

https://dspace.org/

The evaluation of point of care testing and dried blood spots to assess diagnostic accuracy of HCV infection as part of Sero-Molecular surveillance in people most-at-risk for hepatitis C virus

Gaelejwe, Lucinda Rebaone

2018

https://hdl.handle.net/10539/27723

Gaelejwe, Lucinda Rebaone (2018) The evaluation of point of care testing and dried blood spots to assess diagnostic accuracy of HCV infection as part of Sero-Molecular surveillance in people most-at-risk for hepatitis C virus, University of the Witwatersrand, Johannesburg, https://hdl.handle.net/10539/27723

Downloaded from a DSpace repository. https://dspace.org/

THE EVALUATION OF POINT OF CARE TESTING AND DRIED BLOOD SPOTS TO ASSESS DIAGNOSTIC ACCURACY OF HCV INFECTION AS PART OF SERO-MOLECULAR SURVEILLANCE IN PEOPLE MOST-AT-RISK FOR HEPATITIS C VIRUS

Lucinda Rebaone Gaelejwe

A Dissertation submitted to the Faculty of Health Science, University of the Witwatersrand, in fulfilment of the requirements for the degree of Master of Science in Medicine.

Johannesburg, 08 November 2018

DECLARATION

I **Lucinda Rebaone Gaelejwe**, declare that this dissertation is my own work, unaided. It is being submitted for the Degree of Master of Science in Medicine to the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

_____

Lucinda Gaelejwe

_____ day of _____ 2018 in _____.

DEDICATION

I would like to dedicate this dissertation to my Son, Lwandile Skhosana, my mother, brother, sister, and all my family members. Most importantly I would like to thank the LORD ALMIGHTY for his continuous support, loyalty and for showing me the right path even in hard times.

To my mother for all her kind and supportive words, always pushing me to the best in everything I do. My Son and family, for bringing me joy, happiness, allowing me to be what I have always wanted to be and continuously supporting me throughout the hard times and good times, thank you for your kindness and always being there for me.

PRESENTATIONS ARISING FROM THIS STUDY

1. Poster presentation number 186 at the SA AIDS conference 2017; **Preliminary evaluation of the utility and accuracy of viral hepatitis C point-of-care testing and characterisation of hepatitis C infections among people who inject drugs, sex workers and men who have sex with men in seven South African cities.**
2. Poster and flash presentation at Wits Postgraduate symposium 2017, won third place in faculty of Health Sciences; **Preliminary evaluation of Dried Blood Spots to assess diagnostic accuracy of HCV infection as part of a Sero-Molecular surveillance in people most-at-risk for hepatitis C Virus.**
3. An Oral presentation at the 7th Federation of Infectious Diseases Societies of Southern Africa (FIDSSA) 2017; **Preliminary evaluation of Dried Blood Spots to test for hepatitis C virus (HCV) antibodies among people who inject drugs, sexual workers and men who have sex with men in Cape Town.**

ABSTRACT

There are gaps in the knowledge of the burden of HCV infection in high-risk communities in South Africa. We evaluated HCV POCT and DBS samples for diagnostic accuracy as potential strategies for access to healthcare services and to determine the sero-molecular epidemiology of HCV in high-risk groups.

This study formed a sub-study on a major study entitled “The viral hepatitis C initiative for most at risk populations in South Africa” in collaboration with TB HIV Care (THC), Anova Health Institute (Anova), OUT Wellbeing (OUT), the Division of Hepatology at the University of Cape Town (UCT) and the National Institute of Communicable Diseases (NICD). The study was based on the integrated services of existing HIV prevention programmes for men who have sex with men (MSM), sex workers (SW) and people who use drugs, including people who inject drugs (PWUDs/PWIDs) across seven South African cities. This is a first study in South Africa to provide information on hepatitis C prevalence data among these key populations and assessing the usefulness and validity of point of care hepatitis C testing in the South African context.

The study was conducted from August 2016 - October 2017, in seven cities in South Africa and enrolled a total of 3509 participants. HCV POCT was used for diagnostic testing at research sites. A total of 532 PWUDs/PWIDs, 66 MSM and 115 SWs. Samples with positive HCV tests were sent to the NICD laboratory for confirmation of POCT results using the automated Architect *i* system for anti-HCV testing. All antibody positive samples were followed up with viral load testing on the automated COBAS® Ampliprep/TaqMan® and genotyping using the Versant HCV genotyping 2.0 Assay. Furthermore, from Cape Town sites, DBS and plasma paired specimens were collected to assess the accuracy of POCT testing and to assess the accuracy of viral testing on DBS. HCV antibody was tested on both DBS and plasma paired samples on the Architect *i* system. HCV viral loads were tested on paired plasma and DBS samples using the automated COBAS® Ampliprep/TaqMan® 48 analyser. The STARD approach was used in reporting the accuracy of POCT. Sensitivity and specificity, positive predictive and negative predictive values were calculated for antibody tests. For viral load comparison between DBS and plasma, agreement of methods was determined by Linear Regression and Bland Altman plot.

At the end of the study, 713 plasma samples and 240 DBS were received at the NICD laboratory. Plasma samples (N=705) and DBS (N=239) were tested. HCV POCT testing had a sensitivity of 98.5% and specificity of 97.6%. DBS testing had a sensitivity of 96.3% and specificity of 97.4%. The linear regression was R^2=0.918 and there was no difference between methods using the Bland Altman analysis. Viral load on plasma ranged from 1.2 log IU/ml to 7.7 log IU/ml. The prevalent genotypes were genotype 1a (73.93%), 3a (14.79%), and 3 (3.51%).

HCV POCT can be used with a high degree accuracy. DBS can be used as an alternative sample matrix for HCV serology and RNA testing among high-risk groups. These tools are recommend in the South Africa setting to provide access to diagnosis and treatment as part of the WHO 2030 HCV elimination strategy.

ACKNOWLEGDMENTS

To the Centre of Vaccine and Immunology (CVI) staff and Human Immunodeficiency virus (HIV) Serology staff at NICD, I would like to say thank you for your kindness and assistance throughout my studies, for being kind enough for me to use your facilities and special equipment to aid in my research.

I would like to acknowledge all the investigators; Wendy Spearman, Harry Hausler, Mark Sonderup, Kevin Rebe, Andrew Lambert, Andrew Scheibe, Dawie Nel and co-investigators.

I would also like to acknowledge all the participants at the study sites and the study sites: The Division of Hepatology, Department of Medicine, University of Cape Town, Faculty of Health Sciences, University of Cape Town TB/HIV Care Association (THCA), OUT LGBT Wellbeing (OUT), Anova Health Institute (ANOVA) and National Institutes for Communicable Diseases (NICD) and partnering organisations.

Lillian, Susan I would like to say THANK YOU, for being my mentors, teachers and big sisters. You have taught me so much about lab techniques, work ethics and how to handle pressures from work; you showed me kindness and overabundance of love. Thank you for everything. I will forever be grateful.

I would like to acknowledge National Research Foundation (NRF), Poliomyelitis Research Foundation (PRF) and Bristol Myers Squibb Foundation (BMSF) for their generous financial assistance towards my studies.

Lastly, to my supervisors, Prof. Adrian Puren (Supervisor) and Dr. Nishi Prabdial-Sing (Co-Supervisor), I would like to say THANK YOU, for your support, kindness and patience with me, you have offered me so much, I have learned so much from the both of you. I will carry all the knowledge that you instilled in me for the rest of my life.

TABLE OF CONTENTS

DECLARATION ii
DEDICATION iii
PRESENTATIONS ARISING FROM THIS STUDY iv
ABSTRACT v
ACKNOWLEGDMENTS vii
TABLE OF CONTENTS viii
LIST OF ABBREVIATIONS AND SYMBOLS xii
LIST OF FIGURES xvi
LIST OF TABLES xvii
CHAPTER ONE – INTRODUCTION 1
1.1. Hepatitis C Virus (HCV) Historical Background 1
1.2. HCV Structure 1
1.3. HCV Genome 1
1.4. HCV Genotypes 2
1.5. HCV Prevalence 4
1.5.1. HCV prevalence in high-risk groups 5
1.5.2. Population at risk in South Africa 6
1.6. Natural History Of HCV Infection 7
1.7. HCV Transmission 8
1.7.1. Blood transfusions and iatrogenic exposures 8
1.7.2. Occupational and perinatal exposure 9
1.7.3. Sexual transmission 9
1.7.4. Intravenous drug users (IDUs) 11
1.7.5. Other routes of HCV transmission 12

1.8. Prevention Of HCV Transmission 12
1.9. HCV Diagnostic Testing 14
1.9.1. Immunoassays 14
1.9.2. Molecular techniques 15
1.9.3. Molecular genotyping 15
1.9.4. HCV rapid assays 16
1.9.5. DBS 20
1.10. HCV Treatment 23
1.11. Problem Statement 24
1.12. Purpose Of The Study 26
CHAPTER TWO - MATERIALS AND METHODS 27
2.1. Study Design 27
2.2. Ethics 27
2.3. Study Population 27
2.4. Sample Size Calculation 28
2.5. Samples Collection 28
2.6. Data Collection 30
2.7. Laboratory Methods 30
2.7.1. OraQuick® HCV rapid antibody test 30
2.7.2. Processing of samples 31
2.7.3. Anti-HCV ELISA 32
2.7.4. HCV viral load assay 33
2.7.5. HCV RNA extraction for genotyping plasma samples. 35
2.7.6. HCV Amplification for genotyping. 37
2.7.7. Agarose gel electrophoresis 38
2.7.8. HCV genotyping 2.0. (LiPA) 39
2.7.9 Normalization Coefficient calculation 41

2.7.10. Statistics....42
CHAPTER THREE - RESULTS....44
3.1. STARD Diagram....44
3.2. HCV POCT Sensitivity and Specificity....47
3.2.1. HCV POCT OraSure (whole blood) serology accuracy....48
3.2.2. HCV POCT OraSure (oral fluid) serology accuracy....50
3.3. HCV DBS Serology Accuracy....51
3.4. HCV Viral Loads on HCV DBS....52
3.4.1. Linear regression....53
3.4.2. Bland-Altman plot....55
3.5. HCV Plasma Viral Loads Per High-Risk Groups....57
3.6. HCV Amplification For Plasma Genotyping....58
3.7. HCV Genotyping....59
3.7.1. Prevalent genotypes....59
CHAPTER FOUR – DISCUSSION....61
4.1. HCV POCT....61
4.2. DBS Immunoassay Results....64
4.3. HCV Viral Load on HCV DBS....64
4.4. HCV Viral Loads on Plasma....66
4.5. HCV Genotypes on Plasma....66
4.6. Limitations....68
4.7. Conclusions....69
5. REFERENCES....70
6. APPENDICES....99
APPENDIX A: UNIVERSITY OF WITWATERSRAND HREC COMMITTEE APPROVAL....99
APPENDIX B: UNIVERSITY OF CAPE TOWN HREC COMMITTEE APPROVAL....100

APPENDIX C: INFORMATION SHEET AND CONSENT FORM: STUDY 101
APPENDIX D: INFORMED CONSENT FORM: BLOOD STORAGE.....103
APPENDIX E: HEPATITIS SURVELLANCE LABORATORY REQUEST FORM 105
APPENDIX F: VIRAL HEPATITIS POINT OF CARE TESTING INSTRUCTIONS
ORAQUICK HCV TESTING INSTRUCTIONS 107
APPENDIX G: TURNITIN REPORT.....108

LIST OF ABBREVIATIONS AND SYMBOLS

%	Percentage
~	Approximately
°C	Celsius degrees
<	Less than
≥	Greater or equal to
5'UTR	5 prime untranslated region
aa	Amino acid
ab	Antibody
AMPL CTRL 1	Amplification Control 1
AMPL CTRL 2	Amplification Control 2
Anti-HCV	Antibody to hepatitis C virus
BCIP/NBT tetrazolium	5-Bromo-4-chloro-3-indolyl phosphate/ nitro blue
bp	Base pairs
Buffer AVL:	Lysis Buffer
Buffer AW1:	Wash buffer 1
Buffer AW2:	Wash buffer 2
C	Control
cDNA	Complementary DNA
CI	Confidence Interval
CMIAs	Chemiluminescent Immunoassays
CONJ CTRL	Conjugate Control
CVI	Center of Vaccine and Immunology
DBS	Dried Blood Spots
DNA	Deoxyribonucleic Acid
dNTP	Deoxyribonucleotide triphosphate
dt	Deoxy-thymidine nucleotides
dUTPs	Deoxyuridine 5-triphosphate.

e	Exponent
EDTA	Ethylenediaminetetraacetate Acid
EIA	Enzyme Immunoassay
ELISA	Enzyme-Linked Immunoassay
FDA	Food and Drug Administration
HAV	Hepatitis A Virus
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
IDU	Injecting Drug Use
IDUs	Injecting Drug Users
IVDU	Intravenous Drug Use
IgG	Immunoglobulin G
IU/ml	International units per millilitres
LPC	Low Positive Control
$MgCl_2$	Magnesium Chloride
mins	Minutes
ml	Millilitres
MSM	Men Who Have Sex with Men
MW	Molecular Weight
NaCl	Sodium Chloride
NANBH	Non-A, non-B hepatitis
NATs	Nucleic acid tests
NC	Negative Control
NICD	National Institute of Communicable Diseases
NPC	Negative Positive Control
NPV	Negative Predictive Value
NS	Non-Structural
NSP	Needle/Syringe Provision

nts	Nucleotides
OF	Oral Fluid
ORF	Open Reading Frame
OST	Opioid Substitution Therapy
PBS	Phosphate-Buffered Saline
PC	Positive Control
PCR	Polymerase Chain Reaction
PID	Participant Identification
POC	Point Of Care
POCT	Point-of-Care Tests
PPV	Positive Predictive Value
PWIDs	People who Inject Drugs
PWUDs	People who Use Drugs
RIBAs	Recombinant Immunoblot Assays
RLUs	Relative Light Units
RNA	Ribonucleic Acid
RNAses	Ribonucleases
rpm	Revolutions per minute
RT	Reverse transcription
RT-PCR	Reverse transcription-polymerase chain reaction
S/CO	Sample /Cutoff
SD	Standard Deviation
SPEX buffer	Sample Pre-Extraction buffer
STARD	Standards for Reporting of Diagnostic Accuracy Studies
S-tube	Sample tube
SW	Sexual Worker
TAE buffer	Tris-acetate-EDTA
TBE buffer	Tris/Borate/EDTA buffer
TMA	Transcription-mediated Amplification
Tris	Trimethamine

UCT HREC	University of Cape Town Human Research Ethics Committee
USA	United States of America
UV	Ultra violet
V	Volts
VI	Six
WITS HREC	University of Witwatersrand Human Research Ethics Committee
X	Times
µL	Microliters
TaqMan probes	dye-labelled oligonucleotide probes
g	Grams

LIST OF FIGURES

CHAPTER ONE

Figure 1.1; The HCV genome with different viral proteins……2

CHAPTER THREE

Figure 3.1; Specimen and test flow to assess the accuracy of HCV POCT and DBS matrices……43

Figure 3.2; The linear regression graph of HCV viral load of DBS (log IU/mL) and HCV of viral load plasma (log IU/mL) without application of normalization coefficient to raw DBS results……52

Figure 3.3; The linear regression of the corrected HCV viral load of DBS (log IU/mL) and the HCV viral load of plasma (log IU/mL) ……53

Figure 3.4; The Bland Altman Plot of HCV viral load of DBS (log IU/mL) and HCV viral load of plasma (log IU/mL) without application of normalization coefficient……54

Figure 3.5; The level of agreement between HCV viral load of DBS (log IU/mL) and the corrected HCV viral load of plasma (log IU/mL) viral load after volume normalization……..55

Figure 3.6; Agarose gel documentation of the HCV PCR products…….56

Figure 3.7; The HCV genotyping test strips showing different types of HCV genotypes……57

LIST OF TABLES

CHAPTER THREE

Table 3.1; Demographic characteristics of high risk groups……44

Table 3.2; The 2x2 table of HCV POCT OraSure (whole blood and oral fluid) and reference test (ELISA) results…….45

Table 3.3; The statistical analysis of both POCT OraSure (whole blood and oral fluid) was compared to reference test (ELISA) results. ……46

Table 3.4; The 2x2 table of POCT OraSure (whole blood) and reference test (ELISA) results.…….47

Table 3.5; The statistical analysis of POCT OraSure (whole blood) was compared to reference test (ELISA) results…….47

Table 3.6; The 2x2 table of HCV POCT OraSure (oral fluid) and reference test (ELISA) results……...48

Table 3.7; The statistical analysis of HCV POCT (oral fluid) compared to reference test (ELISA) results……48

Table 3.8; The 2x2 table of HCV DBS compared to reference test (ELISA) results.49

Table 3.9; The statistical analysis of DBS compared to reference test (ELISA) results…….50

Table 3.10; HCV plasma viral load (log IU/ml) per high-risk group…….56

Table 3.11; The distribution of HCV genotypes among various the high-risk groups…….58

CHAPTER ONE – INTRODUCTION

1.1. Hepatitis C Virus (HCV) Historical Background

It was through serological tests of hepatitis A (HAV) and hepatitis B (HBV) viruses which lead to the discovery of the first non-A, non-B viral hepatitis (NANBH) in 1975 (Tang, 1991). These tests demonstrated that cases of transfusion-associated hepatitis lacked serological markers of either HAV or HBV (Feinstone et al., 1975, Alter et al., 1975b). It was later discovered that 10% of transfusions resulted in NANBH (Berman et al., 1979, Aach et al., 1981), which would later cause persistent liver damage that leads to cirrhosis in as many as 20% of chronic infections. Approximately 90% of NANBH was attributed to hepatitis C virus infections (Kuo et al., 1989). It was through these discoveries that the development of more sensitive and specific diagnostic assays for the detection of anti-hepatitis C antibodies in 1989 was launched (Hoofnagle and Alter et al., 1985).

1.2. HCV Structure

Hepatitis C Virus (HCV) is a small, enveloped virus particle (36-62nm), spherical in shape with an icosahedral capsid encompassing the single-stranded positive-sense ribonucleic acid (RNA) genome (Choo et al., 1991a). It was identified and sequenced in 1989 (Choo et al., 1989b) and the RNA genome is ~ 9500 nucleotides in length and encodes a large polyprotein of 3010 amino acids (Choo et al., 1991a). The coding region is flanked by two non-coding regions at the 5' and 3' ends. HCV belongs to the family *Flaviviridae* and genus *Hepacivirus* (Kuo et al 1989, Choo et al., 1991a, International Committee of the Taxonomy of Viruses, 2015).

1.3. HCV Genome

The genome has a large open reading frame (ORF) which encodes a precursor polyprotein of 3008-3037 amino acids (Choo et al., 1991a). The HCV 5'untranslated region (5'UTR) is 342 nucleotides (nts) and the most conserved region of the genome

located upstream of the ORF (Choo et al., 1991a). The 3'URT contains 225 nts that are organized into three regions (Choo et al., 1991a). The HCV ORF encodes 11 proteins, including 3 structural proteins, a small protein, p7 and 6 non-structural (NS) proteins (NS2, NS3, NS4A, NS4B, NS5A and NS5B) (Choo et al., 1991a). The structural protein includes the core, E1, and E2 which are located in the N-terminal. The non-structural protein resides at the C-terminal part (Choo et al., 1991a).

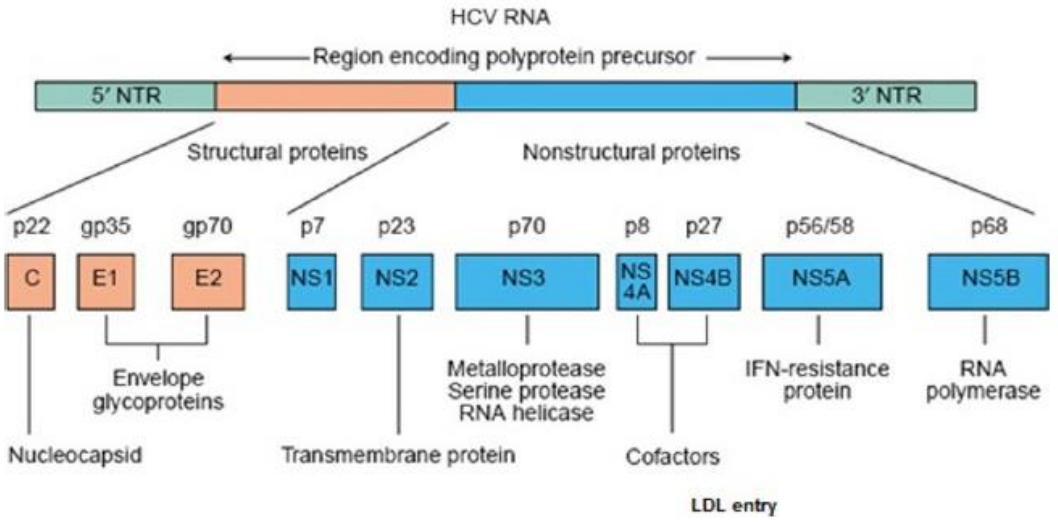


Figure 1.1; The HCV genome with the different coded proteins. Adopted from Ashfaq et al., 2011

1.4. HCV Genotypes

Seven genotypes have been described for HCV by phylogenetic analyses of > 95% of the coding region (Simmonds et al., 1993c, Smith et al., 2014a). HCV genotypes show a nucleotide divergence of 30% - 35% whilst subtypes are defined by a nucleotide divergence of 20% - 25% (Simmonds et al., 1993c). There are 67 confirmed subtypes (Smith et al., 2014a) and numerous quasi-species variants, based on scattered and local variations of the genome that exist within each subtype (Jamalidoust et al., 2014). The distributions of the HCV genotypes reflect the epidemiology and the routes of transmission of HCV (Pawlotsky et al., 1995b).

HCV genotypes and subtypes are geographically located worldwide (Dusheiko et al., 1994). Globally, genotype 1 is the most common (42.6%), followed by genotype 3 (30.1%) and genotype 2, genotype 4 and genotype 6 account for about (22.8%) of all HCV cases worldwide (Messina et al., 2015). Genotype 5 represents less than 1% of the global HCV cases (Messina et al., 2015).

Genotype 1a is most common worldwide and mostly prevalent in the United States and Northern Europe (Simmonds, 2004b). Genotype 2a and 2b are prevalent in Europe and Japan, subtype 2c is most predominant in Northern Italy (Zein, 2000, Simmonds, 2001a) and West Africa (Messina et al., 2015) and genotypes 3a and 3b are most commonly found in Pakistan, North America, South America and Europe (Idrees and Riazuddin, 2008, WHO., 2009a). Genotype 1a, 1b, and 3a have a wide distribution due the result of blood transfusions and needle sharing among people who use drugs and people who inject drugs (PWUDs/PWIUs) respectively (Simmonds, 2004b, Jamalidoust et al., 2014).

The common cause of chronic hepatitis C in the Middle-East, North Africa, and sub-Saharan Africa is genotype 4. Genotype 4 accounts for 90% of all HCV cases in Egypt (Bukh et al., 1993, Stuyver et al., 1993, EMOH, 2007). There has been documented increase in the prevalence of genotype 4 in France, Italy, and Belgium (Antaki et al., 2009a). In sub-Saharan Africa genotype 4 is mainly transmitted via scarification, circumcision practices and sexual activity (Xu et al., 1997, Mudawi et al., 2007). In the European borders, the transmission of genotype 4 is due to immigration and movement of intravenous drug users (IVDUs) (Nguyen and Keeff, 2005, Antaki et al., 2009a). HCV subtype 4a and 4d are most predominant in Egypt, Europe and Africa respectively (Antaki et al., 2009a). IVDUs in Europe are mostly infected with subtype 4c/4d (Delwaide et al. 2006b).

Approximately 40% of all genotype 5 HCV cases are confined in the Northern parts of South Africa (Ohno et al., 1994a, Smuts and Kannemyer, 1995, Ohno et al., 1997b, Prabdial-Sing et al., 2016). Genotype 5 is also found in other parts of the world such as France, Spain, Syria, and Belgium (Jover et al., 2001, Delwaide et al., 2005a, Antaki et al., 2008b). It has been reported that genotype 5 originated in South Africa; however,

there is speculation that HCV genotype 5 viruses could have possibly originated in Central Africa and spread through via human migration (Verbeeck et al., 2006a). The main route of HCV genotype 5 transmission was through blood transfusions and unsafe therapeutic injections in South Africa (Tanaka et al., 2006) and France (Abergel et al., 2007). However, to date there has not been any studies that characterized HCV genotypes in high-risk groups.

Genotype 6 is mostly confined in South Asia (Zhang et al., 1995, Prescott et al., 1996), among Asian Americans and Asian Australians (Antaki et al., 2009a). It mainly transmitted via unsafe therapeutic injections, IVDU, and blood transfusions. Genotype 7 is a novel genotype found in Democratic Republic of Congo (Murphy et al., 2015).

1.5. HCV Prevalence

Hepatitis C is considered a major public health burden in the world (Sy and Jamal, 2006). It is estimated that 3% of the world's population, approximately 185 million people, are infected with hepatitis C virus (WHO, 2009a, Mohd Hanafiah et al., 2013). An estimated 80% are from developing countries (Graham and Swan, 2015) with 399 000 deaths annually being HCV-related (WHO, 2002d). A large majority of people still remain underdiagnosed and unaware of their HCV status (Kwiatkowski et al., 2002). HCV prevalence varies worldwide with 14% in Egypt (El-Zanaty and Way, 2009) to 0.5% - 1.5% reported in Europe (Gower et al., 2014) and India (EMI Guidelines, 2014). Prevalence's of 3.2% and 6.5% have been reported in China and Pakistan respectively (EMI Guidelines, 2014). In the Central Africa region, Cameroon has the highest HCV prevalence of 13.8% followed by 11.3% in Burundi (Karoney and Siika, 2013); in West Africa and Guinea the highest HCV prevalence is 5.5% (Madhava et al., 2002). The estimated HCV prevalence in Africa is 5.3% (Pybus et al., 2003) with 2.1% - 2.8% prevalence reported in Sub-Saharan Africa (Layden et al., 2014), and a seroprevalence of 0.1% - 1.7% has been described in South Africa (Abuelhassan, 2012).

1.5.1. HCV prevalence in high-risk groups

High-risk groups are the groups of individuals that have a greater chance of contracting HCV infection due to their risk behaviours. These include people who engage in injecting risk behaviours by needle sharing, people who engage in risky sex behaviour with inconsistent condom use with casual partners, people who are homeless, have been incarcerated (Degenhardt et al., 2017). The estimated HCV prevalence for high-risk groups in Central Africa ranges from 11% to 55% and 2.1% to 38% in West Africa and in South and East Africa the HCV prevalence ranges from 1.3% to 65% (Madhava et al., 2002) and in southern Africa 0.91% (Rao et al., 2015). Other countries such as Zimbabwe and Kenya have HCV prevalence in high-risk groups of 1.3% and 1.7% respectively (WHO, 1999c). The HCV seroprevalence in South Africa among blood donors is 1.78%, pregnant women is 2.51%, people living with HIV 3.57% and the general population 5.41% (Sonderup et al., 2017).

The PWID high-risk group in Tanzania have an HCV prevalence of 27.7% (Bowring et al., 2013) and 14% prevalence in Men who have sex with Men (MSM) (Dahoma et al., 2011). High-risk populations who are recipients of blood or blood products in Western Africa have the highest HCV seroprevalence 15.69%, followed by Central African (16.26%) and Southern Africa (6.40%). South Africa has a low HCV prevalence in the general population; however, to date there is inconsistent data in the different populations and regions. In Gauteng, Western Cape, Limpopo, KwaZulu-Natal the HCV seroprevalence among HIV positive individuals is 1.2% (Gededzha et al., 2010a), 3.2% (Gogela et al., 2013), 0.8% (Barth et al., 2011) and 5.1% (Tiathiah et al., 2014) respectively. Only a few studies have been conducted in South Africa among PWUDs/PWIDs, MSM and SWs.

1.5.2. Population at risk in South Africa

People Who Inject Drugs And People Who Use Drugs (PWUDs/PWIDs)

HCV prevalence among PWID is estimated to be greater than 90% in South Africa (Hernandez et al., 2011), however there is a lack of HCV data among SWs who use or inject drugs. Almost half of the PWID groups have always shared their needles and syringes with other people (United Nations office on Drugs & Crime, 2015).
There have been reported high rates of HCV in HIV-positive MSM in KZN and Cape Town, South Africa (Tathiah et al., 2014, Parboosing et al., 2008).
There was a 24% HCV prevalence among 241 PWID in 2013/14 (Sediba Hope medical Centre, 2013) and Cape Town has the highest prevalence of drug use in the country.

Men who have sex with Men (MSM)
There is no estimated data on the HCV prevalence of MSM in South Africa. In a survey it was reported that 56% of MSM interviewed have more than two sexual partners in the last month and about 23% of MSM were involved in transactional sex in the past six months (Cloete et al., 2014).

Sex Workers (SWs)
There is limited reported data on the HCV prevalence of SWs among various cities in South Africa. In the Eastern Cape there was an estimated 13 408 of female sex workers (SWEAT, 2013) and approximately 6294 sex workers have been reported, in Durban (SWEAT, 2013). An estimated 7500 female sex workers in Cape Town metropolitan area with a HIV prevalence of 7% (SWEAT, 2013), with a HIV prevalence of 52% and 65% has been reported in Durban and in Pietermaritzburg respectively (SWEAT, 2013).

A detailed epidemiology and understanding of HCV disease burden among high risk groups is absent (Barth et al., 2014). Currently the HCV epidemics are concentrated in specific populations and not generalized across all populations. Therefore, there is a

greater need for studies to focus on HCV screening and to provide key services among these groups (Semugoma et al., 2017).

1.6. Natural History Of HCV Infection

HCV is a major cause of chronic liver disease and cirrhosis in the United States (Strahotin and Babich, 2012). According to the Global Burden of Disease study, liver cancer and cirrhosis together contribute to one of the top 20 causes of mortality (Thursz and Lacombe, 2016). Chronic hepatitis C causes the development of hepatocellular carcinoma (HCC), resulting in approximately half a million new cases of liver cancer occurring each year (WHO, 1996b).

Acute hepatitis C infection is challenging to diagnose because 70% - 80% of HCV infected persons are asymptomatic (McCaughan et al., 1992); the incubation period is from 3 to 12 weeks after exposure (Seeff, 2009) and 20% to 30% of those acutely infected may develop mild clinical symptoms (Alter et al., 1992f). Symptoms include anorexia, jaundice, malaise, and weakness (Chen and Morgan, 2006). Acute HCV infection can be severe, resulting in fulminant liver failure, (Farci et al., 1996). HCV infections are only cleared in 15% - 45% of cases and usually within six months of acquiring the infection (WHO, 2002d).

Chronic infection develops in 75% to 85% of infected patients and this is indicated by persistence of HCV RNA in the blood for 6 months after acute infection (Chen and Morgan, 2006). Chronic hepatitis is affected by various factors. The rate of chronic hepatitis C infection appears to be lower in younger individuals (Chen and Morgan, 2006). Alcohol consumption in high levels does contribute to the development of progressive liver disease (Peters and Terrault, 2002) and the rate of chronic HCV is lower in individuals who progress to jaundice in the acute onset of HCV relative to those who develop symptoms later on after infection (Chen and Morgan, 2006). An estimated 10% - 20% of chronic HCV infection will develop end-stage liver diseases after 20 years (Chen and Morgan, 2006).

1.7. HCV Transmission

HCV is a blood borne pathogen. It is commonly transmitted through parenteral exposure of infected blood or blood products such as contaminated injections and organ transplants from an infected individual. Other routes include vertical exposure, sexual transmission, perinatal exposure and occupational exposure via needle stick injury (Chen and Morgen, 2006). High-risk behaviours that are common for transmitting HCV include intravenous drug use (IDU) (Chen and Morgen, 2006), needle sharing for immunizations, tattooing and scarification techniques (Lavanchy, 2011). However, in Africa and other parts of the globe, there is a significant proportion where HCV transmission risk is unknown (Zeuzem et al., 2006a) mainly due to limited information about HCV transmission and limited HCV screening facilities, resulting in people not aware of their HCV status.

1.7.1. Blood transfusions and iatrogenic exposures

The screening of all blood transfusion and organ transplants from donors began in 1990 (Leary, 1990). The countries which started HCV screening in the 1990s managed to eradicate HCV in their general population (Alter, 1997c). Today, we know that prior to HCV screening in all blood products, transfusions accounted for the majority of the HCV transmission cases worldwide (Alter, 1997c, Alter, 2007d).

Iatrogenic exposures is the use of unsterilized medical equipment/s, unsafe therapeutic injections utilized in hospitals and dental settings (Hauri et al., 2004) which have contributed to HCV transmission. This was due to a mass government campaign, during parenteral administration of the antischistosomal therapy in Egypt (Frank et al., 2000). The cause of the increase in HCV prevalence in Cameron, Gabon and the Central African Republic was due to large-scale intervention program required for the control of tropical diseases using intravenous (IV) drugs needed for treatment (Njouom et al., 2007b, Ndong - Atome et al., 2008, Pépin and Labbé, 2008, Njouom et al.,2009a).

1.7.2. Occupational and perinatal exposure

Occupational exposure is a result of injuries from contaminated injections in a healthcare facility. Health care workers are the most at risk individuals for HCV infection with an average rate of transmission of 0.5% (Jagger et al., 2002). There is a 1.8% incidence of healthcare workers who sustained percutaneous exposure from anti-HCV positive patients (CDC, 1995a, Puro et al., 1995), with an estimated 400 000 percutaneous related injuries which occur annually in hospital employees worldwide (Panlilio et al., 2004). Primary and secondary prevention practices are the most important methods in preventing HCV transmission among healthcare workers (Hughes and Henderson, 2016).

Perinatal transmission is uncommon and HCV transmission depends on the detectable HCV RNA levels above 10^6 copies per mL (Roberts and Yeung, 2002). There is an estimated 4% - 7% rate of mother-to-infant transmission per pregnancy provided that there are detectable HCV RNA in the maternal serum during delivery (Roberts and Yeung, 2002, Alter, 2007d).

Breastfeeding has also been associated with HCV transmission. There is a high potential of HCV transmission in symptomatic anti-HCV positive mothers with high viral loads and little to no chance of mother-to-infant transmission in asymptomatic anti-HCV positive mothers with HCV RNA in their maternal milk (Rachana and Shahul, 1998). It has been suggested that if there are no abrasions of the nipple due to suckling, HCV cannot be transmitted vertically, through milk. Furthermore, the low levels of HCV RNA in the colostrum can easily get inactivated by the gastrointestinal tract, thus decreasing the potential of mother-to-infant transmission (Rachana and Shahul, 1998).

1.7.3. Sexual transmission

Sexual transmission of HCV occurs via infected body-fluids that are exchanged across the mucosal surface. There are various factors that influence sexual transmission of HCV and include the presence of HCV RNA in bodily fluids (Liou et al., 1992), HCV RNA levels, the integrity of the mucosal surface (Terrault et al.,2013c), engaging in risky sexual activity with multiple sexual partners (Alter et al., 1997c). The ability to detect

HCV RNA in bodily fluids alone does not imply possible transmission (Terrault et al., 2013c) and there is still a relatively lower risk of sexual transmission of HCV (Tahan et al., 2005).

HIV infection increases HCV RNA levels (Sherman et al., 2005) and promotes the shedding of HCV in semen (Pasquier et al., 2003, Briat et al., 2005). The HIV status and presence of ulcers caused by sexually transmitted diseases (STI) facilitate the transmission of HCV (Rauch et al., 2005, Danta et al., 2007, CDC, 2011d, Schmidt et al., 2011). The ulcers in the genital region interrupt the mucosal integrity of the genitourinary tract and encourage HCV transmission (Danta et al., 2007, CDC, 2011d, Schmidt et al., 2011, Larsen et al., 2011, Wandeler et al., 2012, Apers et al., 2015, Wong et al., 2015). Low virus titres in the genital secretions may be one reason that HCV is transmitted less efficiently than HBV (Qian et al., 2005) or HIV (Shepard et al., 2002). HCV is 10 times more infectious than HIV (Crofts et al., 2001). It is through the early phase of acute infection, high virus concentrations and no antibody to complex the antigen that HCV is most likely to be transmitted (Alter et al., 2007d).

The sexual transmission of HCV remains a controversial topic due to inconsistent data (Terrault et al., 2013). The first documented case-control study from persons with hepatitis C showed strong evidence that heterosexual activity was a high risk of transmitting HCV infection (Alter et al., 1989g). However, the association of heterosexual persons in long-term monogamous relationships with a partner chronically infected with HCV virus has not been established (CDC, 1998b).

There is 1% - 7% anti-HCV prevalence among MSM who report to not have a history of drug use and 25% - 50% anti-HCV prevalence among MSM who have a history of IDU (Buchbinder et al., 1994, Bodsworth et al., 1996, van der Laar et al., 2010, Raymond et al., 2012, Seaberg et al., 2014). Previous studies report that HCV is frequently found among HIV positive MSM (Tor et al., 1990, Ricchi et al., 1992, Buchbinder et al., 1994, Bodsworth et al., 1996, Ndimbie et al. 1996, Van der Laar et al., 2010, Kouyos et al., 2014). The transmission of HCV among HIV positive MSM individuals is predominantly via permucosal transmission routes (Van der Laar et al., 2010, Bradshaw et al., 2013).

Previous studies have demonstrated the role of cofactors in sexual transmission among MSM, and these include co-infection with HIV (Danta et al., 2007, CDC, 2011d, Schmidt et al., 2011), presence of ulcers caused by Sexually Transmitted Infections (STIs) (Rauch et al., 2005) and drug use (Page and Nelson et al, 2016). High risk sexual behaviours involving the use of mucosal administered drugs, such as methamphetamines, ketamine, gamma-hydroxybutyrate and lysergic acid diethylamide, increase HCV transmission among MSM (Ndimbie et al., 1996, Rauch et al., 2005, Danta et al., 2007, Giraudon et al.,2008, CDC, 2011d, Schmidt et al., 2011, Larsen et al., 2011). The use of recreational drugs such as crystal methamphetamine and mephedrone is known as "chemsex", can also increase HCV transmission among MSM (Page et al., 2016).

1.7.4. Intravenous drug users (IDUs)

HCV transmission is through blood-to-blood contact, direct and indirect sharing of needles or injecting equipment (CDC, 2016f). HCV transmission in sharing of needles depends on the quantity of blood inoculation and the HCV viral load (Migard et al., 2016). Ancillary injecting equipment and practices such as sharing cotton, filters spoons, cookers, and back loading and rinse water have also been associated with HCV transmission (Thrope et al., 2002, Migard et al., 2016). These practices have been associated with an increased risk of HCV infection and proportion of new infections (Pouget et al., 2012, Palmateer et al., 2013a), however, it has a lesser probability of HCV transmission than sharing needles and syringes (Migard et al., 2016).

HCV is the most common viral infection that affects the injecting drug users (IDUs) (Nelson et al., 2011, Hajarizadeh et al., 2013, Bruggmann et al., 2014). The global prevalence of IDU is estimated to be 14.0 million (Kaya et al., 2004) and over the past 40 years IDU has been the main mode of HCV transmission in high-income countries (Kaya et al., 2004, Alter, 2007d, Geraghty, 2011, Lavanchy, 2011). There is an estimated 67% anti-HCV prevalence among PWIDs, with Europe and Asia having the highest reported PWID populations (Nelson et al., 2011), followed by China, Russia, and

USA (Nelson et al., 2011). The estimated viraemia prevalence in Europe is between 53% - 97% (Wiessing et al., 2014).

1.7.5. Other routes of HCV transmission

The use of unsterilized instruments, and supplies to perform health-related procedures by non-medical professionals and traditional healers such as e.g. circumcision, excision, and scarification are associated with an increased risk of HCV infection (Inhorn, 1998, El Katsha et al., 2006). Other activities such as breaking the skin (e.g. tattooing, body piercing, and acupuncture) increase HCV transmission (Alter, 2007d). All these practices are a potential for HCV infection due to the use of contaminated instruments.

1.8. Prevention Of HCV Transmission

Primary prevention of hepatitis C includes the reduction of transmission by the implementation of educational and social programs in community outreach programmes and special centres for PWID group (Daw et al., 2012). Behavioural interventions (Martin et al., 2013) along with repeated risk reduction counselling programs throughout the course of treatment can facilitate the decrease of HCV transmission among MSM (Martin et al., 2016). Extra attention should be introduced in high-risk populations such as those in correctional institutions, drug therapy programs and HIV counselling and testing sites (Sy and Jamal, 2006). Health care workers should practice more strict rules such as practice better sterilization and safer injection use (Sy and Jamal, 2006).

The increase in HCV therapy and harm reduction programs among PWID is important in decreasing HCV prevention (Martin et al., 2013). The increase in harm reduction interventions, such as the use of opioid substitution therapy (OST) and supply of sterile needle/syringe provision (NSP) (Midgard et al., 2016) could reduce HCV prevalence among high-risk groups.

Opioid Substitution Therapy (OST) is the supply of replacement drugs to PWID, this includes prescribed medicine such as methadone or buprenorphine usually in a clinical

setting (Kermode et al., 2011). High-income countries have demonstrated the effectiveness of OST (WHO, 2009a). However, there are still more prevention measures to be implemented and phased in especially in resource-limited settings (Hartan and Das, 2010).

Needle/syringe provision (NSP) is the packaging of injecting equipment which consists of sterile needles and syringes in the aim of distributing them to individuals who inject drugs (PWIDs groups) (WHO, 2004e). There are various NSP programs that exist in different countries, and some countries use the one-for-one exchange of needles and syringes, while others, use the exchange of needles only with 100% return rate (WHO, 2004e). The secondary exchange is the collection of a large number of clean syringes and needles and distributing them to IDUs, who in turn distribute to other IDUs who they interact with (WHO, 2004e).

The implementation of both OST and NSP programs can decrease the risk of HCV transmission among IDUs. In those countries (e.g. Australia and New Zealand) (Mathers et al., 2010) which implemented the programs and achieved high levels of harm reduction interventions, reported that they could only manage to reduce HCV transmission, however, could not fully control it (Mehta et al., 2011, Palmateer et al., 2014b). There have to be many stringent implementations on the harm reduction communications and better access to drug treatment (Daw et al., 2012). There is still more research required to investigate whether the scaling-up of such programs could possibly reduce HCV prevalence in high-risk populations (Turner et al., 2011).

Reducing HCV transmission can decrease the morbidity and mortality caused by hepatitis C virus infection. Furthermore, effective prevention interventions have a potential to reduce HCV transmission and the impact caused in chronically infected individuals (Daw et al., 2012).

1.9. HCV Diagnostic Testing

1.9.1. Immunoassays

Serological assays detect specific antibodies to HCV (anti-HCV) against various HCV epitopes that are located in the core, non-structural proteins such as the NS3, NS4, and NS5 (Pawlotsky, 2002, Marwaha and Sachedev, 2014). The common approach using serological assays for HCV infection screening is the use of Enzyme Immunoassay (EIA) for the detection of Immunoglobulin G antibody (IgG antibodies) to HCV (Gao et al., 2014). However, antibody tests cannot distinguish between acute or chronic HCV infection (Alter et al., 2003e).

The United States Food and Drug Administration (FDA) licensed the first anti-HCV enzyme-linked Immunoassay (ELISA) in 1990 (CDC, 2003c). The 1st generation assays used a Yeast-recombinant protein that contained an epitope from the non-structural (NS4) region of the HCV genome and gave false positive results. It detected anti-HCV IgG in only 80% of patients and lacked sensitivity and specificity (Barrera et al., 1991a). Since then newer versions of these tests have been developed and approved by the FDA which all have been widely used in the clinical diagnosis and in the screening of symptomatic persons (WHO, 2002d, CDC, 2003c).

The Second-Generation assay use various recombinant antigens from the core, NS3 and NS4 regions of HCV genome, and allows for the detection of various epitopes (Ellethy et al., 2012). The 3rd generation assays added an extra antigen, (NS5) (Barrera et al., 1995b). This anti-HCV test reduced the window period and detected antibodies earlier compared to other assays. The 3rd generation assays have a high specificity of greater than 99% (Colin et al., 2001). False-negative results can be obtained in patients undergoing haemodialysis and those with a weakened immune system (Ghany et al., 2009). In addition, these anti-HCV tests can produce low positive predictive values in areas were HCV prevalence is low (<10%) (CDC, 1998b). High false positive rates (Tucker et al., 1997, Seremba et al., 2010, Mullis et al., 2013) occur as HCV antibody tests cannot distinguish between resolved and active infection. Also, the late appearance

of HCV antibodies 8-12 weeks after infection (CDC, 2016f) indicates that those recently infected may be missed until repeat testing 6-8 weeks later (CDC, 2016f).

1.9.2. Molecular techniques

1.9.2.1. HCV RNA detection and quantification

Nucleic acid tests (NATs) are molecular tests that can detect and characterize HCV RNA as early as one week after the initial exposure and they are sensitive and specific enough to detect active HCV infection (Saldanha et al., 2005). For these reasons, NATs remain the gold standard for HCV confirmatory diagnostic testing (WHO, 2002d, CDC, 2003c, Chavaliez and Pawlotsky, 2006). These tests include tests that detect the presence of HCV RNA by detecting active HCV infection (Kamili et al., 2012), amplifying products and hybridizing amplicons onto specific probes (e.g. Polymerase chain reaction (PCR) and Transcription-Mediated Amplification (TMA) (Pawlotsky, 2002a). Other tests that assess the quantity of HCV RNA (HCV viral load) in serum or plasma includes real-time PCR amplification, COBAS® Ampliprep/COBAS® TaqMan 48 analyser Automated test (Roche Molecular Systems) and Abbott RealTime™ HCV Assay (Abbott Diagnostics) (Pawlotsky, 2002a).

1.9.3. Molecular genotyping

Furthermore, there are tests that determine the genetic nature of HCV genome (e.g. HCV genotyping tests). Restriction fragment length polymorphism (Smith et al., 1995b), probe hybridization (Verbeeck et al., 2008b), genotype-specific real-time PCR or Sanger and next-generation sequencing (Simmonds et al., 1993c, Cai et al., 2013) are methods used for HCV genotyping. However, the determination of HCV genotype 6 is challenging mainly due to similarity to genotype 1 and the large sequence diversity, thus increasing mistyping when using earlier versions of the line probe assay (Inno-LiPA HCV 1.0) (Chinchai et al., 2003, Noppornpanth et al., 2006). The Versant HCV Genotype 2.0 (LiPA 2.0) assay, announced the incorporation of the core sequence which allows for an

improved and more accurate distinction of HCV genotype 1a-1c and genotype 6 and between subtypes a and b of genotype 1 (Verbeeck et al. 2008b). Recently there have been more specific and accurate tests such as Abbott Realtime HCV Genotype II assay (Abbott Diagnostics) have been launched. The assay uses probes specific for 5' untranslated region (5' UTR) and NS5B to determine genotype 1 to 6 and subtypes 1a and 1b respectively (Yang et al., 2014).

1.9.4. HCV rapid assays

HCV diagnosis is costly; requires specialized equipment and the need for highly trained operators (Johannessen, 2015). Cheaper options with less invasive methods of sampling and time to result have to be investigated. The use of minimal instrumentation that is simple such as a rapid Point-of-Care Tests (POCT) for HCV is a vital tool which can be used to assist in the detection of HCV, especially in different settings. Some of the advantages of POCT are; results are quickly available (Drain et al., 2014), allow for rapid clinical response (Drain et al., 2014), reduces patient loss to follow-up (Brooks et al., 2008) and the easiness of POCT may increase screening, especially in large populations (Lee et al., 2011a). This could include, settings with high rates of HCV under-diagnosis or difficult to reach populations. (Lee et al., 2010b). Point-of-care testing can contribute to the routine screening practice if there are satisfactory performance levels of the conventional laboratory testing compared to POC tests (Shivkumar et al., 2012).

There are various types of HCV rapid tests that are available; these include the OraQuick® HCV rapid Antibody test (Orasure Technologies, Inc), Chembio DPP® HCV Test (Chembio Diagnostics Systems, Inc), the Multiplo Rapid HIV/HCV Antibody Test (MedMira Laboratories, Inc) and the Multiplo Rapid HBV/HIV/HCV Antibody Test (MedMira Laboratories, Inc). All these rapid tests were provided to the CDC for evaluation to detect anti-HCV and recently, the SD Bioline HCV rapid test (Standard Diagnostics Inc, South Korea) was validated by WHO.

The OraQuick® HCV Rapid Antibody Test utilizes a lateral flow immunoassay procedure which is intended to detect the presence and or absence of structural and non-structural HCV proteins in the sample. The assay test strip uses synthetic peptides and recombinant antigens from the core, NS3 and NS4 regions of the HCV genome all immobilized in the test line. The test results are visualized by a protein A labelled colloidal gold. The test is interpreted between 20 to 40 minutes (Smith et al., 2011, http://www.orasure.com/products-infectious/products-infectious-oraquick-hcv.asp).

The OraQuick® HCV is the first rapid test to be approved by the FDA and used as a first-line screening test in the United States (Khuroo et al., 2015). The OraQuick® test can be performed on various samples such as whole blood, fingerstick (capillary blood), serum, plasma and oral fluid (Cha et al., 2013, Khuroo et al., 2015). The OraQuick® HCV rapid test has been shown to have sensitivities 100% and specificities 100% when using plasma or whole blood as a specimen which is equivalent to results reported in laboratory-based tests, even in the presence of low antibody levels (Lee et al., 2010b, O'Connell et al., 2013).The OraQuick® tests also showed to have higher sensitivities of 97.8% and specificities of 99.6% when compared to other rapid tests such as Chembio DPP® and MedMira rapid tests against the Centres for Disease Control and Prevention (CDC) reference testing algorithm (Smith et al., 2011).

The Chembio DPP® HCV Test (Chembio Diagnostics Systems, Inc) is a screening assay for the detection of anti-HCV in whole blood, serum, plasma and oral fluid specimens. Chembio rapid test uses a Dual Path Platform chromatographic immunoassay, which uses recombinant multiepitope chimeric HCV antigen that contains structural (core) and non-structural (NS3, NS4 and NS5) of HCV proteins. The assay provides visual reading of results between 15-30 minutes (Smith et al., 2011, http://www. Chembio.com/newtechnologies.html).

The Multiplo Rapid HIV/HCV Antibody Test (MedMira Laboratories, Inc) is a flow-through device with a nitrocellulose membrane for qualitative detection of antibodies of HIV type1/2 and HCV in serum, plasma or whole blood specimens. The nitrocellose

membrane is embedded with conserved regions of the surface glycoproteins of HIV virus and core and NS3 of the HCV proteins. The results were visualized by a reaction with protein A labelled colloidal gold. The time required to perform the assay is 3 minutes, with immediate reading of the results (smith et al., 2011, http://www.medmira.com/multiplex/).

The Multiplo rapid HBV/HIV/HCV Antibody Test (MedMira Laboratories, Inc) is a flow-through device with a nitrocellulose membrane for simultaneous detect of antibodies for HIV1/2, hepatitis B and hepatitis C in a single test. The assay can detect HBV/HIV/HCV in whole blood, serum or plasma. The time required to perform the assay is 3 minutes, with immediate reading of the results. (medmira.com/wp-content/uploads/.../Multiplo-HBc-HIV-HCV-Product-Sheet_EN.pdf).

In an evaluation study of HCV by Smith et al., on three different rapid assays, both Chembio (96.2%) and MedMira Rapid HIV/HCV Antibody Test (86.8%) had a sensitivity that were lower than that observed in OraSure (97.8%) rapid test (Smith et al., 2011). However, the Chemo DPP® HCV Test (99.8%) and MedMira Rapid HIV/HCV Antibody Test (99.8%) had a higher specificity compared to OraSure (97.8%) (Smith et al., 2011). The MedMira assay yielded the highest proportion of false-negative results between 8% - 19% as compared to the Chembio that had (0.4% - 7%) and OraSure (0% and 6%) (Smith et al., 2011). It was also observed that in a laboratory setting both the Chembio and MedMira were more likely to provide false negative results if specimens were HIV positive (Smith et al., 2011, Fisher et al., 2015). Similar results were also observed in other studies (Kendrick et al., 2005, Lubelchek et al., 2005, Metcalf et al., 2005).

The SD BIOLINE HCV (Standard Diagnostics Inc, South Korea) is an inviro immunochromatographic rapid assay designed for qualitative detection of HCV antibodies in serum, plasma or venous whole blood. This assay is intended for use in population with high HCV prevalence or those with a history of HCV risk exposure/behavior including women. The performance of the assay has not been

established in infants and children. The assay contains a nitrocellulose membrane strip, which is pre-coated with recombinant HCV capture antigen (core, NS3, NS4 and NS5) at the test line region (T). The SD BIOLINE HCV was evaluated by WHO in 2016, it had a sensitivity of 98.8% and specificity of 100% when compared to reference laboratory testing method and it takes 5 minutes to result. (www.who.int/.../diagnostics.../hcv/161216_final_amended_report_0257_012_00.pdf).

1.9.4.1. POC molecular test

There are various strategies in improving the HCV testing and diagnosis via venepuncture (Zhou et al., 2016), dried blood spot testing, (Easterbrook, 2016) and point-of-care HCV testing (Morano et al., 2014). These includes various advantages to this assay, it is very sensitive, requires minimal technical training to run the test, has quick turnaround time (less than 2 hours), uses one cartilage and less bench space and is free from sample handling (Cepheid, 2017, Gupta et al., 2017).

HCV POCT rapid tests that use finger stick or oral fluid have been developed, although, these tests are restricted to certain areas and only test for HCV antibodies (past infection) and not HCV RNA (recent infection or chronic infection). As per the WHO guidance on HCV testing (Easterbrook, 2016), there is a need for a nucleic acid test that detects HCV RNA at or near the point of care sites inorder to increase early diagnosis, and for speedy access to treatment and monitoring (Grebely et al., 2017). Recently in 2015, commercially available point-of-care molecular test, Xpert® HCV Viral Load assay (Cepheid, 2017) was launched. The Xpert® is a fully automated real-time reverse transcriptase polymerase chain reaction (qRT-PCR) assay (Cepheid, 2017). The Xpert® HCV Viral Load assay combines sample preparation, nucleic acid extraction, amplification and detection of the target sequence in one cartridge (Cepheid, 2017).

1.9.5. DBS

The use of polymerase chain reaction techniques for HCV testing using DBS has demonstrated to be accurate, reproducible and achievable in resource-poor settings (Ross et al., 2013). The standard method for blood collection in HCV testing is via venepuncture (Muzembo et al., 2017); challenges exist with blood collection and processing of venous blood samples especially in resource-limited settings (Muzembo et al., 2017).

Dried blood spots (DBS) have been used for antibody and molecular detection for many pathogens, such as HIV, HBV, and HCV and analytes such as nucleic acids have been shown to remain stable for much longer on the DBS than in serum or plasma (Greenman et al., 2015). DBS are more cost-effective with regard to human resources, offers a practical solution to blood collection by using capillary blood, offers easy transportation from distant clinics to centralized laboratories without refrigeration. They have the ability to retain the integrity of the samples because blood samples remain stable at room temperature (Vinikoor et al., 2015). They also offer limited processing e.g. no centrifugation, especially in resource-poor settings with no to little laboratory equipment (Ross et al., 2013, Ostler et al., 2014), minimal invasive procedure for donors and reduced risk to health care workers (McDade et al., 2007). The use of whole blood DBS for HCV viral load quantification has been demonstrated to be an efficient sample matrix. A recent study Marins et al., 2017, observed good correlation of R^2=0.97 (y= -0.0081x -0.3154) when comparing the use of DBS to plasma as a sample matrix for quantification of HCV viral load. In another study, they investigated the use of serum and DBS samples for HCV viral load quantification, they observed a good correlation of R^2=0.94 (Tuaillan et al., 2017).

A study comparison between two quantitative real-time PCR assays with DBS and serum for HCV RNA testing has demonstrated to be significant. A positive correlation was observed between DBS R^2= 0.90 and serum R^2= 0.89 for HCV RNA levels (Soulier et al., 2016). The use of DBS for anti-HCV testing as an alternative sample matrix has been investigated, and proven to be reliable, with significant correlation (R^2=0.631) (Lee

et al., 2011). The excellent correlation between DBS and plasma (Marins et al., 2017) and DBS with serum (Tuaillan et al., 2017) has shown that the use of DBS as an alternative method for anti-HCV and HCV RNA testing has proven to reliable.
However, there are reports of that the use of DBS from capillary blood or whole blood for HCV testing involves samples to be sent to centralized laboratory sites and requires patients to return for results. This remains a challenge, as there are marginalized populations in remote areas (Grebely et al., 2017).

1.9.4.1. POCT and DBS testing for other viruses.

Point-of-care tests (POCT) have been validated in various settings and counties for HIV testing (Kania et al., 2013). POC testing can offer the opportunity to screen HBV, HIV and syphilis testing and offer counselling concerning the infection and an opportunity to communicate the results to the patient at the same time (Kania et al., 2013). The use of rapid POCT will play an important role in the scale-up of community-based and large-scale screening, treatment and prevention of HBV in sub-Saharan Africa settings (Caroline et al., 2018). Rapid tests for HBsAg have demonstrated a good sensitivity (87.9%) and specificity (99.7%) when using Determine™ HBsAg (Alere Inc., MA USA) compared to ELISA as a gold standard (Chisenga et al., 2018). In a South African study, a specificity of 100% was reported when using when using Determine™ HBsAg (Alere Inc., MA USA) point of care test in pregnant women, (Chotun et al., 2017).

HIV rapid tests have demonstrated to be excellent in screening among different populations. In a South African study, the use of rapid testing in a population of MSM showed a sensitivity of 92.6% and a specificity of 99.4% against fourth generation EIA assay (Kufa et al., 2017). In another South African study, Alere q½ Detect (Alere technologies GmbH, Jena, Germany) was compared to the Roche COBAS® Ampliprep/COBAS® Taqman® (CAP/CTM) HIV-1 qualitative assay (Roche diagnostics, Branchburg, New Jersery, USA), and Alere rapid test demonstrated a sensitivity of 90% and specificity of 100% (Dunning et al., 2017). There are different POCT available on

the market for syphilis rapid testing, these includes the SD Bioline syphilis 3.0 (Standard Diagnostics, South Korea) and chembio DPP Syphilis Screen & Confirm Assay (Chembio Diagnostics Systems, USA). These rapid tests detect antibodies of all isotypes (IgG, IgM, IgA) against the *Treponema pallidum* bacterium (https://www.alere.com/en/home/product-details/sd-bioline-hiv-syphilis-duo.html).

DBS have been demonstrated to be used for both serological and molecular diagnosis of Human Immunodeficiency virus (Cassol et al. 1996, Bigger at al., 1997, Mössner et al., 2016, Barin et al., 2006, Marconi et al., 2009). In Sub-Saharan Africa the use of DBS has been used in HIV care programs, DBS samples are collected in HIV-exposed infants and routinely used for HIV DNA testing using automated assays (Smith et al., 2014). However, the use of DBS in HBV testing has been mostly limited to research settings. In North America, Europe, and Africa there have been studies that demonstrated the ability of testing both serological and virological markers for HBV infection using DBS samples (Kania et al., 2013, Mohamed et al., 2013).

A previous study validated HBV DNA quantification using DBS samples and a high correlation (R^2=0.966, P<0.001) between DBS and plasma was observed (Vinikoor et al., 2015), similar findings (R^2= 0.9646, P<0.0001) were reported in a validation study of HBV DNA quantification between DBS and serum (Jardi et al., 2004). The use of DBS for HIV screening is reported to be effective (sensitivity of 100% and specificity of 100%) and an excellent sample matrix for HIV testing (Ross et al., 2013, Kania et al., 2013). The use of DBS for HIV RNA testing in early infant diagnosis in South Africa has also demonstrated to be effective and practical. Several studies have validated the use of DBS for HIV RNA testing and high correlations above R^2=0.995, were observed when using DBS (Govender et al., 2016, Tang et al., 2017).

1.10. HCV Treatment

Due to the increased mortality and morbidity that is associated with hepatitis C infection, there has been an urgent need to achieve viral clearance (Burstow et al., 2017). Viral clearance reduced progression to fibrosis and reverses cirrhosis (Poynard et al., 2002). Virological clearance with undetectable HCV RNA in serum in 3-6 months after completing antiviral treatment is referred to as Sustained Virological Response (SVR) (NIH, 1997, Swain, 2010, Martinot-Peignoux, 2010). HCV genotyping allows for virological monitoring, monitoring of treatment duration and the dosage of medication to be administrated (Hadziyannis et al., 2004).

There was no available treatment for chronic hepatitis C until 1990 (Burstow et al., 2017). This has since lead to the development of the recommended treatment regimen which consists of 24-28 week of interferon-alpha 2a or 2b, depending on the genotype (NIH, 1997). The outcomes of this treatment included three weekly injections, however, there were poor results, with less than 10% success rate of HCV clearance (Carithers, 1997). Thus this led to the addition of ribavirin (RBV) to interferon-alfa therapy and contributed to the increased SRV rate by 30% - 40% (McHutchison, 1998).

The standard treatment for chronic hepatitis is a 24 or 48 week course of pegylated interferon (PEG-IFN)- alfa 2a and 2b in the combination of and ribavirin (NIH, 1997) (Blight,1998). The development of PEG-IFN-alfa 2a and 2b offered single weekly injections (Di Bisceglie, 2002), which increased SRV rates to 80% in genotypes 2, 3, 5 and 6 (EASL, 2015, Antaki, 2010c). The poor outcomes in genotypes 1 drove to the development of protease inhibitors (PIs) boceprevir and telaprevir (Burstow et al., 2017). Boceprevir and telaprevir are NS3 and NS4A inhibitors, and play a role in cleaving the viral polyprotein during HCV replication (Blight, 1998). These protease inhibitors significantly improved the treatment outcomes of genotype 1 (Burstow et al., 2017) and increased SVR by 75% (Bacon et al., 2011, Jacobson et al., 2011, Poordad et al., 2011, Zeuzem et al., 2011b). There we documented poor outcomes in patients with progressive fibrosis and cirrhosis (Bacon et al., 2011, Jacobson et al., 2011, Poordad et al., 2011, Zeuzem et al., 2011b). The treatment regimen was limited to patients with HCV genotype 1 and had common sides effects (Poordad et al., 2011, Bacon et al.,

2011, Jacobson et al., 2011, Zeuzem et al., 2011b). Consequently, it was evident that there was a need for more tolerable and effective chronic hepatitis treatments (Burstow et al., 2017). A number of NS3/4A (e.g. Simeprevir, Grazoprevir) and NS5A/5B (Daclatasvir, Sofosbuvir) inhibitors have been licensed for treatment (Burstow et al., 2017).

Greater rates SVR and shorter treatment durations have been documented, however, the use of the 'triple therapy' included interferon includes the frequent side effects and weekly injections (Burstow et al., 2017). This has led to interferon-free regimens which depend on genotype and presence of cirrhosis (Zhang et al 2016). Since then there has been an emergence of first fixed-dose of a combination of pangenotyptic regimen of sofosbuvir and velpatasvir that was approved by the FDA (FDA, 2016). This new type of regimen will potentially simplify management by removing the need for genotype determination prior treatment (Burstow et al., 2017).

1.11. Problem Statement

Currently, HCV infection is underdiagnosed in Africa and that has left many individuals, including high-risk groups (Gupta et al., 2014) unaware of their HCV infection status (Tuaillon et al., 2010). Information on the prevalence of HCV in individuals most-at-risk, including SWs, MSM and PWUDs/PWIDs is limited in South Africa (Semugoma et al., 2017). Identifying high-risk populations is difficult due to the stigma and discrimination attached to HCV positivity. This study is part of a larger collaboration, consisting of several non-governmental organizations and clinicians who are working with populations-at-risk as part of an established HIV program. Reaching out to these populations for hepatitis C testing is thus feasible.

Serological tests remain the first line of screening because of the relatively low costs. For reasons mentioned above, it is imperative to confirm infection by NAT. However, in South Africa, NAT is only performed in large cities. Centralised testing poses challenges in terms of time for specimens to reach the testing laboratory, maintaining the integrity of

the specimens (Layden et al., 2014), time to return results to the facility and the patient not returning for results. Sampling for testing may prove problematic with regard to fear/pain of venepuncture and volumes of blood needed, especially in populations such as PWUDs/PWIDs who may have scarred or damaged veins. It is vital that cheap, reliable tests are developed requiring little or no equipment, providing ease-of-use in resource-limited settings and that can be performed at the site of patient care (POC setting) (Johannessen, 2015). Diagnostic accuracies of OraQuick® POCT range from high clinical and analytical sensitivity of 99.5% and specificity of 99.8% (Khuroo et al., 2015) to lower sensitivities of 97.8% (Chai et al., 2013) and 92.7% (Fischer et al., 2015).

DBS has been used in several settings in South Africa for HIV diagnosis (Govender et al., 2016). A study comparing serum samples to DBS found a sensitivity of 93.8% - 100% and specificity of 94.0% - 100% and HCV RNA on DBS was detected in 100% of HCV positive samples (Bennett et al., 2012). DBS can be used for HCV detection, monitoring, and HCV treatment and genotype of HCV (De Crignis et al., 2010). This includes the use of automatic modern platform (e.g. Abbott Architect) which can perform a high production of testing (Ross et al., 2013, Soulier et al., 2016). Better sampling matrices can impact on screening and access to care for HCV as they have for HIV. However, it remains imperative that POCT and DBS tests for HCV are properly validated against standard tests to improve testing algorithms, reduce the costs of unnecessary testing and improve access to care for those that test positive.

The sixty-ninth World Health Assembly approved for the first time a strategy for viral hepatitis. This included a 30% reduction in new cases of hepatitis B and C by 2030; a 10% reduction in mortality and 65% decrease in mortality from end-stage liver disease; and a further 90% reduction of new infections by 2030 (WHO, 2016f, Sonderup et al., 2017). They also aimed at improving injection, blood and surgical safety; “harm reduction” for people who inject drugs; and increased access to treatment for hepatitis B and C (WHO, 2016f). To meet these goals, there is a need to improve access to the HCV diagnostic testing, to increase screening, to determine prevalence of HCV, to have epidemiological data on people infected with HCV, to assess risk behaviours, to create

public awareness, to implement prevention control plans and policies and, lastly, to provide treatment and care to all those infected.

1.12. Purpose Of The Study

Research question/s

1. How do different tests (POCT) and sampling matrices (DBS) compare to that of reference laboratory-based HCV diagnostic tests using plasma samples in South Africa?
2. What are the hepatitis C virus genotypes and viral load in individuals most-at-risk of hepatitis C infection in South Africa?

Aims of the study

1. To assess the accuracy of a rapid HCV POCT when compared to laboratory-reference testing.
2. To assess the use of DBS as an alternative matrix for HCV laboratory-based diagnostic testing.
3. To describe the HCV genotypes and viral load in most-at-risk groups in South Africa.

Objectives

1a. To compare the sensitivity and specificity of HCV POCT to that of immunoassay which is used as laboratory reference tests on the plasma.

1b. To compare the sensitivity and specificity of HCV DBS to that of immunoassay laboratory reference tests on the plasma.

1c. To compare the sensitivity and specificity of HCV DBS to that of viral load laboratory reference tests on the plasma.

1d. To describe the genotypes and viral loads on HCV PCR positive samples.

CHAPTER TWO - MATERIALS AND METHODS

2.1. Study Design

This was a descriptive study that investigated the HCV seroprevalence, viral load, and genotypes in individuals most-at-risk including SWs, MSM, and PWUDs/PWIDs. The study investigated the diagnostic accuracy of POCT and further explored the use of DBS as an alternative sample matrix to that of plasma for standard routine tests for HCV on a subset of the larger cohort. This study was part of a larger collaborative study entitled "Viral Hepatitis C initiative for most-at-risk populations in South Africa".

2.2. Ethics

Ethics approval has been received from WITS (HREC REF: M170698) 04 July 2017, see Appendix A. Ethics approval has been received from UCT (HREC REF: 004/2016) 4 July 2016, see Appendix B.

2.3. Study Population

A total of 3509 participants who are most-at-risk (SWs, MSM, and PWUDs/PWIDs) across the seven cities in South Africa namely: Durban (DBN), Pietermaritzburg (PMB), Mthatha (ORT), Port Elizabeth (NMB), Cape Town (CPT), Johannesburg (JHB), and Pretoria (PTA) were enrolled in the study. The study included the community-based and clinic-based screening activities which are linked to community-based education. People accessing HIV testing, counselling and other services provided by the study collaborators were invited to participate in the study. Each participant had a unique participant identification (PID) number. Whole blood specimens from all sites and, DBS cards (GE Healthcare, Piscataway, NJ) from Cape Town sites only, that tested positive and negative were sent to NICD reference laboratory for further serological and molecular testing and for validation purposes.

2.4. Sample Size Calculation

The sample size calculation was performed on OpenEpi version 3.01 (Dean[a] et al., 2016). A population size of 750 in MSM (250 from Cape Town, Pretoria and Johannesburg) assuming assumed a frequency of 5% and a confidence interval of 95%, provided an estimated sample size of 67. A population size of 1 550 in SWs (400 in Cape Town and Durban and 250 in Pietermaritzburg, Port Elizabeth, and Mthatha) assuming assumed a frequency of 5% and a confidence interval of 95%, the estimated sample size was calculated to be 134. A population size of 1 200 PWUDs/PWIDs (400 in Cape Town, Durban and Pretoria) assuming a frequency of 5% and a confidence interval of 95% the estimated sample size is 228. A total of 429 sample size from different sites of high-risk population would have statistical power for the study.

2.5. Samples Collection

A trained nurse or counsellor provided the participant with an information sheet that outlined who was doing the study, why the study was being done, the potential risks and benefits or participation, the duration and details of the activities and contact details for additional questions (Appendix C). Participants were also requested to provide consent for blood to be stored for future testing around hepatitis and other infections affecting key populations (Appendix D). The trained nurse or counsellor assisted recruited participants at the sites to fill in the (Appendix E).

All participants recruited from all sites except Cape Town sites had two 5ml EDTA tubes of whole blood collected by venepuncture, after consent was provided. One EDTA tube was used for POC testing using the test kit sampler. All participants recruited from sites in Cape Town had an oral fluid sample taken as part of the OraQuick® testing procedure using the test kit sampler. Also from Cape Town sites, 50 µL of blood from an EDTA tube was spotted onto filter paper for DBS testing, see Figure 3.1 for details on which samples were collected and tested at the different sites collected All samples were collected at a one-time point, only the participants that have signed the consent form and agreed for their samples to be used for further research study were included. After

sample collection a total of 532 PWUD/PWID, 66 MSM and 115 SWs samples were collected from high-risk groups. All EDTA tubes were marked with participant identification code, initials, and date of birth.

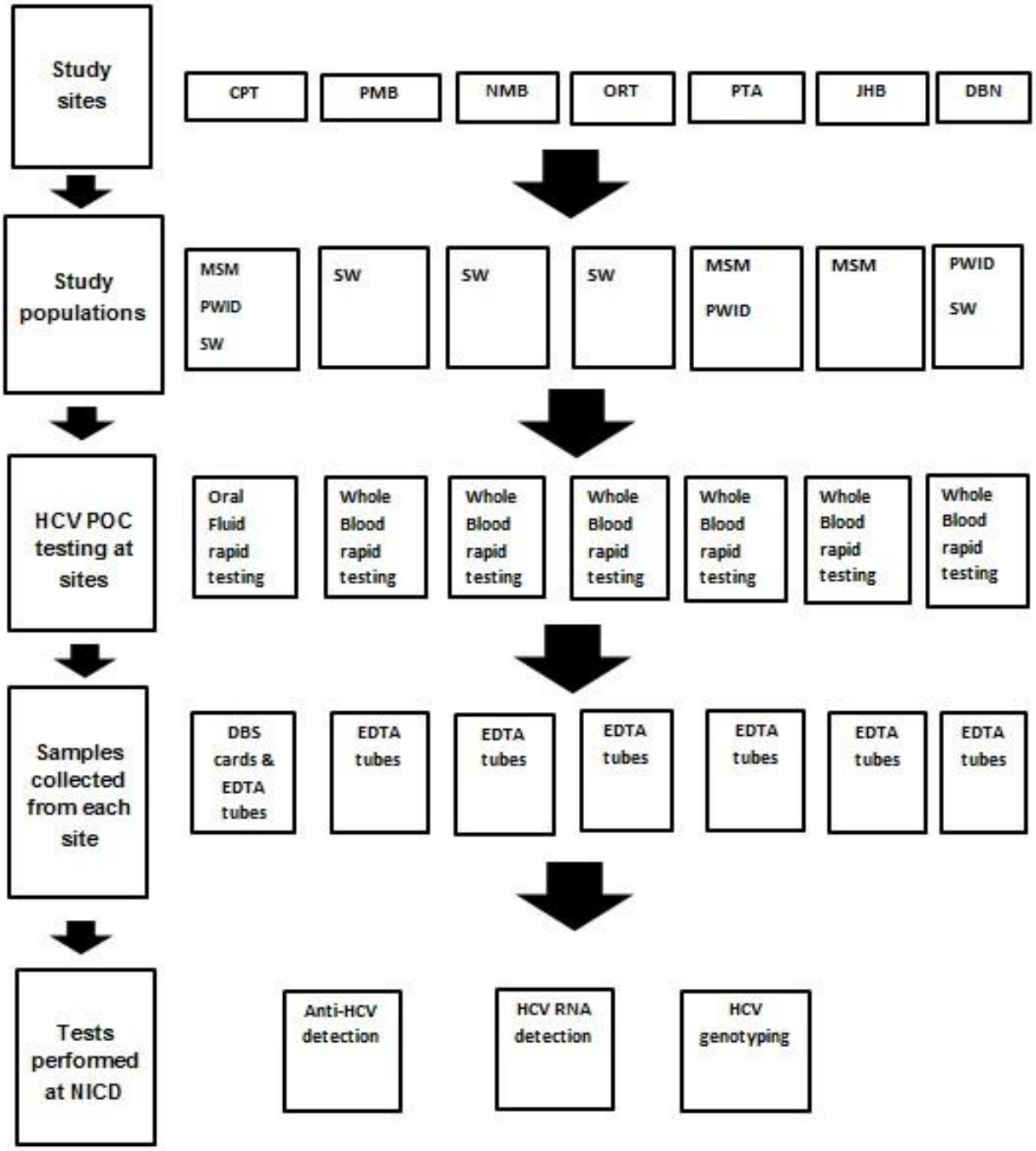


Figure 3.1; Study sites, sample collection, and tests performed at various sites.

EDTA; Ethylenediaminetetraacetate acid, DBS; Dried Blood Spots, Anti-HCV; Antibodies to HCV, POC; Point- of -care, PWID/PWUD; People who Inject Drugs/ People who Use Drugs, MSM; Men who have sex with men, SW; Sex workers, CPT; Cape Town, PMB; Pietermaritzburg, ORT; OR Tambo, NMB; Mthatha, PTA; Pretoria, JHB; Johannesburg, DBN; Durban

2.6. Data Collection

All recruited participants at the sites filled in a hepatitis surveillance laboratory request form (Appendix E). There were different sections on the request form that had to be filled in including Client Information, Point of Care Results and Laboratory Specimen Details. The information on the request form was then captured on an HCV database using Epi info version 3.2.4. (Dean et al., 2011a). All results were recorded on the Epinfo version 3.2.4. Software (Dean et al., 2011a). All data was collected onto Excel spreadsheets using Microsoft Windows.

2.7. Laboratory Methods

2.7.1. OraQuick® HCV rapid antibody test

HCV Point-Of-Care test was performed using OraQuick® HCV Rapid Antibody Test (OraSure Technologies, Bethlehem) (Appendix F).

Principle

The OraQuick® HCV Rapid test is performed manually. The test uses visual reading of the qualitative detection of Immunoglobulin (IgG) to hepatitis C virus HCV antibodies (anti-HCV) in human finger stick, whole blood, and oral fluid. The OraQuick® HCV Rapid Antibody Test detects the presence and or absence of structural and non-structural HCV proteins in the sample (OraSure Technologies, Bethlehem) (Appendix F).

Method

This test was performed at the seven different sites. A trained nurse followed the manufacturer's instructions when performing the OraQuick® HCV Rapid Antibody Test. The POC testing methods are detailed in (Appendix F). At Cape Town sites oral fluid

samples, DBS cards and whole blood samples from one participant were collected. The Oral fluid was tested using the OraQuick® HCV Rapid Antibody test (Appendix F) at the site and the DBS card and two EDTA whole blood tube were sent to NICD At other sites, (PTA, DBN, ORT, PMB) two tubes of EDTA whole blood samples were collected from one participant. One whole blood tube was used for rapid testing using the HCV Rapid test and the other EDTA whole blood tube was sent to NICD (Appendix F). The OraQuick® HCV Rapid Antibody test results obtained at the sites were recorded on the hepatitis surveillance laboratory request form (Appendix E) and sent with samples to NICD.

2.7.2. Processing of samples

2.7.2.1. Labelling and processing of whole Blood samples

On arrival of whole blood samples at NICD, whole blood samples were labelled and processed. The whole blood samples were centrifuged (UNICEN 20, Madrid) with a bench top centrifuge at 3000 rpm for 10 minutes at room temperature. Plasma from the EDTA tubes was transferred into new 1.5 ml Screw Cap microtubes (SARSTEDT, Germany) and labelled with unique laboratory numbers for traceability. The plasma was then stored at -70˚C in the freezer in labelled racks.

2.7.2.2. Labelling and processing of DBS

On arrival of DBS samples, the Whatman 903 protein saver cards (GE Healthcare, Piscataway, NJ) (DBS) cards were labelled with a unique laboratory number from NICD that correspond to that of the plasma and stored in -20˚C freezer. After labelling and processing of samples, the testing all samples were tested at NICD.

2.7.2.3. Elution buffer preparation

The elution buffer was made up with 50 ml of 1X Phosphate-Buffered Saline (PBS) buffer (Lonza, Belgium) and 25 µL of 0.05% Tween® 20 (Merck, Germany) which was then stored at 4 - 8˚C.

2.7.3. Anti-HCV ELISA

The ARCHITECT HCV Ab assay was performed at NICD laboratory using the automated ARCHITECT *i1000SR* CMIA system (Abbott Laboratories, Diagnostics Division, Abbott Park, IL).

Principle

The ARCHITECT Anti-HCV assay (Abbott Laboratories, Diagnostics Division, Abbott Park, IL) is a two-step immunoassay. It uses the chemiluminescent microparticle immunoassay (CMIA) technology, for qualitative detection of immunoglobulin G (IgG) and immunoglobulin M (IgM) antibodies to hepatitis C virus (anti-HCV) in human serum and plasma. The ARCHITECT Anti-HCV assay is intended to detect antibodies to structural and non-structural proteins of the HCV genome. The chemiluminescent reaction is measured as relative light units (RLUs). The RLUs are compared to the chemiluminescent signal and reaction cut-off signal. The results are interpreted as follows, the S/CO values that are less than 1.0 are non-reactive and those S/O values greater or equal to one are reactive (Abbott Laboratories, Diagnostics Division, Abbott Park, IL).

Testing plasma on ARCHITECT *i System**

Plasma samples were thawed, vortexed for 8 seconds and with a macro centrifuged at 3000 rpm for 1 minute (Merck, South Africa) at room temperature. The minimum sample cup volume of 150 µL is required to perform a single anti-HCV test on the ARCHITECT *i** System and an additional 20 µL will be required for an additional anti-HCV test from the same sample cup. One hundred and seventy microliters of plasma were then processed on the ARCHITECT *i** System following manufacturer's instructions (Abbott Laboratories, Diagnostics Division, Abbott Park, IL).

Testing DBS *on* ARCHITECT *i System**

One spot (50 µL - 75 µL) from the DBS card (GE Healthcare, Piscataway, NJ) was eluted in 400 µl (Gavin Cloherty, Abbott diagnostics, personal communication) of 1X PBS buffer (Lonza, Belgium) and 0.05% of Tween® 20 (Merck, Germany) (Ross et al., 2013). The DBS were incubated at room temperature with continuous agitation on a laboratory shaker (Labex, South Africa) at maximum speed 300 motions per minute for 1 hour. The eluate was then centrifuged with a macro centrifuge (Merck, South Africa) at 3000 rpm for 1 minute prior to testing. One hundred and seventy microliters of DBS eluate was then processed on the ARCHITECT *i1000SR* system following manufacturer's instructions (Abbott Laboratories, Diagnostics Division, Abbott Park, IL).

2.7.4. HCV viral load assay

Molecular detection of HCV RNA was performed at NICD laboratory using the automated COBAS® Ampliprep Instrument for amplification and automated COBAS® TaqMan® 48 analyzer for detection (Roche Molecular Systems, Pleasanton, California, USA).

Principle

The COBAS® AmpliPrep/COBAS® TaqMan® HCV Quantitative Test v2.0 is an automated, real-time, nucleic acid amplification test used to quantify HCV RNA in human serum or plasma of patients infected with HCV genotypes 1-6 (Deeks, 2015). The test uses primers specific to the 5'UTR of the HCV genome.

The COBAS® AmpliPrep/COBAS® TaqMan® 48 analyser HCV Quantitative Test v2.0 (Roche Molecular Systems, Pleasanton, California, USA) is an automated system that extracts, amplify and detect HCV RNA on board. The preparation of specimens is based on the automated Reverse Transcription Polymerase Chain. The plasma samples were labelled and processed as specified in 2.7.2.1. Six hundred and fifty microliters of samples and controls were pipetted into sample tubes (s-tube) with corresponding

barcode clips. The samples and controls were loaded in the following order: Negative Roche control (NC), Low Positive Roche control (LPC) and High Positive control (HPC) followed by 21 samples on the COBAS® Ampliprep Instrument. The Roche reagents were loaded onto the reagent racks of the COBAS® Ampliprep Instrument and samples were extracted, amplified and HCV RNA was detected on the COBAS® Ampliprep/COBAS® TaqMan® Instrument following manufacturer's instructions. For the interpretation of results, each run had to have valid controls with no flags or invalid results in the comments section (Roche Molecular Systems, Pleasanton, California, USA). The Upper and Lower limits of detection are <1.50E+01 IU/ml and >1.00E+08 IU/ml. For interpretation, details refer to (Appendix H) (Roche Molecular Systems, Pleasanton, California, USA).

2.7.4.1. Testing of plasma samples on the COBAS® Ampliprep/ COBAS® TaqMan ® 48 Analyser

Six hundred and fifty microliters of plasma samples were briefly centrifuged (Merck, South Africa) with a macro centrifuge for 1 minute at 3000 rpm and were tested on the COBAS® Ampliprep/COBAS® TaqMan® 48 Analyser following manufacturer's instructions (Roche Molecular Systems, Pleasanton, California, USA).

2.7.4.2. DBS elution for viral load testing

The DBS was processed as specified in 2.7.2.2. Two spots (50 µL-75 µL per spot) from DBS were eluted in 1.0 ml of Sample Pre-Extraction (SPEX) buffer (Roche Molecular Systems, Pleasanton, California, USA). The tubes containing the DBS in buffer were incubated in a dry cooling/heating block Eppendorf® Thermomixer® R (Sigma, South Africa), at 56°C with continuous shaking for 10 minutes (Vinikoor et al., 2015, Roche Molecular Systems, Pleasanton, California, USA).

2.7.4.3. Testing of DBS on the COBAS® Ampliprep/COBAS® TaqMan® Analyser

To detect and quantify HCV RNA by RT-PCR, 650 µL of DBS eluate was tested on the COBAS® Ampliprep/COBAS® Taqman® Analyser following manufacturer's instructions (Roche Molecular Systems, Pleasanton, California, USA).

2.7.5. HCV RNA extraction for genotyping plasma samples.

The HCV RNA extraction was performed at NICD laboratory using the QIAamp® Viral RNA Mini Kit (Qiagen, Hilden, Germany).

Principle

QIAamp® Viral RNA Mini Kit is used to purify viral RNA either from plasma, serum or cell-free body fluids. The kit uses selective binding properties of the silica-based membrane with a vacuum technology. The sample is lysed in high denaturing conditions that will inactivate the RNAses and ensure the isolation of intact viral RNA. The buffering conditions are adjusted to offer optimal binding of the RNA to the QIAamp® membrane. Once the RNA is bound to the membrane the contaminants are efficiently washed away in a two-step process using two different wash buffers. The RNase free buffer is used to directly elute RNA which is then stored in a -70 ˚C freezer. The purified RNA is free of any protein, nucleases, contaminants, and inhibitors (Qiagen, Hilden, Germany).

Method

The plasma was thawed, vortexed for 15 seconds and briefly centrifuged for 1 minute at 8000 rpm with a macro centrifuge (Merck, South Africa. Wash buffer 1 (Buffer AW1) was supplied as a concentrate; it was prepared by adding 25 ml of ethanol (96% - 100%) and mixed by inverting 10 times and stored at room temperature. Wash buffer 2 (Buffer AW2) was supplied as a concentrate, it was also prepared by adding 30 ml of ethanol (96% - 100%) and mixed by inverting 10 times and stored at room temperature. Three hundred and ten microliters of Buffer AVE was added into carrier RNA. Buffer

AVE dissolved the lyophilized carrier RNA thoroughly, resulting in Buffer AVI (Buffer AVE and dissolved lyophilized carrier RNA). Buffer AVI was then divided into sized aliquots and stored in -30 ˚C to -15 ˚C.

Five hundred and sixty microliters of prepared Buffer AVI was pipetted into a 1.5 ml labelled microcentrifuge tube and 140 µL of plasma was added to the Buffer AVI in the microcentrifuge tube. The mixture was mixed with pulse-vortexing for 15 seconds. This was followed by incubation at room temperature for 10 minutes. The mixture was then briefly centrifuged at 8000 rpm with a macro centrifuge (Merck, South Africa) for 1 minute. Five hundred and sixty microliters of ethanol (96% - 100%) was added to the sample and mixed by pulse-vortexing for 15 seconds then followed by centrifugation at 8000 rpm with a macro centrifuge (Merck, South Africa) for 1 minute. From the homogeneous solution, 630 µL was pipetted into the QIAamp® Mini column without wetting the rim and briefly centrifuged at 8000 rpm with a macro centrifuge (Merck, South Africa) for 1 minute. The filtrate and the collection tube were discarded and the QIAamp® Mini column was placed into new clean 2 ml collection tube. The remaining solution was carefully added to the existing QIAamp® Mini column. The sample was then centrifuged at 8000 rpm with a macro centrifuge (Merck, South Africa) for 1 minute. The QIAamp® Mini column was placed into a new collection tube and the old collection tube with the filtrate was discarded. Five hundred microliters of Buffer AW1 was added into in the QIAamp® Mini column and centrifuged at 8000 rpm with a macro centrifuge (Merck, South Africa) for 1 minute. The collection tube and the filtrate were discarded and replaced with a clean 2 ml collection tube which was placed in the QIAamp® Mini column. Five hundred microliters of buffer AW2 was pipetted into the QIAamp® Mini column and centrifuged at 14000 rpm for 3 minutes. The collection tube was discarded along with the filtrate, replaced with a new 2 ml collection tube and centrifuged again at 14 000 rpm with a macro centrifuge (Merck, South Africa) for 3 minutes. The QIAamp® Mini column was placed in a clean labelled 1.5 ml Eppendorf tube. The collection tube which contained the filtrate was discarded. Fifty microliters of distilled water were added to the column, and incubated for 1 minute at room temperature. Then centrifuged at 8000 rpm with a macro centrifuge (Merck, South Africa) for 1 minute. The column was

discarded and the eluate was stored in a labelled 1.5 ml Eppendorf tube in -70 ˚C freezer (Qiagen, Hilden, Germany).

2.7.6. HCV Amplification for genotyping.

The HCV amplification was performed at NICD laboratory using the Versant HCV amplification 2.0 kit (manufactured by Innogenetics, Ghent, Belgium, for Siemens, Tarrytown, NY, USA) (Siemens Diagnostics).

Principle

The amplification 2.0 kit amplifies fragments in the 5'UTR and the core region of the HCV genome (Verbeeck et al., 2008b). The amplification 2.0 kit contains enzyme mix reagent and amplification mix reagent. The amplification has a two-step process the of reverse transcription-polymerase chain reaction (RT- PCR).

The viral RNA is added to the enzymes (Uracil-N-Glycosylase, Hot Start Taq Polymerase, Forward and Reverse Transcriptases) and amplification mix (oligonucleotides in buffer with deoxyribonucleotide triphosphate (dNTP), deoxyuridine 5-triphosphate (dUTP) and magnesium chloride ($MgCl_2$). The genomic RNA is reversely transcribed into complementary DNA (cDNA) using HCV-specific primers. The DNA polymerase is activated by heating the reverse transcriptase for the PCR amplification step. Two sets primers of the 5'UTR and the core regions of HCV genome were used to amplify two DNA fragments of the 240 and 270 base pairs (Siemens Healthcare Diagnostics, Belgium).

Method

The Amplification Mix reagent was placed on ice for 30 minutes to thaw. Fifty microliters of RT-PCR reaction master mix was calculated using the HCV PCR amplification worksheets. PCR micro-tubes (0.2 ml) and 1.5 ml Eppendorf tubes were labelled. The master mix reaction was prepared in the clean laboratory by adding the following

reagents; 26 µL of Amplification mix and 4 µL of enzymes according to manufacturer's instruction. In the specimen laboratory, 20 µL of extracted template (RNA) was added into the RT-PCR master mix making a total of 50 µL reaction. The mixture was briefly centrifuged with a microcentrifuge Merck, South Africa) for 15 seconds at maximum speed (14 000 rpm). The PCR tubes with the reaction mixture were incubated for 10 minutes on ice. The following thermal profile was used, reverse transcriptase at 50 ˚C for 30 minutes, the initial PCR activation step 95 ˚C for 15 minutes; denaturation step at 95 ˚C for 30 seconds; annealing step at 50 ˚C for 30 seconds and the extension step at 72 ˚C for 15 seconds. The denaturation, annealing and extension steps are repeated 40X followed by the elongation step which was performed for 2 minutes at 72 ˚C and a holding step at 4 ˚C. After the run, all the amplicons were then stored at 4 ˚C (Siemens Healthcare Diagnostics, Belgium) in the post PCR laboratory.

2.7.7. Agarose gel electrophoresis

Principle

The separation of nucleic acid by agarose gel electrophoresis is a procedure that is used routinely. The nucleic acid molecules are separated by an electric field. Since nucleic acid possesses a negative charge due to the phosphate backbone of the DNA. The negatively charged DNA migrates through an agarose gel matrix towards a positive electrode. The agarose gel consists of seaweed genera *Gelidium* and *Gracilaria*, and repeated agarobiose (L - and D-galactose) subunits (Kirkpatrick, 1991). The subunits form a matrix that acts as a sieve that will separate molecules such as DNA, proteins, and lipids based on their size, shape, and charge (Lee et al., 2012). These characteristics, together with the buffer conditions, gel concentrations and the voltage affect the mobility of molecules in the agarose gels. The nucleic acid is stained with a loading dye and ethidium bromide which consists of glycerol. Glycerol gives the DNA density in order to enable it to sink into the pre-cast wells and not float (Lee et al., 2012) and ethidium bromide (an intercalating agent that fluorescence under light). The nucleic

acid is visualized in the agarose gels through the illumination of the agarose gels with 300nm UV light (Yilmaz et al., 2012).

Methods

The expected HCV amplified PCR product was 240 bp - 270 bp long. The amplified HCV products were detected using the agarose gel electrophoresis. The agarose gel was made up with 100 ml of 0.5 Tris/Borate/EDTA buffer (TBE buffer) (Darmstadt, Germany), 4 grams of agarose powder (Leuven, Belgium) and 1 µL of a 2% ethidium bromide (Bio-Rad Laboratories, Inc; United States). Five microliters of PCR products, negative and positive controls and 3 µL the molecular weight marker VI (Roche Diagnostics, Germany) were stained with 1 µL of Fermentas 6X Orange DNA Loading Dye (Thermo Fisher Scientific Inc, South Africa). The gel was run at 120 V for 30 minutes in 1X Tris/Borate/EDTA (TBE buffer) (Qiagen, Hilden, Germany). The gel was viewed under a transilluminator (Vacutec, Johannesburg, South Africa).

2.7.8. HCV genotyping 2.0. (LiPA)

The HCV genotyping was performed at NICD laboratory using Versant HCV genotyping 2.0 Assay (LiPA) (manufactured by Innogenetics, Ghent, Belgium, for Siemens, Tarrytown, NY, USA) (Siemens Diagnostics).

Principle

The VERSANT HCV Genotyping 2.0 Assay (Siemens Healthcare Diagnostics, Belgium) is manually performed. It uses reverse hybridization to generate biotinylated DNA PCR products by RT-PCR amplification of the 5’ UTR and core region of HCV RNA. This allows for the proper distinction between the HCV genotype 1-6 and the subtypes a, b and c found among the genotype 1-6 (Verbeeck et al., 2008b)

The RNA is hybridized to immobilize the oligonucleotide probes. The probes are bonded to a nitrocellulose strip by a poly (dt) tail that is specific for the 5' UTR and the core region of the various HCV genotypes (Verbeeck et al., 2008b). In the hybridization step unhybridized PCR products are washed from the strip and the alkaline phosphatase labelled streptavidin (conjugate) is bound to the biotinylated hybrid. The substrate chromogen 5-Bromo-4-chloro-3-indolyl phosphate/nitro blue tetrazolium chloride (BCIP/NBT) reacts with the streptavidin-alkaline phosphatase complex to form a purple/brown precipitate which results in a visible banding pattern on the strip (Cai et al., 2013). The Versant HCV Genotype Assay contains 20 5’ untranslated region (5’ UTR) and 4 core specific probe lines. The 5’ UTR oligonucleotides allow for the detection and classification of HCV genotypes and subtypes 1-6a/b. The core specific probe lines are designed for more reliable discrimination of subtypes 1a and 1b and recognition of subtypes 6c-l (Ross et al., 2007).

Method

Equal volumes of 10 µL biotinylated PCR amplicons and the denaturation solution (1.7 % sodium hydroxide) were added and incubated at room temperature for 5 minutes. The test strips were marked with test numbers and placed in each trough. The 2 ml hybridization/stringent wash buffer solution (sodium chloride, sodium citrate buffer with detergent and preservatives) was dispensed into the troughs. The test strips were incubated for 1 hour at 50 - 55 ˚C in a shaking water bath. The solution was aspirated from each trough followed by an addition of 2 ml wash buffer solution and incubated for 1 minute at room temperature. The wash buffer solution was aspirated from each trough and 2 ml of the wash buffer was added to each trough. The mixture was then incubated for 30 minutes in 50˚C water bath. The wash buffer solution was then aspirated and 2 ml of diluted rinse 5X solution (phosphate buffer with 0.5% 2-chloroacetamide, NaCl, detergent and other preservatives) was added to each test trough and incubated for 1 minute in a shaker at room temperature. Two millilitres of the diluted conjugate (phosphate buffer with 0.1% 2-chloroacetamide detergents, protein stabilizers and other preservatives) and diluted rinse 5X solution was aspirated. Alkaline phosphatase

labelled streptavidin conjugate was added to the trough and diluted in standard conjugate solution incubated for 30 minutes at room temperature.

The diluted streptavidin conjugate was aspirated and 2 ml of rinse solution was used to wash the strips twice followed by washing once with standard substrate buffer (TRIS buffer with 0.1% 2-chloroacetamide, $MgCl_2$, NaCl and other preservatives). Colour development was initiated by addition of 2 ml of diluted substrate (5-bromo-4-chloro-3 indolyl phosphate) (BCID/NBT) to the strips and incubated for 30 minutes on a shaker at room temperature. The substrate reacted with the conjugate forming a purple/brown precipitate which resulted in a visible line pattern on each test strip that was specific for each genotype. The colour reaction was stopped by aspiration of the substrate buffer and addition of 2 ml distilled water and incubated for 3 minutes. The strips were removed from the trough and dried immediately followed by result interpretation using the reading card over the strip and aligning the green line and marker line on the card.

2.7.9 Normalization Coefficient calculation

We accounted for the volume difference and sample type in the DBS. Fifty microliters of whole blood was spotted onto the Whatman 903 card (GE Healthcare, Piscataway, NJ). Two circles of DBS were used for viral load analysis and we assumed that there is 50% volume of plasma in DBS. Thus 2 X 50 µL of whole blood X 50% (100 µL x 50% = 50 µL of plasma).

The DBS dilution factor was calculated as follows. Two circles of 50 µL whole blood were spotted on DBS cards and 1000 µL of SPEX buffer was added, providing a total volume of 1100 µL. A total volume of plasma in DBS would give us the dilution factor (1100 µL / 50 µL = 22), normalization coefficient (c) was 22. When correcting for volume difference and sample type, the DBS results were multiplied with the normalization coefficient (c=22) (Ed Marins, Dept. of Virology, Roche diagnostics personal communication).

2.7.10. Statistics

Sensitivity, specificity and Negative Predictive Value, Positive Predictive Value analysis

The STARD approach was adopted in reporting the accuracy of serology testing (Simel et al., 2008). The accuracy of POCT immunoassay results were analysed using the 2x2 tables and the sensitivity, specificity, negative predictive value (NPV) and positive predictive value (PPV) were determined against the laboratory reference test. The following standardised definitions were used (Šimundić., 2009). The sensitivity was expressed in percentage and it defines 'the proportion of true positive subjects with the disease in a total group of subjects with the disease' which is defined by this formulae (TP/TP+FN). Specificity was defined as a 'proportion of subjects without the disease with negative test result in total of subjects without disease' which is defined by this formulae (TN/TN+FP). The true positive (TP) was defined as 'the subjects with the disease with the value of a parameter of interest above the cut-off'. The false positive (FP) was 'defined as subjects without the disease with the value of a parameter of interest above the cut-off'. The true negative (TN) was 'defined as subjects without the disease with the value of a parameter of interest below the cut-off' and the false negative (FN) was 'defined as the subjects with the disease with the value of a parameter of interest below the cut-off' (Šimundić., 2009). The Positive Predictive Value (PPV) defined as the 'proportion of people that are positive and test positive with a reference test' which is defined by this formulae (TP/TP+FP). The Negative Predictive Value (NPV) was' defined as the proportion of people that are negative and test negative with a reference test' which is defined by this formulae (TN/TN+FN) (Šimundić., 2009). Online statistical algorithm tool MedCalc for windows (MedCalc Software, Ostend, Belgium) (Dean et al., 2016b) was used to analyse the sensitivity and specificity and the confidence intervals.

Linear regression plots and Bland Altman graphs.

The linear regression analysis was performed to determine the association of DBS and plasma RNA levels in $\log_{10}$. To determine the agreement between the DBS and Plasma

the Bland Altman plot was used (Cutler, 2013). Data analysis for HCV viral loads were analysed using the statistical software XL STAT Biomed Perpetual version 18.06 (XL STAT, 2017).

Statistical analysis on plasma genotype and viral load.

The STATA version 13 (StataCorp, 2013) was used to describe the statistical analysis of the genotypes and viral loads in the plasma within the various high-risk groups.

CHAPTER THREE - RESULTS

3.1. STARD Diagram

At the end of the study a total of 713 plasma samples and 240 DBS samples were obtained from the study sites from 10 August 2016 - 31 October 2017. Two whole blood EDTA tubes and one DBS card was collected from each participant at one-time point period. Testing of the plasma samples and DBS was performed as illustrated by a flow diagram using the Standard for Reporting Diagnostic accuracy studies (STARD) approach that aims at the completeness and transparency of reporting studies for diagnostic accuracy (Simel et al., 2008) (Figure 3.2).

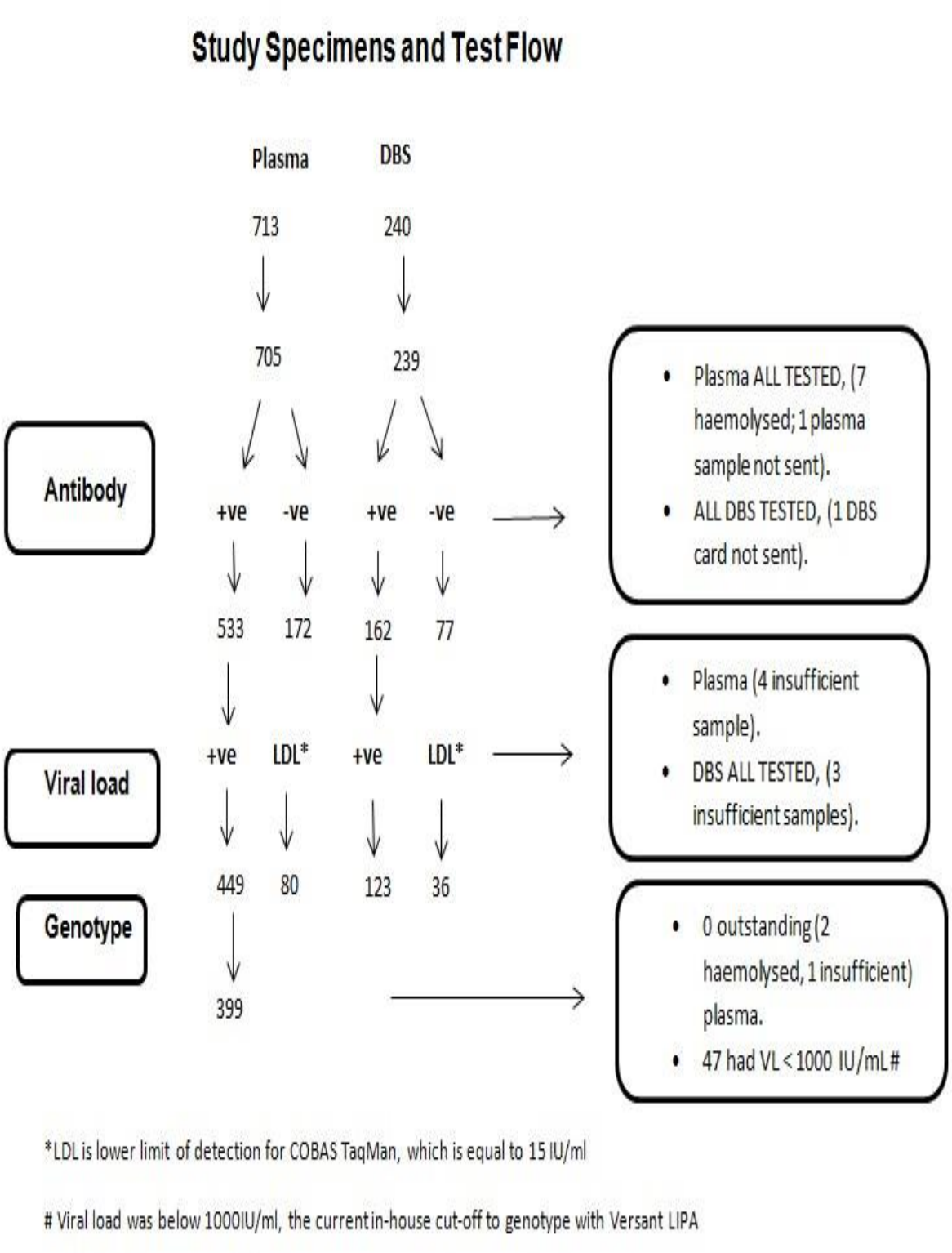


Figure3.2; Specimen and test flow to assess the accuracy of HCV POCT and DBS matrices.

A higher percentage of PWUDs/PWIDs was observed among the male population. The black population was found to be the majority among the high-risk groups (PWUDs/PWIDs, MSM, SWs) followed by white, coloured and Indian race groups. A significant number of PWUDs/PWIDs were found in major cities, Cape Town (CPT), Pretoria (PTA) and Durban (DBN) (Table 3.1) in South Africa. A total of 713 samples were collected from 532 PWUDs/PWIDs, 66 MSM and 115 SWs (Table 3.1).

Table 3.1; Demographic characteristics of high-risk-groups

	PWUDs/PWIDs[a] N=532	MSM[b] N=66	SWs[c] N=115
Median age (year)	30	32	29
IQR[d]	9	14.5	4
Males	461 (86.7%)	66 (100%)	19 (17%)
Females	68 (12.8%)	0 (0%)	94 (82%)
Transgender Female	3 (0.6%)	0 (0%)	2 (1.74%)
Population groups **Blacks** **White** **Coloured** **Indian** **Other**	236 (44.36%) 166 (31.20%) 122 (22.93%) 8 (1.5%) 0 (0%)	25 (37.88%) 25 (37.88%) 13 (19.69%) 1 (1.51%) 2 (3.03%)	70 (60.86%) 35 (30.43%) 10 (8.70%) 0 (0%) 0 (0%)
Co-Infection with HCV **HBV[e]** **HIV[f]**	5.26% 27.63%	7.58% 42.42%	8.62% 36.2%
Sites **CPT[g]** **JHB[h]** **PTA[i]** **NMB[j]** **PMB[k]** **DBN[l]** **ORT[m]**	146 4 266 0 0 116 0	40 11 15 0 0 0 0	53 0 0 12 12 22 16

[a] PWUDs/PWIDs: People who inject drugs/ People who use drugs
[b] MSM: Men who have sex with Men
[c] SWs: Sexual Workers
[d] IQR: Interquartile range
[h] JHB: Johannesburg
[l] PTA: Pretoria
[e] HBV: Hepatitis B Virus
[f] HIV: Human Immunodeficiency Virus
[g] CPT: Cape Town
[j] NMB: Port Elizabeth
[k] PMB: Pietermaritzburg
[l] DBN: Durban
[m] ORT: Mthatha

3.2. HCV POCT Sensitivity and Specificity

The aim was to assess accuracy of POCT OraSure (whole blood and oral fluid) relative to a reference ELISA method. Seven hundred and five plasma samples were tested by the ELISA using the ARCHITECT *i1000SR* system.

A total of (533/705; 75.60%) were positive and (172/705; 24.39%) were negative for anti-HCV. Eight samples were excluded from testing and analysis (Figure 3.1). There were, 4 false positives (FP) and 8 false negatives (FN) specimen's (Table 3.2). The combined data for whole blood and oral fluid are presented (Table 3.3). The accuracy was determined by sensitivity, specificity, positive predictive value (PPV) and the negative predictive value (NPV) (Table 3.3).

Table 3.2; The 2x2 table of HCV POCT OraSure (whole blood and oral fluid) and reference test (ELISA) results.

	ELISA[a] Positive	ELISA Negative
POCT[b] Positive	**TP[c]** 525	**FP[e]** 4
POCT Negative	**FN[d]** 8	**TN[f]** 168

a ELISA: Enzyme-Linked Immunoassay
b POCT: Point-of-Care Test
c TP: True Positive
d FN: False Negative
e FP: False Positive
f TN: True Negative

Table 3.3; The statistical analysis of both POCT OraSure (whole blood and oral fluid) was compared to reference test (ELISA) results.

Statistical analysis	POCT[a] OraSure (whole blood and Oral fluid) and ELISA[b]
Sensitivity	98.5% (95% CI[c]: 97.1 - 99.4%)
Specificity	97.7% (95% CI: 94.2 - 99.4%)
Positive Predictive Value	99.2% (95% CI: 98 - 99.7%)
Negative Predictive Value	95.5% (95% CI: 91.3 - 97.7%)

a POCT: Point-of-Care Test

b ELISA: Enzyme-Linked Immunoassay

c CI: Confidence Interval

3.2.1. HCV POCT OraSure (whole blood) serology accuracy

Four hundred and seventy-three whole blood samples were tested using the OraQuick® HCV Rapid Antibody Test. There were 3 false positives (FP) and 4 false negatives (NP) (Table 3.4). We also evaluated the accuracy of POCT OraSure (whole blood) results with the reference test (ELISA) results as illustrated by the (Table 3.5). A total of (372/473; 78.64%) were positive and (101/473; 21.35%) were negative for anti-HCV.

Table 3.4; The 2x2 table of POCT OraSure (whole blood) and reference test (ELISA) results.

	ELISA[a] Positive	ELISA Negative
POCT[b] Positive	TP[c] 368	FP[e] 3
POCT Negative	FN[d] 4	TN[f] 98

a ELISA: Enzyme-Linked Immunoassay
b POCT: Point-of-Care Test
c TP: True Positive
d FN: False Negative
e FP: False Positive
f TN: True Negative

Table 3.5; The statistical analysis of POCT OraSure (whole blood) was compared to reference test (ELISA) results.

Statistical analysis	POCT[a] OraSure (Whole Blood) and ELISA[b]
Sensitivity	98.9% (95% CI[c]: 97.2 - 99.7%)
Specificity	97.0% (95% CI: 91.2 - 99.4%)
Positive Predictive Value	99.2% (95% CI: 97.6 - 99.7%)
Negative Predictive Value	96.1% (95% CI: 90.3 - 98.5%)

a POCT: Point-of-Care Test
b ELISA: Enzyme-Linked Immunoassay
c CI: Confidence Interval

3.2.2. HCV POCT OraSure (oral fluid) serology accuracy

A total of (157/232; 67.67%) were positive and (75/232; 32.32%) were negative for anti-HCV. There was 1 false positive (FP) and 4 false negatives (FN) (Table 3.6). Two hundred and thirty-two oral fluid samples were tested using the OraQuick® HCV Rapid antibody test, POCT OraSure oral fluid was compared with the ELISA reference test results as illustrated by the (Table 3.7). The statistical results of POCT OraSure oral fluid versus the ELISA were analysed.

Table 3.6; The 2x2 table of HCV POCT OraSure (oral fluid) and reference test (ELISA) results.

	ELISA[a] Positive	ELISA Negative
POCT[b] Positive	**TP**[c] 153	**FP**[e] 1
POCT Negative	**FN**[d] 4	**TN**[f] 74

a ELISA: Enzyme-Linked Immunoassay
b POCT: Point-of-Care Test
c TP: True Positive
d FN: False Negative
e FP: False Positive
f TN: True Negative

Table 3.7; The statistical analysis of HCV POCT (oral fluid) compared to reference test (ELISA) results.

Statistical analysis	POCT[a] OraSure oral fluid and ELISA[b]
Sensitivity	97.5% (95% CI[c]: 93.6 - 99.3%)
Specificity	98.7% (95% CI: 92.8 - 100%)
Positive Predictive Value	99.4% (95% CI: 95.6 - 99.9%)
Negative Predictive Value	94.9% (95% CI: 87.5 - 98%)

a POCT: Point-of-Care Test
b ELISA: Enzyme-Linked Immunoassay
c CI: Confidence Interval

3.3. HCV DBS Serology Accuracy

Serology tests were performed on DBS. The evaluation of the accuracy of DBS was performed by comparing the DBS with the reference test (ELISA) results (Table 3.8). A total of (162/239; 67.78%) were positive and (77/239; 32.21%) were negative for anti-HCV. There were 2 false positives (FP) and 6 false negatives (FN) (Table 3.8). Two hundred and thirty-nine DBS samples were tested using the ARCHITECT *i1000SR* system and the results were compared with ELISA reference results using plasma as illustrated by the (Table 3.9).

Table 3.8; The 2x2 table of HCV DBS compared to reference test (ELISA) results.

	ELISA[a] Positive	ELISA Negative
DBS[b] Positive	TP[c] 156	FP[e] 2
DBS Negative	FN[d] 6	TN[f] 75

a ELISA: Enzyme-Linked Immunoassay
b DBS: Dried Blood Spot
c TP: True Positive
d FN: False Negative
e FP: False Positive
f TN: True Negative

Table 3.9; The statistical analysis of DBS compared to reference test (ELISA) results.

Statistical analysis	DBS[a] and ELISA[b]
Sensitivity	96.3% (95% CI[c]: 92.1 - 98.6%)
Specificity	97.4% (95% CI: 90.9 - 99.7%)
Positive Predictive Value	98.7% (95% CI: 95.2 - 99.7%)
Negative Predictive Value	92.6% (95% CI: 85.1 - 96.5%)

a DBS: Dried Blood Spot
b ELISA: Enzyme-Linked Immunoassay
c CI; Confidence Interval

3.4. HCV Viral Loads on HCV DBS

The DBS viral load results were analysed and compared with the reference standard molecular test using plasma samples. Two hundred and thirty-nine paired plasma and DBS samples were sent to NICD (Figure 3.2). One hundred and sixty-two DBS samples

tested positive for HCV antibodies, 123 had detectable HCV RNA levels, and 36 specimens were below the lower limit of HCV RNA detection (Figure 3.2). Only 116 plasma results were compared to DBS viral loads results because 7 plasma samples were haemolysed and could not be tested for viral load and were excluded from analysis (Figure 3.2).

Paired plasma and DBS samples were statistically analysed on XL STAT Biomed Perpetual version 18.06 (XLSTATA. 2017) (Figure 3.2). The regression line graph was used to investigate if there is a relationship between the DBS and plasma variables and the Bland-Altman plot was used to measure the agreement between the two assays (Cutler, 2013). A normalization coefficient (c=22) (Marins et al., 2017, Ed Marins, Dept. of Virology, Roche Diagnostics, personal communication) was determined which considered the volume difference and sample type in DBS and plasma. The normalization coefficient was applied to the raw DBS results. The linear regression and Bland Altman plots were constructed with the corrected DBS in (log IU/mL) and plasma in (log IU/mL) results.

3.4.1. Linear regression

The linear regression graph **without** normalization coefficient application to raw DBS shows correlation coefficient of R^2=0.879 ($p<0.0001$) and a regression equation of y=0.722x (Figure 3.3).

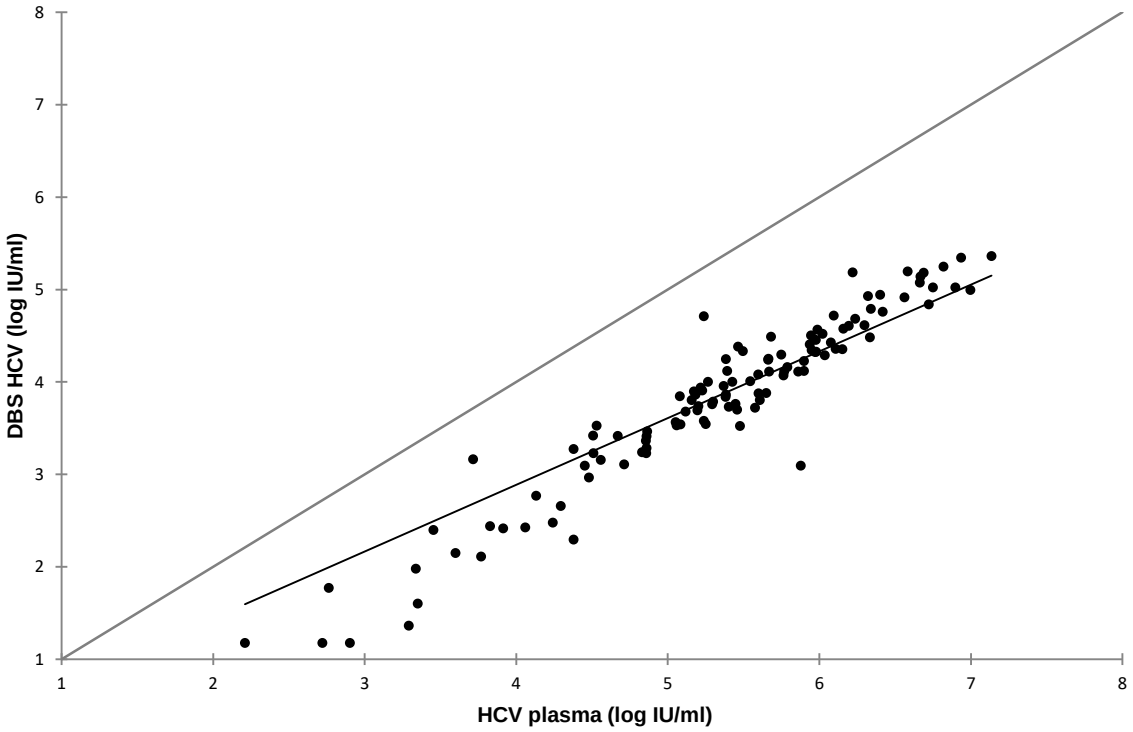


Figure 3.3; The linear regression graph of HCV viral load of DBS (log IU/mL) and HCV of viral load plasma (log IU/mL) without application of normalization coefficient to raw DBS results.

The results from the linear regression graph **after** application of the normalization coefficients to raw DBS results shows a correlation coefficient of R^2=0.918 ($p<0.0001$) and a regression equation of y=0.965x (Figure 3.4).

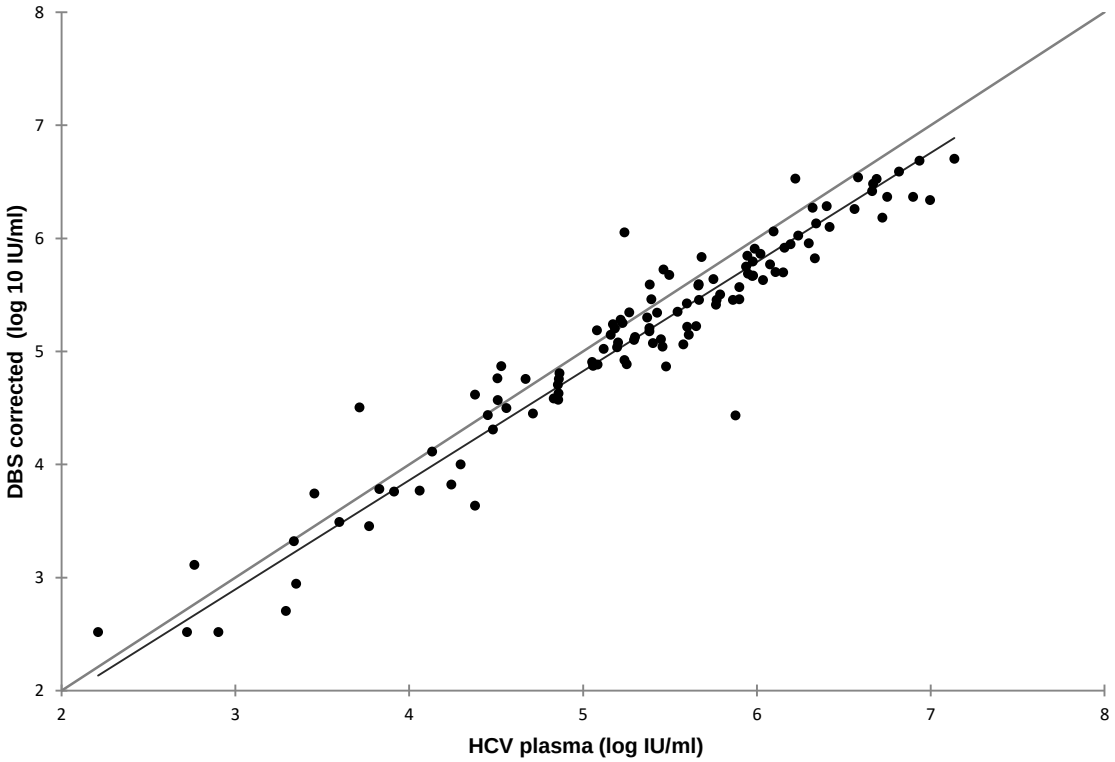


Figure 3.4; The linear regression of the corrected HCV viral load of DBS (log IU/mL) and the HCV viral load of plasma (log IU/mL).

3.4.2. Bland-Altman plot

The results of the Bland-Altman plot **without** application of the normalization coefficient to the DBS raw results showed limits of agreement of [-2.069 log_{10} IU/ml, -0.965 log_{10} IU/ml] (Figure 3.5).

The mean [SD] for plasma viral load was 5.325 IU/ml [1.00 IU/ml]. The mean [SD] for DBS viral load was 3.808 IU/ml [0.951 IU/ml]. The Bias was -1.517 with SD of 0.282 and the 95% mean CI Bias was [-1.569, -1.465].

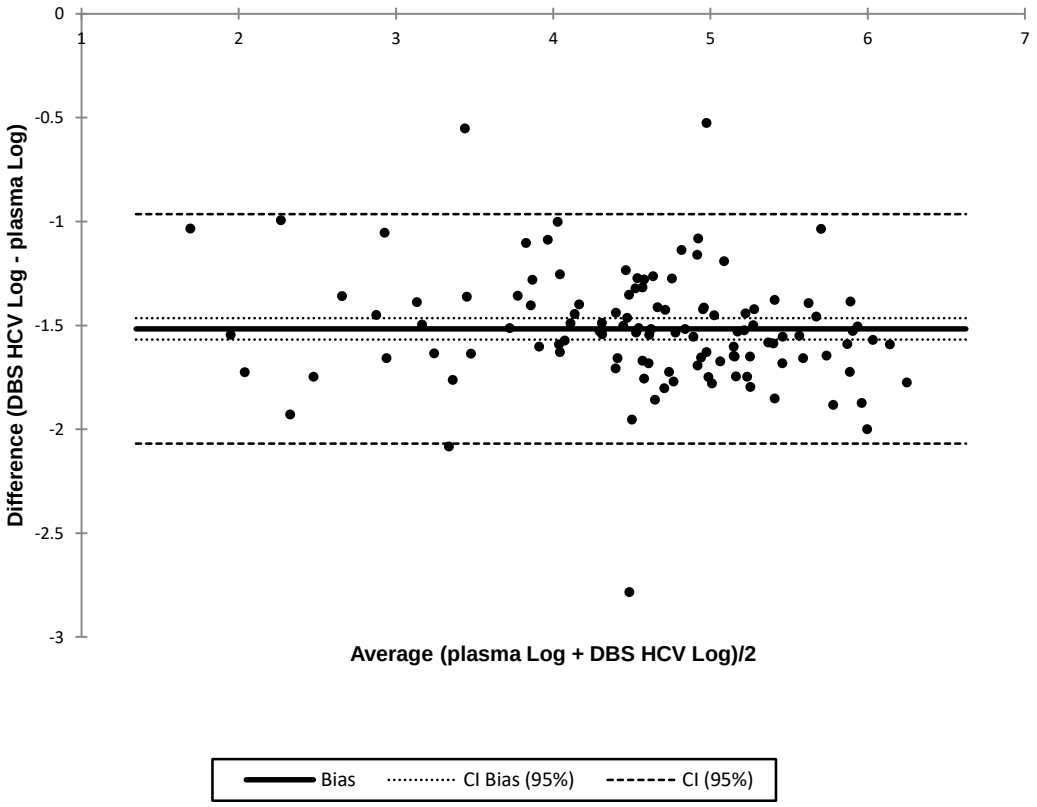


Figure 3.5; The Bland Altman Plot of HCV viral load of DBS (log IU/mL) and HCV viral load of plasma (log IU/mL) without application of normalization coefficient.

The Bland-Altman plot results **after** application of the normalization coefficient to the DBS raw results shows the limits of agreement of [– 0.726 log_{10} IU/ml, 0.377 log_{10} IU/ml] (Figure 3.6).

The mean [SD] for plasma viral load was 5.325 IU/ml [SD 1.0IU/ml] and the mean [SD] for DBS viral load was 5.151 IU/ml [SD of 0.95 IU/ml]. The Bias was -0.175, SD of 0.282 and the 95% mean CI Bias was [-0.226, -0.123] with a P<0.0001. Therefore, after the application of the normalization coefficient, the use of DBS as a sample matrix for HCV viral load testing give similar results as the use of Plasma sample matrix. This demonstrates that the two testing sample matrices are not different from each other

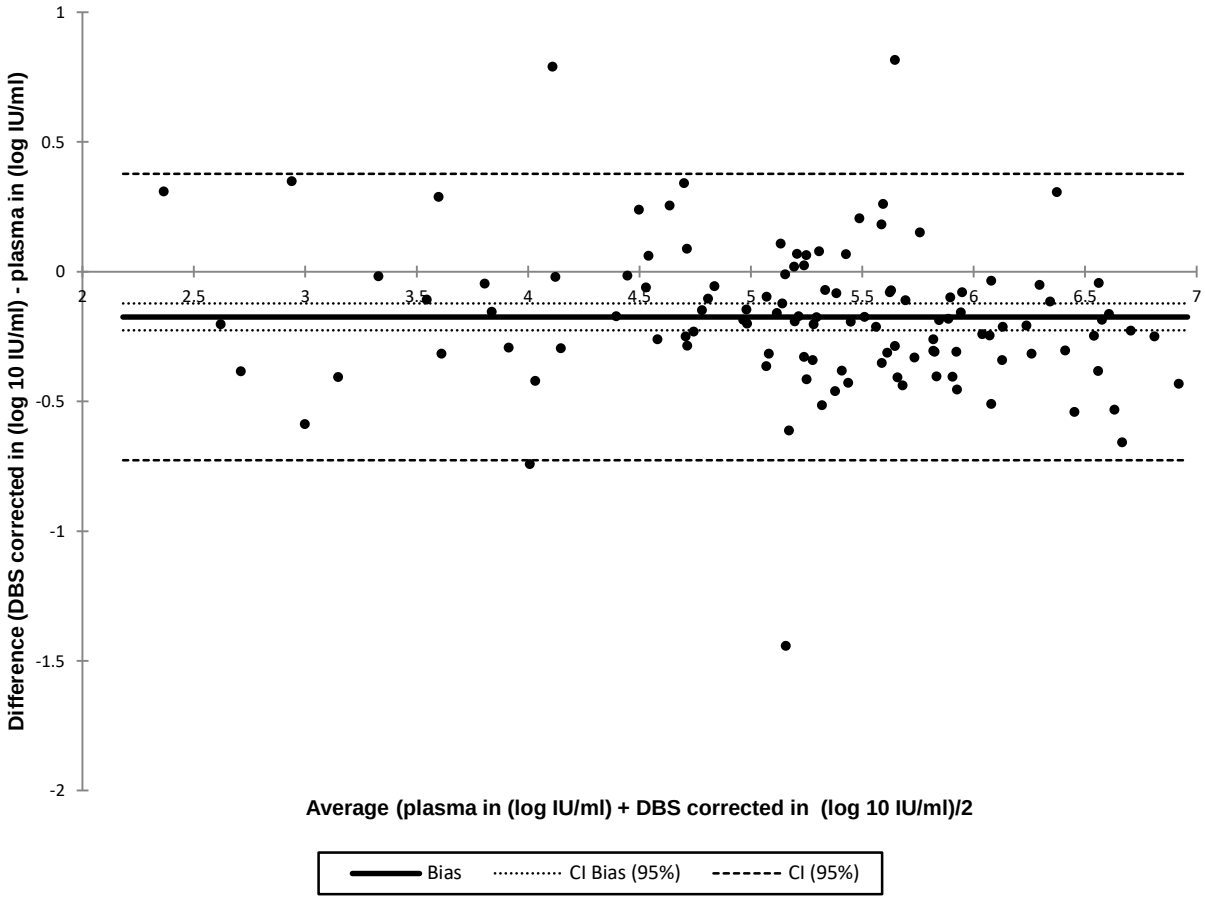


Figure 3.6; The level of agreement between HCV viral load of DBS (log IU/mL) and the corrected HCV viral load of plasma (log IU/mL) viral load after volume normalization.

3.5. HCV Plasma Viral Loads Per High-Risk Groups

Of the 705 plasma specimens, 449 (63.69%) had detectable viral loads and 80 (11.35%) had undetectable viral loads.

Table 3.10; HCV plasma viral load (log IU/ml) per high-risk group

High-risk group	Number of samples	HCV RNA (log IU/ml)	Mean HCV RNA [SD] (log IU/ml)
PWUDs/PWIDs	420	1.17 - 7.7	5.44 [1.23]
MSM	17	1.69 - 6.99	5.12 [1.31]
SW	12	2.20 - 6.74	5.30 [1.32]
Total	449	1.2 - 7.7	4.69 [1.23]

3.6. HCV Amplification For Plasma Genotyping

The desired amplified PCR product of approximately 240bp and 270bp in size representing the 5' untranslated region (5' UTR) and core regions using the Versant HCV amplification 2.0 kit was observed (Figure 3.7).

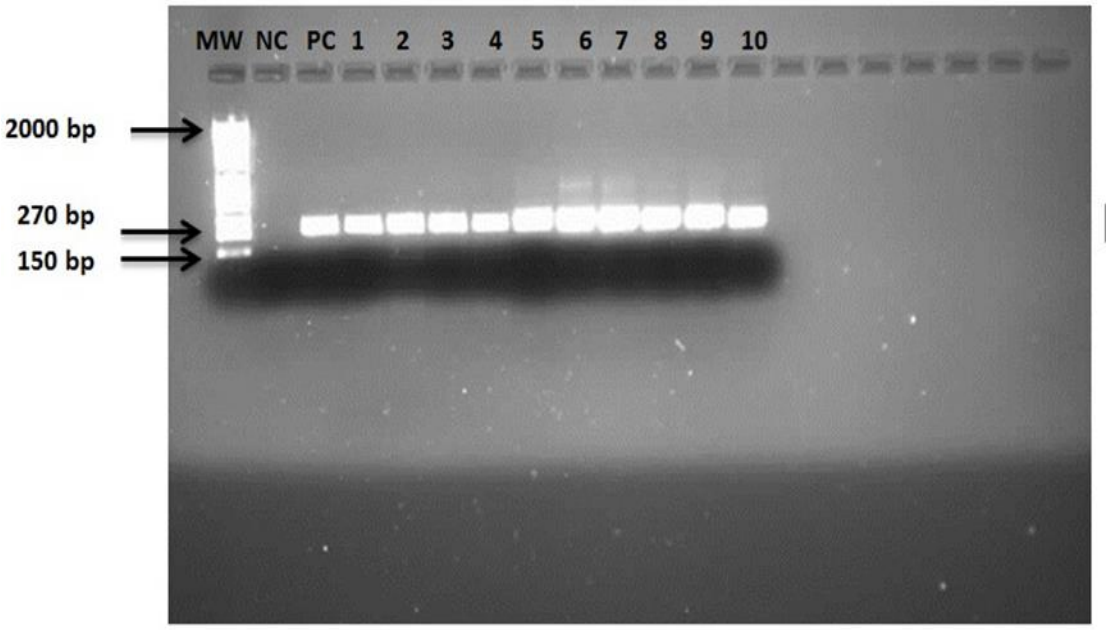


Figure 3.7; Agarose gel documentation of the HCV PCR products.
MW= VI kbp (MW) Molecular weight marker. 1=Negative Control (NC), 2=Positive Control (PC), and 1-10=Study Samples.

3.7. HCV Genotyping

Of the 449 plasma samples that had detectable HCV RNA, 399 from those were genotyped (Figure 3.2). Forty-seven plasma samples had a viral load less than 1000 IU/ml and could not be genotyped. Two samples were haemolysed and 1 had insufficient sample for HCV genotyping. For the detection of the HCV genotypes, each strip has to be recorded and interpreted as shown in (Figure 3.8).

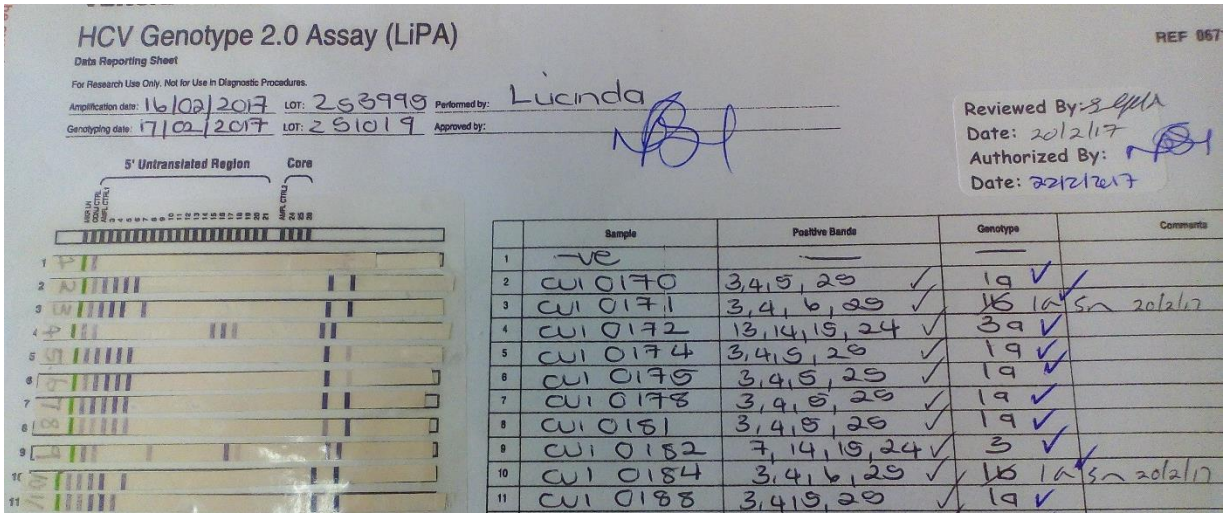


Figure 3.8; The HCV genotyping test strips showing different HCV genotypes.
Line 1= negative control, line 2-11 = test samples.

3.7.1. Prevalent genotypes

The most prevalent genotypes were genotype 1a (73.93%), genotype 3a (14.79%), and genotype 3 (3.51%). Most genotypes were found amongst the people who use drugs and inject drugs (PWUDs/PWIDs) as shown in (Table 3.11). Genotype 1a is the most common genotype amongst PWUDs/PWIDs high-risk group.

Table 3.11; The distribution of HCV genotypes among the various the high-risk groups

Genotypes	1	1a	1a +3	1a +3a	1a+3 c	1b	1b+ 3a	3	3a	3c	4	4a/4c/4 d	Total
PWUDs/PWIDs	6	280	1	9	1	5	1	13	53	3	1	0	373
MSM	1	11	0	0	0	0	0	1	0	0	1	2	16
SWs	0	4	0	0	0	0	0	0	6	0	0	0	10
Total	7	295	1	9	1	5	1	14	59	3	2	2	**399**

CHAPTER FOUR – DISCUSSION

HCV infection is a public health problem of global significance with an estimated 130-150 million people chronically infected. There is a lack of information on the performance of HCV virological assays (Soulier et al., 2016) and data on HCV prevalence among high-risk groups with many cases of acute and chronic HCV infection that are underdiagnosed (Dokubo et al., 2014). The WHO has established new measures of Hepatitis C elimination by ending transmission and reducing the burden of diseases among those that are already infected (WHO, 2016f). The WHO can achieve hepatitis elimination target by increasing diagnosis and therapy, however, due to poor linkage in health care services this offers a major challenge especially within high-risk groups (Scott et al., 2017).

4.1. HCV POCT

The use of anti-HCV rapid assays can be implemented in various settings to benefit hard-to-reach populations and high-risk groups, for example, PWUDs/PWIDs, MSM, and SWs. These individuals are mostly hard to reach as they are marginalized and/or stigmatized and unaware of their infection status making it difficult for them to be screened for infectious diseases such as HIV, HBV, and HCV by conventional clinical services and laboratory testing. There is also the likelihood of loss of follow up in routine services as most individuals are likely not to return for their results (Keenan et al., 2001, Lubelchek et al., 2005).

The evaluation of POC testing using whole blood and oral fluid assays in this study, based in South Africa found a sensitivity of 98.93% and specificity of 97.00% (Table 3.5) and a sensitivity of 97.45% and specificity of 98.67% (Table 3.7), respectively. These results are similar to a previous study (Lee et al., 2010b) which reported that when using OraQuick® rapid HCV test the specificity on oral fluid was 100%. By contrast, these results differ from a study which compared OraSure HCV rapid Antibody Test using

whole blood and HCV EIA laboratory test. Fisher et al. (2015) found a lower sensitivity of 92.7% and a higher specificity of 99.8%.

We also reported a positive predictive value (PPV) of 99.19% and 99.35% (Table 3.5), and a negative predictive value (NPV) of 96.04% and 94.87% (Table 3.7), for whole blood and oral fluid respectively. These results differ to those observed by a previous study, which reported a good sensitivity 94.4% and specificity 96.94% with a NPV of 99.9% and a PPV of 72.7% when using OraQuick® with venepuncture from whole blood by comparing this to serum by EIA (Gao et al., 2014).

There have been reported association between obtaining false-negative results when using rapid tests and HIV positive status (Smith et al. 2011c). There is a 22.4% chance of obtaining false-negative results when using rapid tests among HIV-positive individuals (Lubelchek et al., 2005). Approximately 3-6% of HIV positive persons have reduced HCV antibody responses (Marcellin et al., 1994, Bonacini et al., 2001). It is also assumed that when using rapid assays in HIV-positive persons the HCV titres can be low and undetectable, but remain detectable by the reference testing (Fisher et al., 2015). Other than an impaired immune system (Vermeersch et al., 2008), other possible reasons to explain the false results obtained in HIV-positive persons include: recent infection and the 'window period' i.e. titres are in evolution and not sufficiently high enough titres to be detected (Ridzon et al., 1997, Bendinelli et al., 1999, Bux-Gewehr et al., 2001), resolved infection and decreasing titre levels (Alter et al., 2000a).

The other reason for our false negative results could be that the performance levels in field studies and clinical sensitivity of rapid tests are lower as compared to conventional laboratory-based assays (Kendrick et al., 2005, Metcalf et al., 2005, Scheiblauer et al., 2006, Cha et al. 2013). The quality control measures performed by the operator when using POCT could also possibly impact POC results thus increasing false results (Cha et al. 2013). However, OraQuick® has been reported to have an excellent operator agreement in result interpretation, irrespective of who reads the results (Smith et al., 2011c).

Our study was based on an HIV community-based study, with a significant number of high-risk individuals. Previous studies show the HIV status has a direct influence in the detection of anti-HCV when using rapid assays (Smith et al., 2011c, Cha et al., 2013, Fisher et al., 2015). However, when we looked closely at our false negative results, we found only one sample that had co-infection with HIV. It has been documented that the sensitivity of the rapid tests is based on the number of true positives that the test can accurately detect (Kendrick et al., 2005, Metcalf et al., 2005, Scheiblauer et al., 2006, Cha et al. 2013). In our study, the field operators read the HCV results five minutes prior to the recommended reading time. Thus possibly explaining the number of false negative results we obtained. It is also highly recommended that more studies evaluate the performance of rapid assays among high-risk-groups with HIV-positive individuals (Smith et al., 2011c).

From our POCT results, we recommend that POC testing using OraQuick® could be a feasible option. Even though there were false negative results, with education and training of field operators and nurses at different sites there can be an improvement in POCT performance and the potential to decrease in false negative results. POC testing must be integrated into existing HIV testing services and organizations that are effective in reaching HIV-positive, high-risk persons (Smith et al., 2011c). The use of POCT in PWUDs/PWIDs could assist in decreasing HCV prevalence by increased testing and help initiate programs such the harm-reduction.

The breakthrough on molecular testing of POCT (Xpert® HCV Viral Load assay) may also make a significant contribution to HCV diagnosis and treatment monitoring by linking HCV testing to on-site testing and by simplifying sample collection, through increased sensitivity, reduced turn-around time, and ease of performance (Grebely et al., 2017, Gupta et al 2017). The Xpert HCV Viral Load assay may be a key step towards public health approach in rapid HCV diagnosis and in referral to care and HCV elimination (Gupta et al 2017).

4.2. DBS Immunoassay Results

We reported a sensitivity of 96.30% and specificity of 97.40% (Table 3.9) when comparing DBS from whole blood with ELISA laboratory test and a PPV of 98.73% and NPV of 92.59% (Table 3.9) These findings are similar to other studies which have reported very good sensitivity and specificity when comparing DBS with plasma (Kania et al., 2013, Mössner et al., 2016, Soulier et al., 2016). There have been reported differences shown across other studies with higher PPVs and NPVs reported (Kania et al., 2013, Dokubo et al., 2014, Soulier et al., 2016, Mössner et al., 2016). In our study, we reported on a high prevalence of HCV among PWUDs/PWIDs, which could account for the high PPV (Dokubo et al., 2014).

Our study recommends that DBS be used as an alternative sample matrix for anti-HCV testing by means of commercial laboratory methods. Similar findings have been reported (Brandão et al., 2013, Soulier et al., 2016). Previous studies demonstrate clear evidence that whole blood sample collection using DBS offers a better alternative to conventional blood collection methods (Bennett et al., 2012, Soulier et al., 2016) and will increase the numbers of HCV screening, diagnosis and treatment especially amongst high-risk-groups (Bennett et al., 2012, Coats and Dillon, 2015). DBS also offers minimal blood collection for testing as compared to the conventional collection of plasma and whole blood specimens (Lee et al., 2011a). Much work still remains to be done in terms of standardizing the methods when using DBS for HCV testing and it is crucial to be aware that the volume of blood in DBS collection is limited with the risk of false-negative and false-positive reporting (Muzembo et al., 2017).

4.3. HCV Viral Load on HCV DBS

Our study examined the use of DBS as a sample matrix for HCV RNA quantification amongst high-risk-populations (PWUDs/PWIDs, MSM, and SWs). Our study is the only national and international study to report on a sample size of more than 100 paired DBS and plasma samples (Figure 3.2). Our study provided a working methodology on how to

perform HCV testing on DBS and included steps on how to adjust for sample type, volume difference and also to apply the normalization coefficient to the DBS results.

Other studies (Tuaillon et al., 2010, Marins et al., 2017) have successfully quantified HCV RNA using the COBAS® AmpliPrep/COBAS® Taqman® Instrument. However, they had limited information on a working methodology, on how they adjusted for their sample type and volume difference. In addition, these studies had low sample size of 48 (Marins et al., 2017) and 62 (Tuaillon et al., 2010) respectively. In our study a strong correlation of (R^2= 0.918) (Figure 3.4) and good agreement was observed between DBS and plasma samples, this demonstrates that the two testing sample matrices are not different from each other. Similar results with a strong correlation of (R^2= 0.97) were reported (Marins et al., 2017). The regression plot demonstrates that the sample matrices have a positive linear relationship and the correlation significantly improved the Linear Regression Plot from R^2=0.879 (Figure 3.3) to R^2=0.918 (Figure 3.4) by correcting for sample type, volume and also applying the normalization coefficient.

A negative offset of approximately 2.0 $\log_{10}$ IU/ml was observed between the DBS and plasma results. This proposes that applying the normalization to the DBS results did not resolve the correlation, since there was no perfect linear correlation after correcting for sample type and volume differences, similar findings have also been reported (Marins et al., 2017). Outliers were observed in the Bland Altman analysis; these outliers could be a result of the overcorrected DBS results since some observations on the DBS had HCV viral loads higher than in plasma (Figure 3.6).

The low HCV RNA titres observed in DBS can be due to the different input volumes of whole blood (50µl) spots and plasma (650 µl) when testing on the COBAS® Ampliprep/COBAS® Taqman® 48 analyser Instrument. A possibility of nucleic acid entrapment in the DBS cards and suboptimal elution of the nucleic acid from the filter paper can be another explanation for the difference in HCV RNA levels observed in DBS. It is important to know that there might be less volume of blood extracted on the DBS filter paper as compared to plasma and serum (De Crignis et al., 2010). Increasing

the number of DBS spots (+/- 50µl) used during a test could possibly be a feasible solution (Tuaillon et al., 2010, Marins et al., 2017). The use of cold chain transportation 72 hours after sample collection and freezing DBS cards at -20˚C for long term storage could yield better extraction of HCV RNA (Tuaillon et al., 2010) and retaining the stability, preventing degradation of the nucleic acid material (Tuaillon et al., 2010). Our study is the first to report on the quantification of HCV using DBS amongst high-risk groups in the Sub-Saharan Africa. From our results, we strongly recommend that the DBS be used as a sample matrix for both serology and molecular testing in high-risk-populations.

4.4. HCV Viral Loads on Plasma

The HCV viral load showed a small range of data among PWUDs/PWIDs, MSM, and SWs. A HCV RNA baseline range of 2.6 - 7.4 log IU/ml (Table 3.10) observed among the high-risk group was demonstrated in a previous study (Ticehurst et al., 2007) and another former study found a higher HCV RNA range of 5.6 - 26.0 log IU/ml amongst PWUDs/PWIDs group (Smith et al., 2016). However, we could not perform a statistical analysis of the plasma viral loads between PWUDs/PWIDs, MSM, and SW due to small sample size found in MSM and SW high-risk-groups.

4.5. HCV Genotypes on Plasma

HCV genotype 5 has been reported as the most predominant genotype in South Africa followed by genotype 1, 4, 3 and 2 (Prabdial-Sing et al., 2005a, Gededzha et al., 2012b, Prabdial-sing et al., 2016b). There has been only two full length genomes of genotype 5a reported in South Africa (Gededzha et al., 2010a). Thus due to the restricted data on genotype 5 sequences in South Africa there has been limited phylogenetic analysis, poor confirmation of new HCV genotypes (Simmonds et al., 2005) and knowledge on the current circulating HCV genotypes in different high-risk groups in South Africa. There is limited information on HCV genotype 5 with little to no studies conducted on the origin, endemic history, diseases severity and treatment outcome of HCV genotype 5 infections (Gededzha et al., 2010a), although in one study they have made attempts on

documenting the association between HCV genotype 5 and severe liver disease (Pozzato et al., 1994). To date, there are no reports on the circulating HCV genotypes among high-risk groups in South Africa. We attempted to report on HCV genotypes that are circulating among high-risk groups in South Africa. We observed HCV genotypes 1a, 3a and 3 as the most prevalent genotypes among PWUDs/PWIDs followed by MSM and SW (Table 3.11), with similar findings reported in previous studies (Verbeeck et al., 2006, Jamalidoust et al., 2014).

We also observed a low prevalence of genotype 4, 1 and 3, and genotype 2, 5 and 6 were not observed amongst our high risk groups (Table 3.11). There have been documented similar results with genotype 4 and genotype 1+3 found to be less prevalent among the PWUDs/PWIDs group (Jamalidoust et al., 2014) and genotype 2, 4 and 6 are rare and account for one-quarter of all HCV cases globally in their study findings and (Chowdhury et al., 2003, Messina et al., 2015).

The paucity in identifying genotype 5 in our study contradicts reports that genotype 5 is most predominant in South Africa (Ohno et al., 1997b, Prabdial-Sing et al., 2005a). This could be due to restricted circulation of HCV genotype 5 amongst populations with low HCV prevalence (Gededzha et al., 2010a) and that this HCV genotype could be possibly circulating amongst the older population (Prabdial-Sing et al., 2016b). It is important to acknowledge that all previous studies have collected their samples from blood donors, patients with chronic renal failures, haemophiliacs and those attending liver disease clinics (Prabdial-Sing et al., 2005a, Ohno et al., 1997b, Smuts et al., 1995) and none from high risk groups. This reflects a predominance of HCV genotype 5 among blood donor populations in South Africa; however, the reason for this still remains unclear and could be due to the number of those collected blood products which happened to be administered to this group at that period (Smuts et al., 1995).

HCV genotype 1a was the only genotype observed among sex workers in our study (Table 3.11). The association between sexual transmission of HCV in high-risk groups such as SWs and MSM have not been conducted. Previous studies have also reported

that there is a generally lower risk of sexual transmission of HCV (Terrault, 2005b) and sexual transmission of HCV is rare in high-risk groups (Alary et al., 2004, Hahn, 2007). The risk of sexual transmission of HCV is dependent on multiple reasons; co-infection with HIV, HCV RNA levels, integrity of mucosal surface (Terrault et al., 2013c), risky sexual behaviour such as multiple sex partners (Alter et al., 1997c), practice of IDU (Alter.,1997) and type of sexual relationship (Terrault., 2002a). Studies conducted among MSM and SWs reported that there was no direct association of HCV with sexually transmitted infections (STIs) and numbers of sex partners (Osmond et al., 1993, Ndimbie et al., 1996, Mesquite et al., 1997). However, an increased risk of sexual HCV transmission among HIV-positive MSM has been documented (Browne et al., 2004).

4.6. Limitations

The limitations of the study included not optimizing the volume of whole blood to be spotted on the DBS cards and, not optimizing for the optimum dilutions of the eluate. Other limitations included the use of venepuncture blood instead of finger stick blood as a type of sample. However, it has been observed that there is no difference in results when using different samples (venepuncture blood or finger stick blood) (Lee et al., 2010b), although, it is desirable to use finger prick when evaluating rapid tests. The use of finger prick compared to venepuncture has advantages among PWUDs/PWIDs who may have scarred or damaged veins.

Our major limitation was not establishing the serum cut-offs and lower limits of detection of DBS values prior to DBS testing, as the manufacturer's serum cut-off were used. This possibly increased the number of false negative results in our study. The manufacturer signal-to-cut off value increases the number of false-negative results (Soulier et al., 2016). It is important that studies perform DBS optimization prior to testing because an increase in false positive results will result in a decrease in the sensitivity of the test (Soulier et al., 2016). The receiver operating characteristics curves (ROC) curve could have been used to identify the optimal corresponding DBS threshold for both anti-HCV

and HCV RNA detection. However, regardless of our limitations our results show the feasibility of using DBS for anti-HCV and HCV RNA detection.

4.7. Conclusions

Our study confirms that the OraQuick® HCV POC rapid test can be used as a point-of-care test with high accuracy. Thus HCV POCT can be phased into already existing medical settings such as the HIV/AIDS community programs, mobile clinics situated at universities, rural areas, and sites which are frequented by hard-to-reach groups. The use of POCT would offer an opportunity for free-for-service HCV screening and screen for persons without access to medical insurance (Smith et al., 2011c) especially in those areas with limited laboratory resources. Alternatively, DBS cards can be used to test anti-HCV antibodies and HCV RNA using automated laboratory techniques. The collection of DBS samples can be used outside health-care settings and in settings that use point-of-care testing. DBS can also be used to diagnose and promote the uptake of establishing national programs for HCV screening among high-risk groups and surveillance of circulating genotypes.

5. REFERENCES

- Aach, R.D., Szmuness, W., Mosley, J.W., et al. (1981). Serum alanine aminotransferase of donors in relation to the risk of non-A, non-B hepatitis in recipients: the transfusion-transmitted viruses study. *New England Journal of Medicine*. 304, 989-994.
- Abbot Laboratories. Architecy package insert. Puerto Rico: Abbot Laboratories; 2006.
- Abergel, A., Ushetto, S., Dubost, C., et al. (2007). The epidemiology and virology of hepatitis C virus genotype 5 in central France. *Alimentary Pharmacology and Therapeutic.* 26,1437-1446.
- Abuelhassan, W. (2012). Hepatitis C Virus infection in 2012 and beyond. *South African Journal of Epidemiology and Infectious Diseases*. 27, 93-97.
- Alary, M., Joly, J.R., Vincelette, J., et al. (2004). Lack of Evidence of sexual Transmission of Hepatitis C virus in a Prospective Cohort Study of Men Who Have Sex with Men. *American Journal of Public Health*. 95 (3), 502-505.
- Alter, H.J. and Seeff, L.B. (2000). Recovery, persistence, and sequaele in hepatitis C virus infection: a prospective on long-term outcome. *Seminars in Liver Diseases*. 20, 17-35.
- Alter, H.J., Holland, P.V., Morrow, A.G., et al. (1975). Clinical and serological analysis of transfusion-associated hepatitis. *Lancet*. 2, 838-841.
- Alter, H.J., Purcell, R.H., Shih, J.W., et al. (1989).Detection of Antibody to Hepatitis C Virus in Prospectively Followed Transfusion Recipients with Acute and Chronic Non-A, Non-B Hepatitis. *New England Journal of Medicine*. 321,1494-1500.
- Alter, M.J. (1997). Epidemiology of hepatitis C. Hepatology. 26(3), 625-655.
- Alter, M.J. (2007). Epidemiology of hepatitis C virus infection. *World Journal of Gastroenterology*. 148, 56–82.
- Alter, M.J., Kuhnert W.L., Fineli L. (2003). Guidelines for laboratory Testing and Results Reporting of Antibody to hepatitis C virus. *Center for Disease Control*. 52, 1-6.

- Alter, M.J., Margdis, H.S., Krzysztof, K., et al. (1992). The Natural History of Community-Acquired Hepatitis C in the United States. *The new England Journal of Medicine*. 327,1899-1905.
- Antaki, N., Crax, A., Kamal, S., et al. (2009). The Neglected Hepatitis C virus genotypes 4,5 and 6: an international consensus report. *Liver International*. 30(3), 342-355.
- Antaki, N., Craxi, A., Kamal, S., et al. (2010). The neglected hepatitis C virus genotypes 4, 5 and 6: an international consensus report. *Liver International*. 30(3), 342-355.
- Antaki, N., Haddad, M., Kebbewar, K., et al. (2008). The unexpected discovery of a focus of hepatitis C virus genotype 5 in a Syrian province. *Epidemiology and Infection*. 17, 1-6.
- Apers, L., Vanden Berghe, W., De Wit, S., et al. (2015). Risk factors for HCV acquisition among HIV-positive MSM in Belgium. *Journal of Acquired Immune Deficiency Syndrome*. 68, 585-593.
- Ashfaq, U. A., Javed, T., Rehman S., et al., (2011). An overview of HCV molecular biology, replication and immune responses. Virology Journal. 8,1-10.
- Bacon, B.R., Gordon, S.C., Lawitz, E., et al. (2011). HCV RESPOND-2 Investigators. Boceprevir for previously treated chronic HCV genotype 1 infection. *New England Journal of Medicine*. 364 (13), 1207-1217.
- Barin, F., Plantier, J.C., Brand, D., et al. (2006). Human Immunodeficiency virus serotyping on dried serum spots as a screening tool for the surveillance of the AIDS epidemic. *Journal of Medical Virology.* 78(1), 13-18.
- Barrera, J.M., Bruguera, M., Ercilla, M.G., et al. (1991). Incidence of non-A, non-B hepatitis after screening blood donors for antibodies to hepatitis C virus and surrogate markers. *Annals of Internal of Medicine*. 115, 596-600.
- Barrera, J.M., Francis, B., Ercilla, M.G., et al. (1995). Improved detection of anti-HCV in post-transfusion hepatitis by a third-generation ELISA. *Vox Sanguinis.* 68, 15-18.
- Barth, R.E., Huijgen, Q., Taljaard, J., et al. (2014). Hepatitis B/C and HIV in sub-Saharhan Africa; an association between highly prevalent infectious diseases. A

systematic review and meta-analysis. *International Journal of Disease*.14, 1024 -1031.

- Barth, R.E.,Huijgen, Q., Tempelman, H.A., et al. (2011).Presence of occult HBV, but near absence of active HBV and HCV infections in peole infected with HIV in rural South Africa.*Journal of Medical Virology.* 83(6) ,929-934.
- Bendinelli, M., Vatteroni, M.L., Maggi, F., et al. (1999). Hepatitis C virus: biology, pathogenesis, epidemiology, clinical description, and diagnosis. In: Specter S edition. Viral Hepatitis: Diagnosis, Therapy, and Prevention. Totawa, NJ: Humana Press Inc. pp 65-127.
- Bennett, S., Gunson, R.N., McAllister, G.E., et al. (2012). Detection of hepatitis C virus RNA in dried blood spots. *The Journal of Clinical Virology*. 54 (2), 106-109.
- Berman, M., Alter, H.J., Ishak, K.G., et al. (1979). The chronic sequelae of non-A, non-B hepatitis. *Annals of Internal Medicine*. 91, 1–6.
- Biggar, R.J., Miley, W., Miotti, P., et al. (1997). Blood collection on filter paper: a practical approach to sample collection for studies of perinatal HIV transmission. *Journal of Acquired Immune Deficiency Syndrome Research and Human Retroviruses*.14, 398-373.
- Blight, K.J., Kolykhalov, A.A., Reed, K.E., et al. (1998). Molecular virology of hepatitis C virus: an update with respect to potential antiviral targets. *Antiviral Therapy*. 3 (3), 71–81.
- Bodsworth, N.J, Cunningham, P., Kaldor, J., et al. (1996). Hepatitis C virus infection in a large cohort of homosexually active men: independent associations with HIV-1 infection and injecting drug use but not sexual behaviour. *Archive of Genitourinary Medicine*. 72, 118–122.
- Bonacini, M., Lin, H.J., Hollinger, F.B. (2001). Effects of coexisting HIV-1 infection on the diagnosis and evaluation of hepatitis C virus. *Journal of Acquired Immune Deficiency Syndromes*. 26, 340-344.
- Bowring, A.L., Luhmann, N., Pont, S., et al. (2013). An urgent need to scale-up injecting drug harm reduction services in Tanzania: prevalence of blood-borne

viruses among drug users in Temeke District, Dar-es-Salam 2011. *International Journal of Drug Policy*. 24, 78-81.

- Bradshaw, D., Matthews, G., Danta, M. (2013). Sexually transmitted hepatitis C infection: the new epidemic in MSM? *Current Opinions of Infectious Diseases*. 26, 66-72.
- Brandão, C.P., Marques, V.A., Marques, C.A., et al. (2013). Simultaneous detection of hepatitis C virus antigen and antibodies in dried blood spots. *Journal of Clinical Virology*. 57 (2), 92-102.
- Briat, A., Dulioust, E., Galimand, J., et al. (2005). Hepatitis C virus in the semen of men coinfected with HIV-1: prevalence and origin. *Journal of Acquired Immune Deficiency Syndrome*.19, 1827-1835.
- Brooks, R., Reynolds, G.L., Napper, L.E., et al. (2008). Failure to return for HIV, hepatitis, and STI test results in Los Angeles County, California. In: American Public Health Association 136th Annual Meeting and Exposition. San Diego, CA; 2008.
- Browne, R., Asboe, D., Gilleece, Y., et al. (2004). Increased numbers of acute hepatitis C infection in HIV positive homosexual men: is sexual transmission feeding the increase? *Sexual transmitted Infections*. 80 (4), 326-327.
- Bruggmann, P., Berg, T., Ovrehus, A.L., et al. (2014). Historical epidemiology of hepatitis C virus (HCV) in selected countries. *Journal of Viral Hepatology*. 21, 5-33.
- Buchbinder, S.P., Katz, M.H., Hessol, N.A., et al. (1994). Hepatitis C virus infection in sexually active homosexual men. *Journal of Infection*. 29, 263-269.
- Bukh, J., Purcell, R. H., Miller, R. H. (1993). At least 12 genotypes of hepatitis C virus predicted by sequence analysis of the putative E1 gene of isolates collected worldwide. *Proceedings of the National Academy of Science of United States America.* 90; 8234-8238.
- Burstow, N.J., Mohamed, Z., Gomaa, A.I., et al. (2017). Hepatitis C treatment where are we now? *International Journal of General Medicine*. 10.39-52.
- Bux-Gewehr, I., Schmandt, S., Zotz, R.B., et al. (2001). Long-term hepatitis C seroconversion in a blood donor. *Transfusion*. 41,427.

- Cai, Q., Zhao, Z., Liu, Y., et al. (2013). Comparison of three different HCV genotyping methods: core, NS5B sequence analysis and line probe assay. *Journal of Virological Methods*. 31, 347-352.
- Carithers, R.L. Jr. and Emerson, S.S. (1997). Therapy of hepatitis C: meta-analysis of interferon alfa-2b trials. *Hepatology*. 26(3), 83–88.
- Cassol, S., Weniger, B.G., Babu P.G., et al. (1996). Detection of HIV type 1 env subtypes A, B, C and E in Asia using dried blood spots: a new surveillance tool for molecular epidemiology. *AIDS Research and Human Retroviruses*. 12,1435-1441.
- CDC. (1995). Exposure to Blood. What Healthcare Personnel Need to Know. http//:www.cdc.gov/hepatitis. Updated on 2003. Accessed 14.10.2017.
- CDC. (1998). Centres for Diseases Control and Prevention. Recommendations for prevention and control of hepatitis C virus (HCV) infection and HCV-related chronic diseases. Morbidity and Mortality Weekly Report. 47(19),1-33.
- CDC. (2003). Guidelines for laboratory testing and the results reporting of antibody to hepatitis C virus. Morbidity and Mortality Weekly Report. 52, 1-3.
- CDC. (2011). Centers for disease, control prevention, sexual transmission of hepatitis C virus among HIV-infected men who have sex with men–New York City, 2005–2010. *Morbidity and Mortality Weekly Report*. 60(28), 945-950.
- CDC. (2016). Viral hepatitis- laboratory testing. http://www.cdc.gov/hepatitis/hcv.labtesting.htm. Accessed 10.06.2016.
- Cepheid Xpert HCV Viral Load, Cepheid (2017). http://www.cepheid.com/en/cepheid-solutions-uk/clinical-ivd-tests/virology/xpert-hcv-viral-load. Accessed 20.02.17.
- Cha, Y.J., Park, Q., Kang, E.S., et al. (2013). Performance Evaluation of OraQuick Hepatitis C Virus Rapid Antibody Test. *Annals of Laboratory Medicine*. 33, 184-189.
- Chavaliez, S. and Pawlotsky, J.M. (2006). Hepatitis C Virus Serological and Virological Tests and Clinical Diagnosis of HCV- Related Liver Disease. *International Journal of Medical Sciences*. 3, 35-40.

- Chembio DPP® HCV Test (Chembio Diagnostics Systems, Inc). (2012). Updated on 19 June 2012. http://www. Chembio.com/newtechnologies.html. Accessed 18.06.2018.
- Chen, S.L. and Morgen, T.R. (2006). The Natural History of Hepatitis C Virus (HCV) Infection. *International Journal of Medical Sciences*.3,47-52.
- Chinchai, T., Labout, J., Noppornpanth, S., et al. (2003). Comparative study of different methods to genotype hepatitis C virus type 6 variants. *Journal of Virological Methods*. 109,195-201.
- Chisenga, C.C., Musukuma, K., Chilengi, R., et al. (2018). Field performance of the Determine HBsAg point-of-care test for diagnosis of hepatitis B virus co-infection among HIV patients in Zambia. Journal of clinical virology : the official publication of the Pan American Society for Clinical Virology. 98:5-7.
- Chisenga, C.C., Musukuma, K., Chilengi,R., et al. (2017). Field performance of the Determine HBsAg point- of- care tests for diagnosis of hepatitis B virus co-infection among HIV patients in Zambia. Journal of Clinical Virology. 98, 5-7.
- Choo, O.L., Richman, K.H., Han J.H., et al. (1991). Genetic organization and diversity of the hepatitis C virus. *Proceedings of National Academy of science of the United States of America.* 88, 2451-2455.
- Choo, Q. L., Kuo, G., Weiner, A. J., et al. (1989). Isolation of a cDNA derived from a blood borne non-A, non-B hepatitis genome. *Science*. 244. 359-362.
- Chotun, N., Preiser, W., van Rensburg., C.J., et al. (2017). Point-of-care screening for hepatitis B virus infection in pregnant women at an antenatal clinic: A South African experience. PLOS ONE 12(7): e0181267.
- Chowdhury, A., Santra, A., Chaudhuri, S., (2003). Hepatitis C virus infection in the general population: a community-based study in West Begal. *Hepatology*. 37(4), 802-809.
- Cloete A, Simbayi L, Rehle T, Jooste S, Mabaso M, Townsend L, et al. The South African Marang Men's Project: HIV bio-behavioural surveys using respondent-driven sampling conducted among men who have sex with men. Cape Town: *Human Science Reseach Coiuncil Press*. 2014.
- Cloherty G. (2016). Abbott Diagnostics. Personal Communications.

- Coats, J.T. and Dillion, J.F. (2015). The effect of introducing point-of-care or dried blood spot analysis on the uptake of hepatitis C virus testing in high-risk population: A Systematic review of the literature. *International Journal of Drug Policy*. 26 (11), 1050-1055.
- COBAS® AmpliPrep / COBAS® TaqMan® system.2016. https://usdiagnostics.roche.com/en/instrument/COBAS-ampliprep-taqman-system.html. Accessed 10.06.2016.
- Colin, C., Lanoir, D., Touzet, S., et al. (2001). Sensitivity and specificity of third-generation hepatitis C virus antibody detection assays: an analysis of the literature. *Journal of Viral Hepatology*. 8,87-95.
- Crofts, N., Dore G., Locarnini, S. (2001) Hepatitis C: an Australian perspective. Melbourne.
- Cutler, E. (2013). Validation of test methods and equipment used in the molecular lab. SOP. NHLS.
- Dahoma, M, Johnson, L.G., Holman, A., et al. (2011). HIV and related risk behavior among men who have sex with men in Zanzibar, Tanzania: results of a behavior surveillance survey. *AIDS and Behavior*. 15,186-192.
- Danta, M., Brown D, Bhagani S, et al. (2007). Recent epidemic of acute hepatitis C virus in HIV-positive men who have sex with men linked to high-risk sexual behaviors. *Journal of Acquired Immune Deficiency Syndrome*. 21, 983-991.
- Daw, M.A. Dau, A.A., Agnan, M.M. (2012). Influence of healthcare-associated factors on the efficacy of hepatitis C therapy. *Scientific World Journal*. 580216.
- De Crignis, E., Re, M.C., Cimatti, L., et al. (2010). HIV-1 and HCV detection in dried blood spots by SYBR Green multiplex real-time RT-PCR. *The Journal of Virological Methods*.165, 51-56.
- Dean, A.G., Arner, T.G., Sunki ,G.G., et al. (2011). Epi Info™, a database and statistics program for public health professionals.CDC, Atlanta,GA,USA.
- Dean, A.G., Sullivan, K.M., Soe, M.M.(2016). OpenEpi: Open Source Epidemiology Statistics for Public Health, Verion.www. OpenEpi.com. Updated 2013/04/06. Accessed 2016/04/15.

- Deeks, E.D. (2015). COBAS® AmpliPrep/COBAS® Taqman® HCV Quantification Test, version 2.0: an invitro test for hepatitis C virus RNA qauntification.19 (1),1-7.
- Degenhardt, L, Peacock, A., Colledge, S., et al. (2017). Global prevalence of injecting drug use and sociodemographic characteristics and prevalence of HIV, HBV, and HCV in people who inject drugs: a multistage systematic review. The Lancet Global Health. 5, (12).
- Delwaide, J., Gerard,C ., Reenaers, C., et al. (2005). Hepatitis C virus genotype 5 in Southern Belgium: epidemiological characteristics and response to therapy. Digestive Diseases and Science. 50, 2348-2351.
- Delwaide, J., Reenaers, C., Gerard, C., et al. (2006). HCV genotype 4 in Belgium: three distinct patterns among patients from European and African origin. European Journal of Gastroenterology and Hepatology. 18, 707-712.
- Di Bisceglie, A.M. and Hoofnagle, J.H.(2002). Optimal therapy of hepatitis C. Hepatology. 36(5),121-127.
- Dokubo, E.K, Evans, J., Winkelman, V., et al. (2014). Comparison of Hepatitis C Virus RNA and antibody detection in dried blood spots and plasma specimens. Journal of Clinical Virology. 59(4), 1-5.
- Drain, P.K., Hyle, E.P., Noubary, F., et al. (2014). Diagnostic point-of-care tests in resource-limited settings. Lancet Infectious Diseases. 14, 239-249.
- Dunning, L., Kroon, M., Hsiao, N-y., et al. (2017). Field evaluation of HIV point-of-care testing for early infant diagnosis in Cape Town, South Africa. PLoS ONE.12(12),e0189226.
- Dusheiko, G., Schmilovitz-Weiss, H., Brown, D., et al. (1994). Hepatitis C genotypes: an investigation of type-specific differences in geographic origin and disease. *Hepatology.* 19, 13-18.
- EASL.(2015). European Association for Study of Liver. EASL Recommendations on Treatment of Hepatitis C 2015. *Journal of Hepatology*. 63(1),199-236.

- Easterbrook, P.J. (2016). WHO Guidelines Development Group. Who to test and how to test for chronic hepatitis C infection-2016 who testing guidelines for low-and middle-income countries. *Journal of Hepatology*. 12,193-194.
- El Katsha, S., Labeeb, S., Watts, S., et al. (2006). Informal health providers and the transmission of hepatitis C virus: pilot study in two Egyptian villages. *Eastern Mediterranean Health Journal*. 12: 758-767.
- Ellethy, A.T., Sliem, H.A., Hassan, G.M.A., (2012). Update Molecular Diagnosis of Chronic Hepatitis C. *Journal of Gastroenterology and Hepatology Research*.1(8), 147-152.
- El-Zanaty, F. and Way, A. (2009). Egypt Demographic and Health Survey 2008. Cairo, Egypt: Ministry of Health, El-Zanaty and Associates, and Macro International.
- EMI Guidelines-Appendix 23. Hepatitis C virus. (2014). Epidemiology and transmission risks. 71-74. http//; www.hpsc.ie \ A-Z \ EMI Toolkit \ appendics/app23. Accessed 20.05.2016.
- EMOH. European Harm Reduction Network. (2007). www.eurohrn.eu . Accessed 7.06.2016.
- Farci, P., Alter, H.J., Wong, D., et al. (1996). A long-term study of Hepatitis C virus replication in non-A, non-B Hepatitis C virus-associated fulminant hepatic failure. *New England Journal of Medicine*. 335(9), 631-634.
- FDA. U.S. Food & Drug Administration. FDA approves Epclusa for treatment of chronic Hepatitis C virus infection. Available from: http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm508915.htm. Accessed 12.11. 2017.
- Feinstone, S.M., Kapikian, A.Z., Purcell, R.H., et al. (1975). Transfusion-associated hepatitis not due to viral hepatitistype A or B. *New England Journal of Medicine*. 292, 767-70.
- Fisher, D.G., Hess, K.L., Erlyana, E., et al. (2015). Comparison of Rapid Point-of-Care for Detection of Antibodies to Hepatitis C Virus. *Open Forum Infectious Diseases*.2(3), 1-6.

- Frank, C., Mohamed, M.K., Strickland, G., et al. (2000). The role of parenteral Vantischistosomal therapy in the spread of hepatitis C virus in Egypt. *Lancet*. 355, 887-891.
- Gao, F., Talbot, E.A., Loring, C.H., et al. (2014). Performance of the OraQuick HCV Rapid Antibody Test for Screening Exposed Patients in a Hepatitis C Outbreak Investigation. *Journal of Clinical Microbiology*. 52, 2650-2652.
- Gededzha, M.P., Mphahlele, M.J., Lukhwareni, L., et al. (2010). Should routine serology screening for HCV be mandatory in HIV/AIDS patients enr olling for HAART in South africa? *South African Medical Journal*.100(12),814-815.
- Gededzha, M.P., Selabe, S.G., Kyaw, T., et al. (2012).Introduction of new subtypes and variants of hepatitis C virus genotype 4 in South Africa. *Journal of Medical Virology*. 84, 601–607.
- Geraghty, J. (2011). Drug policy, intravenous drug use, and heroin addiction in the UK. *British Journal of Nursing*. 20, 884.
- Ghany, M.G., Strader, D.B., Thomas, D.L., et al. (2009). Diagnosis, management, and treatment of hepatiis C: an update. *Hepatology*. 49,1335-1374.
- Giraudon, I., Ruf, M., Maguire, H., et al. (2008). Increase in diagnosed newly acquired hepatitis C in HIV-positive men who have sex with men across London and Brighton, 2002–2006: is this an outbreak? *Sexually Transmitted Infectious*. 84,111-115.
- Gogela, N., Sonderup, M., Rebe, K., et al. (2013). The sero-prevalence of hepatitis C in an HIV infected population of heterosexual and men who have sex with men (MSM). *South African Medical Journal.*103(8),568-575.
- Govender, K., Parboosing, R., Siyaca, N., et al. (2016). Dried blood spot specimen quality and validation of a new pre-analytical processing method for qualitative HIV-1 PCR, KwaZulu-Natal, South Africa. *African Journal of Laboratory Medicine.* 5(1), 1-6.
- Gower, E., Estes, C., Blach, S., et al. (2014). Global epidemiology and distribution of the hepatitis C virus infection. *Journal of Hepatology*. 61, 45-57.

- Graham, C.S. and Swan, T.A. (2015). A path to eradication of hepatitis C in low and middle income countries. *Antiviral Research*.119, 89-96.
- Grebely, J., Applegate, T.L., Cunningham, P., et al. (2017). Hepatitis C point-of-care iagnostics in search of a single visit diagnosis. *Journal of Expert Review of Molecular Diagnostics*. 17(12), 1109-1115.
- Greenman, J., Roberts, T., Cohn, J., et al. (2015). Dried Blood Spot in the genotyping, quantification and storage of HCV RNA; A systematic literature review. *Journal of Viral Hepatitis*.22, 353-361.
- Gupta, E., Agarwala, P., Kumar, G., et al., (2017). Point-of-care testing (POCT) in molecular diagnostics: performance evaluation of GeneXpert HCV RNA test in diagnosing and monitoring of HCV infection. *Journal of Clinical Virology*. 88, 46-51.
- Gupta, E., Bajpai, M., Choudhary, A. (2014). Hepatitis C virus: Screening, diagnosis, and interpretation of laboratory assays. *Asian Journal of Transfusion Science*. 8(1), 19-25.
- Hadziyannis, S.J., Sette, H.Jr., Morgan, T.R., et al., (2004). Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Annals of Internal Medicine*. 140, 364-355.
- Hahn, J.A. (2007). Sex, Drugs, and Hepatitis C Virus. *The Journal of Infectious Diseases*. 195,1556-1559.
- Hajarizadeh, B., Grebely, J., Dore, G.J., (2013). Epidemiology and natural history of HCV infection. *Nature Reviews Gastroenterology and Hepatology*.10, 553-562.
- Harton, R. and Das, P. (2010). Rescuing people with HIV who use drugs. *Lancet.* 376, 207-208.
- Hauri, A.M., Armstrong, G.L., Hutin, Y.J.F. (2004). The global burden of disease attributed to contaminated injections given in healthcare settings. *International Journal of STD&AIDS*. 15 (1), 7-16.

- Hernandez, M.D and Shermnan, K.E. (2011). HIV/ hepatitis C co-infection natural history and diseases progression. Current Opinion of HIVAIDS. 6(6), 478-482.
- Hoofnagle, J.H. and Alter, H.J. (1985). Chronic non-A, non-B hepatitis. *Progress in Clinical and Biological Research*.182, 63–9.
- Hughes, H.Y. and Henderson, D.K. (2016). Postexposure prophylaxis after hepatitis C occupational exposure in the interferon-free era. *Current Opinion of Infectious Diseases*. 29(4), 373-380.
- Idrees, M. and Riazuddin, S. (2008). Frequency distribution of hepatitis C virus genotypes in different geographical regions of Pakistan and their possible routes of transmission. *BMC Infectious Diseases*. 8,69.
- Inhorn, M.C. (1998). Urban Egyptian women in the informal health care sector. In: Lobban RA, ed. Middle Eastern women and the invisible economy. Gainesville, FL: University Press of Florida.
- International Committee on Taxonomy of Viruses. (2015). Virus Taxonomy: 2015 Release. Accessed 16.05.2016.
- Jacobson, I.M., McHutchison, J.G., Dusheiko, G., et al., (2011). ADVANCE Study Team. Telaprevir for previously untreated chronic hepatitis C virusinfection. *New England Journal of Medicine*. 364(25), 2405-2416.
- Jagger, J., Puro, V, De Carli, G. (2002). Occupational transmission of hepatitis C virus. *Journal of the American Medical Association*. 288,1469.
- Jamalidoust, M., Namayandeh, M., Asaei, S., et al. (2014). Determining hepatitis C virus genotype distribution among high-risk groups in Iran using real-time PCR. *World Journal of Gastroenterology*. 20 (19), 5897-5902.
- Jardi, R., Rodriguez-Friasi, F, Buti, M., et al. (2004). Usefulness of dried blood samples for quantification and molecular characterization of HBV-DNA. *Hepatology.*40(1), 195-208.
- Johannessen, A. (2015). Where We Are With Point Of Care Testing. *Journal of Viral Hepatitis*. 22, 362-365.

- Jover, R., P´erez-Serra, J., de Vera, F., et al. (2001). Infection by genotype 5a of HCV in a district of southeast Spain. *American Journal of Gastroenterology*. 96, 3042-3043.
- Kamili, S., Drobeniuc, J., Araujo, A.C., et al. (2012). Laboratory diagnostics for hepatitis C virus infection. Clinical Infectious Diseases. 55, 43-8.
- Kania, D., Bekalé, A.M., Nagot, N., et al. (2013). Combining rapid diagnostic tests and dried blood spot assays for point-of-care testing of human immune-deficiency virus, hepatitis B and hepatitis C infections in Burkina Faso, West Africa. *Clinical Microbiology and Infection*.19, 533-541.
- Karoney, M.J. and Siika, A.M. (2013). Hepatitis C virus (HCV) infection in Africa: a review. *Pan African Medical Journal*. 14, 44.
- Kaya, C.Y., Tugai, Y., Filar, J.A, et al. (2004). Heroin users in Australia: population trends. *Drug and Alcohol Review*. 23,107–116.
- Keenan, P.A., Keenan, J.M. (2001). Rapid hiv testing in urban outreach: a strategy for improving posttest counselling rates. A*IDS Education and Prevention*.13, 541-550.
- Kendrick, S.R., Kroc, K.A., Withum, D., et al. (2005). Outcomes of offering rapid point-of-care HIV testing in a sexually transmitted disease clinic. *Journal of Acquired Immune Deficiency Syndromes*. 38, 142-146.
- Kermode, M., Crofts, N., Kumar, M.S. (2011). Opioid Substitution therapy in resource –poor settings. *Bulletin of the World Health Organization*. 89, 243-243.
- Khuroo, M.S., Khuroo, N.S., Khuro,o M.S. (2015). Diagnostic Accuracy of Point of Care Tests for Hepatitis C virus Infection: A systematic Review and Meta-Analysis. *PloS One*. 10,1-22.
- Kirkpatrick, F.H. (1991). Overview of agarose gel properties. Electrophoresis of large DNA molecules: theory and applications 9-22. Kouyos, R.D., Rauch, A., Braun. D.L., et al. (2014). Higher risk of incident hepatitis C virus coinfection among men who have sex with men, in whom the HIV genetic bottleneck at transmission was wide. *Journal of Infectious Diseases*. 210, 1555–1561.

- Kufa, T., Lane, T., Manyuchi, A., et al. (2017). The accuracy of HIV rapid testing in integrated bio-behavioral surveys of men who have sex with men across 5 Provinces in South Africa. *Medicine.* 96 (28), 1-7.
- Kuo, G., Choo, Q. L., Alter, H. J., et al. (1989). An assay for circulating antibodies to a major etiologic virus of human non-A, non-B hepatitis. *Science*. 244,362-364.
- Kwiatkowski, C.F., Fortuin-Corsey, K., Brooth, R.E. (2002). The association between knowledge of hepatitis c virus status and risk behaviours in injecting drug users. *Addiction.* 97,1289-1294.
- Larsen, C., Chaix, M.L., Le Strat ,Y., et al. (2011). Gaining greater insight into HCV emergence in HIV-infected men who have sex with men: the HEPAIG Study. *PLoS One*. 6 e29322..
- Lavanchy, D. (2011). Evolving epidemiology of hepatitis C virus. *Clinical Microbiology and Infection*. 17, 107-115.
- Layden, J.E., Phillips, R., Opare-Sem, O., et al. (2014). Hepatitis C in Sub-Saharan Africa: Urgent Need for Attention. *Open forum of Infectious Diseases*. 1(2),1-3.
- Leary, W.E. (1990). The New York Times. U.S approves Test to screen Blood for Hepatitis C. http//: www.nytimes.com. Updated 03.05.1990. Accessed 4.10.2017.
- Lee, C.E., Sri Ponnampalavanor, S., Syed Omar, S.F., et al. (2011). Evaluation of Dried Blood Spot (DBS) Collection Method as a Tool for Detection of HIV Ag/Ab, HBsAg, anti-HBs and anti-HCV in a Malaysian Tertiary Referral Hospital. *Annals Academy of Medicine*. 40 (10), 448-453.
- Lee, P.Y., Costumbrado, J., Hsu, C.Y. (2012). Agarose gel electrophoresis for the Separation of DNA Fragments. *Journal of Visual Experiments*. 62, 3923.
- Lee, S.R., Kardos, K.W., Schiff, E, et al. (2011). Evaluation of a new, rapid test for detecting HCV infection, suitable for use with blood or oral fluid. *Journal of Virological Methods*.172, 27–31.

- Lee, S.R., Yearwood, G.D., Guillon, G.B., et al. (2010). Evaluation of rapid, point-of-care test device for the diagnosis of hepatitis C infection. *Journal of Clinical Virology.* 48,15-17.
- Liou, T.C., Cang, T.T., Young, K.C. (1992). Detection of HCV RNA in Saliva, Urine, Seminal fluid, and ascites. *Journal of Medical Virology*. 37(3). 197-202.
- Lubelchek, R., Kroc, K., Sharief, R., et al. (2005). The role of rapid vs conventional human immunodeficiency virus testing for inpatients: effects on quality of care. *Archives of Internal Medicine*. 165, 1956-1960.
- Madhava, V., Burgees, C., Drucker, E. (2002). Epidemiology of chronic hepatitis C virus infection in sub-Saharan Africa. *Lancet Infectious Diseases*. 2, 293-302.
- Marcellin, P., Martinot-Peignoux, M., Elias, A., et al., (1994). Hepatitis C Virus (HCV) viremia in human immunodeficiency virus-seronegative and –seropositive patients with indeterminate HCV recombinant immunoblot assay. *Journal of Infectious Diseases*. 170(2), 433-435.
- Marconi, A., Balestrieri, M., Comastri, G., et al. (2009). Evaluation of the Abbott Real-Time HIV-1 quantitative assay with dried blood spot specimens. *Clinical Microbiology and Infection*. 15, 93-97.
- Marins E. (2017). Dept. of Virology. Personal Communications.
- Marins, E.G., Bodinaidu, K., Lin, M., et al. (2017). Evaluation of the COBAS® Ampliprep/COBAS® TaqMan® HCV Test v2.0 for HCV viral load monitoring using dried blood spot specimens. *Journal of Virological Methods*. 247, 77-80.
- Martin , N.K., Thornton, A., Hickman, M., et al. (2016). Can Hepatitis C Virus (HCV) Direct-Acting Antiviral Treatment as Prevention Reverse the HCV Epidemic Among Men Who Have Sex With Men in the United Kingdom? Epidemiological and Modeling Insights. *Clinical Infectious Diseases*. 62, 1072-1080.
- Martin , T.C, Martin, N.K, Hickman, M., et al. (2013). Hepatitis C virus reinfection incidence and treatment outcome among HIV positive MSM. *Journal of Acquired Immune Deficiency Syndrome*. 27, 2551-2557.
- Martinot-Peignoux M, Stern C, Maylin S, et al.(2010). Twelve weeks posttreatment follow-up is as relevant as 24 weeks to determine the sustained

virological response in patients with hepatitis C virus receiving pegylated interferon and ribavirin. *Hepatology*. 51(4),1122-1126.

- Marwaha, N. and Sachdev, S. (2014). Current testing strategies for hepatitis C virus infection in blood donors and the way farward. *World Journal of Gastroenterology*. 20, 2948-2954.
- Mathers, B.M., Degenhardt, L., Ali, H., et al. (2010). HIV prevention, treatment, and care services for people who inject drugs: a systematic review of global, regional, and national coverage. *Lancet*. 375, 1014-1028.
- McCaughan, G.W., McGuinness, P.H., Bishop, G.A., et al. (1992). Clinical assessment and incidence of hepatitis C RNA in 50 consecutive RIBA-positive volunteer blood donors. *Medical journal of Australia*. 157(4), 231-233.
- McDade, T.W., Williams, S., Snodgrass, J.J. (2007). What a drop can do: dried blood spots as a minimally invasive method for integrating biomarkers into population-based research. *Demography*. 44, 899-925.
- McHutchison JG, Gordon SC, Schiff ER, et al. (1998). Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *New England Journal of Medicine.*339(21),1485-1492.
- MedCalc Software, Ostend,Belgium. www.medcalc.org. (2016). Accessed 2017.10.22.
- Mehta, S.H., Astemborski, J., Kirk, G.D., et al. (2011). Changes in blood-borne infection risk among injection drug users. *Journal of Infectious Diseases*. 203, 587-594.
- Mesquita, P.E., Granato, C.F., Castelo, A. (1997). Risk factors associated with hepatitis C virus (HCV) infection among prostitutes and their clients in the city of santos, San Poulo State, Brazil. *The Journal of Medical Virology*. 51, 338-343.
- Messina, J.P., Humphreys, I., Flaxman, A., et al. (2015). Global distribution and prevalence of hepatitis C virus genotypes. *Hepatology*. 61, 77-87.
- Metcalf, C.A., Douglous, J.M., Malotte, C.K., et al. (2005). Relative efficiency of prevention counseling with rapid and standard HIV testing: a randomized, controlled trial (RESPECT-2). *Sexual Transmission Diseases*. 32, 130-138.

- Midgard, H., Weir, A., Palmateer, N., et al. (2016). HCV epidemiology in high-risk groups and the risk of reinfection. *Journal of Hepatology*. 65, 33-65.
- Mohammed, S., Raimondo, A., Penaranda, G., et al. (2013). Dried blood spot sampling for hepatitis B virus serology and molecular testing.PLoS ONE. 8(4),e61077.
- Mohd Hanafiah, K., Groeger J., Flaximan M.D., et al. (2013). Global Epidemiology of hepatitis C virus infection; new estimates of age specific antibody to HCV seroprevalence. *Hepatology*. 57,1333-1342.
- Morano, J.P., Zelenev, A., Lombard, A., et al. (2014). Strategies for hepatitis C testing and linkage to care for vulnerable populations: point-of-care and standard HCV testing in a mobile medical clinic. *Journal of Community Health*. 39, 922-934.
- Mössner, B.K., Staugaard, B., Jensen, J., et al. (2016). Dried blood spots, valid screening for viral hepatitis and human immunodeficiency virus in real-life. *World Journal of Gastroenterology*. 22, 7604-7012.
- Mossner, B.K., Staugaard, B., Jensen,J., et al. (2016). Dried bloos spots, valid screening for viral hepatitis and human immunodeficiency virus in real-life. *World Journal of Gastroenterology*. 22(33), 7604-7612.
- Mudawi, H.M., Smith, H.M., Fletcher, I.A., et al. (2007). Prevalence and common genotypes of HCV infection in Sudanese patients with hepatosplenic schistosomiasis. *Journal of Medical Virology*. 79, 1322-1324.
- Mullis, C.E., Laeyendecker O., Reynolds S.J., et al. (2013). High frequency of false-positive hepatitis C virus enzyme-linked immunosorbent assay in Rakai, Uganda. *Clinical Infection of Diseases*. 57, 1747-1750.
- Multiplo Rapid HBc/HIV/HCV Antibody Test (MedMira Laboratories, Inc). (medmira.com/wp-content/uploads/.../Multiplo-HBc-HIV-HCV-Product-Sheet_EN.pdf). Accessed 18.06.2018.
- Multiplo Rapid HIV/HCV Antibody Test (MedMira Laboratories, Inc). http://www.medmira.com/multiplex/. Accessed 18.06.2018.

- Murphy, D.G., Sablan, E., Chamberland, J., et al. (2015). Hepatitis C virus Genotype, a New Genotype 7 Originating from Central Africa. *Journal of Clinical Microbiology*.53, 967-972.
- Muzembo, B.A., Mbendi, N.C., Nakayama, S.F. (2017). Systematic review with meta-analysis: performance of dried blood spots for hepatitis C antibodies detection. *Public Health*. 152,128-136.
- Ndimbie, O.K., Kingsley, L.A., Nedjar, S., et al. (1996). Hepatitis C virus infection in a male homosexual cohort: risk factor analysis. *Genitourinary Medicine*.72 (3), 213-216.
- Ndong-Atome, G., Makuwa, M., Ouwe-Missi-Oukem-Boyer, O., et al. (2008). High prevalence of hepatitis C virus infection and predominance of genotype 4 in rural Gabon. *Journal of Medical Virology*. 80, 1581-1587.
- Nelson, P.K., Mathers, B.M., Cowie, B., et al. (2011). Global epidemiology of hepatitis B and hepatitis C in people who inject drugs: results of systematic reviews. *Lancet*. 378, 571-583.
- Nguyen, M.H. and Keeffe, E.B. (2005). Prevalence and treatment of hepatitis C virus genotypes 4, 5, and 6. *Clinical Gastroenterology and Hepatology*. 3(2), 97-101.
- NIH. (1997). National Institutes of Health Consensus Development Conference Panel statement: management of hepatitis C. *Hepatology*. 26(3), 2-10.
- Njouom, R., Frost, E., Deslandes, S., et al. (2009). Predominance of hepatitis C genotype 4 infection and rapid transmission between 1935 and 1965 in the Central African Republic. *Journal of General Virology*. 90, 2452–2456.
- Njouom, R., Nerrienet, E., Dubois, M., et al. (2007). The hepatitis C virus epidemic in Cameroon: genetic evidence for rapid transmission between 1920 and 1960. *Infection Genetics and Evolution*. 7, 361-367.
- Noppornpanth, S., Sablon, E., De Nys, K., et al. (2006). Genotyping hepatitis C viruses from Southeast Asia by a novel line probe assay that simultaneously detects core and 5’ untranslated regions. *Journal of clinical Microbiology*. 44, 3969-3974.

- O' Connell, R.J., Gates, R.G., Bautista, C.T., et al. (2013). Laboratory evaluation of rapid test kits to detect hepatitis C antibody for use in pre donation screening in emergency setting. *Transfusion*. 53, 505-517.
- Ohno , T., Mizokami, M., Tibbs, C.J, et al. (1994). New genotype of hepatitis C virus in South Africa. *Journal of Medical Virology*. 42, 409-13.
- Ohno, T., Mizokami, M., Rong-Rong, W.U., et al. (1997). New Hepatitis C Virus (HCV) Genotyping System That Allows for Identification of HCV Genotypes 1a, 1b, 2a, 2b, 3a, 3b, 4, 5a, and 6a. *Journal of Clinical and Microbiology*. 35, 201-207.
- OraQuick® Rapid Antibody Test. (2013). Updated 2013/01/21. http://www,orasure.com/products-infectious/products. Accessed 2017/04/13.
- Osmond, D.H., Charlebois, E., Sheppard, H.W., et al. (1993). Comparison of risk factors for hepatitis C and hepatitis B virus infect ion in homosexual men. *Journal of Infectious Diseases*. 167(1), 66-71.
- Ostler, M.W., Porter, J.H. (2014). Dried blood spot collection of health biomarkers to maximize participation in population studies. *Journal of Visual Experiments*. 28, e50973.
- Page, E.E, Nelson, M. (2016). Hepatitis C and sex. *Clinical Medicine (London)* .16, 189-192.
- Palmateer , N.E., Hutchinson, S.J., Innes, H., et al. (2013). Review and meta-analysis of the association between self-reported sharing of needles/syringes and hepatitis C virus prevalence and incidence among people who inject drugs in Europe. *International Journal of Drug Policy*. 24, 85-100.
- Palmateer , N.E., Taylor, A., Goldberg, D.J., et al. (2014). Rapid decline in HCV incidence among people who inject drugs associated with national scale-up in coverage of a combination of harm reduction interventions. *PLoS One*.9 e104515.
- Panlilio, A.L., Orelien, J.G., Srivastava, P.U., et al. (2004). Estimation of the annual number of percutaneous injuries among hospital-based healthcare workers in the United States, 1997-1998. *Infection Control and Hospital Epidemiology*.25(7), 556-5624.

- Parboosing, R., Paruk, I., Lalloo, U.G. (2008). Hepatitis Cvirus seropositivity in a South African cohort of HIV co-infected, ARV naïve patients is associated with renal insufficiency and increased mortality. *Journal of Medical Virology.*80(9),1530-1536.
- Pasquier, C., Bujan, L., Daudin, M., et al. (2003). Intermittent detection of hepatitis C virus (HCV) in semen from men with human immunodeficiency virus type 1 (HIV-1) and HCV. *Journal of Medical Virology*. 69, 344-349.
- Pawlotsky J.M. (2002). Molecular diagnosis of viral hepatitis. *Gasteroenterology*.122, 1554-1568.
- Pawlotsky, J.M., Tsakiris, L., Roudot-Thoraval, F., et al. (1995). Relationship between hepatitis C virus genotypes and sources of infection in patients with chronic hepatitis C. *Journal of Infectious Diseases*.171, 1607-1610.
- Pépin, J. and Labbe´, AC. (2008). Noble goals, unforeseen consequences: the control of tropical diseases in colonial Central Africa and the iatrogenic transmission of blood-borne viruses. *Tropical Medicine and International Health* 13, 744-753.
- Peters, M.G. and Terrault, N.A. (2002). Alcohol use and hepatitis C. *Hepatology*. 36(1), 2220-2225.
- Poordad, F., McCone, J Jr., Bacon, B.R., et al., (2011). SPRINT-2 Investigators. Boceprevir for untreated chronic HCV genotype 1 infection. *New England Journal of Medicine*. 364(13), 1195-1206.
- Pouget, E.R., Hagan, H., Des Jarlais, D.C. (2012). Meta-analysis of hepatitis C seroconversion in relation to shared syringes and drug preparation equipment. *Addiction*.107, 1057-1065.
- Poynard T, McHutchison J, Manns M, et al.(2002). Impact of pegylated interferon alfa-2b and ribavirin on liver fibrosis in patients with chronic hepatitis C. *Gastroenterology*. 122(5), 1303-1313.
- Pozzato, G.S., Kaneko, M., Moretti, L., et al. (1994). Different genotypes of hepatitis C virus are associated with different severity of chronic liver disease.*Journal of Medical Virology.* 43,291-296.

- Prabdial-Sing, N., Chirwa, T., Thaver, J., et al. (2016). Hepatitis C genotype distribution in patient and blood donar sample in South Africa for the period 2008-2012. *Journal of viral hepatitis.* 23(11),881-888.
- Prabdial-Sing, N., Chirwa, T., Thaver, J., et al. (2016). Hepatitis C genotype distribution in patient and blood donor samples in South Africa for the period 2008-2012. *Journal of Viral Hepatitis*. 23(11), 881-888.
- Prabdial-sing, N., Chirwa,T., Thaver J., et al. (2016). Hepatitis C Genotype distribution in patient and blood donar sample in South Africa for the period 2008-2012. *Journal of Viral Hepatitis*. 23(11). 881-888.
- Prescott, L.E., Simmonds P., Lai C.L., et al., (1996). Detection and clinical features of hepatitis C virus type 6 infections in blood donors from Hong Kong. *Journal of Medical Virology*. 50, 168-175.
- Puro, V., Petrosillo, N., Ippolito, G., et al. (1995). Occupational hepatitis C virus infection in Italian health care workers. Italian Study group on Occupational Risk of Blood borne Infections. *American Journal of Public Health*. 85(9),1272-1275.
- Pybus, O.G., Drummond, A.J., Nakano, T., et al. (2003). The epidemiology and iatrogenic transmission of hepatitis C virus in Egypt: a Bayesian coalescent approach. *Molecular Biology Evolution*. 20(3), 381-387.
- QIAamp Viral RNA Mini Kit, Qiagen, Hilden, Germany. https://www.qiagen.com/za/. Accessed 11.06.2017.
- Qian, W-P., Tan, Y-Q., Chen, Y., et al. (2005). Rapid Quantification of semen hepatitis B virus DNA by real-time polymerase chain reaction. *World Journal of Gastroenterology*. 11(34), 5385-5389.
- Rao, V.B., Johari, N., du Cros, P., et al. (2015).Hepatitis C seroprevalence and HIV co-infection in sub-saharan Africa: A systematic review and meta-analysis. *Lancet Infectious Diseases.*15(7),819-824.
- Rauch, A., Rickenbach, M., Weber, R., et al. (2005). Unsafe sex and increased incidence of hepatitis C virus infection among HIV infected men who have sex with men: the Swiss HIV Cohort Study. *Clinical Infectious Diseases*. 41, 395-402.

- Rauchana, M.K. and Shahul, S. (1998). Role of in transmission of hepatitis C virus to infants of HCV-infected mothers. *Journal of Hepatology*. 29(2), 191-197.
- Raymond, H.F., Chu, P., Nieves-Rivera, I., et al. (2012). Hepatitis C infection among men who have sex with men, San Francisco, 2011. *Sexual Transmitted Diseases*. 39, 985-986.
- Ricchi, E., Borderi, M., Costigliola, P., et al. (1992). Antihepatitis C virus antibodies amongst Italian homo-bisexual males. *European Journal of Epidemiology*. 8, 804–807.
- Ridzon, R., Gallagher, K., Ciesieiski, C., et al. (1997). Simultaneous transmission of human immunodeficiency virus and hepatitis C virus from a needle stick injury. *New England Journal of Medicine*. 336, 919-922.
- Roberts, E.A. and Yeung, L. (2002). Maternal- infant transmission of hepatiis C virus infection. Hepatology. 36,106-113.
- Ross, S. R., Stombouli, O., Gruner, N., et al. (2013). Detection of infections with hepatitis B virus, hepatitis C virus, and human immunodeficiency virus by analysis of dried blood spots- performance characteristics of ARCHITECT system and two commercial assays for nucleic acid amplification. *Virology Journal*. 10, 1-8.
- Saldanha, J., Heath, A., Aberham, C.M., et al. (2005). World Health Organization collaborative study to establish a replacement WHO international standard for hepatitis C virus RNA nucleic acid amplification technology assays. *Vox Sanguinis*. 88, 202-204.
- Scheiblauer, H., El-Nageh, M., Nick, S., et al. (2006). Evaluation of the performance of 44 assays used in countries with limited resources for the detection of antibodies to hepatitis C virus. *Transfusion*. 46, 708-718.
- Schmidt, A.J., Rockstroh, J.K., Vogel, M., et al. (2011). Trouble with bleeding: risk factors for acute hepatitis C among HIV-positive gay men from Germany–a case-control study. *PLoS One*.6 e17781.
- Scott, N., Doyle, J.S., Wilson, D.P. (2017). Reaching hepatitis C virus elimination targets requires health system interventions to enhance the care cascade. *The International Journal of Drug Policy*. 47,107-116.

- SD BIOLINE HCV (Standard Diagnostic, Inc) (2016). (Updated on 20 December 2016). (www.who.int/.../diagnostics.../hcv/161216_final_amended_report_0257_012_00.pdf). {Last accessed on 18.06.2018}.
- SD Bioline syphilis 3.0 (Standard Diagnostics, South Korea). (https://www.alere.com/en/home/product-details/sd-bioline-hiv-syphilis-duo.html). {Date accessed 18.06.2018}.
- Seaberg, E.C., Witt ,M.D., Jacobson, L.P., et al. (2014). Differences in hepatitis C virus prevalence and clearance by mode of acquisition among men who have sex with men. *Journal of Viral Hepatology*. 21,696-705.
- Sediba Hope Medical Centre. Viral hepatitis sceening. Pretoria: Sediba Hope Medical Centre; 2013.
- Seeff LB. (2009).The history of the “natural history” of hepatitis C(1968–2009). Liver international : *Journal of the International Association for the Study of the Liver.* 29(1), 89-99.
- Semugoma, N.P., K Rebe,K., M W Sonderup, M.W., et al. (2017). Hepatitis C: A South African literature review and results from a burden of disease study among a cohort of drug-using men who have sex with men in Cape Town, South Africa. *South African Medical Journal*. 107(12),1116-1120.
- Seremba, E., Ocama, P., Opio, C.K., et al. (2010). Poor performance of hepatitis C antibody tests in hospital patients in Uganda. *Journal of Medical Virology*. 82, 1371-1378.
- Shepard, R.N., Schock, J., Robertson, K., et al. (2002). Quantitation of Human Immunodeficiency Virus Type 1 RNA in Different Biological Compartments. *Journal of Clinical Microbiology*. 38(4), 1414-1418.
- Sherman, G.G., Cooper, P.A., Coovadia, A.H., et al. (2005). Polymerase chain reaction for diagnosis of human immunodeficiency virus infection in infancy in low resource settings. *Paediatric Infectious Diseases*. 24(11), 993-997.
- Sherman, K.E., Shire, N.J., Rouster, S.D., et al. (2005). Viral kinetics in hepatitis C or hepatitis C/human immunodeficiency virus infected patients. *Gastroenterology*.128, 313-327.

- Shivkumar, S., Peeling, R., Jafari, Y., et al. (2012). Accuracy of rapid and point-of-care screening tests for hepatitis C: a systematic review and meta-analysis. *Annals of International Medicine*.157, 558-566.
- Simel, D.L., Rennie, D., Bossuyt, P.M.M. (2008). The STARD Statement for Reporting Diagnostic accuracy studies: Application to the history and Physical Examination. *Journal of General Internal Medicine*. 23(6), 768-774.
- Simmonds, P. (2001). The origin and evolution of hepatitis viruses in humans. *Journal of General Virology* .82,693-712.
- Simmonds, P. (2004). Genetic diversity and evolution of hepatitis C virus- 15 years on. *Journal of General Virology*. 85, 3173-3188.
- Simmonds, P., Buck, J., Combet, C., et al. (2005). Consensus proposals for a unified system of nomenclature of hepatitis C virus genotypes. *Hepatology*. 42, 962–973.
- Simmonds, P., Holmes, E. C., Cha, T.A., et al. (1993). Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *Journal of General Virology*. 74; 2391-2399.
- Šimundić, A.-M. (2009). Measures of Diagnostic Accuracy: Basic Definitions. *EJIFCC*, 19(4), 203-211.
- Smit, P.W., Sollis, K.A., Fiscus, S., et al. (2014) Systematic Review of the Use of Dried Blood Spots for Monitoring HIV Viral Load and for Early Infant Diagnosis. PLOS ONE 9(3): e86461
- Smith, B. D., Combellick, J., Jordan, A.E., et al., (2016).Hepatitis C Virus (HCV) disease progression in people who inject drugs (PWID): A systematic review and meta-analysis.*International Journal of Drug Policy*. 26(10), 911-921.
- Smith, B.D., Bukh, J., Kuiken, C., et al. (2014). Expanded Classification of Hepatitis C Virus Into 7 Genotypes and 67 Subtypes: Updated Criteria and Genotype Assignment Web Resource. *Hepatology*.59,318-327.
- Smith, B.D., Drobeniuc, J., Jewtt, A., et al. (2011). Evaluation of Three Rapid Screening Assays for Detection of Antibodies to Hepatitis C Virus. *The Journal of Infectious Diseases*. 204, 825-831.

- Smith, B.D., Jewett, A., Drobeniuc, J., et al. (2018). Rapid diagnostic HCV antibody assays. *Antiviral Therapy*. 17 (7), 1409-1413.
- Smith, B.D., Mellor, J., Jarvis, L.M., et al. (1995). Variation of the hepatitis C virus 5' non-coding region: implications for secondary structure, virus detection and typing. The international HCV Collaborative Study Group. *Journal of General Virology*.76, 1749-1761.
- Smuts, H.E. and Kannemeyer, J. (1995). Genotyping of hepatitis C virus in South Africa. *Journal of Clinical Microbiology*. 33,1679–81.
- Sonderup, M.W., Afihene, M., Ally, R., (2017). Hepatitis C in sub-Saharan Africa: the current status and recommendations for achieving elimination by 2030. *Lancet Gastroenterology and Hepatology*. 2(12), 910-919.
- Sonderup,M.W., Afihene, M., Ally, A., et al. (2017).Hepatitis C in sub-Saharan Africa: the current status and recommendations for achieveing elimination by 2030. *Lancet Gastroenterology and Hepatology*. 2,910-919.
- Soulier, A., Poiteau, L., Rosa, I., et al. (2016). Dried Blood Spots: A Tool To Ensure Broad Access to Hepatitis C Screening, Diagnosis, and Treatment Monitory. *Journal of Infectious Diseases*. 213, 1087-1095.
- StataCorp.2013. Stata Statistical Software: Release 13. College Station,TX: StataCorp. LP
- Strahotin, C.S. and Babich, M. (2012). Hepatitis C variability, patterns of resistance, and impact on therapy. *Advanced Virology*. 12,267483.
- Stuyver, L., Rossau, R., Wyseur, A., et al. (1993). Typing of hepatitis C virus isolates and characterization of new subtypes using a line probe assay. Journal of General of Virology. 74,1093–1102.
- Suthar, A.B and Harries, A.D. (2015). A public health approch to hepatitis C control in low-and middle-income countries. PLoS Medicine.12(3).e1001795.
- Swain MG, Lai MY, Shiffman ML, et al.(2010). A sustained virologic response is durable in patients with chronic hepatitis C treated with peginterferon alfa-2a and ribavirin. Gastroenterology. 139(5),1593–1601.
- SWEAT, Impact Consulting. SEX WORKERS IN SOUTH AFRICA : A RAPID POPULATION SIZE ESTIMATION. Cape Town; 2013.

- Sy, T. and Jamal M.M. (2006). Epidemiology of Hepatitis C Virus (HCV) Infection. *International Journal of Medical Sciences*. 3(2), 41-46.
- Tanaka, Y. Kurbanov, F., Mono, S., et al. (2006). Molecular tracing of the global hepatitis C virus epidemic predicts regional patterns of hepatocellular carcinoma martality. *Gastroenterology*. 130,703-714.
- Tang, E. (1991). Hepatitis C Virus. A Review. *West Journal of Medicine.* 155,164-168.
- Tang, N., Pahalawatta, V., Fank, A., et al. (2017). HIV-1 viral load measuremnt in venous blood and fingerprink blood using Abbott RealTime HIV-1 DBS assay. *Journal of clinical Virology.* 92, 56-61.
- Tiathiah, N., Parboosing, R., Singh, L., et al. (2014).Human Immunodeficency virus and hepatitis B or C co-infection in KwaZulu Natal:retrospective analysis of a laboratory database.*South African Journal of Infectious Diseases.*29(1),19-22.
- Terrault, N.A. (2002). Sexual activity as a risk factor for hepatitis C. *Hepatology.* 36, 99-105.
- Terrault, N.A. (2005). Sex and hepatitis C. *The American Journal of Gastroenterology*. 100, 825-826.
- Terrault, N.A., Dodge, J.L., Murphy, E.L., et al. (2013). Sexual transmission of hepatitis C virus among monogamous heterosexual couples: the HCV partners study. *Hepatology*. 57(3), 881-889.
- Tahan, V., Karaca, C., Yildirim, B. (2005). Sexual Transmision of HCV between spouses. *American Journal of Gatroenetrology*. 100,821-824.
- Thrope, L.E., Ouellet, L.J., Hershow,R., et al. (2002). Risk of hepatitis C virus infection among young adult injection drug users who share injection equipment. *American Journal of Epidemiology*. 155(7),645-653.
- Thursz, M. and Lacombe, K. (2016). Breaking Down Barriers to care in Hepatitis C virus Infection. *Journal of Infectious Diseases*. 213,1055-1056.
- Ticehurst, J.R., Homzeh,F.M., Thomas, D.L. (2007). Factors affecting serum concentrations of Hepatitis C Virus (HCV) RNA in HCV Genotype 1-Infected Patients with Chronic Hepatitis. *Journal of Clinical Microbiology*.45(8),2426-2433.

- Tor, J., Llibre, J.M., Carbonell, M., et al. (1990). Sexual transmission of hepatitis C virus and its relation with hepatitis B virus and HIV. *British Medical Journal.* 301, 1130–1133.
- Tuaillon, E., Mandain, A.M., Meroveh, F., et al. (2010).. Dried Blood Spots for Hepatitis C virus serology and Molecular Testing. *Hepatology*. 51, 752-756.
- Tucker, T.J., Voigt, M., Bird, A.,et al. (1997) Hepatitis C virus infection rate in volunteer blood donors from the Western Cape-comparison of screening tests and PCR. *South African Medical Journal*. 8, 603-605.
- Turner, K.M., Hutchinson, S., Vickerman, P. (2011). The impact of the needle and syringe provision and opiate substitution therapy on the incidence of hepatitis C virus in injecting drug users: pooling of UK evidence. *Addiction.* 106 (11), 1978-1988.
- United Nations Office on Drugs and Crime. Rapid assessment of HIV and related risk factors among people who inject drugs from five South African cities. Final report. Pretoria: UNODC; 2015.
- van de Laar, T.J., Matthews, G.V., Prins, M. et al. (2010). Acute hepatitis C in HIV infected men who have sex with men: an emerging sexually transmitted infection. Journal of Acquired Immune Deficiency Syndrome.24,1799–1812.
- Verbeeck, J., Maes, P., Lemey, P., et al. (2006). Investigating the Origin and Spread of Hepatitis C Virus Genotype 5a. *Journal of Virology*. 80 (9), 4220-4226.
- Verbeeck, J., Stanley, M.J., Shieh, J., et al. (2008). Evaluation of Versant Hepatitis C Virus Genotype Assay (LiPA) 2.0. *Journal of Clinical Microbiology*. 46 (6), 1901-1906.
- Vermeersch, P., Van Ranst, M., Lagrou, K. (2008). Validation of strategy for HCV antibody testing with two enzyme immunoassays in a routine clinical laboratory. *Journal of Clinical Virology*. 42, 394-398.
- Versant HCV amplification 2.0 kit, Siemens Diagnostics. https://www.healthcare.siemens.com/. {Date accessed on 15.06.2017}.

- Vinikoor, M.J., Zürcher, S., Musukuma, K., et al. (2015). Hepatitis B viral load in dried blood spots: A validation study in Zambia. *Journal of Clinical Virology*. 72, 20-24.
- Wandeler, G., Gsponer, T., Bregenzer, A., et al. (2012). Hepatitis C virus infections in the Swiss HIV Cohort Study: a rapidly evolving epidemic. *Clinical Infectious Diseases*. 55, 1408-1416.
- WHO (2009). World Health Organization. Hepatitis C. WHO fact sheet 164. Available at http://www.who.int/mediacentre/fact sheets/fs164/en/print.html (updated on 10.2017) {Date accessed on 21 .01. 2016}.
- WHO. (1996). World Health Organization. World Health Report. Geneva, Switzerland. http://www.who.int/whr/1996/en/whr96_en.pdf {Date accessed on 22.10.2017}.
- WHO. (1999). Weekly Epidemiology Record. 49(10). www.who.int/ {Date accessed on 22.10.2017}.
- WHO. (2002). Hepatitis C. www.who.int/csr/disease/heaptitis/Hep C.pdf. {Date accessed on 20.05.2016}.
- WHO. (2004). Evidence for action on HIV/AIDS and injecting drug use. WHO. http;//www. Who.int>WHO_HIV_2004. (Updated 13.07.2004). {Accessed on 3.02.2018}.
- WHO. (2016). Sixty-ninth World Health Assembly closes. http://www.who.int/mediacentre/news/releases/2016/wha69-28-may-2016/en/ {Accessed on 6.6.2016}.
- Wiessing, L., Ferri, M., Grady, B., et al. (2014). Hepatitis C virus infection epidemiology among people who inject drugs in Europe: a systematic review of data for scaling up treatment and prevention. *PLoS One*. 9 e103345.
- Wong, J., Moore, D., Kanters, S., et al. (2015). Seroprevalence of hepatitis C and correlates of seropositivity among men who have sex with men in Vancouver, Canada: a cross-sectional survey. *Sexually Transmitted Infections*. 9, 430-433.
- XL STAT 2017: Data Analysis ans Statistical Solution for Microsoft Excel. Addinsoft, Paris, France (2017).

- Xu, L-Z., Larzul, D., Delaporte, E., et al. (1997). Hepatitis C virus genotype 4 is highly prevalent in central Africa (Gabon). *Journal of General Virology*. 75, 2393-8.
- Yang, R., Cong, Xu., Du, S., et al. (2014). Performance Comparison of the Versant HCV Genotype 2.0 Assay (LiPA) and the Abbott Realtime HCV Genotype II Asay for Detecting Hepatitis C Virus Genotype 6. *Journal of Clinical Microbiology.* 52 (10), 3685-3692.
- Yazdanpanah, Y., De Carli, G., Migueres, B., et al. (2005). Risk factors for hepatitis C virus transmission to health care workers after occupational exposure: a European case-control study. *Clinical Infectious Diseases*. 41, 1423-1430.
- Yilmaz, M., Ozic, C., Gok, I. (2012). Principles of Nucleic Acid Separation by Agorose Gel Electrophoresis. pp33-40.
- Zein, N.N. (2000). Clinical significance of hepatitis C virus genotypes. *Clinical Microbiology Review*. 13,223-35.
- Zeuzem, S., Alberti, A., Rosenberg, W., et al. (2006). Review article: management of patients with chronic hepatitis C virus infection and 'normal' alanine aminotransferase activity. *Alimentary Pharmacology & Therapeutics*. 24, 1133-1149.
- Zeuzem, S., Andreone, P., Pol, S., et al. (2011). REALIZE Study Team. Telaprevir for retreatment of HCV infection. *New England Journal of Medicine*. 364(25), 2417-2428.
- Zhang, J., Nguyen, D., Hu, K.Q. (2016). Chronic hepatitis C virus infection: a review of current direct-acting antiviral treatment strategies. *New American Journal of Medical Science (Boston)*. 9(2),47-54.
- Zhang, Y.Y., Lok, A.S., Chan, D.T., et al. (1995). Greater diversity of hepatitis C virus genotypes found in Hong Kong than in mainland China. *Journal of Clinical Microbiology*. 33, 2931-2943.
- Zhou, K., Fitzpatrick, T., Walsh, N., et al. (2016). Interventions to optimise the care continuum for chronic viral hepatitis: a systematic review and meta-analyses. *Lancet Infectious Diseases*.16, 1409–1422.

6. APPENDICES

APPENDIX A: UNIVERSITY OF WITWATERSRAND HREC COMMITTEE APPROVAL

R14/49 Ms Lucinda Gaelejwe et al

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M170698

NAME: (Principal Investigator)	Ms Lucinda Gaelejwe et al
DEPARTMENT:	Pathology National Institute for Communicable Diseases
PROJECT TITLE:	The Evalaution of Point of Care Testing and Dried Blood Spots to Assess Diaganostic Accuracy of Hepatitis C Viral Infection as Part of Sero-Molecular Surveillance in People Most-at-risk for Hepatitis C Virus
DATE CONSIDERED:	Adhoc
DECISION:	Approved unconditionally
CONDITIONS:	Sub-Study under Primary Study (M160510)
SUPERVISOR:	Dr Nishi Prabdial-Sing
APPROVED BY:	Professor A. Woodiwiss, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	04/07/2017

This clearance certificate is valid for 5 years from date of approval. Extension may be applied for.

DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary in Room 10004,10th floor, Senate House/3rd floor, Phillip Tobias Building, Parktown, University of the Witwatersrand. I/We fully understand the conditions under which I am/we are authorised to carry out the above-mentioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit to the Committee. **I agree to submit a yearly progress report**. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. in this case, the study was initially review June and will therefore be due in the month of June each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature Date

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

APPENDIX B: UNIVERSITY OF CAPE TOWN HREC COMMITTEE APPROVAL

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E52-24 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6338 • **Facsimile** [021] 406 6411
Email: shuretta.thomas@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

18 March 2016

HREC REF: 004/2016

Prof CWN Spearman
Hepatology
K47 OMB

Dear Prof Spearman

PROJECT TITLE: VIRAL HEPATITIS C INITIATIVE FOR MOST AT RISK POPULATIONS IN SOUTH AFRICA

Thank you for your response to the Faculty of Health Sciences Human Research Ethics Committee dated 29 February 2016.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 30th March 2017.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our **website:** www.health.uct.ac.za/fhs/research/humanethics/forms)

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval before the research may occur.

The following documentation is noted and approved:

- Research Protocol version 1.1 dated 3 March 2016 (clean copy and tracked changes)
- Study Synopsis

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE
Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

HREC REF 004/2016

APPENDIX C: INFORMATION SHEET AND CONSENT FORM: STUDY

Study title: Viral hepatitis C initiative for most at risk populations in South Africa[1]

What is this study about?

[1] The Flesch-Kincaid reading ease test was used to develop the information content of this form using Readability-Score.com (https://readability-score.com). Reading ease score is 63 (out of a possible range between 0 and 100). The grade level is 8.1 (scores of 22 or more or higher reflect the level for university graduates).

This is a research project being conducted by TB/HIV Care Association in partnership with OUT LGBT Wellbeing, Anova Health Institute, National Institute for Communicable Diseases and the Division of Hepatology, Department of Medicine, University of Cape Town and Groote Schuur Hospital.

We are finding out more about Hepatitis B and C in South Africa. We are trying to finding out how many people have hepatitis B, hepatitis C and HIV in certain high-risk populations. We would like to be able to better understand what behaviours may increase the risk of hepatitis C. We would like to explore ways of testing for HCV and we want to increase access to HCV prevention, screening and referral for treatment for these most at risk populations. We would then like to use this information to advocate for improved access to HCV related services for these populations.

"Most at risk populations" for the purposes of this study include sex workers (SW), men who have sex with men (MSM), people who use drugs (PWUD) and people who inject drugs (PWID).

What is Hepatitis C?
Hepatitis C, or HCV, is a virus that infects the liver. It is spread through blood by sharing needles and sexual contact, particularly if there are multiple sexual partners or there are other infections such as HIV or other types of sexually transmitted infections. HCV can lead to liver damage and eventually liver failure.

Can HCV be cured?
Yes. Recently medicines have become available that are able to cure most cases of HCV. One of the reasons we are doing this study is to see how we can help people get the medicines and which populations need to be given these medicines. Please note that just because you take part in the study does not mean you will automatically be able to get the medicines if you have Hepatitis C. The study itself does not provide treatment, but we will refer you to services that may be able to offer treatment.

Unfortunately the medications used to treat HCV are not currently available in the public health sector in the Eastern Cape. Participants from this province will be referred to either Livingstone Hospital (Port Elizabeth) or Nelson Mandela Academic Hospital (Mthatha) for assessment. This project will include efforts to make these medications more available.

What is hepatitis B and can it be treated?
Hepatitis B is a different virus that infects the liver. Hepatitis B can be prevented with a vaccine. Three vaccine injections are needed and are given over a few months. The South African government gives the vaccine to all children. Hepatitis B can be treated.

Due to limited money for this study, we will only test to see if people may have an active hepatitis B infection using a rapid ("finger prick") test. We will not do all of the tests that are needed to see if you are immune (cannot get the infection) to hepatitis B. We will refer people with a positive hepatitis B screening test to a local hospital or public treatment centre to be further assessed. We will refer people who have a negative hepatitis B screening test to a local clinic asking for vaccination, however, the vaccination of adults is not presently part of standard service delivery in the public health sector.

APPENDIX D: INFORMED CONSENT FORM: BLOOD STORAGE

Title of research study:
Viral Hepatitis C initiative for most at risk populations in South Africa.

Principle Investigators:

Prof. Harry Hausler
Extraordinary Professor School of Public Health, UWC
CEO, TB/HIV Care Association
8th Floor Absa House 25 St. Georges Mall
Cape Town, 8001
021 425 0050
hhausler@tbhivcare.org

A/Prof. Wendy Spearman
Division of Hepatology
Department of Medicine
University of Cape Town/ Groote Schuur Hospital
K47 Old Main building, Groot Schuur Hospital
Cape Town, South Africa
021 406 6394
wendy.spearman@uct.ac.za

A/Prof. Mark Sonderop
Division of Hepatology
Department of Medicine
University of Cape Town/ Groote Schuur Hospital
K47 Old Main building, Groot Schuur Hospital
Cape Town, South Africa
021 406 6394
msonderup@samedical.co.za

This document is an additional agreement that notes if you have or have not agreed to have any blood remaining after testing to be stored after the study is complete.

What will happen to my blood?
In the study, about four teaspoons (20ml) of blood will be taken. Some of this blood will be used to do a quick test for HIV and hepatitis B and C and to do confirmatory tests at a laboratory at the National Institutes of Infectious Diseases (NIDC). Any remaining blood will be frozen and stored at the NICD laboratory for future testing. Future tests will be done around hepatitis and other health conditions affecting sex workers, men who have sex with men and people who use drugs.

How long will my blood be stored?
Blood samples will be stored for up to 5 years and then they will be destroyed.

How will you protect my privacy?
The samples of blood will have a unique code on them, so that people handling these samples will not know that they belong to you.

Can I withdraw once the study or process has started?
You may withdraw your agreement to participate in this study at any time you no longer feel comfortable with your participation in this study. You can also decide to only take part in some parts of the study.

What will happen to my blood samples after the study is complete?
You have a choice as to what happens to your samples (tick one):

☐ Please destroy my blood samples after the study

☐ You can keep my blood sample for up to 5 years

Will you use my sample again?
The choice is yours (tick all that apply):

☐ I give my consent for my blood sample to be stored and used for future hepatitis research

☐ I give consent for my blood sample to be stored and used for research on other diseases affecting sex workers, men who have sex with men and people who use drugs.

Do you understand this document and agree to have your blood samples stored? Do you have any further questions you would like to ask about this study?

Informed consent to have my blood samples stored
I have read and understand that my blood sample will be taken and stored as part of this study. I have been given the opportunity to ask questions. I hereby give consent:

Signed _____ Date_____

Investigator
I have explained this consent to the participant and answered their questions. In my opinion the participant understands the purpose, procedures, risks and benefits of participation
Signed _____ Date_____

This study has received ethical approval from the Human Research Ethics Committee (HREC REF: 004/2016). For further information about your rights or to report any problems you may encounter, please contact:

Prof M Blockman
Chairperson, FHS Human Research Ethics Committee, University of Cape Town
Old Main Building
Groote Schuur Hospital
Observatory, 7925
Telephone: 021 406 6338
e-mail: Shuretta.thomas@uct.ac.za

Peter Cleaton-Jones
Chairperson, HREC (Medical), University of the Witwatersrand
Email: peter.cleaton-jones1@wits.ac.za
Administrators - Ms Zanele Ndlovu/ Mr Rhulani Mkansi/ Mr Lebo Moeng Tel 011 717 2700/2656/1234/1252
email: HREC-Medical.ResearchOffice@wits.ac.za

APPENDIX E: HEPATITIS SURVELLANCE LABORATORY REQUEST FORM

NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

Centre for Vaccine and Immunology
1 Modderfontein Road, Sandringham, 2031
Tel: +27 (0)11 386 6400
Reference: Hepatitis surveillance

Hepatitis surveillance laboratory Request Form

A. Client information																					
													1. Participant ID								
2. Gender	Male		Female		TransM		TransF														
3. D.O.B	d	d	m	m	y	y	y	y					Org. AHI / TBS / TBP / OUT		City CPT/DBN/ JHB/PTA/NMB/ PMB/ORT		First 2 letters of mother's name		Last 2 digits of client's birth year		Last 2 letters of client's birth name
4. Race	Black		Coloured		White		Indian		other												
5. Site Name:																					

B. Point of care results:							
1. HCV rapid results	Pos	Neg		2. HBV rapid results	Pos	Neg	
3. HIV status	Pos	Neg					

C. Laboratory specimen details																	
1. Date Specime Collected	d	d	m	m	y	y	y	y	2. Date specimen send to Lab	d	d	m	m	y	y	y	y
3. Type of specimen	Blood			DBS													
4. Test requested	Anti-HCV			HCV viral load		HCV Genotype			5. Participant consent Blood storage			Yes		No			
6. Sender's Name				7. Email:					8. Tel/Cell no.								

Site Label

APPENDIX F: VIRAL HEPATITIS POINT OF CARE TESTING INSTRUCTIONS

ORAQUICK HCV TESTING INSTRUCTIONS

OraQuick® Rapid Antibody Test HCV

REF 1001-0181 25 tests 1001-0180 100 tests

Read this Package Insert completely before using the product. Follow the instructions carefully when performing testing. Failure to do so may result in inaccurate test results.

INTENDED USE

IVD For use by healthcare professionals only.

The OraQuick® HCV Rapid Antibody Test is a single-use, anti-HCV assay. It is an immunoassay for the qualitative detection of immunoglobin G (IgG) antibodies to hepatitis C virus (anti-HCV) in oral fluid, fingerstick whole blood, venipuncture whole blood, plasma specimens (EDTA, sodium heparin, lithium heparin, and sodium citrate), and serum (Serum Separator Tube), and from individuals 11 years or older. The OraQuick® HCV Rapid Antibody Test assay results may be used to provide presumptive evidence of infection with HCV in individuals with signs and symptoms of hepatitis and in individuals at risk for hepatitis C infection.

Warning: Not intended for use in screening whole blood, plasma, or tissue donors. The effectiveness of the OraQuick® HCV Rapid Antibody Test for use in screening whole blood, plasma, or tissue donors has not been established.

SUMMARY AND EXPLANATION OF THE TEST

Hepatitis C virus (HCV) is the causative agent for most, if not all, non-A, non-B hepatitis.2 The presence of antibodies to HCV indicates that the individual may be currently infected and capable of transmitting the virus.

PRINCIPLES OF THE TEST

The OraQuick® HCV Rapid Antibody Test is a manually performed, visually read, 20-minute immunoassay for the qualitative detection of HCV antibodies. The assay test strip contains synthetic peptides and recombinant proteins from the core, NS3, and NS4 regions of the HCV genome (test line) and a goat anti-human IgG (control line) immobilized onto a nitrocellulose membrane.

MATERIALS PROVIDED (REF 1001-0181 25 tests, REF 1001-180 100 tests)

- Divided pouch contains OraQuick® HCV Rapid Antibody Test plus desiccant and OraQuick HCV Developer Solution: vial containing 0.75 mL phosphate buffered saline solution containing polymers and an antimicrobial agent.
- Reusable test stands
- Collection loops
- Package Insert

MATERIALS REQUIRED, AVAILABLE AS AN ACCESSORY TO THE KIT

OraQuick® HCV Rapid Antibody Test Kit Controls (REF 1001-0182)

MATERIALS REQUIRED BUT NOT PROVIDED

Timer capable of timing 20 to 40 minutes
Biohazard waste container

Additional Items Required for Fingerstick and Venipuncture Specimens

Antiseptic wipe, sterile lancet or venipuncture supplies, disposable gloves (optional for oral fluid testing), sterile gauze pads, centrifuge

WARNINGS

For in vitro Diagnostic Use. For use by healthcare professionals only.

- Read the Package Insert completely before using the product.
- Follow the instructions carefully when performing the OraQuick® HCV Rapid Antibody Test, failure to do so may cause an inaccurate test result.
- This test kit has been approved for use with oral fluid, finger-stick whole blood, venous whole blood, serum and plasma specimens only. Use with other specimen types may cause inaccurate results.
- If the test kit is exposed to temperatures outside of the recommended storage temperature (2°-30°C, 36°-86°F), or is tested outside of the operating temperature (15°-37°C, 59°-99°F), use the Kit Controls to ensure performance of the test.

PRECAUTIONS

- Handle specimens and materials in contact with specimens as if capable of transmitting infectious agents.
- Wear disposable gloves while handling and testing blood specimens. Change gloves and wash hands thoroughly after performing each test. Dispose of used gloves in a biohazard waste container.
- Oral fluid is not considered potentially infectious unless it contains blood.4 Use of gloves is optional for oral fluid testing.
- Do not reuse blood collection loops, tests or developer solution. Dispose of these components properly.
- Do not use the test beyond the expiration date printed on the pouch.

STORAGE

- Store unused OraQuick® HCV Rapid Antibody Tests unopened at 2°-30°C (36°-86°F).
- Do not open the pouch until you are ready to perform a test.
- If stored refrigerated, ensure that the pouch is brought to operating temperature (15°-37°C, 59°-99°F) before opening.

SPECIMEN HANDLING

- Oral Fluid: Do not eat, drink or chew gum for at least 10 minutes prior to testing. Collect specimen and place in Developer Solution immediately.
- Whole blood, plasma or serum: insert the test device into the Developer Solution within 60 minutes of adding the sample.
- Whole blood, serum or plasma may be stored at 15-30°C (59-86°F) for up to 3 days or at 2-8°C (36-46°F) for up to 7 days. Invert the tube several times to mix.
- Serum or plasma: Centrifuge at 1000-1300 x g for approximately 5 minutes.
- Serum and plasma specimens stored frozen at -20°C (-4°F) may have up to 3 freeze-thaw cycles.

DIRECTIONS FOR USE

GENERAL TEST PREPARATION

- Allow all components to come operating temperature (15°-37°C, 59°-99°F).
- Place the reusable test stand on your work space. Use only the stand provided with the OraQuick® HCV Kit.
- Place the OraQuick® HCV Developer Solution Vial into the test stand. Hold the vial firmly in the stand and remove the cap by rocking it back and forth while pulling it off.
- Do not open the pouch until you are ready to perform a test. Check the pouch for damage or holes. Discard the pouch if it is damaged.
- After opening the pouch, check for a desiccant packet. If it is not present of appears damaged, discard the pouch and open a new one.
- DO NOT cover the 2 holes on the back of the test with labels or other materials. Blocking the holes may cause an invalid result.

1. SAMPLE COLLECTION

1a. Oral Fluid

- Do not eat, drink or chew gum for at least 10 minutes prior to testing.
- Remove the OraQuick® HCV Rapid Antibody Test from the pouch. DO NOT touch the Flat Pad.
- Swab completely around the lower and upper outer gums **ONE TIME**. DO NOT swab the roof of the month, cheeks or tongue.



1b. Fingerstick Whole Blood

- Cleanse finger. Air dry.
- Puncture finger with a sterile lancet. Hold the finger downward and apply gentle pressure beside the point of puncture. Avoid squeezing the finger to make it bleed.
- Fill the collection loop. Immediately insert the Loop into the Developer Solution. Mix with Loop.
- If the Loop is dropped or contacts any other surface, discard it. Use a new Loop to collect the blood.

1c. Venipuncture Whole Blood

- Collect the specimen using standard phlebotomy procedures into a tube containing EDTA, sodium heparin, lithium heparin, or sodium citrate. Other anticoagulants have not been tested and may cause an incorrect result.
- Mix the blood by inversion. Fill the collection loop. Immediately insert the Loop into the Developer Solution. Mix with Loop.

1d. Serum or plasma

- Plasma: Collect the specimen using standard phlebotomy procedures into a tube containing EDTA, sodium heparin, lithium heparin, or sodium citrate. Serum: Collect into SST tube. Other anticoagulants have not been tested and may cause an incorrect result.
- Centrifuge at 1000-1300 x g for approximately 5 minutes.
- Fill the collection loop. Immediately insert the Loop into the Developer Solution. Mix with Loop.

2. RUN TEST

- Insert the test device into the Developer Solution.
- Set the timer for 20 to 40 minutes.

TEST RESULT AND INTERPRETATION

Refer to the Result Window on the Test Device.

NON-REACTIVE

A test is Non-Reactive if a line appears in the C Zone and NO line appears in the T Zone. A Non-Reactive test result means that HCV antibodies were not detected in the specimen. Patient is presumed not to be infected with HCV.



REACTIVE

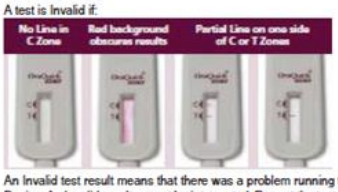
A test is Reactive if a line in the C Zone and a line appears in the T Zone. Lines may vary in intensity. The test is reactive regardless how faint these lines are. A Reactive test result means that HCV antibodies have been detected in the specimen. Patient is presumed to be infected with HCV.



Follow appropriate guidelines for supplemental testing.

INVALID

A test is Invalid if:



An Invalid test result means that there was a problem running the test, either related to the specimen or to the Device. An Invalid result cannot be interpreted. Repeat the test with a new Pouch and a new specimen. Contact OraSure Technologies' Customer Service if you are unable to get a valid test result upon repeat testing.

APPENDIX G: TURNITIN REPORT