AN INVESTIGATION OF THE IMPACT OF UNIVERSAL HEPATITIS B VIRUS (HBV) VACCINATION AMONG YOUNG BLOOD DONORS IN SOUTH AFRICA

Wendy Sykes

Submitted in partial fulfilment of the requirement for the degree of Master of Health Science in Medical Laboratory Science in the Department of Biomedical and Clinical Technology at the Durban University of Technology

Supervisor:

Dr Pavitra Pillay
Senior Lecturer
Department of Biomedical and Clinical Technology
Faculty of Health Sciences
Durban University of Technology

Co-Supervisor:

Marion Vermeulen
Senior Manager, Operations Testing
South African National Blood Service

DECLARATION

I, Wendy Sykes, declare that this is my own work and that it has not been submitted for any degree or examination at any other university.

Signature: Date: 30 November 2020

DEDICATION

This study is dedicated to the Blood Services in South Africa for the work they do in saving lives through the gift of life, and to the blood donors who selflessly give of themselves to save others.

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I would like to thank my supervisor Dr Pavitra Pillay for her guidance and encouragement to keep going. You have patiently steered me through the process and I am forever grateful.

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Lastly, to my parents, Herby and Jenny Edwards, thank you for always believing in me and encouraging me to believe in myself. Thank you for setting the example I try to follow and for loving me unconditionally. I miss you every day.

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Anti-HBc	Antibody to Hepatitis B core antigen
Anti-HBs	Antibody to Hepatitis B surface antigen
DNA	Deoxyribonucleic acid
ECZ	Eastern Cape Zone
EZ	Egoli Zone
EPI	Expanded Programme on immunisation
FS/NC	Free State/Northern Cape
HBcAg	Hepatitis B core antigen
HBeAg	Hepatitis B e antigen
HBsAg	Hepatitis B surface antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HCW	Health care workers
HepB3	Hepatitis B vaccine 3-dose
HIV	Human Immunodeficiency Virus
ICT	Information communication technology
ISBT	International Society for Blood Transfusion
IU/L	International Units / litre
KZN	KwaZulu-Natal
MpZ	Mpumalanga Zone
nm	Nano meters
NZ	Northern Zone
OBI	Occult HBV infection
RNA	Ribonucleic acid
SA	South Africa
SANBS	South African National Blood Service
USA	United States of America
VZ	Vaal Zone
WCBS	Western Cape Blood Service
WHO	World Health Organisation

ABSTRACT

Background

Hepatitis B Virus (HBV) is endemic in South Africa (SA) since more than 70% of the population is exposed, and this poses a significant risk to the South African blood supply. In April 1995, SA introduced universal HBV vaccinations for newborns, who became eligible to donate blood in 2011. The South African National Blood Service (SANBS) reported a 69% decline in the HBV rate in blood donors <20 years of age between 2010 and 2015, while the HBV rate remained relatively unchanged in older donors. The aim of this study was therefore to determine the rate of HBV vaccination in South African blood donors and thereby determine whether the decrease in HBV rates in younger blood donors can be attributed to HBV vaccination. Blood donors are routinely screened for Hepatitis B Virus, Hepatitis C Virus and Human Immunodeficiency Virus using both molecular and serological techniques. In order to determine the HBV vaccination rate, Hepatitis B negative first time blood donors were also tested for antibodies to Hepatitis B surface antigen (Anti-HBs) and antibodies to Hepatitis B core antigen (Anti-HBc).

Methods

A total of 1072 routine blood donors from SANBS and the Western Cape Blood Service (WCBS) were included in the study. These blood donors were stratified into two groups: the pre-vaccination era donors included 536 donors aged 24-28 years who were born before the introduction of universal HBV vaccination in April 1995, and the post-vaccination era donors included 536 donors aged 18-24 years born after April 1995. The two groups were matched for geographic location, gender and ethnic group. Donors with an Anti-HBs titre greater than 10 IU/L and negative for Anti-HBc were deemed vaccinated. Significance was determined using the Chi square test and multivariable logistic regression.

Results

Of the 1072 donors included in the study, 275 (25.7%) tested Anti-HBs titre > 10 IU/L and Anti-HBc negative and were therefore deemed HBV vaccinated. There were 87/538 (16.2%) in the pre-vaccination era donors and 188/536 (35.1%) in the post-vaccination era donors. In the pre-vaccination era, vaccination rates were highest among White donors (22.5%) and donors from the Free State/Northern Cape (37.1%). In contrast, Asian donors (54.8%) and those from the Northern Zone (41.5%) had the highest vaccination rates in the post-vaccination era. All differences were significant ($p<0.0001$). Male and female donors had similar vaccination rates in both periods (pre-vaccination group 16.8%, 15.7% ($p=0.82$), post-vaccination group: 34.8%, 35.4% ($p=0.96$) respectively. Multivariable analysis, after controlling for geography, gender,

and ethnicity showed that donors from the post-vaccination era had a 2.9 times greater odds of being vaccinated than donors born in the pre-vaccination era (OR 2.89, 95%CI, 2.16-3.89). Compared to Coloured donors, White donors had 2.1 times greater odds of being vaccinated (OR 2.1, 95% CI, 1.21-3.65). No statistically significant odds were noted for geography and gender.

Summary / Conclusions

A quarter of the donors tested showed evidence of being vaccinated for HBV. The HBV vaccination rate increased significantly in younger donors born after the 1995 introduction of universal HBV vaccination in South Africa, indicating programme efficacy. Vaccination rates increased in all population groups in the post-vaccination era, with the greatest increases among Asian and Coloured donors, suggesting better uptake of the programme among these groups. Other than being born in the vaccination era, ethnicity was the only factor independently associated with being vaccinated. As young vaccinated donors make up more and more of the donor panel, it is expected that there will be a significant decrease in HBV rates and a concomitant increase in blood safety. HBV however remains an important public health and blood transfusion issue and continued efforts are required to strengthen the implementation and coverage of HBV vaccination programmes targeting all population groups, including the most vulnerable groups and people living with HIV. In addition, consideration of birth-dose vaccination to prevent new infections and access to affordable treatment options are also key to achieving the goals for HBV elimination in South Africa.

CHAPTER 1: INTRODUCTION

1.1 BACKGROUND

The Hepatitis B Virus (HBV) is endemic in South Africa (SA), with an estimated sero-prevalence of between 8 - 10%. At least 70% of the SA population will be exposed to HBV during their lifetime and approximately 10% will become chronic carriers (Tsebe *et al.* 2001). Infection with HBV can lead to acute or chronic hepatitis, with an estimated 25% of chronic carriers expected to die from liver disease, cirrhosis or hepatocellular carcinoma (HCC) (Burnett *et al.* 2012). Hepatitis B is a vaccine preventable disease, with vaccination being the most effective way to control the disease (Tsebe *et al.* 2001). Infection with this virus is highly contagious and it is estimated to be 100 times more infectious than Human Immunodeficiency Virus (HIV) (Spearman *et al.* 2013). HBV can be transmitted parenterally, or through exposure to infected blood and blood products (Shepard *et al.* 2006). In highly endemic areas like SA, HBV can be transmitted horizontally by close personal contact especially among young children and there is a high risk of progression to chronic HBV and death (Büchner *et al.* 2014; Spearman and Sonderup 2014).

Globally over two billion people are infected with HBV, with 250 – 400 million chronic carriers (Shepard *et al.* 2006). The World Health Organisation (WHO) estimates that over 100 million people have chronic HBV infection in Africa (Breakwell *et al.* 2017), with more than 65 million in sub-Saharan Africa (Spearman and Sonderup 2014). It is estimated that without treatment 15 - 25% of these will die from cirrhosis and HCC (Burnett *et al.* 2012; Breakwell *et al.* 2017). The age of infection with HBV affects progression to chronic disease; 70-90% of infants infected before 1 year of age, 20-50% of children infected at 1-5 years of age and 5-10% of those infected after age 5 will develop chronic HBV infection. Between 500 000 and 1.2 million HBV related deaths occur annually (Spearman and Sonderup 2014), with HCC being the third leading cause of cancer related deaths globally (Spearman and Sonderup 2014).

Hepatitis B Virus

HBV is a small circular, partially double-stranded deoxyribonucleic acid (DNA) virus belonging to the Hepadnaviridae family (Spearman *et al.* 2013). The infectious virion, also known as the Dane Particle, is 42 - 47nm in diameter and consists of a lipid envelope containing spherical or tubular hepatitis B surface antigen (HBsAg) surrounding an inner core composed of hepatitis B core antigen (HBcAg), hepatitis B e antigen (HBeAg), a double stranded DNA molecule and DNA polymerase, as seen in Figure 1.1 (Shepard *et al.* 2006; Liang 2009).

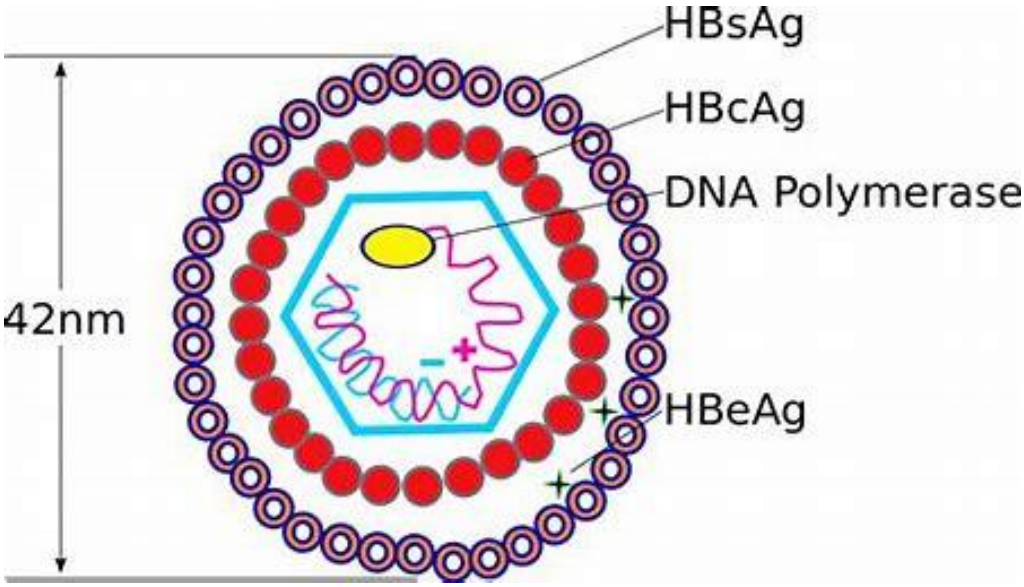

Figure 1.1: Structure of the Hepatitis B Virus
Source: Colm (2007)

HBV DNA, HBsAg and antibodies to HBcAg (Anti-HBc) are the first serological markers to become detectable in an acute HBV infection, as seen in Figure 1.2. Total Anti-HBc persists for life (representing core IgG antibody) and is an indicator of a past infection with exposure to HBV, while Anti-HBc IgM becomes undetectable 6 – 12 months' post infection. Total Anti-HBc is usually present in chronic carriers and in people who have recovered from an HBV infection. Antibodies to HBsAg (Anti-HBs) may indicate immunity with titre levels >10 IU/L, and this can be due to vaccination or due to a naturally resolved infection (in which case Anti-HBc will be present as well) (Shepard *et al.* 2006).

Table 1.1: Markers present in acute and chronic HBV infection

	HBsAg	Anti-HBc Total	Anti-HBc IgM	Anti-HBs	HBV DNA
Acute Infection	Positive	Positive	Positive	Negative	Positive
Chronic Infection	Positive	Positive	Negative	Negative	Positive

Source:(Centre for Disease Control and prevention 2012)

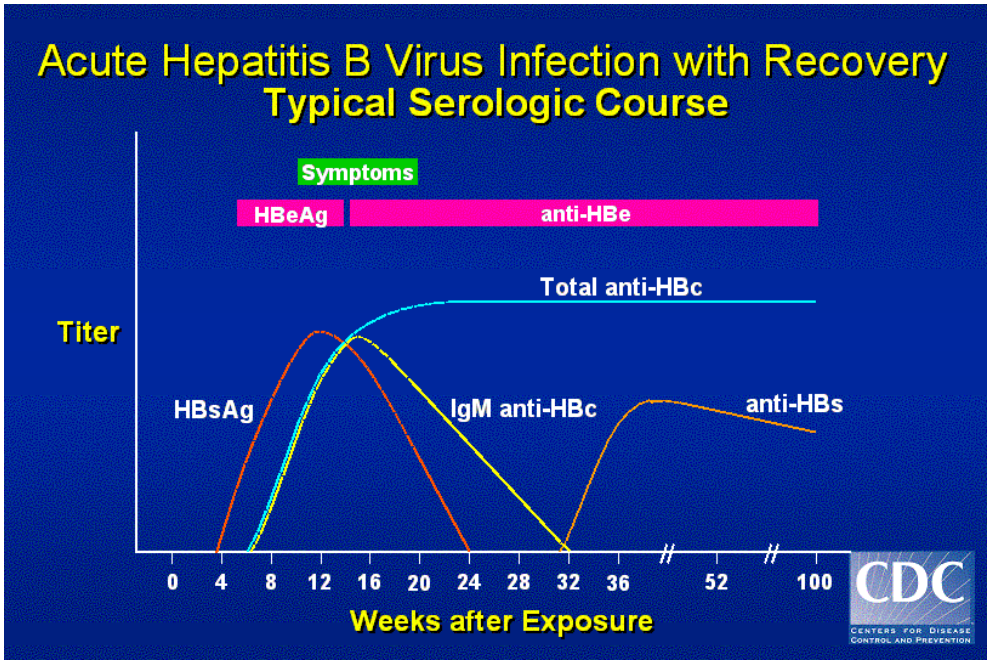

Figure 1.2: Serological course of an acute HBV infection

Source: (Centre for Disease Control and prevention 2012)

In a chronic HBV infection, HBsAg persists for at least 6 months or is detectable in the absence of Anti-HBc IgM and Anti-HBs, as seen in Figure 1.3. Fifteen to twenty-five percent of those chronically infected with HBV die prematurely from cirrhosis or HCC (Shepard *et al.* 2006).

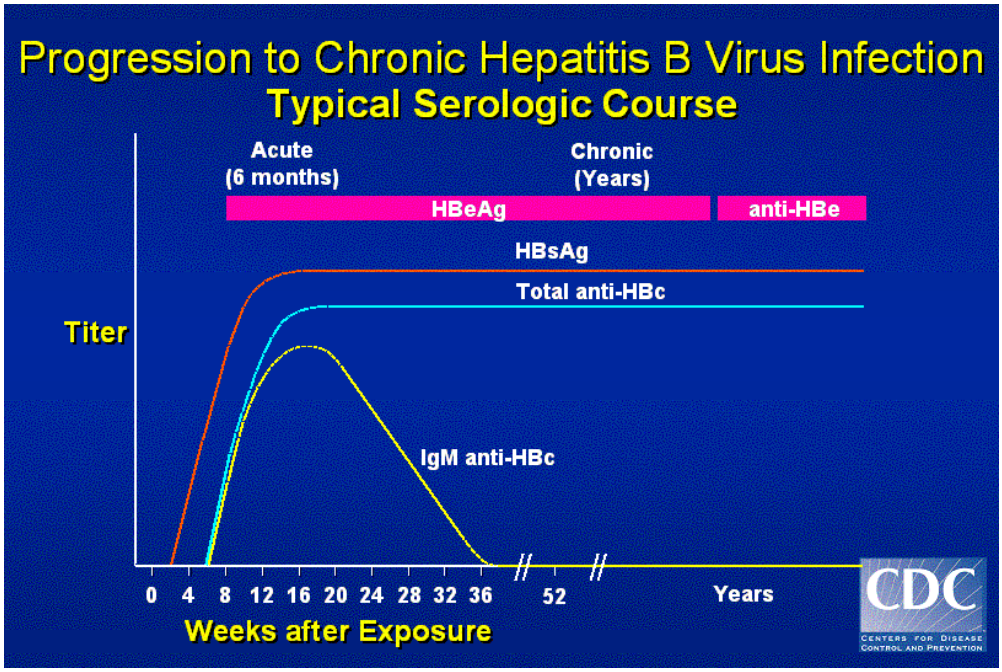

Figure 1.3: Serological course of a chronic HBV infection

Source: (Centre for Disease Control and prevention 2012)

HBV Vaccines

The World Health Organisation (WHO) recommended the incorporation of universal HBV vaccination into the Expanded Programme of Immunisation (EPI) in 1991 as a means to reduce the incidence of HBV infection (Lavanchy 2004). In April 1995, SA introduced universal HBV vaccination to new-borns (at 6, 10, and 14 weeks of age) as part of the EPI-SA. The aim of the EPI-SA is to prevent deaths and reduce suffering from vaccine preventable diseases such as measles, polio, diphtheria, whooping cough (pertussis), tetanus, hepatitis B, *Haemophilus influenza* type b, rotavirus diarrhoea, pneumococcal diseases and tuberculosis (Dlamini and Maja 2016).

Prior to the introduction of universal HBV vaccination, the sero-prevalence of HBsAg in SA was between 0.2 to 10%, and those with evidence of past exposure (HBsAg negative, Anti-HBc positive) between 5 and 76% (Spearman and Sonderup 2014). Sero-prevalence of HBsAg was found to be higher in rural areas compared to urban areas, with marked differences between ethnic groups and gender (Spearman and Sonderup 2014).

The first HBV vaccines developed in the late 1970's by the Unites States of America (USA) and France were highly immunogenic and safe plasma derived vaccines manufactured using the plasma from HBV infected patients and containing purified HBsAg (Shouval 2003). China and Korea later produced similar vaccines. Second generation non-infectious genetically engineered recombinant DNA vaccines were later produced due to inconsistency in the raw material for the plasma derived vaccines as well as advances in recombinant DNA technology e.g. Engerix-B (SmithKline Biologicals, Belgium) and RECOMBIVAX HB-Vax II (Merck & Co., USA) (Shouval 2003).

In South Africa the plasma derived Hepaccine B vaccine (Cheil Foods and Chemicals, Seoul, South Korea) (Burnett *et al.* 2012) was used from 1995 to 1999. This was changed to the more immunogenic genetically reengineered recombinant HB vaccine namely Heberbiovac HB vaccine (Centre for Genetic Engineering and Biotechnology, Havana, Cuba) and Engerix B (GlaxoSmithKline, Belgium) (Burnett *et al.* 2012). A three dose intramuscular immunisation schedule of 10µg at 0, 1 and 6 months is recommended for Engerix B vaccine, with a reported seroprotection rate after one month of 92.6 – 100% and 74.3% after 5 years, and Anti-HBs titres of 85 – 3210.9 IU/L (Keating and Noble 2003). The newest vaccines are combination vaccines for diphtheria, tetanus, acellular pertussis, inactivated poliomyelitis, *Haemophilus influenza b* and Hepatitis B, namely Infanrix Hexa (GlaxoSmithKline, Belgium) and Hexaxim (Sanofi Pasteur) (National Institute for Communicable Diseases 2016). SA introduced the new

hexavalent (6-in-1) vaccine in 2015, becoming the first country in Africa to do so (Dlamini and Maja 2016). Vaccines are administered by intra muscular injection at 6, 10 and 14 weeks (Tsebe *et al.* 2001).

Blood Transfusion in SA

There are currently two Section 21, not for profit blood transfusion services in South Africa (SA), namely the South African National Blood Service (SANBS) and the Western Cape Blood Service (WCBS). The WCBS operates in the Western Cape Province, while SANBS services the other eight provinces (Northern Cape, North West, Limpopo, Mpumalanga, Gauteng, Free State, KwaZulu-Natal and the Eastern Cape) in SA. The eight provinces are broken down into seven SANBS operational zones that roughly mirror the SA provinces:

- Free State/Northern Cape (FS/NC) Zone servicing the Northern Cape and Free State Provinces
- Mpumalanga Zone (MpZ) servicing the Mpumalanga Province
- KwaZulu-Natal Zone (KZN) servicing the KZN province
- Eastern Cape Zone (ECZ) servicing the Eastern Cape Province
- Gauteng due to its high population is split between 3 zones as per below
- Egoli Zone (EZ) covers the greater Johannesburg areas as well as the northern, southern and western suburbs
- Northern Zone (NZ) servicing Pretoria and the Polokwane area
- Vaal Zone (VZ) servicing parts of the North West Province, the northern Free State and the East Rand area in Gauteng Province and part of the northern Free State

Voluntary non-remunerated blood donors donate over one million units of blood annually in SA. All blood donations are screened for Human Immunodeficiency Virus (HIV), Hepatitis B Virus (HBV), Hepatitis C Virus (HCV) and Syphilis (serology only) using both serological and molecular techniques. The Blood Transfusion Services (BTS) have comprehensive algorithms for dealing with positive donations so that all blood products found to be positive for one of these serological markers are destroyed and the donor is notified and deferred from donating blood in the future. (Vermeulen *et al.* 2009)(Jeffery 2012).

1.2 PROBLEM STATEMENT

A 64% decrease in the HBV rate from 0.171% to 0.061% (Figure 1.4) was noted in younger blood donors (< 20 years of age) between 2010 and 2015 (Sykes *et al.* 2018). The HBV rate in donors 21 – 30 years of age declined slightly, while the HBV rate remained either constant or increased in older donors.

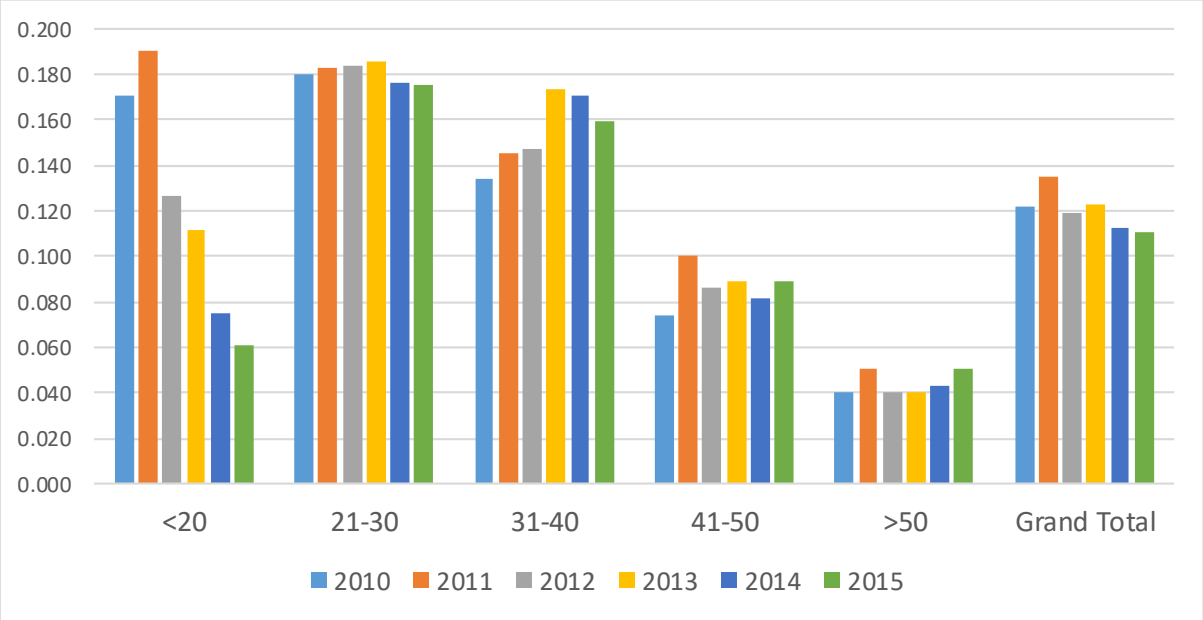

Figure 1.4: HBV infection rates in SANBS blood donors 2010 to 2015

Source: Sykes *et al.* (2018)

It is postulated that the decrease in HBV rate is related to the introduction of universal HBV vaccination in SA in April 1995 as infants vaccinated in 1995 became eligible to donate blood in 2011 at the age of 16.

Although studies have been performed to determine the impact of the introduction of universal HBV immunisation in 1995 on the SA population, there is currently very limited data on the impact on South African blood donors, and blood donors are not asked if they have been vaccinated. Blood donors are generally regarded to be a healthy sub population.

1.3 PURPOSE OF THIS STUDY / AIMS

The aim of this study was to determine the rate of HBV vaccination in South African blood donors and thereby determine whether the decrease in HBV rate in younger blood donors could be attributed to HBV vaccination. This was done by testing blood donors for antibodies to Hepatitis B surface antigen (Anti-HBs) and Hepatitis B core antigen (Anti-HBc). Two groups of HBV DNA and HBsAg negative first time blood donors were tested as follows:

Group 1: Donors born before the implementation of universal HBV vaccination in April 1995 (pre-vaccination).
Group 2: Donors born after the implementation of universal HBV vaccination in April 1995 (post-vaccination), who would have been eligible to receive HBV vaccination.

The Anti-HBs titre provided information on whether the donor had developed immunity (Anti-HBs titre > 10 IU/L) and the Anti-HBc test result provided information on whether the immunity was due to a past HBV infection or as a result of the HBV vaccination (Table 1.2).

Table 1.2: Anti-HBs and Anti-HBc result outcomes

Anti-HBs Titre	Anti-HBc	Probable Outcome
>10 IU/L	Negative	Donor is immune Immunity due to vaccination
>10 IU/L	Positive	Donor is immune Immunity from past HBV infection / exposure
Negative (<10 IU/L)	Negative	No Immunity No exposure to HBV
Negative (<10 IU/L)	Positive	A resolved infection A false positive Anti-HBc result A low level chronic infection A resolving acute infection

Source: (Büchner *et al.* 2014)

The Anti-HBs and Anti-HBc result outcomes were applied in the present study to determine whether the difference in the rate of HBV vaccination (Anti-HBs titre > 10 IU/L, Anti-HBc negative) in Group 2 donors (post-vaccination) was significantly greater than the rate in Group 1 (pre-vaccination) donors. This result would indicate that the decrease in HBV rate in younger blood doors was likely related to HBV vaccination and not to other factors.

A significant decrease in HBV rates in younger blood donors would allow the Blood Transfusion Services to implement strategies to increase donations from these donors, as they would be regarded as a safer population to collect blood from in terms of HBV reactivity.

Aim:

The aim of this study was to investigate whether the decline in HBV rate was related to the introduction of universal HBV vaccination in South Africa in April 1995.

Objectives:

1. To determine the rate of HBV vaccination in SANBS blood donors born before and after the implementation of universal HBV vaccination.

2. To determine whether the decrease in HBV rate in younger blood donors could be attributed to HBV vaccination.
3. To determine the level of HBV immunity in SANBS blood donors by region.
4. To determine whether there was a difference in HBV immunity in HIV positive vs HIV negative blood donors.

Anticipated Outcome:
To provide data on the effects of the introduction of universal HBV immunisation on SA blood donors since there was limited information on this.

1.4 CONCLUSION

Chapter One outlined the background to the study, including HBV and HBV infections, globally and in SA, the introduction of HBV immunisation in SA, the problem statement and discussed the aims and objectives of this study. The rest of the chapters outline the following: Chapter Two: Literature Review; Chapter Three: Research Design / Methods; Chapter Four: Results and Chapter Five: Conclusion and Recommendations.

CHAPTER 2: LITERATURE REVIEW

2.1 INTRODUCTION

This literature review presents an overview of the current knowledge in the field of HBV immunisations in SA and globally. Literature searches were conducted over a period of 24 months using search engines such as PubMed, Google scholar and the World Wide Web. Referenced lists of papers were also used to identify further articles and resources on the subject.

2.2 HBV IN SOUTH AFRICA AND GLOBALLY

HBV prevalence varies globally. Areas such as East and Southeast Asia and sub-Saharan Africa are highly endemic, with 50 – 95% of the population exposed and 8-20% becoming chronic carriers. Northern Europe and Northern America are low endemic areas, with 4-6% of the population exposed and <2% chronic carriers (Keating and Noble 2003). The main modes of transmission in highly emdemic areas are perinatal and vertical such as in child birth and horizontal e.g. from child-to-child. In countries where the endemicity is low, the main transmission modes are sexual and parenteral (Keating and Noble 2003).

HBV is highly endemic in South Africa (SA) with an estimated sero-prevalence of between 8 - 10% (Mayaphi *et al.* 2012). More than 70% of the population has been exposed to HBV, with an estimated 2.5 million chronic carriers. Many of these infections occur before the age of five years (Tsebe *et al.* 2001; Spearman and Sonderup 2014). In SA, HBV prevalence rates are reported to be higher in rural populations than in urban populations e.g. the HBV rate in rural Eastern Cape is reported to be 15.5% as compared to 7.4% and 1.3% in urban areas in Durban and Soweto respectively (Burnett *et al.* 2012; Spearman and Sonderup 2014). It has also been reported that the HBV carrier rate is higher in males than in females, even though both sexes are equally exposed (Burnett *et al.* 2012). Age is an important factor in the risk of developing chronic HBV, with approxiately 90% of infants born to HBV positive mothers, 20-50% of children infected before age 5 but <5% of people infected as adults developing chronic HBV (Spearman *et al.* 2013).

2.3 UNIVERSAL HBV VACCINATION IN SOUTH AFRICA

The most effective way to control HBV infection rates is with universal infant vaccination (Tsebe *et al.* 2001). In April 1995, SA became one of the first ten countries in Africa to

introduce universal HBV vaccination as part of the EPI and this has resulted in a decline in the seroprevalence of HBV from 12.8 to 3% (Spearman and Sonderup 2014). It is estimated that the introduction of universal HBV vaccination in 183 countries worldwide has prevented 1.3 million deaths (Spearman and Sonderup 2014). Prior to 1995 the seroprevalence of HBsAg in the general SA population was between 0.2 - 9.6%, with evidence of past exposure (Anti-HBc positive) between 5 – 76% (Spearman and Sonderup 2014).

A study conducted in SA, which compared a 'post-vaccine introduction population' with a 'pre-vaccine introduction population' nearly 20 years after the introduction of universal HBV immunisation, found that immunity increased from 13% (pre) to 57% (post) vaccine implementation (Amponsah-Dacosta *et al.* 2014). They also showed that the immunity declined with increasing age, that HBV chronic carriage was reduced in the post-vaccine group and that evidence of immunity was higher in HIV negative as compared to HIV positive people (Amponsah-Dacosta *et al.* 2014).

The World Health Organisation (WHO) data on coverage of the HB vaccine 3-dose (HepB3) shows vaccine coverage in SA climbing from 74% in 1997 to 88% in 2000, and 97% in 2010 (Burnett *et al.* 2012). Breakwell *et al*. (2017) reported coverage at 76% in 2011, declining to 71% in 2015. These were, however, estimates and not based on scientific surveys (Burnett *et al.* 2012).

Table 2.1: World Health Organisation estimates on the coverage of the HB vaccine 3-dose (HepB3) in African countries

Country	Year of introduction	2011 (%)	2015 (%)	Administered
South Africa	1995	76	71	6,10,14 weeks
Botswana	1995	95	95	0,2,3,4 months
Gambia	1990	96	97	0,2,3,4 months
Burkina Faso	2006	91	91	8,12,16 weeks

Source: (Breakwell *et al.* 2017)

Various studies have been conducted since the implementation of universal vaccination in SA in 1995 to evaluate the effectiveness of the programme. Hino *et al.* (2001) studied two cohorts of SA children 12 – 24 months of age, one to two years after 1995. One cohort had been

vaccinated and the other was unvaccinated. It was found that HBV DNA was present in 6.5% of the unvaccinated group compared to 0.3% in the vaccinated group, and 84.6% of the vaccinated children had protective levels of Anti-HBs (Hino *et al.* 2001). In a study conducted in rural areas (because HBV is more endemic in rural than urban areas) of all nine provinces in SA, among eighteen month old children, one year after immunisation, it was found that a protective Anti-HBs titre of at least 10 IU/L was present in 87% of the children. Only 0.4% (3/756) were HBsAg positive, showing that the vaccine had been successful in controlling the horizontal spread of HBV among young children (Schoub *et al.* 2002).

In SA, the HBV vaccine is offered free of charge to all infants under one year of age as part of the Expanded Program on Immunisation (EPI). An intramuscular injection is given at 6, 10 and 14 weeks to coincide with existing clinic visits. There is currently no catch up programme or routine vaccination for older age groups and there is no birth dose vaccination as recommended by the WHO (Burnett *et al.* 2012). This may be because in sub-Saharan Africa and SA, horizontal transmission between young children is more common than perinatal transmission (Burnett *et al.* 2012). Unfortunately, with the high prevalence of Human Immunodeficiency Virus (HIV) in SA and the likelihood that pregnant women who are co-infected will have an active HBV infection, there is concern that more mothers will infect their babies perinatally. Babies born to immunosuppressed HIV positive mothers may also not have protective levels of Anti-HBs until their first dose of vaccine at six weeks, leaving them susceptible to infection (Burnett *et al.* 2012). A few breakthrough infections have been reported in HIV-exposed or infected babies (Schoub *et al.* 2002).

2.4 CHALLENGES IN IMPLEMENTING HBV VACCINATION

Although universal HBV immunisation has been available in SA since 1995, 40% of patients tested at a paediatric haematology and oncology centre had no immunity, indicating that they had probably not been immunised or there had been an immunisation failure (Büchner *et al.* 2014). None of the patients showed signs of active infection, but six showed evidence of past infection (Anti-HBc positive) and 80% of HIV positive patients were not immune to HBV (Büchner *et al.* 2014). Shouval *et al.* (2003) showed that immunisation failure may occur despite the efficacy of the second generation vaccines due to improper storage, advanced age, obesity, chronic liver failure, immunosuppression and renal failure (Shouval 2003). This was relevant to the present study because although infants might have been immunised since 1995, there were many factors that could result in immunisation failure that would affect the findings.

Some of the challenges in implementing universal HBV vaccination in Africa are related to lack of sufficient cold chain storage and controls, lack of trained health care workers (HCW) and a high percentage of home births, especially in countries that have a birth dose vaccine (within 24 hours of birth) such as Angola, Botswana and Gambia (Breakwell *et al.* 2017). In SA, one of the challenges relates to differences in the health service quality in different geographic areas within the country. Fadnes *et al.* (2011) conducted a study in three areas in SA, namely Paarl in the Western Cape, Umlazi in KwaZulu-Natal (KZN) and Rietvlei (KZN). They found that there were significant differences in vaccination coverage and timelines (especially for second and third vaccination visits), with wealthy areas such as Paarl performing better than poorer areas such as Rietvlei. They also reported that vaccine coverage was lower if the vaccine was given at an older age (Fadnes *et al.* 2011).

Although universal HBV vaccination was introduced in 1995 at 6, 10 and 14 weeks, no "catch-up" immunisation for older age groups was implemented. The WHO reported that only 56% of babies in SA received all 3 doses of the vaccine and the duration of immunity after the first dose at 6 weeks is unknown (Burnett *et al.* 2012; Büchner *et al.* 2014). Buchner *et al.* (2014) reported that a large number of patients attending a paediatric haematology and oncology unit did not have sufficient immunity against HBV despite having been immunised as part of EPI-SA, and that some became infected with HBV in the paediatric oncology unit due to the intensive chemotherapy adversely affecting immunological functioning. They further recommended that combined active and passive immunisation be considered for oncology patients in SA (Büchner *et al.* 2014).

The challenges faced with the implementation of HBV immunisation in SA could impact on the results of this study. Lower coverage or vaccine uptake would mean fewer donors with protective levels of Anti-HBs and potentially more HBV infections.

2.5 HBV VACCINATION IN THE OTHER COUNTRIES

Peto *et al.* (2014) reported on HBV vaccination in the Gambia. The Gambia Hepatitis Intervention Study (GHIS) was conducted between 1986 and 1990 after which all infants were offered HBV vaccination in a nationwide vaccination programme. A 94% vaccine efficacy and a significant decline in the prevalence of chronic HBV infection, especially in people born in the early 1980s, was found. They further reported that HBsAg prevalence decreased from 1.4% in those born 1990 -1997 to 0.3% in those born 1998 - 2007 (Peto *et al.* 2014).

Chang *et al.* (1997) reported a decline in the HBsAg carrier rate in children in Taiwan from 10% to less than 1% in the 10 years following the launch of their nationwide vaccination programme in 1984. A significant decline in the incidence of childhood HCC was reported in children 6 to 14 years of age, from 0.70 to 0.36 per 100 000, as well as a decline in the mortality rate in children with HCC (Chang *et al.* 1997). A similar decline was seen in China. Prior to the introduction of the vaccination programme in China in 1992, 9.8% of the Chinese population were HBsAg positive. By 1999, vaccine coverage was 70.7% and up to 90% in Beijing, and by 2006 the HBsAg carrier rate had reduced to 7.2% in the general population, to 2.3% in children 5 - 14 years, and to 1% is those younger than age 5 (Luo, Li and Ruan 2012).

2.6 IMPACT OF HBV IMMUNISATION ON BLOOD TRANSFUSION GLOBALLY

There is limited data available on the impact of HBV immunisation on blood donation. Wang *et al*. (2016) reported on the prevalence of HBsAg in blood donors born before and after the implementation of universal HBV vaccination in Shenzhen, China. They reported a 2.3% HBsAg prevalence over a 10-year period from 2005 to 2014, with a higher prevalence in donors 18 – 25 years of age, suggesting that younger donors were still at risk of developing chronic HBV. HBsAg prevalence was found to be higher in first time donors born after 1992 (3.9%) than in those born before 1992 (3.2%). HBV incidence infection was lower in repeat donors born after 1992 (0.27%) than in those born before 1992 (0.6%). Although HBsAg prevalence was higher in younger donors born after implementation of the HBV vaccination (presumed vaccinated), they had a lower incidence of HBsAg seroconversion than older presumed unvaccinated donors. The authors suggested that an HBV booster vaccine for 15 to 17 year olds might help improve blood safety (Wang *et al.* 2016).

Yoshikawa *et al*. (2009) reported on a decrease in HBV rate in first time blood donors (16 – 25 years of age) in Japan following the implementation of a selective vaccination programme in January 1986 that was aimed at preventing mother-to-child HBV transmission. The rate of HBsAg positives decreased from 0.83% to 0.22% between 1996 and 2007 in all donors. HBsAg positive rates decreased from 0.399% to 0.018% and from 0.312% to 0.044% in donors aged 16 and 18 years born in 1980 (before the vaccination programme) and 1991 (after the vaccination programme) respectively (Yoshikawa *et al.* 2009). The authors further suggested that vaccination of blood donors could reduce the risk of post transfusion hepatitis B infection (Yoshikawa *et al.* 2009). A cost-benefit comparison of blood donor screening strategies and vaccination strategies was performed by Fischinger *et al.* (2010) and it was found that HBV vaccination offered a potential cost reduction, as well as greatly reduced transfusion transmitted infections (Fischinger *et al.* 2010).

Seed *et al.* (2012) described two HBV "vaccine breakthrough" infections detected in blood donors by the Australian Red Cross Blood Service. Both donors were screened for HBV DNA using the Ultrio assay and were found to be HBV DNA positive, with the only other marker present being Anti-HBs that was attributed to a full course of vaccinations between eight to ten years earlier (Seed *et al.* 2012). Anti-HBs levels > 10 IU/L are generally considered to be protective but were not in these cases as the levels were 101.2 IU/L and 23.7 IU/L respectively. The authors suggested that donors who were HBV DNA positive with apparently protective levels of Anti-HBs should be considered as having a possible acute HBV infection (Seed *et al.* 2012).

Individual donation Nucleic Acid amplification testing (ID-NAT) is performed for HBV, HCV and HIV in SA blood transfusion services (SANBS and WCBS) as it reduces the window period in comparison to serological screening. A 2018 study of SANBS blood donors born before and after the implementation of universal HBV vaccination in SA in 1995 looked at first time blood donors < 20 years of age in 2010 (donors born before 1995, presumed not vaccinated) and in 2015 (donors born after 1995, presumed vaccinated) and compared HBV infection rates. Just over 40,000 donations were analysed in each group. A higher HBV infection rate of 0.38% was found in the 2010 group, compared to 0.12% ($p<0.00001$) in the 2015 group (Sykes *et al.* 2018). There was a significant decrease in HBV rate in both male and female donors, although the decrease was slightly greater in males, possibly due to a greater impact of the vaccination programme on male donors who started with a higher prevalence in 2010. The authors concluded that the 69% decrease in HBV rate in younger blood donors demonstrated the public health benefit of improved access to health care in terms of the introduction of HBV vaccination in SA. Further investigation was required to prove that the findings were related to the introduction of universal HBV vaccination in 1995 (Sykes *et al.* 2018), in light of the varied reported findings on the impact of HBV immunisation. The present study therefore aimed to investigate whether the decrease in the HBV rate in younger blood donors was related to the introduction of HBV vaccination in SA.

2.7 HBV IMMUNITY IN PEOPLE WHO ARE HIV POSITIVE

Studies in South Africa have shown that 3 - 25% of adults are HBsAg positive, with the highest rates in adults who are also HIV positive (Spearman *et al.* 2017). Mayaphi *et al.* (2012) reported that patients with AIDS (6.5%) had a 3 - fold higher HBsAg prevalence than a control group (2%) and recommended that patients who are HIV positive and do not have HBV immunity receive HBV vaccination to prevent co-infection with HBV (Mayaphi *et al.* 2012).

Anti-HBs protective antibodies are also reported to decline in patients who are HIV positive, with as much as a 40% loss in one year compared to a 5% loss in patients who are HIV negative (Brook 2006). Immunity was found to be higher in HIV negative people than in those who were HIV positive and there were more HBV chronic carriers in HIV positive people (Amponsah-Dacosta *et al.* 2014).

A study of the effectiveness of the expanded programme of immunisation against HBV in KZN children between the ages of 5 and 15 infected with HIV showed that 2.1% of HIV-infected children were HBV positive, while 0% of HIV uninfected children were HBV positive. The response to HBV immunisation was thus significantly higher ($p<0.001$) in uninfected children (61.1%) than in HIV-infected children (15.8%) (Beghin *et al.* 2017).

An HBV seroprevalence study conducted in KZN among men and women aged 15 – 49 years in 2019 found that 6.4% of HIV-positive participants and 2.6% of HIV-negative participants were HBsAg positive. The seroprevalence was higher in HIV-positive men at 8.7% than in HIV positive women at 5.0% (Samsunder *et al.* 2019).

2.8 CONCLUSION

This study aimed to determine the impact of HBV vaccination on and the level of immunisation in young blood donors in South Africa, in light of a decrease in the HBV rate noted by SANBS between 2010 and 2015. Sustained vaccination programmes are required in order to make progress towards eliminating HBV transmissions (Shepard *et al.* 2006). It is also important to be able to measure the impact of such programmes, to improve vaccine coverage and to be able to target high-risk populations. One of the ways to do this is by measuring the impact of HBV immunisations on blood donors, to differentiate between different population groups, geographic locations, gender etc. It was anticipated that this study would provide valuable data that would enable SANBS to develop strategies to target low risk blood donor populations.

CHAPTER 3: RESEARCH DESIGN / METHODS

3.1 INTRODUCTION

This chapter describes the research methodology used in this study. An outline of the study design, study population, sampling, data collection, methodology, data analysis and ethical considerations is presented.

3.2 STUDY DESIGN

This was a cross-sectional quantitative study to determine whether the reduction in HBV prevalence in younger blood donors was due to the implementation of universal HBV vaccination in South Africa in 1995.

Data from two groups of blood donors was analysed:

Group 1:

536 first time blood donors born before April 1995 (24 – 29 years of age).

These donors were born in the pre-vaccination era (pre-vac) i.e. before the implementation of universal HBV vaccination.

Group 2:

536 first time blood donors born after April 1995 (19 – 24 years of age).

These donors were born in the post vaccination era (post-vac) i.e. after the implementation of universal HBV vaccination and would have been eligible for HBV vaccination at birth.

The two groups were matched by geographic location, population group and gender.

3.3 STUDY POPULATION

The blood samples used in this study were from voluntary non-remunerated South African blood donors. Only samples collected from routine blood donors were included, i.e. no new samples were bled for the study. Only donors between the ages of 19 and 29 years of age were selected.

Inclusion criteria: HBV DNA and HBsAg negative, first time blood donors. Only first time donors were included as repeat donors were a pre-screened population.

Exclusion criteria: Donors who tested HBV DNA or HBsAg positive were excluded from both Group 1 and Group 2 as this study was particularly focused on immunity only and not infectivity. Donors who had donated previously i.e. repeat donors were also excluded.

3.4 SAMPLE SIZE

The planned sample size was 960, 480 first time blood donors born before 1995 and 480 first time blood donors born after 1995. This provided 80% power to detect a 50% difference in Anti-HBs positive rate (>10 IU/L), assuming the Anti-HBs prevalence amongst donors born before 1995 was 5%, a two-tailed significance level of 0.05, and using a Chi square test (Figure 3.1). The actual sample size was 1072, with 536 donors in each group.

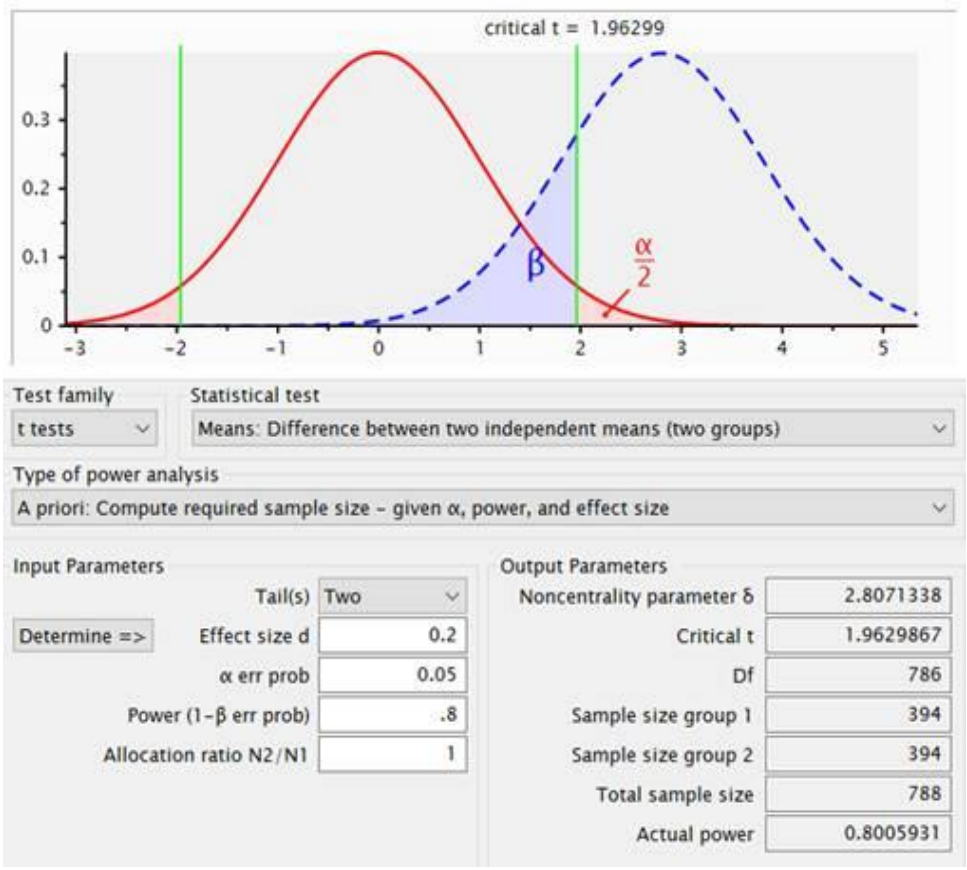

Figure 3.1: Sample size calculation
Source: (miamipsych293 2014)

3.5 DATA COLLECTION

The SANBS and WCBS Information Technology (IT) Departments designed IT programmes to interrogate the donor databases and select samples for the two groups of HBV DNA and HBsAg negative first time blood donors. Donors in Group 1 and 2 were matched by donation

region (zone), population group and gender. Donor demographic data, collected from the donors at the time of donation i.e. date of birth, gender and population group, was collected from the SANBS Business Intelligence (BI) and WCBS IT system for each donor. HIV results for all donors were obtained from the IT systems. All samples were sent to the Donation Testing Department of SANBS in Pinetown, KwaZulu-Natal for testing.

3.6 MEASUREMENTS

Predictors

There were no predictors for the primary prevalence question.
Secondary predictors were age, population group and gender.

Confounders

Each of the secondary predictors were possible confounders for each other, as shown in the table below:

Table 3.1: Predictors and possible confounders

Prevalence vs Predictor	Possible confounder
Age	Gender, population group
Gender	Age, population group
Population group	Age, gender

Other possible confounders

Geographic area

Strategies to overcome confounders

For each of the predictors multivariate analysis using logistic regression that stratified each of the listed possible confounders was performed.

Strategies to reduce errors

Testing was performed on the fully automated Cobas® e411 analyser. Staff performing testing were fully trained and certified as competent to operate the Cobas® e411 analyser. The instruments used had rigorous maintenance and service schedules. Calibrators and controls were run according to the manufacturer's instructions.

3.7 METHODOLOGY

All blood donors were routinely screened using molecular and serological methods. Screening for HBV DNA, HIV RNA and Hepatitis C Virus (HCV) RNA was performed on the Procleix Panther® (Grifols, Barcelona, Spain) and screening for HBsAg, HIV Antigen/Antibody and Anti-HCV was performed on the Alinity S® (Abbott, Wiesbaden, Germany) (SANBS) or Roche Cobas® e 801 (Roche Diagnostics, Basel, Switzerland) (WCBS).

For this study additional testing for antibodies to Hepatitis B surface antigen (Anti-HBs) and antibodies to Hepatitis B core (Anti-HBc) was be performed on the Cobas® e411 analyser (Roche Diagnostics, Basel, Switzerland). All additional testing was performed in the SANBS Donation Testing Laboratory in KwaZulu-Natal (KZN). Samples were shipped from outlying areas to the KZN laboratory for testing. Optimal sample storage and transportation conditions (2-10°C) were maintained. All sample collection was performed in the SANBS Donation Testing Laboratories or the WCBS Donation Testing Laboratory. The test results were sent to the researcher to tabulate and analyse.

HIV results were obtained from SANBS and WCBS data and used to determine whether there was a difference in HBV immunity in HIV positive donors.

3.7.1 Selection of samples

An Information Communication Technology (ICT) programme was used to select 60 random donors in Group 1 and Group 2, from each of the 7 SANBS Zones and 60 from WCBS, making up the 480 blood donors in each group. The programme was not able to match the donors in Group 1 and Group 2 so this was done manually by the investigator. The required sample numbers were sent to the relevant laboratory for sample retrieval. All samples were sent to the Donation Testing (DT) Laboratory in KZN for testing. Due to the manual nature of the sample selection and recording process, an additional 56 samples were selected and tested in each group. This resulted in 536 samples in Group 1 matched with 536 samples in Group 2. Donors in Group 1 and Group 2 were matched by geographic location, population group and gender.

3.7.2 Delinking of Samples

The study samples were delinked (de-identified) from the donors and testing was batched and performed when there were sufficient samples, so as to maximise kit usage.

The required donor demographic information was collected from the SANBS Business Intelligence database or the WCBS database prior to performing any additional testing for the study. The sample barcode was scanned into an Excel spreadsheet containing the donor demographic information. The study barcode was then placed over the sample barcode and scanned into the spreadsheet. The original sample barcode was then deleted, effectively delinking the study sample from the donor.

3.7.3 Testing

The samples, labelled with the unique study barcode and delinked from the donors, were then tested for Anti-HBs and Anti-HBc on the Roche Cobas® 411 analyser. Results were printed out from the instrument and manually entered onto the Excel spreadsheet by the researcher.

3.7.4 Data analysis

Anti-HBs titres and Anti-HBc results were analysed using Microsoft Excel. Data was compared using the Goodness of fit Chi square test, with a significant result indicated by $p \leq 0.05$. Multivariate analysis and logistic regression analysis was used to determine the significance of the findings.

Anti-HBs titres and Anti-HBc results were used to determine the number of donors that developed immunity in Groups 1 and 2 (Anti-HBs titre > 10 IU/L), and whether immunity was due to immunisation (Anti-HBc negative) or past infection (Anti-HBc positive) (Table 3.2).

For the purposes of this study, only donors who had an Anti-HBs titre > 10IU/L and were Anti-HBc negative were regarded as having developed immunity due to vaccination and were termed “deemed vaccinated”. All others were termed “not vaccinated”.

Table 3.2: Anti-HBs titre and Anti-HBc result outcomes

Anti-HBs Titre	Anti-HBc	Probable Outcome	Assumption for results
>10 IU/L	Negative	Donor is immune Immunity due to vaccination	Deemed vaccinated
>10 IU/L	Positive	Donor is immune Immunity from past HBV infection / exposure	Not vaccinated
Negative (<10 IU/L)	Negative	No Immunity, No exposure to HBV	Not vaccinated
Negative (<10 IU/L)	Positive	A resolved infection A false positive Anti-HBc result A low level chronic infection A resolving acute infection	Not vaccinated

Source: (Büchner *et al.* 2014)

3.8 TESTING INSTRUMENTATION

3.8.1 Procleix Panther®

Figure 3.2: Procleix Panther® instrument in use in the NAT Laboratory, SANBS Durban
Source: Researcher (2020)

The Procleix Panther® instrument (Figure 3.2) is a fully automated, versatile and efficient blood screening system. Testing for HBV DNA, HIV RNA and HCV RNA is performed on the system in 3.5 hours.

There are three main steps in the Ultrio Elite assay procedure, namely Sample Preparation / target capture, HBV DNA, HIV RNA and HCV RNA target amplification by Transcription Mediated Amplification (TMA) and Detection of amplicons by Hybridisation Protection Assay (HPA). These steps take place in a single tube and assay performance is monitored by an internal control.

Sample Preparation / Target capture

Viral RNA and DNA are isolated by target capture. A detergent solution is used to solubilise the viral envelope, denature proteins and release viral genomic DNA or RNA. If present in the specimen, capture oligonucleotides are hybridised to the HBV DNA target. After the addition of the sample, Target Enhancer Reagent (TER) is added to each tube to create a transient alkaline shock that disrupts and denatures viral particles and nucleic acids. The hybridised target is then captured onto magnetic microparticles and separated from the specimen in a magnetic field. Washing is used to remove unbound components.

Transcription Mediated Amplification

Two enzymes are used for the transcription-based nucleic acid amplification, namely MMLV reverse transcriptase and T7 RNA polymerase. The reverse transcriptase creates a DNA copy of the target sequence and the RNA polymerase produces multiple copies of the RNA amplicons from the DNA copies.

Detection of amplicons

Single stranded nucleic acid probes with chemiluminescent labels hybridise specifically to the amplicons. The selection reagent differentiates between hybridised and unhybridised probes. The signal produced by the hybridised probes is measured in a luminometer as Relative Light Unites (RLU).

The internal control (IC) in each specimen controls for processing, amplification and detection steps. Calibrators are used to determine the assay cut off and assay run validity.
Discriminatory testing is used to discriminate which marker is present in samples found to be positive in Ultrio Elite testing (Gen-Probe) (Panther 2017).

3.8.2 Abbott Alinity s®

Figure 3.3: Abbott Alinity s® Blood Screening Analyser in use in the SANBS Donation Testing Laboratory, Durban

Source: Researcher (2020)

The Abbott Alinity s® (Figure 3.3) is a fully automated system that uses chemiluminescent microparticle immunoassay technology for the qualitative detection of HBsAg. Human serum or plasma can be used for the detection of HBsAg in this one-step immunoassay. The reaction mixture that consists of sample, Anti-HBs coated magnetic microparticles and an Anti-HBs acridinium labelled conjugate is incubated, allowing time for HBsAg present in the samples to bind with the coated microparticles and conjugate. Following a wash step, ancillary wash buffer is added and incubated. Pre-trigger and Trigger solutions are added following a second wash step. The Alinity s® optics measures the chemiluminescent reaction or relative light units (RLUs). There is a direct relationship between the RLUs detected and the amount of HBsAg in the sample. A cut-off RLU value is determined from active calibration. The sample RLU is compared to this cut-off to determine whether the HBsAg is present in the sample (positive result) or not (negative result) (Abbott 2017).

3.8.3 Roche Cobas® e411 analyser

Figure 3.4: Roche Cobas® e411 (Elecsys) in use in the SANBS Donation Testing Laboratory, Durban

Source: Researcher (2020)

The Roche Cobas® e411 analyser (Figure 3.4) is a fully automated instrument capable of performing an immunoassay for the in vitro determination of antibodies to hepatitis B surface and core antigens in human serum or plasma using an electrochemiluminescence immunoassay, "ECLIA".

Elecsys Anti-HBs II

Anti-HBs can form following infection with hepatitis B or following vaccination against HBV. Anti-HBs assays can be used to check whether vaccination has been successful or is necessary. The sandwich principle assay takes 18 minutes to perform. In the first incubation step, a sandwich complex is formed with Anti-HBs in the sample, biotinylated HBsAg and HBsAg labelled with a ruthenium complex. In the second incubation, the complex becomes

bound to the solid phase after addition of streptavidin-coated microparticles. In the measuring cell the microparticles become magnetically captured onto the electrode before unbound substances are removed in a wash step. A chemilumenescent emission, caused by application of voltage to the electrode, is measured by the photomultiplier. A calibration curve is used to determine if the result is positive or negative (Roche Diagnostics 2016).

Elecsys Anti-HBc II

Anti-HBc appears soon after infection with HBV and usually persists for life, even in people who have recovered from HBV, and is thus a good indicator of existing or past infection. It is not associated with vaccination. The principle of this 27 minute test is a competition principle. Samples are pre-treated with reducing agent in the first incubation. HBcAg is then added and a complex is formed with the Anti-HBc in the samples. Biotinylated antibodies, a ruthenium complex and streptavidin-coated microparticles bind to the free binding sites on HBc antigens. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin. In the measuring cell, the microparticles become magnetically captured onto the electrode before unbound substances are removed in a wash step. A chemilumenescent emission, caused by application of voltage to the electrode, is measured by the photomultiplier. A calibration curve is used to determine if the result is positive or negative (Roche Diagnostics 2018).

CHAPTER 4: RESULTS

4.1 INTRODUCTION

The aim of this study was to investigate whether the decline in HBV rate in young SANBS blood donors was related to the introduction of universal HBV vaccination in South Africa in April 1995. The results are now presented in this chapter.

Results for the two groups of blood donors are presented and compared:

Group 1 – Pre-Vaccination Era (pre-vac): donors born before the implementation of universal HBV vaccination in April 1995 (ages 24 to 29 years).

Group 2 – Post-Vaccination Era (post-vac): donors born after the implementation of universal HBV vaccination in April 1995, who would have been eligible to receive HBV vaccination (ages 19 – 24 years).

Based on the sampling calculation, 960 samples were required to be included, with 120 from each geographic area (60 from donors born before 1995 and 60 from donors born after 1995, stratified by population group and gender). In the process of collecting the samples for testing an additional 112 samples were collected and tested. These were included in the analysis, resulting in the 1072 donations included in the study.

4.2 DEMOGRAPHICS OF THE STUDY POPULATION

A total of 1072 donors were included in the study; 536 in Group 1 (pre-vac era) and 536 in Group 2 (post-vac era). Donors in Group 1 and Group 2 were matched by population group, gender and geographic region at the time the samples were selected for the study.

The minimum number of donors per geographic region was 60. However, in trying to get to the minimum number, additional samples were collected and tested and have been included in the results. The number of donors in each group was dependant on the population of the area and reflected the demographics of the geographic region.

Only 62 Asian donors were included in the study, 31 in Group 1 and 31 in Group 2. There were 42 Asians donors from KwaZulu-Natal and 20 from the SANBS Egoli Zone. There were 124 Coloured donors in the study, 62 in Group 1 and 62 in Group 2. There were no Coloured donors from the Mpumalanga Zone, and only two from the Northern Zone. Black donors made

up the biggest percentage of donors at 56.9%, followed by White donors at 25.7%. There were slightly more female donors (52.2%) than male (47.8%), donors as is seen in Table 4.1.

Table 4.1: Donor demographics by population group, gender and geographic location for Group 1 and Group 2 matched donors

Donor Demographics			Group 1	Group 2
	Total Tested	%	Number Tested	Number Tested
Total	1072		536	536
Population Group				
Asian	62	5.8	31	31
Black	610	56.9	305	305
Coloured	124	11.6	62	62
White	276	25.7	138	138
Gender				
Female	560	52.2	280	280
Male	512	47.8	256	256
Geographic Location				
Eastern Cape	122	11.4	61	61
Egoli	154	14.4	77	77
FS/NC	140	13.1	70	70
KwaZulu-Natal	156	14.6	78	78
Mpumalanga	120	11.2	60	60
Northern	130	12.1	65	65
Vaal	130	12.1	65	65
WCBS	120	11.2	60	60

Table 4.2: Donor demographics by age for Group 1 and Group 2 donors

Age Group					
Group 1			Group 2		
Total	536		Total	536	
Age Group	Number Tested	%	Age Group	Number Tested	%
24	18	3.36	19	31	5.78
25	90	16.79	20	132	24.63
26	109	20.34	21	96	17.91
27	103	19.22	22	97	18.10
28	119	22.20	23	100	18.66
29	97	18.10	24	80	14.93

Only first time donors who were HBsAg and HBV DNA negative were included in the study. Additional testing for Anti-HBs titre and Anti-HBc was performed on all study samples.

4.3 STUDY RESULTS

4.3.1 Rate of HBV Immunity in South African Blood Donors

Of the 1072 donors tested, 323 (30.1%) had an Anti-HBs titre >10 IU/L and were thus deemed to have immunity to HBV, while 749 (69.9%) had an Anti-HBs titre <10 IU/L indicating that they possibly had no immunity to HBV. Results are shown in Table 4.3 and Figure 4.1.

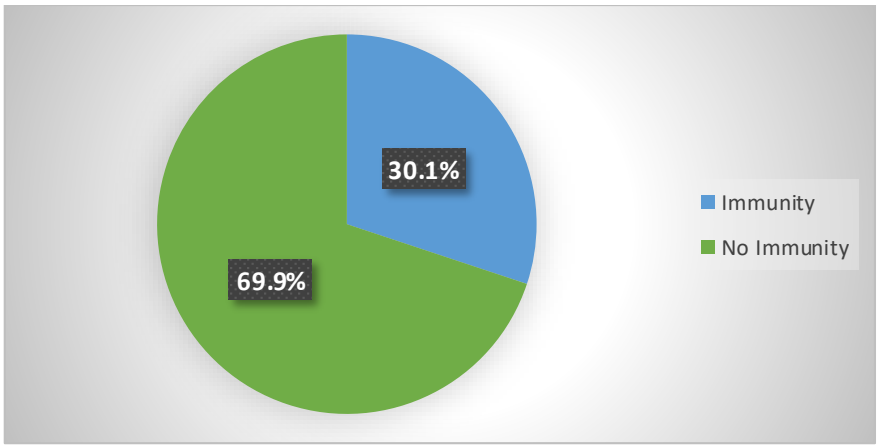

Figure 4.1: Rate of immunity to HBV in SA blood donors

Of the 1072 donors with an Anti-HBs titre >10 IU/L tested, 275 (25.7%) were Anti-HBc negative indicating that immunity to HBV was due to vaccination, and 48 (4.5%) were Anti-HBc positive indicating that they had been exposed to HBV and had developed immunity due to this past exposure, as seen in Table 4.1 and Figure 4.2.

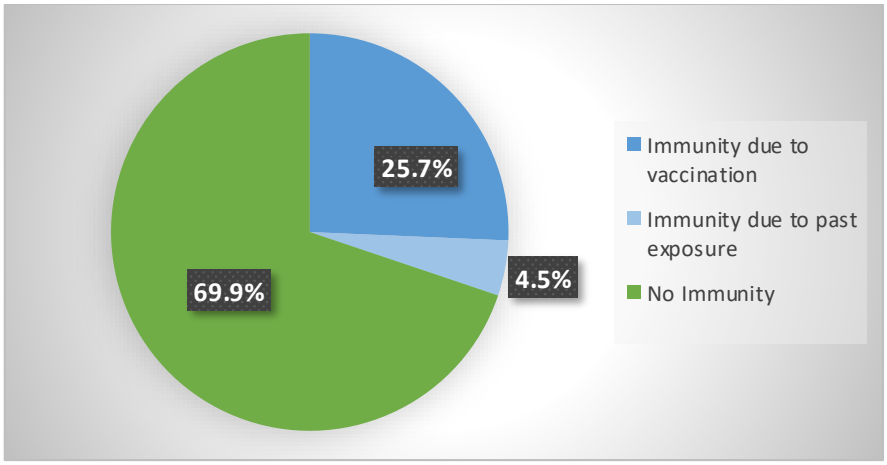

Figure 4.2: Rate of immunity due to vaccination and past exposure to HBV

Immunity was due to vaccination in 85.4% (275/323) of the donors with immunity to HBV, as shown in Table 4.3.

There were seven (0.7%) donors with an Anti-HBs titre <10 IU/L who were Anti-HBc positive, indicating that they had been exposed to HBV (See Table 4.4). These donors could have an occult HBV infection.

Table 4.3: Rate of donors who are vaccinated and not vaccinated

	Tested	Vaccinated		Not Vaccinated		p-value
		Number	%	Number	%	
Immune	323	275	85.4	48	14.9	<0.0001
Not immune	749	0	0	749	100	
Total	1072	275	25.7	797	74.3	

Table 4.4: Rates of donors with immunity and with no immunity

Results	Meaning	Total	%
	TOTAL Donors Tested	1072	
Anti-HBs > 10 IU/L	**Immunity**	**323**	**30.1**
Anti-HBs > 10 IU/L and Anti-HBc negative	Immunity due to vaccination	275	25.7
Anti-HBs > 10 IU/L and Anti-HBc positive	Immunity due to past exposure to HBV	48	4.5
Anti-HBs < 10 IU/L	**No immunity**	**749**	**69.9**
Anti-HBs < 10 IU/L and Anti-HBc negative	No immunity, no past exposure	797	74.3
Anti-HBs < 10 IU/L and Anti-HBc positive	A resolved infection A false positive anti-HBc result A low level chronic infection A resolving acute infection	7	0.7

4.3.1.1 Rate of HBV immunity due to vaccination

The rate of HBV immunity in SA blood donors due to HBV vaccination (Anti-HBs titre > 10 IU/L and Anti-HBc negative) was 25.7%, with 74.3% of donors showing no evidence of HBV vaccination (Anti-HBs titre < 10 IU/L) as shown in Figure 4.3.

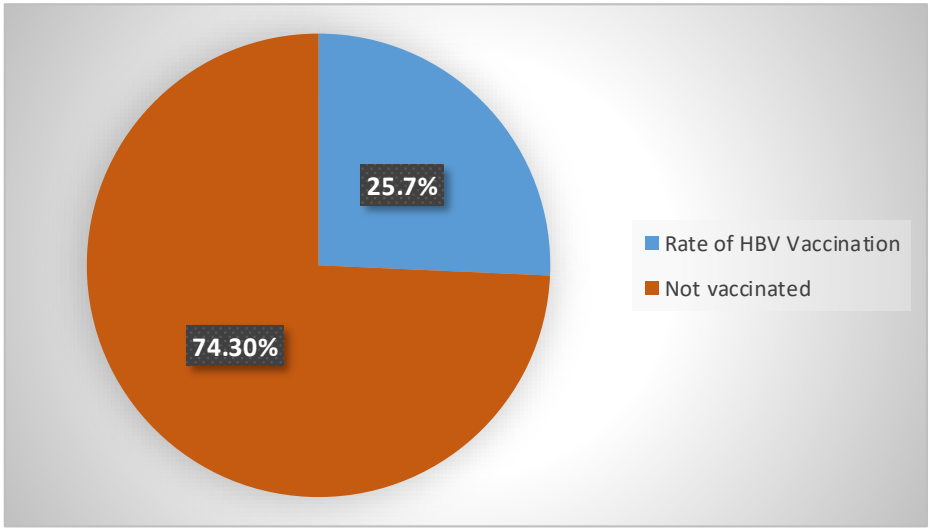

Figure 4.3: Rate of HBV vaccination in SA blood donors

By population group there was a higher rate of Asian donors (32.3%) who were vaccinated than other population groups. Coloured donors had the lowest rate of HBV immunity due to vaccination (18.5%), as seen in Table 4.5. This was statistically significant (p=0.026).

The Free State / Northern Cape (FS/NC) zone had the highest rate of HBV vaccination at 37.9%, followed by Egoli at 30.5%. Mpumalanga, Vaal and the Western Cape had the lowest rates at 20%, as seen in Table 4.5. This was significant (p=0.006).

Male and female donors had similar rates of HBV vaccination at 25.8% and 25.5% (p=0.927) respectively, as seen in Table 4.5.

Table 4.5: Donor demographics by population group, gender and geographic location for donors vaccinated (Anti-HBs titer >10 IU/L, Anti-HBc negative) and not vaccinated (Anti-HBs titer <10 IU/L, Anti-HBc negative)

	Tested	Vaccinated (Anti-HBs titer >10 IU/L, Anti-HBc negative)		Not Vaccinated (Anti-HBs titre <10 IU/L, Anti-HBc negative)		p-value
		Number	%	Number	%	
TOTAL	1072	275	25.7	797	74.3	<0.0001
POPULATION GROUP						
Asian	62	20	**32.3**	42	67.7	0.026
Black	610	147	24.1	463	75.9	
Coloured	124	23	**18.5**	101	81.5	
White	276	85	30.8	191	69.2	
GENDER						
Female	560	143	25.5	417	74.5	0.927
Male	512	132	**25.8**	280	54.7	
GEOGRAPHIC LOCATION						
ECZ	122	32	26.2	90	73.8	0.006
Egoli	154	47	30.5	107	69.5	
FS/NC	140	53	**37.9**	87	62.1	
KZN	156	36	23.1	120	76.9	
MP	120	24	20	96	80	
Northern	130	33	25.4	97	74.6	
Vaal	130	26	20	104	80	
WCBS	120	24	20	96	80	
GROUP						
1 (pre-vac era)	536	87	16.2	449	83.8	<0.0001
2 (post-vac era)	536	188	35.1	348	64.9	

4.3.2 Rate of HBV Immunity in SA Donors Born before (Group 1, Pre-Vaccination) and after (Group 2, Post-Vaccination) the Implementation of Universal HBV Vaccination

Of the 323 (30.1%) donors with immunity to HBV (Anti-HBs titre >10 IU/L), 129 (39.9%) were in Group 1 (pre-vaccination - born before April 1995) and 194 (60.1%) were in Group 2 (post-vaccination - born after April 1995).

4.3.2.1 Rate of HBV vaccination in Group 1 and Group 2 donors

In Group 1 (pre-vac era), 87 of the 536 donors tested **(16.2%)** were found to be vaccinated, while in Group 2 (post-vac era) 188 **(35.1%)** were vaccinated. This was significant with a p value of p<0.00001 (OR 0.359 95% CI 0.268 – 0.479) (See Figure 4.4 and Table 4.5).

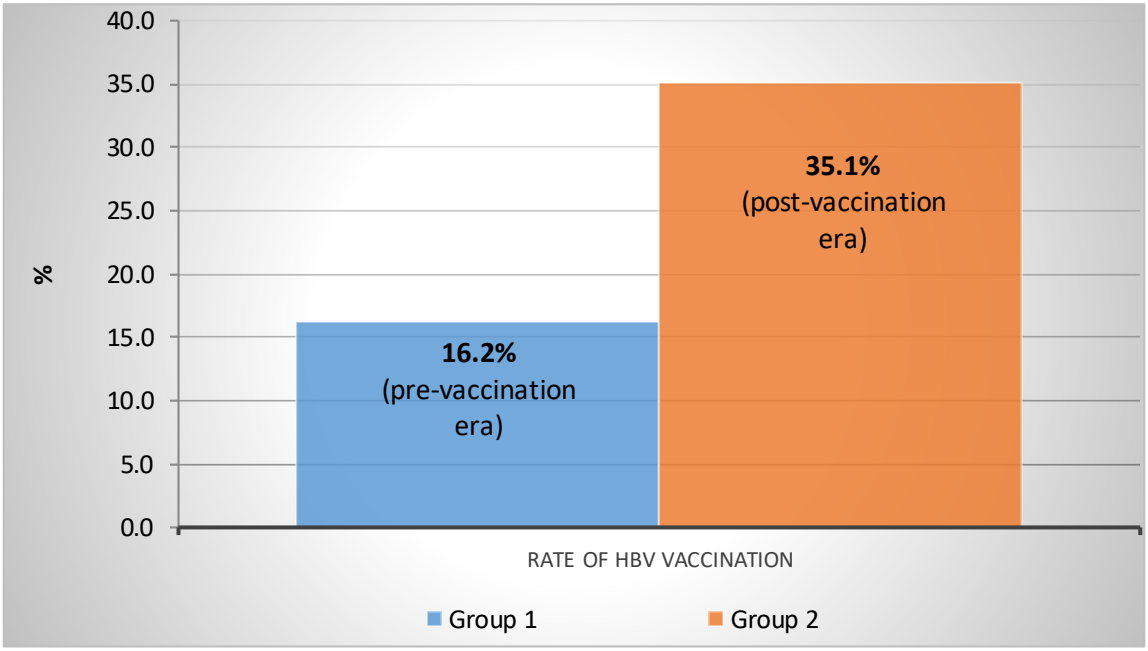

Figure 4.4: Rate of HBV vaccination in Group 1 (pre-vaccination era) and Group 2 (post-vaccination era) donors

4.3.2.2 Donors with past exposure immunity in Group 1 and Group 2 donors

There were 48 (4.5%) donors who had an Anti-HBs titre > 10 IU/L and who were Anti-HBc positive, indicating immunity due to a past exposure to HBV. Of the 536 donors tested in each group, 42 (7.8%) had past exposure immunity in Group 1 (pre-vac), and there were six (6) (1.1%) in Group 2 (post-vac) (p<0.00001) (OR 7.51 95% CI 3.17 – 17.82), as shown in Figure 4.5.

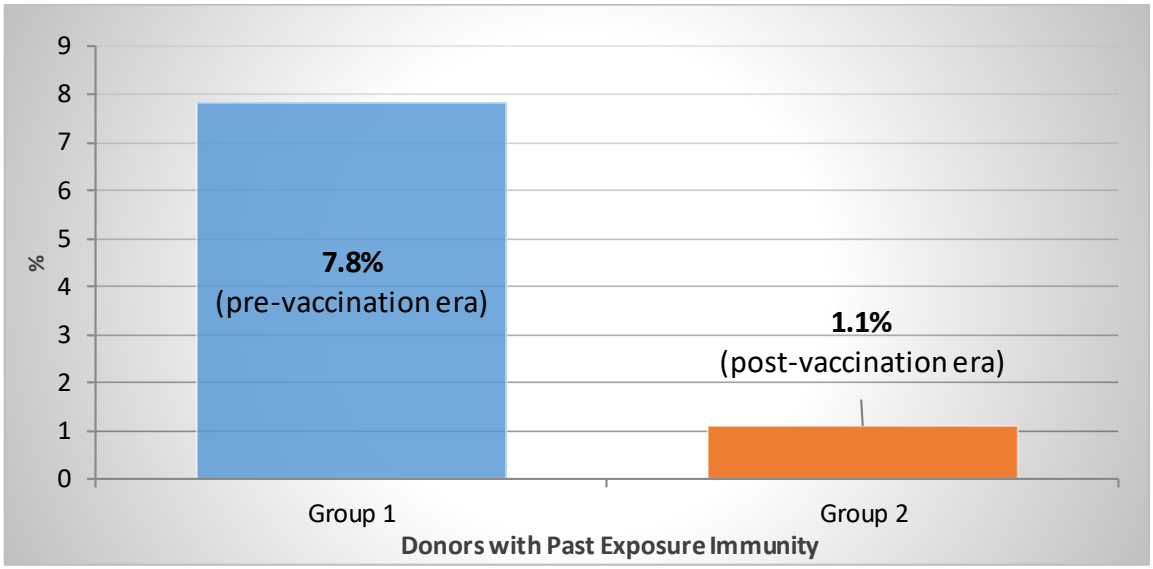

Figure 4.5: Rate of donors with past exposure immunity in Group 1 and Group 2

4.3.2.3 Donors with no immunity in Group 1 and Group 2 donors

There were 749/1072 (69.9 %) donors who had no immunity (Anti-HBs titre < 10 IU/L) and seven (7) (0.7%) who had no immunity with evidence of past exposure to HBV (Anti-HBs titre < 10 IU/L, Anti-HBc positive).

Of the 536 donors tested in each group, 407 (75.9%) had no immunity in Group 1 and 342 (63.8%) had no immunity in Group 2 (Figure 4.6). There were five (5) (0.9%) donors with no immunity but evidence of past exposure to HBV in Group 1 and two (0.4%) in Group 2 (Figure 4.7).

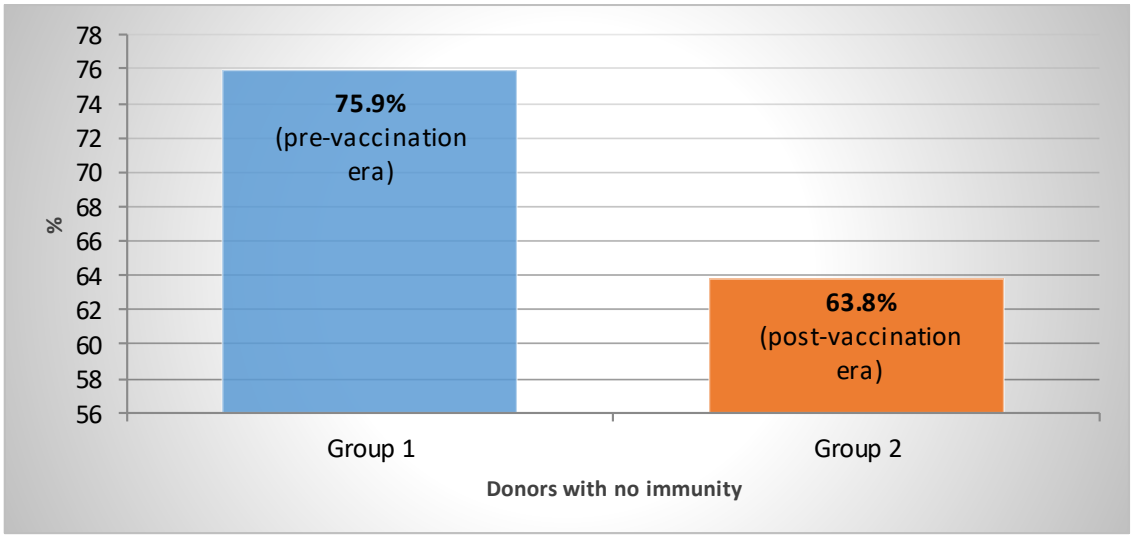

Figure 4.6: Rate of donors with no immunity in Group 1 and Group 2

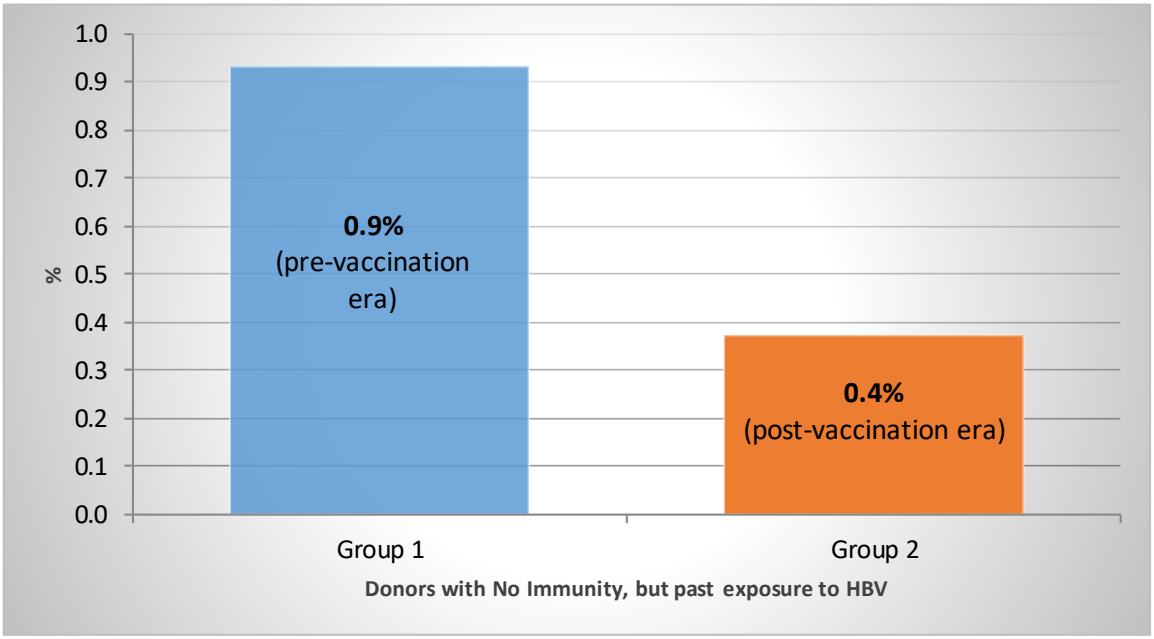

Figure 4.7: Rate of donors with no immunity, but past exposure to HBV

4.3.3 HBV Vaccination Rates by Gender in Group 1 and Group 2 Donors

Male and female blood donors had similar vaccination rates in the pre-vaccination and the post-vaccination eras.

In Group 1 (pre-vac), slightly more male donors were deemed vaccinated (16.8%) than female donors (15.7%), although this was not significant with a p-value of 0.82.

In Group 2 (post-vac), slightly more female donors (35.4%) were deemed vaccinated than male donors (34.8%), although this was not significant with a p-value of 0.96.

In female donors the vaccination rate increased 2.25 fold from 15.7% in the pre-vaccination era to 35.4% in the post-vac era ($p<0.00001$), and in male donors the vaccination rate increased 2.07 fold from 16.8% in the pre-vac era to 34.8% in the post vac era ($p=0.00001$) (Table 4.6, Figure 4.8).

Table 4.6: Bivariate analysis of HBV vaccination in Group 1 and Group 2 by gender

Gender	Total	Group 1 (pre-vaccination)		Group 2 (post-vaccination)			p value
		Number	%	Number	%	Increase (fold)	
Female	280	44	15.7	99	35.4	2.25	<0.00001
Male	256	43	16.8	89	34.8	2.07	0.00001
Total	536	87	16.2	188	35.1	2.16	<0.00001

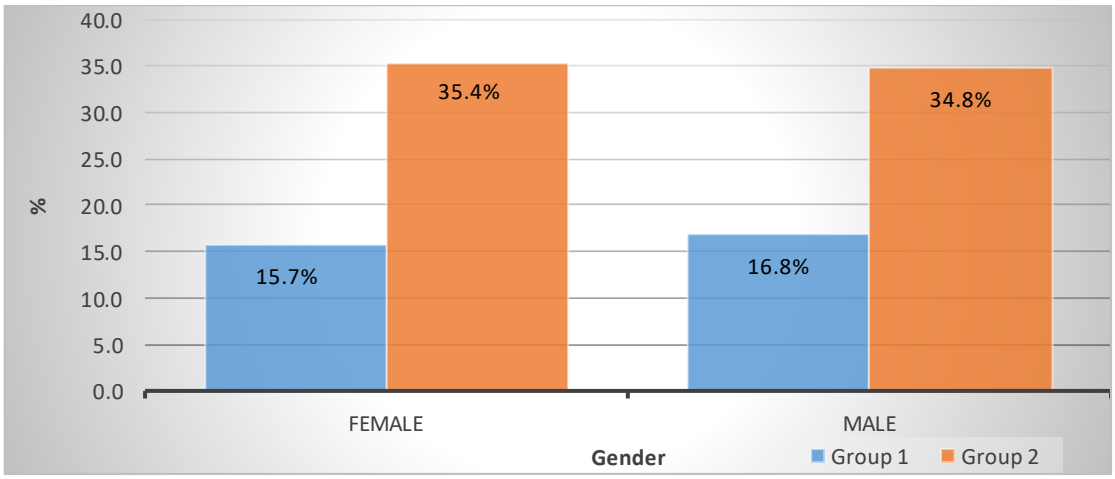

Figure 4.8: Rate of HBV vaccination in Group 1 and Group 2 donors by gender

4.3.4 Vaccination Rates by Population Group

In Group 1 (pre-vac), vaccination rates were highest among White donors (22.5%) and Black donors (16.4%), and lowest in Coloured (4.8%) and Asian (9.7%) donors.

In Group 2 (post-vac), vaccination rates were highest in Asian donors (54.8%) and White donors (39.1%) and lower in Black (31.8%) and Coloured (32.3%) donors.

Vaccination rates increased significantly in all population groups from the pre-vac era to the post-vac era. The greatest increase in vaccination rate was found in Coloured donors (6.7-fold increase) from 4.8% (pre-vac) to 32.3% (post-vac) (p=0.0022), followed by Asian donors with a 5.67-fold increase from 9.7% to 54.8% (p=0.00041). (Table 4.7, Figure 4.9)
All differences were significant (p<0.05).

Table 4.7: Bivariate analysis of HBV vaccination in Group 1 and Group 2 by population group

Population Group	Total in Group	Group 1 (pre-vaccination)		Group 2 (post-vaccination)		Increase (fold)	p-value
		Number	%	Number	%		
Asian	31	3	9.7	17	54.8	5.67	0.00041
Black	305	50	16.4	97	31.8	1.94	0.00001
Coloured	62	3	4.8	20	32.3	6.67	0.00220
White	138	31	22.5	54	39.1	1.74	0.00412
Total	536	87	16.2	188	35.1	2.16	<0.00001

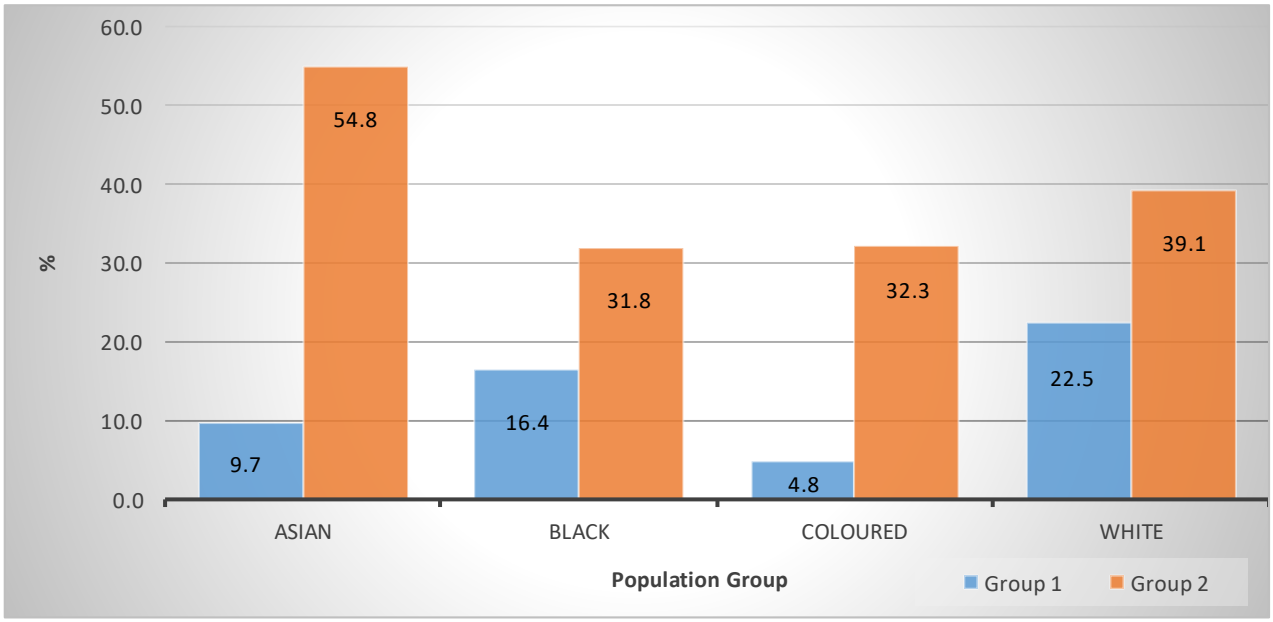

Figure 4.9: HBV vaccination rate in Group 1 and Group 2 by population group

4.3.5 Vaccination Rates by Age Group

Group 1 (pre-vac) was made up of donors born before 1995, aged 24 to 29 years and Group 2 (post-vac) was made up of donors born after 1995, aged 19 to 24 years.

In Group 1, donors aged 24 years had the highest rate of HBV vaccination (22.2%), while donors aged 26 to 28 years had the lowest rates at 12.8%, 14.6% and 14.3% respectively.

In Group 2, donors aged 23 years had the highest rate of HBV vaccination (39.0%), followed by donors aged 19 years at 38.7%. The lowest rate of HBV vaccination was in donors aged 21 years at 27.1% (Table 4.8).

There were no statistically significant differences between vaccinated donors by age in either group. The overall vaccination rate increased 2.2 fold from 87 pre-vaccination to 188 post vaccination (p<0.00001).

Table 4.8: Bivariate analysis of HBV vaccination in Group 1 and Group 2 by age group

Group 1 (pre-vaccination era)					Group 2 (post-vaccination era)				
Age Group	Total Tested	Number Vaccinated	%	p value	Age Group	Total Tested	Number Vaccinated	%	p value
24	18	4	22.2		**19**	31	12	38.7	
25	90	18	20	0.9	**20**	132	45	34.1	0.8
26	109	14	12.8	0.48	**21**	96	26	27.1	0.3
27	103	15	14.6	0.6	**22**	97	36	37.1	0.9
28	119	17	14.3	0.6	**23**	100	39	39	0.8
29	97	19	19.6	0.9	**24**	80	30	37.5	0.9
Total	536	87	16.2		Total	536	188	35.1	<0.00001

4.3.6 Vaccination Rates by Geographic Region

HBV vaccination rates were analysed by geographic region, namely the Western Cape represented by the Western Cape Blood Service (WCBS) and the seven SANBS Zones namely:

- Free State/Northern Cape (FS/NC) Zone servicing the Northern Cape and Free State Provinces
- Mpumalanga Zone (MpZ) servicing the Mpumalanga Province
- KwaZulu-Natal Zone (KZN) servicing the KZN province
- Eastern Cape Zone (ECZ) servicing the Eastern Cape Province
- Gauteng due to its high population is split between 3 zones as per below
- Egoli Zone (EZ) covers the greater Johannesburg areas as well as the northern, southern and western suburbs
- Northern Zone (NZ) servicing Pretoria and the Polokwane area
- Vaal Zone (VZ) servicing parts of the North West Province, the northern Free State and the East Rand area in Gauteng Province and part of the northern Free State

In Group 1 (pre-vac), FS/NC (37.1%) and Egoli (20.8%) had the highest rates of HBV vaccination. In Group 2 (post-vac) the Northern (41.5%) and Egoli (40.3%) Zones had the highest rates of HBV vaccination. The areas with the lowest rates of vaccinated donors were KZN (8.97%) in Group 1 and Vaal (24.6%) in Group 2 (Table 4.9, Figure 4.10).

The greatest increase in vaccination rates from the pre-vac to the post-vac era was found in the Northern Zone, with a 4.5-fold increase from 9.2% to 41.5%. The lowest was in FS/NC, with only one additional donor vaccinated in Group 2 (Table 4.9, Figure 4.10).

Table 4.9: HBV Vaccinated Donors in Group 1 and Group 2 by Geographic Region

Geographic Region	Tested	Group 1 Pre-vaccination era		Group 2 Post-vaccination era		Increase (fold)	p-value
		Number	%	Number	%		
ECZ	61	9	14.8	23	37.7	2.6	0.007
Egoli	77	16	20.8	31	40.3	1.9	0.014
FS/NC	70	26	37.1	27	38.6	1.0	1
KZN	78	7	9.0	29	37.2	4.1	0.00007
MP	60	6	10.0	18	30.0	3.0	0.012
Northern	65	6	9.2	27	41.5	4.5	0.00006
Vaal	65	10	15.4	16	24.6	1.6	0.27
WCBS	60	7	11.7	17	28.3	2.4	0.039
TOTAL	**536**	**87**	**16.2**	**188**	**35.1**	**2.2**	**<0.00001**

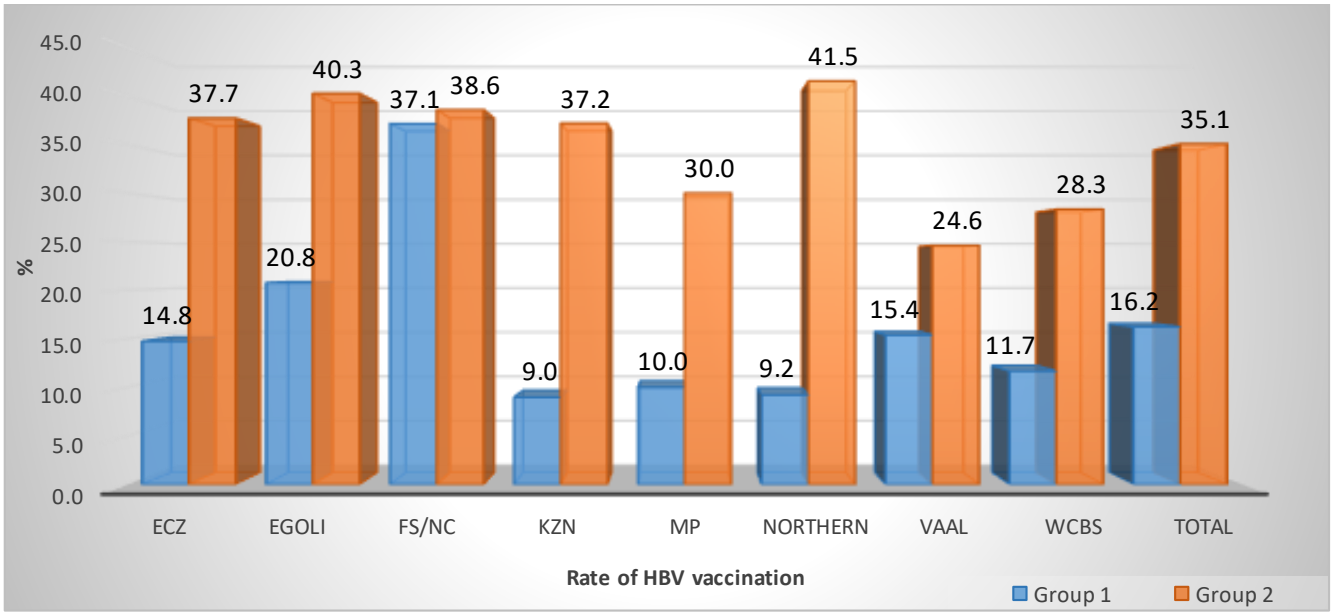

Figure 4.10: Rate of HBV vaccination in Group 1 and Group 2 donors by geographic region

4.3.7 Donors with Past Exposure Immunity

Immunity due to past exposure (Anti-HBs>10IU/L, Anti-HBc positive) was significantly higher in donors born in Group 1 (pre-vaccination era: 7.8%) than in donors born in Group 2 (post-vaccination era: 1.1%) (p<0.00001).

In both pre-vaccination and post-vaccination groups there were more Black donors with past exposure immunity, with a significant decrease in the vaccinated group. In Group 1 there were

11.1% and in Group 2 there were 1.6% (*p<0.00001)*. Asian donors in Group 1 and Group 2 and Coloured donors in Group 2 had no past exposure immunity. Female donors had higher rates of past exposure immunity in Group 1 (8.2%) and in Group 2 (1.4%).
Donors from Mpumalanga had the highest rate of past exposure immunity in both groups; Group 1: 20% and Group 2: 3.3% (0.0155) (Table 4.10).

Table 4.10: Bivariate analysis of donors with immunity due to past exposure to HBV

Donor Demographics	Total Tested per Group	Group 1 (Pre-vaccination)	%	Group 2 (Post-vaccination)	%	p-value
Total	536	42	7.8	6	1.1	<0.00001
Population Group						
Asian	31	0	0.0	0	0.0	
Black	305	34	**11.1**	5	**1.6**	<0.00001
Coloured	62	4	6.5	0	0.0	0.1329
White	138	4	2.9	1	0.7	0.3700
Gender						
Female	280	23	**8.2**	4	**1.4**	0.0004
Male	256	19	7.4	2	0.8	0.0002
Geographic Location						
Eastern Cape	61	4	6.6	0	0.0	0.1329
Egoli	77	1	1.3	1	1.3	0.4791
FS/NC	70	4	5.7	1	1.4	0.1329
KwaZulu-Natal	78	7	9.0	2	2.6	0.1811
Mpumalanga	60	12	**20.0**	2	**3.3**	0.0155
Northern	65	5	7.7	0	0.0	0.0730
Vaal	65	4	6.2	0	0.0	0.1329
WCBS	60	5	8.3	0	0.0	0.0730

4.3.8 Donors with No Immunity or Past Exposure to HBV

There were significantly more donors with no immunity or past exposure in Group 1 (75.9%) than in Group 2 (63.8%) (*p*=0.00002).

4.3.9 Multivariable Analysis: Geography, Gender and Ethnicity

In multivariable analysis, after controlling for geography, gender and ethnicity, donors from the post-vac era had a 2.9 times greater odds of being vaccinated than donors born in the pre-vac era (OR 2.89, 95% CI 2.16 - 3.89), as seen in Table 4.11.

Table 4.11: Multivariable analysis by age group

	Age Group (years)	Total Tested	Number Vaccinated	%	Odds Ratio	95% Confidence interval	
Group 1 (Pre-vaccination)	24-29	536	87	16.2	1		
Group 2 (Post-vaccination)	19-24	536	188	35.1	2.89	2.16	3.89

Compared to Coloured donors, Asian donors had a 2.5 times greater odds (CI 1.14-5.62) of being vaccinated and White donors had a 2.1 times greater odds of being vaccinated (OR 2.1, 95% CI 1.21 - 3.65) as seen in Table 4.12

Table 4.12: Multivariable analysis by population group

	Odds Ratio	95% Confidence interval	
Coloured	1		
Asian	2.53	1.14	5.62
Black	1.15	0.89	2.57
White	2.1	1.21	3.64

No statistically significant odds were noted for geography and gender, as seen in Table 4.13

Table 4.13: Multivariable analysis by geographic location and gender

ZONE	Tested	Vaccinated	Odds Ratio	95% Confidence interval		p-value
Geographic Area						
ECZ	122	32	1	.	.	.
Egoli	154	47	1.086	0.62	1.903	0.773
FS/NC	140	53	1.671	0.964	2.899	0.068
KZN	156	36	0.694	0.378	1.275	0.239
MP	120	24	0.65	0.345	1.225	0.183
Northern	130	33	0.834	0.461	1.506	0.546
Vaal	130	26	0.634	0.343	1.17	0.145
WCBS	120	24	0.672	0.359	1.255	0.212
Gender						
Female	560	143	1	.	.	.
Male	512	132	1.027	0.858	0.77	1.368

4.3.10 HBV Immunity in HIV Positive vs HIV Negative Blood Donors

No HIV positives were detected in the 1072 donors tested. Study samples were selected from donor samples following routine testing i.e. no additional samples were drawn from donors for this study. As standard procedure in the routine testing laboratory (at SANBS and WCBS), all initial viral positive samples are removed following testing in order to perform second line and confirmatory testing. During the process of performing the additional testing, most of the sample is depleted. This resulted in only viral negative samples being available for selection for the purposes of the HBV vaccination study. Determining the correlation between HBV vaccinated donors and HIV was thus beyond the scope of this study.

CHAPTER 5: DISCUSSION

5.1 INTRODUCTION

In this chapter, the study results are discussed relative to the aims and objectives of the study and in relation to the available literature.

5.2 SUMMARY OF AIMS AND OBJECTIVES

In the present study, the impact of the introduction of universal HBV immunisation on South African blood donors was evaluated due to the limited information available on this topic in the current database. The aim of this study was therefore to investigate the rate of HBV vaccination in South African blood donors born before and after 1995 and thereby determine whether the decrease in HBV rate in younger blood donors noted by SANBS could be attributed to the introduction of universal HBV vaccination in South Africa in April 1995. This was done by testing two groups of HBV DNA and HBsAg negative first-time blood donors for antibodies to Hepatitis B surface antigen (Anti-HBs) and antibodies to Hepatitis B core antigen (Anti-HBc).

The main objective of the study was to determine the rate of HBV vaccination in blood donors born before and after the implementation of universal HBV vaccination. Further objectives were to determine the level of HBV immunity by geographic region in SA, to determine whether the decrease in HBV rate in younger blood donors could be attributed to HBV vaccination and to determine whether there was a difference in HBV immunity in HIV positive vs HIV negative blood donors.

5.3 DISCUSSION OF FINDINGS

5.3.1 Study Population

The study included 1072 routine blood donors, 536 donors born before the 1995 introduction of universal HBV vaccination (pre-vaccination era) and 536 blood donors born after 1995 (post-vaccination era). Donations were matched by the areas of South Africa to ensure all areas were included in the study, to give a representative view of HBV vaccination in SA blood donors. Donations were stratified by age, geographic location, population group and gender.

It was found that there were slightly more female than male donors in the study population (52.2% vs 47.8%). This correlated with StatsSA's mid-year population estimates of 51.2%

females vs 48.8% males (StatsSA 2019), and with the SANBS donations for 2019 (51.3% female vs 48.7% male donors) (SANBS Business Intelligence data). Although it could be expected that women would donate less due to lower iron levels than men, a 2018 study of 4,412 SA blood donors found that male donors had twice the odds of iron deficiency compared to female donors (van den Berg *et al.* 2019). This was associated with inter-donation intervals of < 3 months in male donors (van den Berg *et al.* 2019). A study by Swanevelder *et al.* (2019) also found that female donors were also more likely to donate blood and to return for another donation than male donors, and these could explain the slightly higher proportion of female donors in the current study (Swanevelder *et al.* 2019).

By population group, there were more Black donors included in the study (56.9%), followed by White donors at 25.7%, Coloured donors at 11.6% and Asian donors at 5.8%. This correlated with the SANBS donor base for Black and White donors for the year 2019, however, there were more Asian donors bled in 2019 than Coloured donors. According to the SANBS Business Intelligence data, the donor base was made up of 47% Black donors, 38% White donors, 7.7% Asian donors and 5.5% Coloured donors. According to StatsSA (2019), the SA population was made up of 80.7% Black Africans, 8.8% Coloureds, 7.9% Whites and 2.6% Asians. Due to matching of donors by population group and geographic region and including the Western Cape donors in this study, the population groups lost some representation and this could explain the differences noted.

5.3.2 Summary of Study Results - Rate of HBV Vaccination in SA Blood Donors

Of the 1072 donors tested in this study, almost a third showed evidence of having some level of HBV immunity (i.e. having an Anti-HBs titre >10 IU/L). A quarter of the study population had Anti-HBs levels >10 IU/L without Anti-HBc indicating immunity was due to HBV vaccination, while 4.5% of the study population had detectable Anti-HBc indicating immunity was due to a past exposure to HBV.

This study's findings were similar to those of a 2012 study by Mayaphi *et al.* in which 400 patients from a Gauteng hospital were tested and it was found that 39.5% of the HIV positive people and 24% of a control group (HIV negative) showed evidence of immunity to HBV (Mayaphi *et al.* 2012). The findings were, however, much lower than reported vaccine coverage data from a review of the status of HBV control in Africa of between 76% in 2011 to 71% in 2015 (Breakwell *et al.* 2017). They were also lower than those found in a study of 756, 18-month-old children from nine provinces in SA who had been immunised as part of the EPI. In that study, the children were tested one year after receiving the three-dose immunisation

as part of the EPI. It was found that 87% had protective Anti-HBs titres of >10 IU/L, indicating success of the programme (Schoub *et al.* 2002). Another study of 598 babies (with a mean age of 23.3 months) in the Northern Province who received the Hepaccine-B 3 dose vaccination between April 1995 and August 1999 as part of the EPI were found to have a seroprotection rate of 86.8% (Tsebe *et al.* 2001).

There could be a number of reasons why the HBV vaccination rates in this current study were found to be lower than in other studies in SA. These included waning of immunity (the progressive loss of protective antibodies over time) and missing or incomplete immunisation which could result in low antibody titres leaving children at risk of infection with HBV (McNaughton *et al.* 2019). Two of the above studies reported on HBV vaccination rates in babies (younger than 24 months old), while in this study adults were tested (19 – 29 years of age). In a study of 8,733 students in China who had received HBV vaccination (four-dose coverage), almost 17% had lost their immunological memory against HBsAg by age 15 (Hipgrave, Maynard and Biggs 2006). Amponsah-Dacosta *et al.* (2014) reported that HBV chronic carrier rates increased as immunity waned with increasing age (Amponsah-Dacosta *et al.* 2014). A study of HBV sero-prevalence in SA children under 15 years of age found that vaccine induced immunity was higher in younger children and infants but declined in older children, possibly due to waning of immunity, 'biological non-response' or failure to vaccinate (Prabdial-Sing *et al.* 2019). The study found that 67.3% of children tested showed evidence of vaccine induced immunity, and that the rate of immunity was higher in children aged 1-5 years (80.2%) than in those who were 10 – 14 years old (60.3%). Only 2.9% of the children had natural immunity caused by exposure to HBV (Prabdial-Sing *et al.* 2019).

There was no catch up vaccine offered to older children (Kew 1996) and no vaccine offered to adults in SA, thus waning of immunity over time could explain the lower HBV vaccination rate of 25.7% found in this study as compared to the Prabdial-Singh study. Another factor could be that not all adults developed immunity from their first vaccination. Approximately 10% did not develop an Anti-HBs titre >10 IU/L and required repeat vaccination (Spearman *et al.* 2013).

South Africa had no birth-dose or catch-up vaccination programmes in place at the time of this study and full coverage was not always achieved with three doses. This was especially true in rural areas where HBsAg seroprevalence was highest. Spearman and Sonderup (2014) recommended that the introduction of a birth dose Hepatitis B vaccine, added to the current three dose programme would reduce the risk of perinatal transmission and that adding an

adolescent booster dose should be considered to counter waning of immunity (Spearman and Sonderup 2014).

In endemic countries such as South Africa, most infections occurred before age 5 and the risk of developing chronic HBV depended on the age at which the child was infected, whereas acquisition of chronic HBV in adults was relatively low (Spearman and Sonderup 2014). Thus, vaccination of babies and children offered good protection against chronic HBV during this critical time.

There were seven (0.7%) donors who tested Anti-HBc positive and Anti-HBs negative, indicating exposure to HBV. Of these, five were pre-vaccination era donors and two were post vaccination era donors. As HBsAg and HBV DNA positives were excluded from this study, there were four possible interpretations for results that were HBsAg negative, Anti-HBc positive and anti-HBs negative. These could be: a resolved infection; a false positive Anti-HBc result; a low level chronic infection; or a resolving acute infection (World Health Organisation 2005). These results did not form part of this current study.

5.3.3 Rate of HBV Immunisation in SA Donors Born before and after the Implementation of Universal HBV Vaccination

The main objective of this study was to determine the rate of HBV vaccination in SA blood donors born before and after the implementation of universal HBV vaccination in SA. The study results showed a statistically significant ($p<0.00001$) increase in the rate of HBV vaccination in blood donors born after the 1995 introduction of universal HBV vaccination in South Africa, indicating the efficacy and public health benefit of the inclusion of HBV vaccination in the SA EPI.

SANBS noted a 2.8-fold decrease in the HBV infection rate in young blood donors from 2010 to 2015, prompting this study. The 2.2-fold increase in vaccination rate from **16.2%** in donors born before 1995 (pre-vaccination era) to **35.1%** in donors born after 1995 (post vaccination era) proved the hypothesis that the decrease in HBV rate noted by SANBS could be attributed to the introduction of universal HBV vaccination in SA in 1995.

Amponsah-Dacosta *et al.* (2014) described the long-term success of the vaccination programme in SA as being an increase in population immunity and a decrease in the HBV carrier rate. They noted a statistically significant increase in immunity in their study population (made up of 1,206 patients from various health facilities across SA) from 13% in the pre-

vaccination era to 57% in the post vaccination era (Amponsah-Dacosta *et al.* 2014). This 4.4 fold increase in immunity was higher than the 2.2 fold increase seen in blood donors in the present study, and could be explained by the fact that blood donors are a generally healthy population and are pre-screened prior to blood donation. In comparison, the population in the above-mentioned study were in health facilities, presumably with health related issues (i.e. a higher risk population).

In terms of an increase in immunity, the implementation of universal HBV vaccination could be regarded as a success in young blood donors born after 1995. The current study found a significant (2.2-fold) increase in HBV immunity in younger donors born in the post vaccination era. An unpublished 2018 SANBS study (Sykes *et al.* 2018) found a 3.2-fold decrease in the HBV infection rate in young blood donors from 2010 to 2015, and Spearman *et al*. (2017) reported a 4.3-fold decline in HBV sero-prevalence from 12.8% to 3% in children under 5 years of age from 1995 to 2009. Children 5 years of age in 2009 would still have been too young to be blood donors as part of this study, but this showed a definite decline in HBV rates following the implementation of universal HBV vaccination.

Unfortunately, the HBV carrier rate could not be accurately determined from the study results as HBsAg positives were excluded from this study, and an HBV chronic carrier is defined as being HBsAg positive, Anti-HBc positive and Anti-HBs negative (World Health Organisation 2005).

There were more donors in the pre-vaccination group who had past exposure immunity (Anti-HBs titre >10 IU/L and Anti-HBc positive) than in the post vaccination group (7.8% vs 1.2% respectively). This could be explained by the unvaccinated group being more likely to have contracted HBV during childhood and resolved their infection.

Almost 70% of the donors tested had no immunity to HBV (i.e. Anti-HBs titre <10IU/L). There could be a number of reasons for the high percentage of donors tested showing no evidence of HBV vaccination. Spearman and Sonderup (2014) reported that there was most likely an overestimation of HBV vaccination coverage. They stated that the 97% official vaccine coverage rate reported in SA in 2010 was probably too high due to the reduced coverage in rural areas than in urban areas, combined with a WHO reported coverage of only 56% in 2007 (Spearman and Sonderup 2014). Mayaphi *et al.* (2012) reported that 60.5% of people with AIDS and 76% of a control group showed no evidence of immunity to HBV, with the authors suggesting that this could be due to universal HBV vaccination only being introduced in SA in 1995 and not being routinely offered to adults (Mayaphi *et al.* 2012). Waning immunity in older

children and adults (Hipgrave, Maynard and Biggs 2006) could also explain the lower immunity detected in this study of blood donors aged 19 – 29 years of age.

5.3.3.1 Vaccination rates by gender, population group and age

Although the present study found that vaccination rates increased significantly in both male and female donors from the pre-vaccination to post vaccination eras, there was little difference in the vaccination rates between male and female donors. There was a slightly higher increase in the vaccination rate in female donors as compared to male donors (2.25 fold vs 2.07 fold). This was, however, not statistically significant and could be seen as indicative that there was little difference in HBV vaccinations given to male and female babies born in SA, even though males had a higher HBsAg carrier rate than females, with a male to female ratio of 2.6:1 (Burnett *et al.* 2012).

By population group, the results showed an overall higher vaccination rate in Asian and White donors than in Black and Coloured donors. Although vaccination rates were higher in White (22.5%) and Black (16.4%) donors in the pre-vaccination era group, Asian (54.8%) and White (39.1%) donors had the highest rates in the group born in the post-vaccination era. In multivariable analysis after controlling for geography, gender, and ethnicity, compared to Coloured donors Asian donors had a 2.5 (95% CI: 1.14 – 5.62) times greater odds of being vaccinated and White donors had a 2.1 (95% CI: 1.21 – 3.64) times greater odds of being vaccinated. Other than being born in the post-vaccination era, ethnicity was the only factor independently associated with being vaccinated in the blood donors tested.

A number of papers have reported on the challenges of implementing vaccination programmes in rural areas. These may included problems with maintaining the cold chain of HBV vaccines, knowledge of or access to vaccines, and missing or incomplete vaccination (Spearman and Sonderup 2014; Breakwell *et al.* 2017; McNaughton *et al.* 2019). Other challenges included missing vaccinations in children who were born outside of health care systems and a higher number of home births with insufficiently trained health care workers to attend to these home births and conduct postnatal care (Spearman and Sonderup 2014; Breakwell *et al.* 2017; McNaughton *et al.* 2019). These challenges could be possible reasons for the lower vaccination rates seen in Black donors as compared to other population groups in the post-vaccination era donors.

The study found that Black donors had the highest rate of past exposure immunity in the pre-vaccination (11.1%) group, followed by Coloured donors (6.5%), White donors (2.9%) and

Asian donors (0.0%). This correlated with findings reported by Burnett *et al.* (2012) for HBsAg chronic carrier (9.6%) and previous exposure to HBV (76%) rates in Black donors prior to the introduction of HB vaccination in SA (Burnett *et al.* 2012). Kiire (1996) reported that prior to the 1995 introduction of universal HBV vaccination in SA, 9.6% of Black South Africans, 0.2% of Whites and Asians and 0.4-3% of Coloureds were HBV chronic carriers. In terms of exposure to HBV, 76% of Black, 5% of White and Asian and 18-25% of Coloured South Africans showed evidence of previous exposure to HBV (Kiire 1996).

As expected, younger donors born in the post-vaccination era had the highest HBV vaccination rates. Donors aged 19 and 23 years (born 2000 and 1996 respectively) had the highest rates at 39%. Donors aged 26 and 28 (born 1993 and 1991 respectively) had the lowest rates of HBV vaccination (12.8% and 14.3% respectively). In multivariable analysis, after controlling for geography, gender, and ethnicity (possible confounders), younger donors from the post-vaccination era had a 2.9 (95% CI: 2.16 - 3.89) times greater odds of being vaccinated than donors born in the pre-vaccination era. No statistically significant odds were noted for geography or gender.

5.3.3.2 Vaccination rates by geographic region

In terms of geographic region, overall more donors were found to be vaccinated in the FS/NC (37.9%) and Egoli Zones (30.5%). The Mpumalanga Zone, Vaal Zone and the WCBS had the lowest rates of HBV vaccination at 20%. In the pre-vaccination era the FS/NC (37.1%) and Egoli (20.8%) Zones had the highest rates of vaccination, while in the post-vaccination era the Northern (41.5%) and Egoli Zones (40.3%) had the highest rates. KZN (9%) had the lowest rate in the pre-vaccination era, while the Vaal Zone (24.6%) had the lowest rate in the post-vaccination era.

The greatest increases in HBV vaccination rates were seen in donors from the Northern Zone and KZN (4.5 and 4.1 fold respectively). With KZN having the highest HIV rates in SA (Human Sciences Research Council 1 July 2018), there could be a higher level of awareness and more attendance at community clinics, resulting in more babies being vaccinated in this zone. The lowest increase was in the FS/NC donors, with only one additional donor found to be vaccinated. The FS/NC however had a high vaccination rate prior to 1995, which could explain the small increase in vaccination rate in this geographic area after the implementation. The FS/NC Zone is the largest zone serviced by SANBS, with the Northern Cape Province being the largest and most sparsely populated province in South Africa. With most studies performed

in South Africa being done in specific locations, there is limited data with regards to vaccination rates in specific areas and additional studies covering the whole country may be required.

It is important to have a well-functioning health system in order to have a good vaccination programme. Unfortunately studies in SA have previously shown geographic differences in the quality of health services and health outcomes (Jackson *et al.* 2007). A study conducted by Fadnes *et al.* (2011) in Paarl in the Western Cape, Umlazi in KwaZulu-Natal (KZN) and in Rietvlei (KZN) found that there were significant differences in vaccination coverage and timelines (especially for second and third vaccination visits). Wealthy areas such as Paarl performed better than poorer areas such as Rietvlei. They also reported that vaccine coverage was lower if the vaccine was given at an older age (Fadnes *et al.* 2011). These differences in the health system and differences in the socioeconomic circumstances of the different populations could explain the differences in vaccination rates and the uptake of the vaccination programme. It was not known whether the HBV prevalence in SA varied by geographic area, but what was known was that rural areas and coastal locations such as in the Eastern Cape and KZN had a higher risk in terms of HBV prevalence (Mayaphi *et al.* 2012).

KZN was also reported to have the highest HIV prevalence in SA at 27% (95% CI 23.9-30.4), followed by the Free State at 25.5% and the Eastern Cape at 25.2% (Human Sciences Research Council 1 July 2018). The study by Mayaphi *et al.* (2012) showed that patients with AIDS had an increased HBV prevalence and decreased Anti-HBs titres, placing them at higher risk of developing chronic HBV and experiencing more severe disease outcomes. There could be a greater effort to offer HBV vaccinations in areas where HIV prevalence was high, although no evidence to this effect could be found in the literature. People living with HIV and their families could have a greater awareness of treatment options available. This could explain the 4-fold increase in HBV immunity seen in donors in KZN, but required further investigation.

Donors in Mpumalanga had the highest rate of past exposure immunity (Anti-HBs titre > 10IU/L, Anti-HBc positive) in both the pre and post vaccination groups, followed by KZN (Mp: 20% and 3.3%, KZN: 9% and 2.6% respectively). This however did not correlate with HBV positives found by SANBS for the period 2005 to 2015. During this period, the highest rates of HBV positive donors were found in KZN at 0.16% and the ECZ at 0.12%, followed by Mpumalanga at 0.1% (SANBS Business Intelligence). This could warrant further investigation.

5.3.4 HBV Immunity in HIV Positive Blood Donors

One of the objectives of this study was to determine if the rate of HBV immunity was higher in HIV positive vs HIV negative blood donors. Unfortunately, no HIV positives were detected in the 1072 donations tested. It was determined after testing had been completed that due to the manner in which the samples were selected for the study, any viral positive samples would already have been removed. Samples for the study were selected after routine testing of donor samples for HIV, HBV and HCV was performed. As standard procedure in the routine testing laboratory (at SANBS and WCBS), all initial viral positive samples are removed following testing in order to perform second line and confirmatory testing. During the process of performing the additional testing, most of the sample was depleted. This resulted in only viral negative samples being available for selection for the purposes of the HBV vaccination study. It is regrettable that this objective could not be addressed in the present study, and it is recommended that a further study be conducted to determine the rate of HBV immunity in HIV positive vs HIV negative blood donors.

5.4 LIMITATIONS OF THIS STUDY

One of the limitations of the study was that blood donors were a generally healthy population and were pre-screened prior to donation to exclude high-risk groups. This meant that the blood donor population was thus not totally representative of the general South African population, and this could have skewed the results.

It was not possible to get the same number of donors in each category, e.g. there were no Asian donors in the Eastern Cape Zone (ECZ), Free State / Northern Cape (FS/NC), Mpumalanga (MP), Northern and Vaal Zones and Western Cape Blood Service (WCBS), and there were limited numbers of Coloured donors in most areas except the Western Cape. A higher increase in the rate of vaccinations in the Asian and Coloured population groups was found in the study but there were limited donor numbers in these groups and this could warrant further investigation in the future.

5.5. RECOMMENDATIONS

A possible strategy to further improve the rate of immunised donors could be to offer HBV vaccinations to adult donors found to be Anti-HBs negative (Yoshikawa *et al.* 2009; Fischinger *et al.* 2010) or to offer HBV booster vaccinations to young people about to become donors (Wang *et al.* 2016). These strategies could further improve blood safety.

In order to maintain and improve HBV vaccination coverage in South African, the government needs to improve community awareness of HBV vaccination programmes and focus on areas where the vaccination rates are lowest, as shown by this study's results. The introduction of a birth-dose vaccination to prevent new infections, full vaccine coverage and access to affordable treatment options will also be key to achieving the goals for HBV elimination (Spearman *et al.* 2017).

Going forward, the blood services should consider strategies to increase collection of blood from young people born after 1995 by targeting schools and universities. This must be balanced with the risk of HIV in these groups and sensitive screening techniques (including ID-NAT) for other transfusion-transmitted infections.

CHAPTER 6: CONCLUSION

A quarter of the donors tested showed evidence of being vaccinated for HBV, with an additional 4.5% having immunity due to past exposure to HBV. The HBV vaccination rate increased significantly in younger donors born after the 1995 introduction of universal HBV vaccination in South Africa, indicating programme efficacy. Vaccination rates increased in all population groups in the post-vaccination era, with the greatest increases among Asian donors, suggesting better uptake of the programme among this population group. Other than being born in the vaccination era, ethnicity was the only factor independently associated with being vaccinated.

These findings showed that HBV remains an important public health and blood transfusion issue and highlighted the importance of implementing and improving the HBV vaccination programmes and ensuring they reach all population groups, especially the most vulnerable groups and people living with HIV.

As young vaccinated donors will make up more and more of the blood donor panel going forward, it is expected that a significant decrease in HBV rates and a concomitant increase in blood safety will be seen.

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APPENDICES

1. SANBS HREC approval 2016/07 (16 September 2016)
2. SANBS HREC approval update 2016/07 (19 January 2018)
3. DUT IREC approval 147/18 (2 October 2018)

SOUTH AFRICAN NATIONAL BLOOD SERVICE NPC

Human Research Ethics Committee

OHRP Number : IORG0006278
FWA Registration Number : IRB00007553
SA NHREC Registration Number : REC-270606-013

SANBS
South African National Blood Service
Association Incorporated Under Section 21
Registration No. 2000/026390/08

Secretariat: V. Pepping Tel: 011 761 9135 | Fax: 011 761 9135 | 083 708 0569 | veronica.pepping@sanbs.org.za

To : Ms Wendy Sykes
E-mail : Wendy.Sykes@sanbs.org.za

Dear Ms Sykes,

DATE OF COMMITTEE MEETING: 6 September 2016

PROJECT TITLE: A cross sectional study to investigate whether the implementation of universal HBV immunizations in April 1995 has reduced HBV Prevalence in young blood donors (<20 year old) compared with donors aged 20-25 years old.

DECISION OF THE COMMITTEE : Approved

CLEARANCE CERTIFICATE NO : 2016/07

- Execution of the study must be compliant with applicable guidelines and policies.
- Any amendment, extension or other modifications to the protocol must be submitted to this Ethics Committee for approval prior to implementation.
- The Committee must be informed of any serious adverse event, planned and unplanned termination of the study.
- A progress report should be submitted yearly for long-term studies and a final report at completion of both short term and long term studies.
- Kindly refer to the SANBS HREC clearance certificate number on all future correspondence on this study to the HREC secretariat.
- This approval is valid for 5 years from the date stated above.

COMMITTEE GUIDANCE DOCUMENTS:

- International Conference on Harmonization (ICH) Good Clinical Practices (GCP) Guideline (ICH, 1996), Ethics in Health Research: Principles, Structures and Procedures (SA Department of Health, 2004); Guidelines for Good Practice in the Conduct of Clinical Trials in Human Participants in South Africa (SA Department of Health, 2016); Ethical Principles for Medical Research Involving Human: Declaration of Helsinki (World Medical Association, 2013); Reviewing Clinical trials: A Guide For Ethics Committees (Karlberg and Speers, 2010)

15 September 2016

CHAIRPERSON: Prof J.N. Mahlangu **DATE**

SOUTH AFRICAN NATIONAL BLOOD SERVICE NPC

Human Research Ethics Committee

OHRP Number : IORG0006278
FWA Registration Number : IRB00007553
SA NHREC Registration Number : REC-270606-013

SANBS
South African National Blood Service

Association Incorporated Under Section 21
Registration No. 2000/029390/08

Secretariat: Tel: 011 761 9135 | Fax: 011 761 9137 | Cell: 082 523 8523 | felicity.lew@sanbs.org.za

19 January 2018

To : Ms Wendy Sykes
E-mail : Wendy.Sykes@sanbs.org.za

Dear Ms Sykes,

ACKNOWLEDGEMENT OF RECEIPT OF NOTIFICATION

HREC REFERENCE NUMBER : 2016/07

PROTOCOL TITLE : A cross sectional study to investigate whether the implementation of universal HBV immunizations in April 1995 has reduced HBV Prevalence in young blood donors (<20 year old) compared with donors aged 20-25 years old

SANBS HREC hereby acknowledges receipt of your email dated 17 January 2018 in which HREC was notified of the proposed changes to the above-named protocol.

The amendments to be made to the protocol are detailed as follows:

1. Test samples from Western Province Blood Transfusion Service
    - The current protocol includes testing samples from SANBS blood donors. We have an opportunity to include samples from WPBTS. This will enable a complete overview of HBV immunisation in South African blood donors.

2. Access to HIV results
    - A paper by Amponsah-Dacosta *et al.* in 2014 documented that immunity was higher in HIV negative as compared to HIV positive people. It would thus be advantageous to include this aspect in the study and compare HBV immunity in HIV positive and HIV negative blood donors.

2/....

Your requests for the above changes were accompanied by the following documents

1. A signed letter of Notification of expansion to include the above changes;
2. Protocol for Ethics approval general information; and
3. A copy of the Clearance Certificate that was issued.

These documents were reviewed by the Chairman of SANBS HREC who accepted and approved the proposed changes.

The proposed changes are necessary and approved and ca be implemented immediately.

Yours sincerely

Felicity Lew
SANBS HREC Secretariat

Institutional Research Ethics Committee
Research and Postgraduate Support Directorate
2nd Floor, Berwyn Court
Gate 1, Steve Biko Campus
Durban University of Technology

P O Box 1334, Durban, South Africa, 4001

Tel: 031 373 2375
Email: lavishad@dut.ac.za
http://www.dut.ac.za/research/institutional_research_ethics

www.dut.ac.za

2 October 2018

Mrs W A Sykes
15 Barbet Avenue
Gillitts
KZN
3610

Dear Mrs Sykes

An investigation of the impact of universal Hepatitis B Virus (HBV) vaccination among young blood donors in South Africa

I am pleased to inform you that Full Approval has been granted to your proposal.

The Proposal has been allocated the following Ethical Clearance number **IREC 147/18**. Please use this number in all communication with this office.

Approval has been granted for a period of two years, before the expiry of which you are required to apply for safety monitoring and annual recertification. Please use the Safety Monitoring and Annual Recertification Report form which can be found in the Standard Operating Procedures [SOP's] of the IREC. This form must be submitted to the IREC at least 3 months before the ethics approval for the study expires.

Any adverse events [serious or minor] which occur in connection with this study and/or which may alter its ethical consideration must be reported to the IREC according to the IREC SOP's.

Please note that any deviations from the approved proposal require the approval of the IREC as outlined in the IREC SOP's.

Yours Sincerely

Professor J K Adam
Chairperson: IREC

DUT DURBAN UNIVERSITY OF TECHNOLOGY
2018 -10- 02
INSITUTUTIONAL RESEARCH ETHICS COMMITTEE
P O BOX 1334 DURBAN 4000 SOUTH AFRICA