0634	[-0.15 ; 5.42]
Sperm motility: Progressive (%)	50.1	13.7	45.8	16.0	0.2647	[-12.0 ; 3.35]

Table 4.4: HIV-positive study group (n = 60) compared with respect to ARV use for differences in semen parameters. Results reported by mean and standard deviation (SD) along with the p-value and the 95% confidence interval (CI).

	ARV – Yes (n = 39)		ARV – No (n = 21)			
Semen Parameter	**Mean**	**SD**	**Mean**	**SD**	**p-value**	**95% CI**
Volume (ml)	2.8	1.19	2.7	1.30	0.7805	[-0.79 ; 0.60]
Viscosity (% viscose)	38.1	-	25.6	-	0.3810	-
pH	7.4	0.16	7.4	0.30	0.2633	[-0.22 ; 0.06]
Morphology (%)	5.5	2.88	6.2	2.10	0.2691	[-0.58 ; 2.03]
Non-spermatic cells (% >1x10^6)	25.6	-	19.1	-	0.7510	-
Concentration (x10^6/ml)	53.5	54.8	52.4	32.8	0.9221	[-23.8 ; 21.5]
Sperm motility: Immotile (%)	**45.8**	**15.0**	**38.8**	**11.4**	**0.0456**	**[-14.0 ; 0.14]**
Sperm motility: Non-progressive (%)	9.3	6.36	6.8	3.87	0.0613	[-5.17 ; 0.12]
Sperm motility: Progressive (%)	**44.6**	**15.7**	**53.4**	**12.1**	**0.0192**	**[1.50 ; 16.1]**

4.5 Discussion

With HIV no longer being defined as a terminal illness, many serodiscordant couples seek safer conception through medical assisted reproduction. Techniques such as sperm washing diminish the risk of transmission between HIV-positive males and there HIV-negative partners. The data collected from patients attending the decontamination programme agrees with other published literature,[6] that a patient with an undetectable plasma viral load does not always present with an undetectable viral load in their semen (26% of the population group attending the unit's decontamination programme). This discrepancy between plasma and semen viral loads strengthens the need for sperm washing and assisted conception amongst this population group.

The Unit receives many known HIV-positive patients that seek sperm washing through the decontamination programme. The Unit also evaluates patients of unknown HIV status while awaiting the results of their HIV test. Research results from this study (Chapter 2), indicated that 8% of new patients tested positive for HIV and were unaware of their status. Patients are tested, according to protocol, for HIV on their initial visit to the unit. Results are unavailable on commencement of semen analysis. Staff, therefore, have to process samples in the diagnostic semen laboratory without knowing the HIV statuses. Even though all samples are treated as potentially hazardous and universal precautions applied, the identification of HIV-positive samples will streamline treatment of patients and provide added safety to patients and staff.

The consequence of HIV on semen parameters has been reported by several studies with different outcomes. In the current study no statistical difference was noted for semen parameters (volume, viscosity, pH, concentration, immotile sperm, non-progressive sperm, progressive sperm, morphology and non-spermatic cells) when comparing HIV-positive semen samples with HIV-negative samples. When the HIV-positive study group was further analysed according to CD4 cell count, plasma RNA viral load and ARV treatment, no

statistical differences were noted for the following parameters: semen volume, pH, sperm concentration, non-progressive sperm motility, sperm morphology and non-spermatic cells. HIV-positive patients with a detectable plasma viral load showed a statistically significant difference with regards to viscosity when compared to patients with an undetectable viral load. The patients with a detectable viral load had 44.4% viscose samples while the undetectable group had 18.2%. Patients with a detectable viral load may have exhibited more viscose samples due to possible co-infection with other pathogens. Screening of patients for other pathogen infections is not common practice at the Reproductive and Endocrine Unit. However, semen samples at the Unit are sent for microbial sensitivity and culture and *Mycoplasma hominis* and *Ureaplasma urealyticum* analysis. Infections such as *Ureaplasma urealyticum* are known to increase semen viscosity.[7] The microbiology results for the patients in the HIV-positive group were evaluated, of the 16 viscose samples, 11 had a detectable viral load. Furthermore, 45% were positive for *Ureaplasma urealyticum*. In the group of HIV-positive samples that were not viscose only 7% were positive for *Ureaplasma urealyticum.*

The group of patients receiving ARV treatment presented with a decrease in sperm motility when compared to the group not receiving treatment. In the ARV treated group the decrease in motility is shown by a decrease in progressive sperm motility (44.6% vs. 53.4%) and an increase in immotile sperm (45.8% vs. 38.8%). Studies have shown that the use of ARV treatment may elicit sperm mitochondrial DNA damage,[8-11] which may explain the decrease in sperm motility observed in this study population.

Results indicated similar semen parameters from patients that are HIV-positive and HIV-negative (no statistical difference $p>0.05$). The primary aim of this study was to establish if HIV-positive semen samples can be identified from HIV-negative samples through specific characteristics of the semen parameters (as the HIV status is unknown when an initial semen evaluation is performed). Patients on ARV treatment presented with a decrease in motility. As ARV

treatment is only prescribed to HIV-positive patients, the infection status of the semen samples will be known before a semen analysis is performed. For this reason decreased sperm motility cannot be used to identify an HIV-positive semen sample in the diagnostic laboratory. However, there is an indication that patients with detectable RNA viral loads may present with a viscose semen sample. Patients who are HIV-positive, but not yet aware of their HIV status, are likely to have a detectable viral load and their semen sample will enter the diagnostic laboratory before their HIV status is available to both staff and the patient. An increased viscosity in a semen sample may be an indication to do further microbiological investigations on the sample.

In conclusion, there is not a definite profile for HIV-positive semen samples at the Reproductive and Endocrine Unit of SBAH making it difficult to identify such samples on initial semen analysis without further pathology testing. The lack of multiple, definite differences between HIV-positive and HIV-negative semen samples further supports the need for simpler, point-of-care and more accessible HIV screening on initial visit to the unit (refer to Chapter 2).

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Chapter 5: Recommendations

5.1 Recommendations

The aim of the Reproductive and Endocrine Unit is to provide patients in the public sector with the best possible treatment for their specific situation and within the financial constraints faced by these patients. Eight percent of new patients who are not aware of their HIV status before seeking fertility assistance at the Reproductive and Endocrine Unit test HIV-positive. The incorporation of rapid testing for the identification and screening of HIV has proven to be an acceptable, simple, quick, cost effective and plausible option in the setting of an ART programme at the Unit of SBAH. The advantages of rapid point-of-care testing may outweigh the disadvantages in resource-limited ART settings. The limitations of rapid testing need to be clearly understood by health care workers and ART programmes that implement it as a screening option. Rapid tests may have a lower sensitivity when compared to automated laboratory-based fourth generation HIV ELISA assays in certain stages of HIV disease. HIV rapid assays may have a longer window period to detect HIV infection in the acute phase. The window period refers to the period between original exposure to HIV and when the test can reliably detect the presence of HIV-specific antibodies and/or virus in the blood. Patients in the acute phase of HIV infection are often asymptomatic.[1] In antibody-based testing, the window period is reliant on the time required for seroconversion as well as the sensitivity of the assay which can vary considerably for different rapid assays. Fourth generation laboratory-based HIV assays can detect antibodies to HIV-1/2 and p24 Ag simultaneously, and thereby reduce the window period to an average of 2 weeks.[1] An average window period of three to four weeks is described for most rapid HIV tests which only detect HIV antibodies.[1] The Determine® HIV-1/2 Ag/Ab Combo test is the first rapid test to incorporate the detection of the HIV p24 Ag. According to an in-house study performed by Alere Ltd (formally known as Inverness Medical

UK Ltd), the Determine® HIV-1/2 Ag/Ab Combo test detects HIV infection on average 5 days earlier than third generation assays.[2] However, the cost of laboratory-based HIV assays could be up to ten times that of a rapid test. Patients in the public sector may not be able to afford repeated screening during the ART work-up. For this reason it is recommended that rapid tests be used for continual screening during the ART work-up and in conjunction with an initial ELISA HIV test on the first visit. The initial ELISA test will provide accurate results for HIV (but won't be available on the same day) while the rapid test run in conjunction will allow for detection of HIV positive patients before they provide the laboratory with a semen sample. To ensure that seroconversion (especially in serodiscordant couples) is detected during the ART work-up and before an ART cycle, rapid tests can be performed at prescribed intervals (see Chapter 2 for details). The repeated rapid testing will aid in the problem caused by lack of detection during the window period. The window period should not be a reason to defer from HIV testing. Rapid testing can produce decidedly sensitive and specific result in approximately 20 – 30 minutes, making same-day testing a feasible option.[3] Rapid testing in conjunction with more sensitive testing is advised in situation where there is a substantial risk of infection.[4] However, the use of rapid testing is not suitable in all settings. Rapid testing is appropriate in high-prevalence settings, when a rapid turnaround time is desirable and in settings where the patient is unlikely to return for test results.[4]

At present the protocol at the Reproductive and Endocrine Unit does not incorporate rapid testing. The HIV status of patients is often unknown on commencement of semen analysis. This study (refer to Chapter 4) could not identify changes in semen that could suggest the possibility of HIV infection. Although viscous semen samples are found more often in HIV positive men; viscosity in semen can also be attributed to many additional factors and is not exclusively associated with HIV infection. The lack of multiple altered semen parameters in HIV-positive samples further supports the need for simpler, quicker, point-of-care HIV screening at the initial visit. In addition to rapid testing for the identification of HIV-positive samples, before they reach the laboratory,

universal precautions and good laboratory practice should always be adopted when working with bodily fluids such as semen. Every bodily fluid sample (semen, follicular fluid, blood etc.) should be handled as possibly infected.[5]

In an ART laboratory there is a series of high-risk events that can lead to the transmission of infectious agents putting both laboratory staff and potential mothers and their desired offspring at risk.[6] A comprehensive safety programme should be embraced in order to reduce transmission of infectious diseases in the ART laboratory with written safety policies and continues employee training.[6] The treatment of blood-borne virus positive patients (HIV, HBV and HCV) should be executed in laboratories with specifically dedicated areas, in which the proper safety measures are followed.[5] If separate laboratory facilities are not available, patients with BBVs should be batched and allotted to specific sequence or time slots throughout the working day, after which precise cleaning and sterilization of the laboratory must be performed.[5] At the Unit at SBAH diagnostic semen samples of unknown HIV status and known HIV-positive samples are handled in a dedicated area and equipment separate from HIV-negative samples. Furthermore samples (all gametes) for therapeutic ART procedures are handled in two separate laboratories dedicated to i) HIV-positive and ii) HIV-negative patients. Cryopreservation storage units are also separately allocated to HIV-positive and HIV-negative samples. The Unit at SBAH is one of the few ART units in South Africa with a dedicated HIV-positive laboratory.

HBV and HCV are not commonly tested for at the Unit at SBAH. The prevalence of these infections among the population group at the Unit was found to be low and therefore costly laboratory-based assays for detection of these infections is not recommended. However, screening of HBV and HCV is important in an assisted reproduction setting and is recommended by international guidelines. Therefore, rapid testing could be extended to incorporate these infections in the full work-up of a patient attending the Unit in a cost effective manner.

Conversely, the prevalence of *Chlamydia trachomatis* was found to be 31.3% among women seeking reproductive assistance at the Unit of Steve Biko Academic Hospital. The study size and lack of sensitivity coupled with the high rate of positive female samples detected by the rapid testing and lack of positive male samples (possibly due to bias from collection of male urine samples) warrants further investigation into the true prevalence of *Chlamydia trachomatis* in the population group at the Unit. It could be recommended that an alternative rapid test be used that is more user friendly and less bias to time and type of sample collection. Rapid testing would be a cost effective means to detect *Chlamydia trachomatis* infection in patients attending the Reproductive and Endocrine Unit. On detection of *Chlamydia trachomatis* both partners should be treated accordingly, if the male partner is not treated, reinfection could take place.[6] A secondary recommendation, in the absence of an appropriate rapid test, would be that all patients seeking assisted reproduction at the Unit be given prophylactic treatment for *Chlamydia trachomatis* as a preventative action.

The findings and recommendations of this study are summarised in Table 5.1 below. To conclude, a concerted effort should be made to provide guidelines similar to those provided by ESHRE and ASRM for the screening and handling of couples in South African ART units. Guidelines should be specifically geared to the specialised needs of ART clinics in resource-limited and developing countries (such as South Africa) in the throes of the HIV epidemic.

Table 5.1: Summary of study finding and recommendations.

Study section	Findings and Recommendation
HIV rapid testing	• Simple, quick and cost effective • Plausible option for HIV screening in an ART setting • To be performed in conjunction with HIV ELISA assay on initial visit • Feasible for continual screening during the ART work-up and before an ART cycle
HBV and HCV screening	• Screening for BBVs is recommended by international guidelines • Low prevalence in the studied population • Rapid testing could be utilised to add HBV and HCV to the work-up of ART patients in a cost effect manner
***Chlamydia trachomatis* screening**	• Prevalence was high among the female population in this study • Rapid test used was not ideal and cumbersome to use • Infected males were potentially not detected due to collection method utilised in the study • Alternative rapid test should be investigated • Prophylactic treatment could be prescribed in the absence of suitable testing methods
Semen profile of HIV positive samples	• No definite semen alteration were identified • Viscose samples are an indication of co-infection and a potential warning sign for further investigation • Further substantiates the need for rapid testing • ARV treatment is known to effect sperm motility, shown also in this study and warrants further investigation

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Section C: *Addendums*

Addendum A: Draft Article

PROFILING OF HIV-POSITIVE SEMEN SAMPLES IN A SOUTH AFRICAN POPULATION

M. Stander[1], K. Richter[2] & C. Huyser[1]

[1]Reproductive Biology Laboratory, Steve Biko Academic Hospital, Department Obstetrics & Gynaecology, University of Pretoria, South Africa

[2]Department of Medical Virology, University of Pretoria/National Health Laboratory Service, Tshwane Academic Division, South Africa

Key words: HIV-semen-sperm parameters-seropositive male

Correspondence

Melissa Stander, Reproductive Biology Laboratory, Department of Obstetrics and Gynaecology, University of Pretoria, Steve Biko Academic Hospital, Pretoria, South Africa.

Tel.: +27 12 354 5138/2208

Fax: +27 12 329 6258

E-mail: melissa.stander@up.ac.za

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Summary

Human immunodeficiency virus type 1 (HIV-1) is found in semen, however, information on the effect of the virus on sperm parameters, for South African men, is unknown and was investigated in this study. Basic semen analyses were performed on samples from patients participating in the HIV semen decontamination programme at Steve Biko Academic Hospital. Sperm parameters (volume, viscosity, pH, morphology, elevated non-sperm cells, concentration and motility) between HIV-1 seropositive and negative males (n = 60, respectively) were compared. Randomly selected HIV-1 seronegative males who participated in a successful ART cycle (βHCG positive 10 to 14 days after embryo transfer), were included in this study. Furthermore, the effect of antiretroviral therapy on sperm parameters was studied. None of the investigated sperm parameters were impacted by HIV infection ($p > 0.05$). However, progressive motility of spermatozoa was negatively impacted by antiretroviral therapy (44.6%±15.7 vs. 53.4±12.1; $p = 0.0192$). In this study a definite profile for semen samples from HIV seropositive men was not observed, illustrating the importance of effective HIV screening of patients prior to assisted reproductive techniques.

Introduction

An estimated 10% of the South African population is HIV-1 seropositive, with approximately 15.9% of adults (17.4% females and 13.3% males) in their reproductive years (15 – 49 years of age) (SA Stats, 2013). The lifespan of HIV positive persons has increased due to improved antiretroviral (ARV) therapy at a higher CD4 threshold. As a result, the percentage of HIV related deaths in South Africa has decreased from 40.4% to 31.9% in the last 12 years (SA Stats, 2013). In 2013, the estimated life expectancies for males and females are 57.7 and 61.4 years, respectively (SA Stats, 2013). Due to the increased life expectancy and improved quality of life, many HIV-1 seropositive patients desire to have their own genetically related offspring. However, among HIV-

serodiscordant couples where the male partner is HIV positive, unprotected intercourse carries an average viral transmission risk of 0.1% (Gilling-Smith, 2006; Powers *et al*., 2008). The risk of HIV transmission is increased through co-infections enhancing the risk for viral replication, CD4 cell decline and accelerating immune function deterioration (Lawn, 2004). Safer conception among HIV-1 seropositive couples is, therefore, crucial.

HIV-1 seropositive couples can conceive without vertical or horizontal transmission through assisted reproductive techniques (ART), by the employment of effective risk reduction procedures (Bujan *et al*., 2007; Duliost *et al*., 2002; Garrido *et al*., 2005; Shisana *et al*., 2008). Studies have shown that the HIV virus could be present in semen samples, even when HIV blood loads are below the lowest limit of detection (Duliost *et al*., 2002), and can negatively alter semen parameters (Sauer, 2005; Nicopoullos *et al*., 2004). The effect of HIV-1 on semen parameters such as ejaculate volume, viscosity, non-spermatic cells and sperm motility, concentration, and morphology has been addressed in several studies (Bujan *et al*., 2007; Duliost *et al*., 2002; Garrido *et al*., 2005; Van Leeuwen *et al.*, 2004). However, consensus has yet to be established on whether or not these differences are significant. This is the first report (to our knowledge) on the semen parameters of South African HIV-1 seropositive men.

The HIV status of patients undergoing diagnostic evaluation at the Reproductive and Endocrine Unit of Steve Biko Academic Hospital (SBAH) is not always available upon the commencement of basic semen analyses. Consequently, all semen samples are handled as potentially infected and universal precautions are utilized. Still, the need for the identification of infected samples entering the laboratory exists (Huyser *et al*., 2010). The aim of this study was to compare the sperm parameters and characteristics of semen samples from HIV-1 seropositive and negative male patients seeking ART at SBAH.

Materials and Methods

Institutional approval for this study was received from SBAH, and the Research Ethics Committee of the University of Pretoria (protocol number S64/2010).

Study Population

i. Semen samples of HIV-1 seropositive male patients (n = 60) partaking in the semen decontamination programme between March 2010 and May 2012 were evaluated. The study population was subdivided into patients with and without ARV therapy. The mean age of the study population was 39 ±6.14 years (range from 26 to 54 years of age). For a control group (n = 60), randomly selected HIV-1 seronegative males who participated in a successful ART cycle (βHCG positive 10 to 14 days after embryo transfer), were included in this study. The mean age of the control group was 37 ±5.63 years (range from 24 to 47 years of age).

Semen Analyses

Semen samples from male patients participating in the semen decontamination programme were used. Samples were produced after 2-5 days abstinence by means of masturbation. The semen sample was left to liquefy at 37°C for 30 minutes. A basic semen analysis was performed according to the guidelines set out by the World Health Organization's laboratory manual for the examination and processing of human semen (WHO, 2010). The following semen parameters were recorded and compared: semen volume, viscosity, pH, sperm morphology, non-spermatic cells, sperm concentration and sperm motility.

Statistical Analysis

Semen samples from HIV-1 seropositive and negative groups were compared with respect to volume, pH, morphology, concentration and motility, using the Welch t-test for unequal variance. Results are reported using mean and standard deviation along with the p-value and the 95% confidence interval Furthermore, the HIV-1 seropositive and negative groups were also equated

with regards to the binary semen parameters (viscosity and non-spermatic cells) using the Fisher's exact test. Patients in each category group with elevated values are reported as a percentage along with the p-value. The semen parameters from HIV-1 seropositive patients receiving ARV therapy were compared to patients not receiving ARV therapy, through similar statistical analyses.

Results

Results for the semen parameters from HIV-1 seropositive males compared to seronegative males are summarized in Table 1. No significant differences ($p > 0.05$) were found for any of the semen parameters evaluated.

The results of HIV-1 seropositive patients categorized according to patients receiving ARV therapy (n = 39), and ARV-naive (n = 21), are summarized in Table 2. Significantly increased percentages of immotile (45.8%±15 vs. 38.8%±11.4; $p = 0.0456$) and decreased percentages of progressively motile (44.6%±15.7 vs. 53.4%±12.1; $p = 0.0192$) spermatozoa were observed in the group receiving ARV therapy.

Discussion

The presence of HIV in semen samples from men, even when blood viral loads are beneath the lowest limit of detection (Duliost *et al*., 2002), indicates that conception through unprotected intercourse should not be considered as an options for HIV-1 serodiscordant couples (where the male is positive). Procreation by ART, employing methods such as sperm washing to lessen the risk of viral transmission ought to be actively promoted in a health-conscious environment. Therefore, semen samples that could potentially harbour HIV should be identified and treated accordingly. Many semen samples of unknown HIV status are received at the diagnostic laboratory of SBAH. Even though all samples are treated as potentially hazardous and universal precautions are

applied, the identification of HIV-positive samples will improve risk reduction procedures during ART.

Results from this study indicated similar semen parameters from HIV-1 seropositive and negative men ($p > 0.05$). However, the group of patients receiving ARV therapy presented with a decrease in sperm progressive motility when compared to the group not receiving treatment (44.6% vs. 53.4%; $p = 0.0192$). This decrease in sperm motility is in accordance with other reports, and could be a result of damage to sperm mitochondrial DNA caused by specific ARVs (Cote, 2007; Kakud, 2000; Lewis *et al*., 2007; Pinti *et al., 2006).*

No definite profile for semen samples from HIV-1 seropositive men could be established in this study. Attempting to identify semen samples that could potentially contain HIV by performing standard semen analyses would, therefore, be unfeasible, without further pathology testing. Results found emphasises the need for rapid HIV screening of patient's, prior to ART. The early identification of semen samples potentially containing HIV, will improve service delivery and laboratory safety.

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Table 1: Semen parameters of the HIV-positive group compared to the HIV-negative group.

	HIV-positive (n = 60)		HIV-negative (n = 60)			
Semen Parameter	**Mean (range)**	**SD**	**Mean (range)**	**SD**	**p-value**	**95% CI**
Volume (ml)	2.7 (0.7 – 7)	1.27	2.3 (0.3 – 4.5)	0.93	0.0548	[-0.80 ; 0.01]
Viscosity (% viscose)	21.6	-	30	-	0.4040	-
pH	7.4 (6.4 – 7.7)	0.22	7.3 (6.4 – 8)	0.33	0.0943	[-0.19 ; 0.02]
Morphology (%)	6 (1 – 13)	2.96	6.8 (1 – 14)	3.06	0.1683	[-0.35 ; 2.00]
Non-spermatic cells (% >1x10^6)	23.3 (0.1 – 6)	-	15.3 (0.1 – 2.4)	-	0.3540	-
Concentration (x10^6/ml)	53.1 (0.9 – 345)	47.95	40.7 (0.1 – 140)	34.12	0.1073	[-27.4 ; 2.73]
Sperm motility: Immotile (%)	43.4 (4 – 77)	14.78	44.2 (3 – 90)	20.02	0.8051	[-5.49 ; 7.06]
Sperm motility: Non-progressive (%)	8.5 (1 – 30)	5.71	9.2 (1 – 47)	7.57	0.5237	[-1.64 ; 3.21]
Sperm motility: Progressive (%)	47.7 (10 – 72)	15.03	46 (2 – 80)	20.39	0.6007	[-8.20 ; 4.76]

Table 2: Semen parameters of the HIV-positive group compared with respect to the ARV category.

	ARV – Yes (n = 39)		ARV – No (n = 21)			
Semen Parameter	**Mean (range)**	**SD**	**Mean (range)**	**SD**	**p-value**	**95% CI**
Volume (ml)	2.8 (0.9 – 6)	1.19	2.7 (0.7 – 7)	1.30	0.7805	[-0.79 ; 0.60]
Viscosity (% viscose)	38.1	-	25.6	-	0.3810	-
pH	7.4 (7 – 7.7)	0.16	7.4 (6.4 – 7.7)	0.30	0.2633	[-0.22 ; 0.06]
Morphology (%)	5.5 (1 – 13)	2.88	6.2 (1 – 10)	2.10	0.2691	[-0.58 ; 2.03]
Non-spermatic cells (% >1x10^6)	25.6 (0.1 – 3.5)	-	19.1 (0.1 – 6)	-	0.7510	-
Concentration (x10^6/ml)	53.5 (0.9 - 345)	54.8	52.4 (10.2 – 118.5)	32.8	0.9221	[-23.8 ; 21.5]
Sperm motility: Immotile (%)	**45.8 (4 – 77)**	**15.0**	**38.8 (20 – 70)**	**11.4**	***0.0456***	**[-14.0 ; 0.14]**
Sperm motility: Non-progressive (%)	9.3 (2 – 30)	6.36	6.8 (1 – 16)	3.87	0.0613	[-5.17 ; 0.12]
Sperm motility: Progressive (%)	**44.6 (10 – 72)**	**15.7**	**53.4 (20 – 67)**	**12.1**	***0.0192***	**[1.50 ; 16.1]**

Addendum B: Faculty day poster

University of Pretoria, Health Sciences Faculty Day, 20 August 2013

RAPID TESTING: THE CONTINUAL SCREENING OF HIV IN AN ASSISTED REPRODUCTIVE SETTING

Introduction

An assisted reproductive treatment (ART) program is offered at Steve Biko Academic Hospital, with automated combo HIV ELISA testing being performed on all new patients according to current protocol. Positive results are then confirmed with an HIV viral load. HIV-negative patients are retested after six months to one year depending on potential risk behaviour. Regular screening is not performed during or after an ART cycle. An automated HIV test can cost 10 times that of a rapid test. Financial constraints of patients in the public service sector contribute to the lack of follow-up and patient drop-out, preventing HIV-positive patients from receiving their status result and the necessary treatment. Simpler and quicker testing methods for sexually transmitted infections may address this problem. The aim of the study was to evaluate an alternative, more affordable and regular point-of-care HIV screening protocol in an ART setting.

Materials & Methods

- Participants: patients (n = 100) – unaware of their HIV status (mean age was 35 years).
- HIV rapid testing was performed using the Determine® HIV-1/2 Ag/Ab Combo 100 Test kit with accessories.
- Confirmatory rapid testing was performed on the BIO-RAD Multispot HIV-1/HIV-2 rapid test.
- All rapid testing was performed according to the manufacture's specifications (see figure 1 and 2).
- Blood samples were drawn from patients, by a qualified nurse (2 x BD Vacutainer® blood collection tubes per patient):
  - One tube of blood was sent for analysis on the Architect HIV Ag/Ab Combo assay – Abbott (automated combo ELISA test).
  - One tube of blood was utilised on the Determine® rapid test , and BIO-RAD Multispot rapid test (for confirmation of positive results).
- All results obtained from the rapid tests were confirmed through the automated combo ELISA assay. See figure 3 for rapid test confirmation procedure.

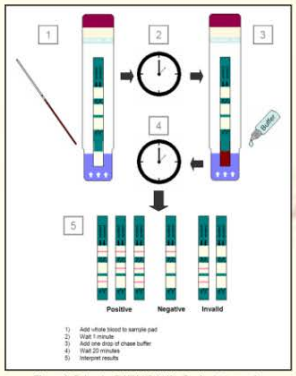

Figure 1: Determine® HIV-1/2 Ag/Ab Combo test procedure illustrating steps 1 – 5 and possible result interpretation.

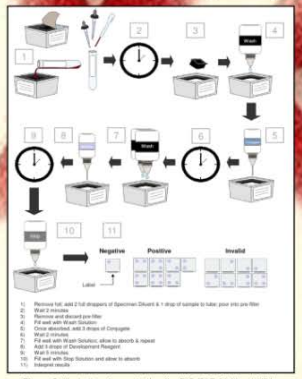

Figure 2: Illustration demonstrating the BIO-RAD Multispot HIV-1/HIV-2 Rapid test procedure and result interpretation.

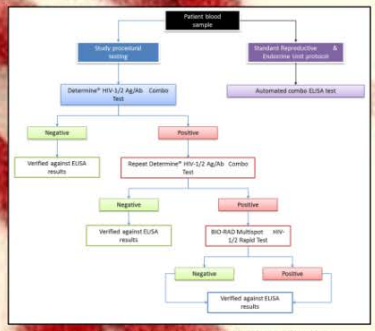

Figure 3: Verification procedure for positive and negative blood results.

Results

- Sample cohort: 53 male and 47 female patients.
- Predominantly South Africans patients tested (see table 1).
- Of the100 samples tested using the Determine® HIV-1/2 Ag/Ab Combo rapid test, 8% were positive for HIV.
- All positive samples came from serodiscordant couples, with 25% of positive patients being male and 75% being female (see table 2).
- In the study population the risk of a female being HIV positive was 3.73 fold that of males.
- All rapid test results were compared to the automated combo ELISA results from the same day.
- No discrepancies were found between the Determine® HIV-1/2 Ag/Ab Combo, BIO-RAD Multispot HIV-1/HIV-2 and automated combo ELISA.

Table 1: Distribution of HIV in sample cohort by country of origin

Country of Origin	Total	HIV-positive	HIV-negative	Percentage HIV-positive
Cameroon	1	0	1	0%
Congo	3	0	3	0%
Malawi	1	0	1	0%
Nigeria	3	0	3	0%
South Africa	89	7	82	7.9%
Zimbabwe	3	1	2	33.3%
Total	***100***	***8***	***92***	***8%***

Table 2: Percentage of positive results in the sampling population

Sample Group	Total	HIV-positive	Percentage HIV-positive
Total study population	100	8	8%
Males	53	2	25%
Females	47	6	75%

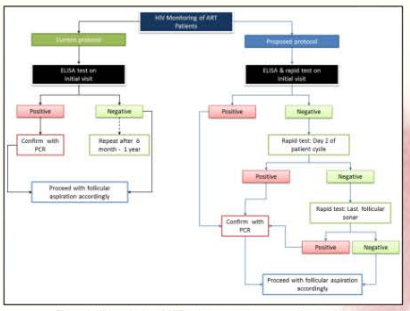

Figure 4: HIV monitoring of ART patients: current vs. proposed protocol.

Discussion

According to the current protocol, patients are not regularly screened for HIV to minimise costs. Point-of-care rapid testing may allow for more regular and affordable identification of patients who become HIV-positive before or during an ART cycle (see figure 4). The lag period before performing ART strengthens the need for continual HIV screening. This will improve the safety of staff and patients. Furthermore, patients will receive the appropriate ART procedure e.g. semen decontamination. In conclusion, early detection of HIV-positive patients and continual screening for seroconversion can be performed at a minimal additional cost through rapid testing.

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UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA
Faculty of Health Sciences

M. Stander[1], K. Richter[2] and C. Huyser[1]

[1]Department Obstetrics & Gynaecology, University of Pretoria, South Africa

[2]Department of Medical Virology, University of Pretoria/National Health Laboratory Service, Tshwane Academic Division, South Africa