

**Evaluation and validation of room temperature biospecimens transportation and storage technologies as an alternative cost effective solution to cold chain logistics and storage within biobanking and/or diagnostics**

By

Dr Fatima Adam Abulfathi

*Thesis presented in partial fulfilment of the requirements for the degree of Master of Science (Hematological Pathology) in the Faculty of Medicine and Health Sciences at Stellenbosch University*



Supervisor: Dr. Carmen Swanepoel

Co-Supervisors: Prof Abayomi and Dr Ravnit Grewal

March 2017

## **Declaration**

By submitting this thesis electronically, I declare that the entirety of the work contained therein is my own, original work, that I am the sole author thereof that reproduction and publication thereof by Stellenbosch University will not infringe any third party rights and that I have not previously in its entirety or in part submitted it for obtaining any qualification.

Signature:

Date: March 2017

Copyright © 2017 Stellenbosch University of Stellenbosch

All rights reserved

## **Abstract**

**Background:** Cold chain management (CCM) is an important aspect of biobanking operation. However challenges such as constant power failure, limited access to dry ice and liquid nitrogen, transport logistics and courier delays especially in Africa becomes a major challenge. Ensuring samples are maintained at the proper temperature throughout all processes is imperative to maximal long term viability and usability. Thus we consider room temperature storage (RTS) technologies as an innovative, cost effective and green alternative to cold chain logistics.

**Methods:** Various room temperature storage technologies were evaluated for the stabilization and storage of whole blood DNA and RNA, buffy coat, genomic DNA and urine DNA. The stabilizers include the Biomatrix liquid gard technology and dry matrix technology as well as DNAGENOTEK Hemagene buffy-coat stabilizers, Paxgene RNA and Norgen urine tubes. Samples were stored with and without a stabilizer under different temperature conditions namely room temperature, 45°C, -80°C, -20°C and liquid nitrogen (-196°C) over different time periods to determine effect on sample integrity and quality. At the end of each time point DNA/RNA was extracted and the integrity of the samples determined by assessing the concentration, purity and integrity. Further downstream analysis such as polymerase chain reaction (PCR), quantitative real time PCR and DNA sequencing was conducted. In addition, a shipping cost analysis between satellite sites in African and our biobank was done to compare frozen and room temperature shipping.

**Results** The study results show that sample integrity/quality for biospecimens stored at room temperature with stabilizers were comparable and more cost effective than cold chain storage systems. In addition some stabilizers showed better stabilizing properties than others.

**Conclusion:** Room temperature storage provides an innovative and cost effective method of storage and shipping to cold chain management systems (CCM). Green technologies forms a small part of biobanking operations however its results would be beneficial as low energy options for biobanking are particular critical in low resource settings which have infrastructural challenges. In turn, it would also be a more cost-effective option for the

transport and storage of human samples collected at various sites all over the world or at difficult out of reach places.

## **Opsomming**

**Agtergrond:** Koue ketting bestuur (KKB) is 'n belangrike aspek van Biobank bedrywighede, maar uitdagings soos konstante kragonderbrekings, beperkte toegang tot droë ys en vloeibare stikstof, vervoer logistiek en koerier verdragings veral in Afrika is 'n groot uitdaging. Om te verseker dat monsters in stand gehou word by die regte temperatuur, in die hele proses, is dit noodsaaklik om lang lewensvatbaarheid en bruikbaarheid van monsters te maksimiseer. Dus kyk ons na kamertemperatuur stoor tegnologie as 'n innoverende, koste-effektiewe en groen alternatief vir koue ketting logistiek.

**Metodes:** Verskeie kamertemperatuur stoor tegnologie is geëvalueer vir die stabilisering en stoor van heelbloed DNA en RNA, “buffy coat”, genomiese DNA en uriene DNA. Die stabiliseerders sluit in die Biomatrix vloeibare beskerm-tegnologie en droë matriks tegnologie asook DNAGENOTek, Hemagene “buffy coat” stabiliseerders, Paxgene RNA en Norgen urienbuis. Monsters is gestoor met en sonder 'n stabiliseerder by verskillende temperature naamlik kamertemperatuur, 45°C, -80°C, -20°C en in vloeibare stikstof (-196°C) oor verskillende tydperke om die uitwerking op monster integriteit en kwaliteit te bepaal. Aan die einde van elke tydperk is DNA / RNA geïsoleer en die integriteit, kwaliteit en konsentrasie van elke monsters is bepaal en geëvalueer. Verdere stroomaf ontleding soos Polimerase Kettingreaksie (PKR), kwantitatiewe “real-time” PKR en DNA volgordebepaling is gedoen. Hierby is 'n koste-ontleding tussen satelliet gebiede in Afrika en ons Biobank gedoen om bevrore en kamertemperatuur monsters wat aangestuur is na die lande te vergelyk.

**Resultate:** Die studie wys dat die integriteit/kwaliteit van monsters wat by kamer temperatuur gestoor was in stabiliseerders, vergelykbaar en meer koste-doeltreffend as koueketting stoor stelsels was. Daarbenewens het 'n paar stabiliseerders beter stabiliserende eienskappe as ander getoon.

**Gevolgtrekking:** Kamertemperatuur berging bied 'n innoverende en meer koste-effektiewe metode vir die stoor en stuur van monsters as koue ketting bestuurstelsels. Groen tegnologie vorm 'n kleindeel van Biobank bedrywighede, maar die resultate sal egter voordelig wees as lae energie-opsies vir Biobank bedrywighede, en is besonder krities in lae hulpbron instellings wat uitdagings met infrastruktuur ervaar. Op sy beurt, sou dit ook 'n meer koste-effektiewe opsie wees vir die vervoer en berging van menslike monsters na verskillende plekke oor die wêreld of in moeilike bereikbare plekke

## **Dedications**

This work is dedicated to my parents, husband and children for their support, love and understanding

## **Acknowledgements**

First of all I want to thank Almighty Allah for giving the opportunity and ability to do this program.

I will like to thank my supervisor Dr Carmen Swanepoel for her hard work, dedication, sacrifice and unprecedented support to see me through my work. My co-supervisors Prof Akin Abayomi and Dr Ravnit Grewal for their constant support. I want to acknowledge Mr. Faghri February for the guidance he provided during this work. Shafieka Isaacs for making sure I always had what I needed. I wish to acknowledge Mr. Timothy Reid, my colleagues and the entire staff and students of the Division of Hematology in one way or the other you all played a role. My entire family and friends for your support and encouragement throughout this academic journey.

## **List of Figures**

**Figure 1.1:** National Institute of Health and Wellcome trust logo

**Figure 1.2:** IATA validated packaging of biospecimens

**Figure 2.1:** Workflow for the collection, stabilization and storage of DNA in whole blood using DNAgard

**Figure 2.2:** Workflow for the collection, stabilization and storage of DNA in buffy coat using HG-BCD

**Figure 2.3:** Workflow for the collection, stabilization and storage of purified DNA using DNASTABLE PLUS

**Figure 3.1:** Agarose gel (0.8%) integrity check of DNA in whole blood with (DG+) and without (DG-) stabilizer (DNAgard) at 3, 6, and 9 months. The red arrow indicates degradation.

**Figure 3.2:** Agarose gel (0.8%) integrity check of DNA in Buffy coat with (HG+) and without (HG-) stabilizer (HEMAGene BUFFY Coat) at 3, 6, and 9 months

**Figure 3.3:** Agarose gel (0.8%) integrity check of purified DNA samples prior to stabilization and storage of 12 samples from lane 2-13.

**Figure 3.4:** Agarose gel (0.8%) integrity check of purified DNA stored with (DS+) and without (DS-) stabilizer (DNASTABLE PLUS) at 3, 6 and 9 months.

**Figure 3.5:** Agarose gel (0.8%) integrity check of DNA in Buffy coat after 3 years of storage for 3 samples with (HEMAGene BUFFY Coat) stabilization at RT, -80°C and 45°C.

**Figure 3.6:** Agarose gel comparison between whole blood and Buffy coat stabilization at 9 months for both DNAgard (DG) and HEMAGene (HG)

**Figure 3.7:** Functional PCR Assay of a sample stored at RT, -80 and 45°C with and without DNAgard stabilization using  $\beta$ - globin housekeeping gene with primer sets: *GH20 + PC04 – 268bp*, *RS42 + KM29 – 536bp*, *RS40 + RS80 – 989bp*, *KM29 + RS80 – 1327bp* at 3 and 9 months.

**Figure 3.8:** Functional PCR Assay of a sample stored at RT, -80 and 45°C with and without HEMAGene Buffy coat stabilization using  $\beta$ - globin housekeeping gene with primer sets: *GH20 + PC04 – 268bp*, *RS42 + KM29 – 536bp*, *RS40 + RS80 – 989bp*, *KM29 + RS80 – 1327bp* at 3 and 9 months.

**Figure 3.9:** Functional PCR Assay of a sample stored at RT, -20°C -80°C and 45°C with and without DNASTable PLUS stabilization using  $\beta$ - globin housekeeping gene with primer sets: *GH20 + PC04 – 268bp, RS42 + KM29 – 536bp, RS40 + RS80 – 989bp, KM29 + RS80 – 1327bp* at 3 and 9 months.

**Figure 3.10:** Functional PCR Assay of a sample stored for 3 years at RT, -80°C and 45°C with HEMAgene Buffy coat stabilization using  $\beta$ - globin housekeeping gene with primer sets: *GH20 + PC04 – 268bp, RS42 + KM29 – 536bp, RS40 + RS80 – 989bp, KM29 + RS80 – 1327bp*

**Figure 3.11:** Agarose gel electrophoresis for DNA extracted from cultured cells with and without stabilization (DNAgard) after 1 month of storage. Lane 1 Molecular marker, 2, 4, 6, 8 and 10 are samples stored at RT. Lanes 2 and 4 protected, 6 NP with water and 8 and 10 in DMSO. Lanes 3, 5, 7, 9 and 11 are samples stored in LN. Lanes 3 and 5 protected, 7 NP in water and 9 and 11 cryopreserved in DMSO

**Figure 3.12:** Functional PCR for DNA from cultured cells stored for 1 month at RT and liquid nitrogen (LN) with and without DNAgard using  $\beta$ -Globin (housekeeping gene) with *Primer Sets: GH20 + PC04 – 268bp, RS42 + KM29 – 536bp, RS40 + RS80 – 989bp, KM29 + RS80 – 1327bp*

**Figure 3.13:** Representative Sequence Trace File for short fragments of  $\beta$ -globin from sample stored in DNAgard for 3 months.

**Figure 3.14A:** Representative Blast result of short fragment of  $\beta$ -globin stored in DNAgard for 3 months

**Figure 3.14B:** Representative blast results of larger fragment  $\beta$ -globin stored in DNAgard for 3 months

**Figure 3.15:** Representative 0.8% gel of  $\beta$ -globin PCR amplification with Primer set *GH20+PC04* from Cdna synthesised after isolation from PAXgene

**Figure 3.16:** Representative 0.8% gel of  $\beta$ -globin PCR amplification with Primer set *GH20+PC04* from Cdna synthesised after isolation from RNAgard

**Figure 3.17:** Amplification of DNA samples stored in DNAgard for 3 months by qRT-PCR

**Figure 3.18:** Standard curve of DNA stored in DNAgard for 3 months by qRT-PCR



**Figure 3.19:** Dissociation curve of DNA samples stored in DNAgard for 3 months by qRT-PCR

**Figure 3.20:** Amplification of RNA samples stored in RNAgard for 7 days by qRT-PCR

**Figure 3.21:** Amplification of RNA samples stored in PAXgene for 7 days by qRT-PCR

**Figure 3.22:** Dissociation curve of RNA samples stored in RNAgard for 7 days by qRT-PCR

**Figure 3.23:** Dissociation curve of RNA samples stored in PAXgene for 7 days by qRT-PCR

**Figure 3.24:** Transportation cost comparison between courier companies, Marken (Red) and DHL Express (blue), for 1kg shipment from satellite sites to NSB-H3A in Cape Town, South Africa at validated ambient temperatures (15-25<sup>0</sup>C) and/or Refrigerated (2-8<sup>0</sup>C) temperatures. The package costing for both validated ambient and refrigerated conditions is the same, thus the same cost

**Figure 3.25:** DHL Express transportation cost for 1kg shipments from satellite sites to NSB-H3A in Cape Town, South Africa at normal ambient temperatures. Bamako in Mali and Cotonou in Benin are the highest priced

**Figure 3.26:** Cost comparison for the shipment of 500 samples to NSB in Cape Town, South Africa at normal ambient (A), normal ambient with the addition of stabilizer (B) and validated ambient /refrigerated conditions (C). The highest priced sites of the 8 satellite sites were chosen to calculate shipping cost per sample, assuming a 1kg shipment can fit ±500 vials. These prices were based on the DHL Express estimates

## **List of Tables**

**Table 2.1:** Primer sequences and annealing temperatures used for the amplification of  $\beta$ -globin gene

**Table of Contents**

<b>Declaration</b> .....	<b>i</b>
<b>Abstract</b> .....	<b>ii</b>
<b>Opsomming</b> .....	<b>iii</b>
<b>Dedications</b> .....	<b>v</b>
<b>Acknowledgements</b> .....	<b>v</b>
<b>List of Figures</b> .....	<b>vi</b>
<b>List of Tables</b> .....	<b>viii</b>
<b>Table of Contents</b> .....	<b>ix</b>
<b>Chapter 1-Introduction and Literature review</b> .....	<b>1</b>
1.1. Introduction .....	1
1.2 Biobanking – a complex science .....	1
1.3. Global Biobanking – a network for harmonization.....	3
1.4. Biobanking in Africa .....	4
1.4.1. Establishment of H3Africa Initiative (H3A Biobank program) .....	4
1.5 Fit for purpose biospecimens – The importance of biospecimen integrity.....	5
1.6. Transport logistics challenges on the African continent.....	6
1.6.1. International Air Transport Association (IATA) dangerous goods regulations	7
1.7. Cold chain management (CCM) and Biobank Storage.....	9
1.8. Room temperature storage technologies.....	11
1.8.1. Biomatrixa ® .....	11
1.8.2. GenVault’sGenTegra .....	13
1.8.3. DNAGenotek HemaGene buffy coat DNA.....	14
1.8.4. Image DNAShell®and RNAShell®.....	15
1.8.5. Urine stabilization via NorgenBiotek Corp .....	15
1.8.6. Qiagen PreAnalytix PaxGene products .....	16
1.8.7. Dried Blood Spots (DBS) .....	17
1.9 The present study.....	18
<b>Chapter 2 Materials and Methods</b> .....	<b>19</b>
2.1 Sample stabilization and nucleic acid isolation .....	19
2.1.1 Stabilization of DNA in whole blood (DNAgard) and Buffy coat (HEMAGene BUFFY COAT) .....	19
2.1.2 Stabilization of purified DNA (DNAstable Plus Stabilizer).....	22
2.1.3 Stabilization of DNA in urine (Norgen Biotek Corp) .....	23
2.1.4 Stabilization of DNA in cultured cells (DNAgard Tissue) .....	24
2.1.5 Stabilization of RNA in whole blood .....	25

2.2 Nucleic acid quality assessment .....	26
2.2.1 DNA quantification using BioDrop $\mu$ LITE .....	26
2.2.2 DNA and RNA quantification using the Qubit 2.0 fluorometer .....	26
2.2.3 Agarose gel electrophoresis .....	26
2.3 Polymerase chain reaction (PCR).....	27
2.3.1 PCR amplification primers and parameters .....	27
2.3.2 Gel electrophoresis for amplified PCR product .....	27
2.4 Quantitative real time Polymerase chain reaction (RT-PCR).....	28
2.4.1 cDNA synthesis .....	28
2.4.2 Quantitative RT-PCR (qRT-PCR).....	28
2.5 DNA Sequencing.....	29
2.5.1 PCR Product purification for sequencing. ....	29
2.5.2 Analysis of sequencing reactions: .....	29
2.6. Transportation cost analysis .....	29
<b>Chapter 3-Results.....</b>	<b>31</b>
3.1 Stabilization of DNA in whole blood, Buffy coat and Purified DNA .....	31
3.1.1 DNA Concentrations of isolated samples .....	31
3.1.2 Determination of DNA integrity by agarose gel electrophoresis.....	31
Figure 3.2: Agarose gel (0.8%) integrity check of DNA in Buffy coat with (HG+) and without (HG-) stabilizer (HEMAGene BUFFY Coat) at 3, 6, and 9 months.32	
3.1.3 Comparison between Whole blood and Buffy coat samples .....	34
3.1.4 Polymerase chain reaction (PCR).....	35
3.2 Stabilization of DNA in cells.....	38
3.3 DNA Sequencing of <i>B-globin</i> gene .....	40
3.4 Stabilization of RNA in whole blood .....	42
3.4.1 Determination of RNA concentration.....	42
3.4.2 Standard PCR on isolated RNA from whole blood cells .....	42
3.4.3 Quantitative Real Time PCR to assess suitability of isolated DNA and RNA in downstream qRT-PCR applications .....	43
3.5: Sample transportation cost analysis for African biobanking/laboratory operations:	47
<b>Chapter 4- Discussion.....</b>	<b>51</b>
4.1 Stabilization of DNA in whole blood, Buffy coat, purified DNA, cells and urine	51
4.1.1. Concentrations of DNA isolated .....	54
4.2 Comparison between DNA in whole blood and Buffy coat .....	54
4.3 Polymerase chain reaction .....	55
4.4 DNA sequencing.....	56
4.5 RNA Quality, Yield and Quantitative Real Time PCR .....	56

4.6 Quantitative PCR with B-globin gene on DNA samples. ....	56
4.7 Transportation cost analysis .....	57
4.8 Limitations of the study .....	58
4.9 Future directions.....	60
4.10 Conclusion .....	60
<b>REFERENCES .....</b>	<b>61</b>
<b>Appendices .....</b>	<b>64</b>
<b>Appendix I: DNA Concentrations of isolated samples.....</b>	<b>64</b>
<b>Appendix II: Samples selected for DNA sequencing.....</b>	<b>96</b>

## **Chapter 1-Introduction and Literature review**

### **1.1. Introduction**

The rapid growth of genomics research has led to a unprecedented need for storage of large numbers of fit for purpose biological specimens, including Deoxyribonucleic Acid(DNA) and Ribonucleic acid(RNA), proteins, cells and tissues (Wan et al. 2010) Current methodologies for maintaining frozen nucleic acid (NA) biospecimens require freezers, space and energy rendering the technology expensive without guaranteeing long term viability if not stored appropriately according to biospecimen type (Clermont et al. 2014). In South Africa (SA) and many African countries transportation and storage of these biospecimens comes with its own challenges as increasing cost, constant power failures and transportation challenges all adds to pre-analytical variables that influence biospecimen integrity. Thus ensuring that biospecimens are maintained at the proper temperatures throughout all pre-analytical and analytical processes is imperative to maximal long-term viability and usability.

In recent years, new technologies for the stabilization and storage of biological biospecimens at room temperature (RT) have been developed. While these technologies differ in their implementation, the overall paradigm remains the same, to provide long-term stabilization and storage of biological biospecimens (Howlett et al. 2014) as an alternative to expensive cold chain management (CCM). Thus, the current study focuses on the evaluation and validation of RTS technologies to provide a cost effective, cheaper and greener alternative to CCM. Stabilization products from Biomatrix, DNAgenotek, Norgen and QIAGEN were evaluated for their stabilization at RT properties. For the purpose of this thesis, a condensed and simplified overview will be given on the importance and need of biobanking infrastructure, the challenges associated with the science of biobanking and transportation globally and within Africa and the various room temperature storage (RTS) products evaluated in order to provide context for the subsequent discussion.

### **1.2 Biobanking – a complex science**

A biobank can be broadly defined as “a facility for the collection, preservation, storage and supply of biological biospecimens and associated data, which follows standardized operating procedures (SOP’s) and provides bio-material for scientific and clinical use” (Watson 2014). Biospecimens from biobanks are used for genomic research applications,

translational research and personalised medicine. A biobank must therefore be consistent as biospecimens need to be processed and stored appropriately for use in later assays over years.

To date, the rapidly expanding era of pharmacogenomics and proteomic research promises tangible solutions to help alleviate health burdens. Genomics research specifically has experienced great advances over the past decade as witnessed by the completion of the human genome in 2003. The field has been driven by the belief that understanding the human genome, that of pathogens, and inter-individual genetic variability would result in radical advances in medicine (Matimba et al. 2008). However, it would require large scale genomics studies of good quality, fit for purpose biospecimens in statistically relevant numbers with its associated clinical data to ensure sustainable research and diagnostics in the era of personalised medicine.

Currently, genomic research studies specifically in SA and Africa is being hindered due to the lack of representation of our unique genetic profiles in the HapMap or 1000 Genome project for example. Despite being grouped into defined populations, high level of human genetic variation have been observed in our African populations and only hints at the number of diverse ethnic populations that reside within the African continent (Warnich et al. 2011; Lu et al. 2014). Therefore, there is a need to establish the genetic and pharmacogenetics profiles of our own unique ethnic populations. Knowledge regarding the genetic diversity, homogeneity and admixture of various population groups within Africa would allow us to understand our evolutionary background which in turn will help to shed light on disease aetiology by translating it in clinical applications which in turn would aid in public health benefits.

This is where biobanks came into play as it forms an integral role as an essential resource. If properly designed, maintained and governed to ensure compliance to global and local standardized ethical, social, and legal policies, procedures and protocols frameworks, a biobank that serves as 'honest brokers' could contribute significantly to addressing important questions on national, continental and global health issues. Furthermore, biobanking has become more than just the storage of biospecimens but has evolved and become a complex science with operations ranging from biospecimen logistic management which include advice on pre-analytical variables, collection, shipping,

processing, and quality control (QC) and storage. A laboratory information management system (LIMS) and quality management system (QMS) underlies all of these operations.

### **1.3. Global Biobanking – a network for harmonization**

To date, there is a global increase in the reliance on biobanks which are seen as huge investment to support research initiatives as access to high quality biospecimens from various populations is required for clinical and basic research but lacks high biospecimen volumes. This is especially observed in the pharma industries that have their own private biobanks for clinical trial purposes but also for drug development initiatives. The importance of such infrastructure is further highlighted by the availability of a number of international resources and societies which promote harmonization of biobanking operational procedures and best practices which is an essential element that enables biobanks to exchange and pool clinical data and biospecimens. Furthermore, this so-called interoperability is the foundation of successful global biobanking. Rather than demanding complete uniformity among biobanks, harmonization is a more flexible approach aimed at ensuring the effective interchange of valid information and biospecimens (Harris *et al.* 2012).

The International Society for Biological and Environmental Repositories (ISBER) is one global forum that addresses harmonization of scientific, technical, legal, and ethical issues relevant to repositories of biological and environmental specimens. ISBER (<http://www.isber.org/>) is a global organization that creates opportunities for sharing ideas and innovations in biobanking and harmonizes approaches to evolving challenges associated with biological and environmental repositories. ISBER fosters collaboration, creates education and training opportunities, provides an international showcase for state-of-the art policies, processes, and research findings, and innovative technologies, products, and services. Together, these activities promote best practices that cut across the broad range of repositories that ISBER serves (Siefers 2014). Thus implementation and adhering to ISBER best practices should be a minimal requirement for all biobanks and/or collections to aid harmonization aspects (Isber.org, 2016). In addition, other international resources and societies which help in standardizing and harmonization of biobanking activities also include the European, Middle Eastern, and African Society of Biopreservation and Biobanking (ESBB- <https://esbb.org/>) a regional chapter of ISBER, National cancer institute (NCI - <https://biospecimens.cancer.gov/practices/>) and the

Biorepositories and Biospecimen Research Branch (BBRB). Recently, the College of American Pathologists (CAP) has also developed a biobanking accreditation program to allow for accreditation of biobanking operations.(Biopreservation And 2012; Anon 2011)

#### **1.4. Biobanking in Africa**

The completion of the Human Genome Project has broadened our understanding of genome biology, genomics and diseases. Similarly, the 1000 genome project shed more light on genetic variants as structural variants are implicated in numerous diseases and make up the majority of varying nucleotides among human genomes (Sudmant et al. 2015). Human history has also advanced tremendously. Technological advances coupled with significant cost reductions in genomic research has yielded novel insights into disease aetiology, diagnosis, and therapy for some of the world's most intractable and devastating diseases including malaria, human immunodeficiency virus/acquired immunodeficiency syndrome (HIV/AIDS), tuberculosis (TB), cancer, and diabetes. SA, with a population of 60 million inhabitants, has one of the highest burdens of infectious disease, predominantly driven by the syndemic of HIV and TB and a growing problem of non-communicable and metabolic disease syndromes. This creates a highly vulnerable and susceptible population that requires a focused research approach in order to find indigenous solutions through national, continental, and international collaborations (Abayomi *et al.* 2013). Yet, despite the burden of infectious diseases and more recently, non-communicable diseases (NCDs) observed in Africa, Africans themselves have only participated minimally in genomics research (Warnich et al. 2011). Of the thousands of genome-wide association studies (GWASs) that have been conducted globally, only seven (for HIV susceptibility, malaria, TB, and podoconiosis) have been conducted exclusively on African participants (Rotimi et al. 2014). This raised concerns for African genetic scientists and Rotimi et al re-emphasised this in 2014 and stated that if the lack of genomics research involving Africans persists, the potential health and economic benefits emanating from genomic science may elude an entire continent (Rotimi et al. 2014).

##### **1.4.1. Establishment of H3Africa Initiative (H3A Biobank program)**

In order to position Africa as a vital resource and a recognized scientific hub to enable genomic research capacity, the Human Heredity and Health in Africa (H3Africa) initiative was founded In June 2010 (<http://h3africa.org/>). This was born out of a partnership among



the U.S. National Institutes of Health (NIH), the UK-based Wellcome Trust and the African Society of Human Genetics (AfSHG) and was based on recommendations in a white paper written by two H3Africa communicable and non-communicable diseases working groups (WG's) following the 2009 AfSHG meeting in Cameroon (H3 Africa Working Group 2011). The H3Africa program provided funding for collaborative centres, research projects, societal implications research as well as biorepositories. Over the period of 2012-2014, The H3Africa biobank program has funded four African biobanks in phase 1 with two being in SA namely, the - National Health Laboratory Services (NHLS) Stellenbosch University Biobank (NSB), the Clinical Laboratory Services (CLS) in Johannesburg, the Institute of Human Virology Nigeria-H3A-Biorepository (I-HAB) and the Integrated Biorepository of H3Africa in Uganda. The H3Africa consortium seeks to harness genomics technologies to investigate diseases pertinent to African patients with the aim of fostering collaboration between scientists in Africa and elsewhere. In addition, of ethical importance is that H3Africa builds equitable partnership between researchers and other key stakeholders which helps in building strong research systems. The initiative was also a means to counter exploitation and promote mutual respect and trust and offer an opportunity to ensure that research is responsive to local health needs and that data interpretation is contextualised (de Vries *et al.* 2015). This consortium not only improves infrastructure and promote research within Africa, but may also lead to increased collaboration both within Africa and the rest of the world. As the H3Africa project intends to increase the number of biobanks across Africa, there is a need to ensure that there are harmonious legal and ethical guidelines on the storage of biological biospecimens across the African continent. Efforts are also made to ensure that there is uniformity of governance of biobanks throughout Africa which would allow for easy transfer of biological biospecimens throughout the continent and ultimately encourage collaboration (Staunton & Moodley 2016; H3 Africa Working Group 2011)

### **1.5 Fit for purpose biospecimens – The importance of biospecimen integrity**

As stated above fit for purpose biospecimens in statistically relevant numbers is essential for genomic and transcriptional research. Thus, maintaining sample integrity is very critical for a biobank. with horrible downstream effects if the biospecimen integrity was to be affected by continuous power failures and/or transportation challenges for example. Literature clearly shows for example that cancer researchers only obtain 39% samples of sufficient numbers while 47% of samples are of satisfactory quality (Masset *et al.* 2011). Given this

statistics, one can conclude that any variable that can be introduced during processes such as biospecimens transportation, collection, processing, storage and analysis are all sources of bias in research that can lead to distorted results. In turn, these effects due to low quality samples cause researchers to question their findings. This highlights the importance of sample integrity and maintaining these precious biological resources especially from a biobank perspective. Loss of sample integrity is greatly increased by chemical and enzymatic activity, freeze thaw cycles, microorganism activity as well as harsh environmental factors such as UV light exposure, humidity and high temperatures. However the degree and spectrum of sample integrity loss would depend on the sample source and the type of environment to which it was exposed to. Thus to ensure that biospecimens stability is maintained, factors such as biospecimens type, time of collection, containers used, preservatives and other additives, transport means, length of transit time and storage of biological biospecimens need to be taken into account. Quality is the conformance to standards and biological specimens must have quality control (QC) checks to determine their purity for downstream analysis and storage. Thus, biospecimen integrity must be maintained to ensure good quality specimens for analysis. Appropriate size for aliquots must be determined before storage to limit the frequent freeze-thaw cycles. Incorrect and incomplete purification procedures can also leave residual nucleic tissue behind that can interfere with the accuracy of a given assay. Any form of degradation, corruption, or damage can reduce the number of intact DNA templates until the biospecimen size is too small for amplification. Like DNA, the accuracy of gene expression evaluation is recognised to be influenced by the quantity and quality of starting RNA. Purity and integrity of RNA are critical elements for the overall success of RNA-based analyses. Starting with low quality RNA may strongly compromise the results of downstream applications which are often labour-intensive, time-consuming and highly expensive (Fleige & Pfaffl 2006).

## **1.6. Transport logistics challenges on the African continent**

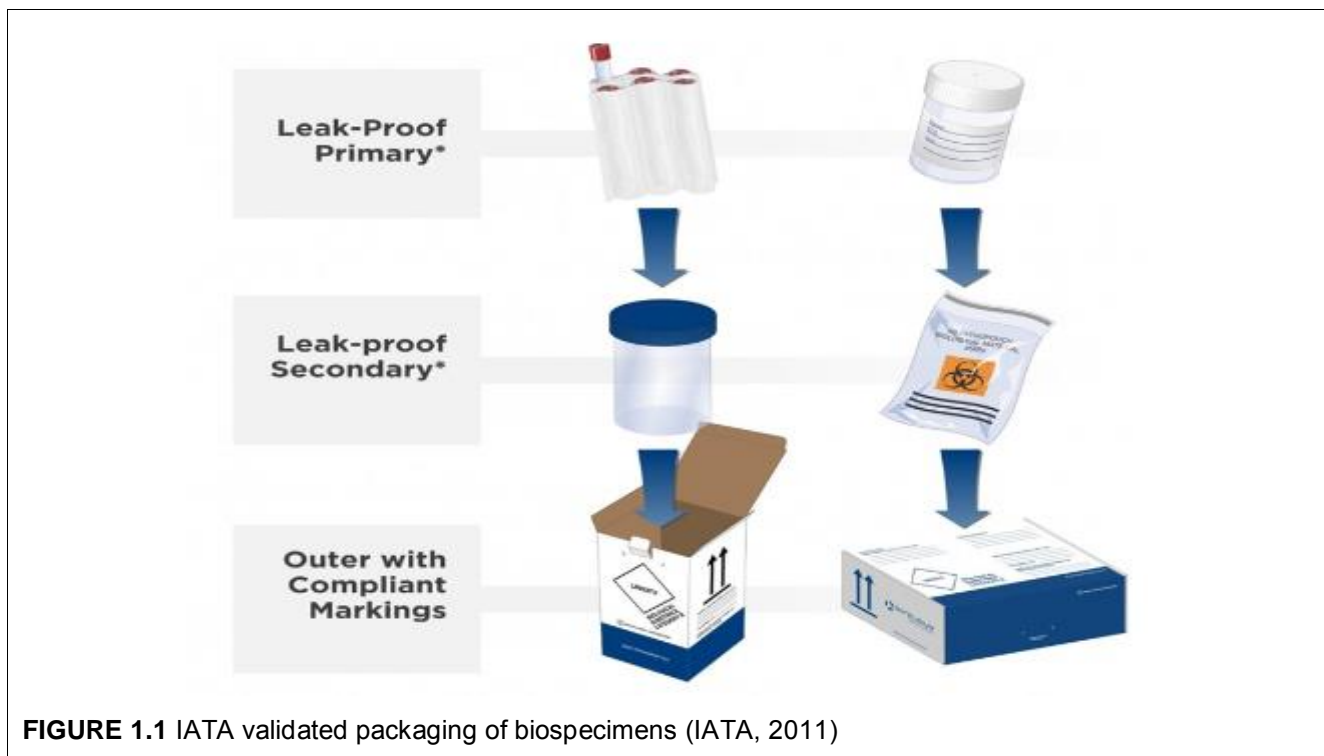
Environmental factors plays a big role in loss of sample integrity, one can imagine that transport logistics of biological biospecimens globally but more specifically on the African landscape would be a big challenge as the climate ranges from tropical to subarctic on its highest peaks. In turn, 60% of the entire land surface consists of drylands and deserts making Africa the hottest continent on earth. In addition, poor infrastructure such as problematic road transportation, lack of regular power supply, limited access to proper cold

storage facilities, extreme weather conditions, the lack of suitable transportation packaging material and refrigerants such as dry ice, the lack of experience and knowledge of correct methods for packaging biological material, long delays to obtain necessary permits to export/import biological material, unreliable and inconsistent custom's situations, lack of proper cold storage facilities at some airports, high-priced transport costs, irregular flight schedules, as well as a lack of International Air Transport Association (IATA) trained airline and biobank staff are all factors that may hinder smooth and easy sample transportation within Africa. Thus when seeking to regulate biospecimen temperature during shipping, the shipping time, distance, climate, season, method of transportation, and regulations as well as the type of biospecimens and their intended use should be considered (Anon 2011). Climate change is an environmental factor that cannot be controlled during shipping of biological specimen and according to FedEx, environmental temperature can be as high as 60°C depending on the time of the year (Howlett *et al.* 2014). Thus to ensure controlled temperature of the package itself, proper packaging is essential to maintain biospecimen integrity during transportation. Validated packaging material and efficient packaging techniques protect biospecimens during transit, unexpected flight cancellations as well as custom delays (Howlett *et al.* 2014). These packaging materials have been validated and tested according to IATA regulations and are thus very expensive.

### **1.6.1. International Air Transport Association (IATA) dangerous goods regulations**

IATA is the air industry's global trade association with a mission to represent, lead and serve the airline industry (lata.org, 2016). IATA has regulations on packaging and transport of biological specimens. Most biological biospecimens are classified in the Infectious substances in Category B, exempt or Category C. Human or animal material including but not limited to excreta, secreta, blood and its components, tissue and tissue fluids, and body parts, being transported for purposes of research, diagnosis, investigational activities, disease treatment and prevention are assigned to UN3373 and their proper shipping name is 'Biological Substances, Category B' and IATA Packing Instruction 650 needs to be followed for UN3373 shipments. Packaging must be of good quality, strong enough to withstand the shocks and loadings normally encountered during transport. Packaging must be constructed and closed so as to prevent any loss of contents that might be caused under normal conditions of transport, by vibration, or by changes in temperature, humidity or pressure. The packaging must consist of three components: a primary receptacle, a secondary packaging and a rigid outer packaging (Figure 1). Primary

receptacles must be packed in secondary packaging in such a way that under normal conditions of transport, they cannot break, be punctured or leak their contents into the secondary packaging. Secondary packaging must be secured in outer packaging with suitable cushioning material. Any leakage of the contents must not compromise the integrity of the cushioning material or of the outer packaging.



With regards to transport of biological specimens using the cold chain management (CCM) systems, IATA ensures that ice or dry ice must be placed outside the secondary packaging or in the outer packaging or an over pack. Interior supports must be provided to secure the secondary packaging in the original position after the ice or dry ice has dissipated. If ice is used, the outside packaging or over pack must be leak proof. If dry ice is used, the packaging must be designed and constructed to permit the release of carbon dioxide gas to prevent a build-up of pressure that could rupture the packaging. The primary receptacle and the secondary packaging must maintain their integrity at the temperature of the refrigerant used as well as the temperatures and the pressures, which could result if refrigeration were to be lost (IATA, 2011). These rules are compulsory and need to be adhered to otherwise it could result in enormous fines to researchers and institutions. Thus it is compulsory for all employees/researchers/laboratory staff and those involved in clinical trials who pack dangerous goods for air transport to be aware of the requirements of the IATA Dangerous Goods Regulations before packaging these substances. All staff thus needs to undergo accredited training before performing relevant duties (renewable every 2

years). Furthermore, training records must be maintained and be available on request by the Civil Aviation Safety Authority.

In Africa, lack of packaging materials, lack of experience and knowledge on how to package biological materials and the cost associated with packaging materials poses a significant problem to the transport logistics. Although, IATA provides training on how to package and transport biospecimens, these type of training are still very expensive. An alternative way of transportation of biological biospecimens other than CCM is needed thus the investigations into room temperature storage stabilization. Room temperature storage and/or transportation alternatives will aid in improving biospecimens stability and protection but also helps removes the need for dangerous refrigerants such as dry ice thus, making packaging easier and more affordable to biobanks and/or diagnostic laboratories within Africa but also globally.

### **1.7. Cold chain management (CCM) and Biobank Storage**

Ensuring biospecimens are maintained at the proper temperature throughout all pre-analytical and analytical processes are imperative to maximal long-term viability and usability. Likewise, the movement of biospecimens between physical locations is a necessary part of laboratory/biobanking, whether by walking, road, air and/or sea. Thus the necessary steps should also be in place to maintain the required constant temperatures depending on the biospecimen type. Traditionally, for CCM one requires ultra-low freezers (-80°C) or liquid nitrogen (LN<sub>2</sub>, -196°C) dewars or freezers to maintain long term integrity of biospecimens. Cold inhibits destructive chemical reactions such as oxidation as well as degradation caused by enzyme activity. Cold also inhibits the growth of any contaminating bacteria and molds (Howlett et al. 2014). Thus to ensure proper preservation and protection of valuable biospecimens, a well-developed and reliable infrastructure that adheres to international guidelines and best practices as mentioned above is required.

Key infrastructure needs for biobanking operations include the availability of, efficient transport logistics, the availability of LN<sub>2</sub> and dry ice as well as the location of the biobank in terms of climate conditions and constant power. In case of short-term electricity shortages, backup generators and energy storage devices are important to ensure a stable power supply for biorepository operations. In order to minimize the risk of

degradation and/or loss of biological samples a risk management plan needs to be in place. Thus, all critical equipment, LN2 and mechanical storage must be connected to alarms, back-up generators and UPS, which would be under constant surveillance to allow for intervention in cases of power failure and other mechanical emergencies. As is the case of SA and many of the African countries where power supply is unstable and power failure has become a major problem. In SA, loadshedding has become a way of life as Eskom tries to cope with electricity demands; however this poses a risk for public health facilities as well as the biobanks, especially for the cold chain management aspect of operations. In general, the colder the better for long term storage. However, CCM can be non-practical and expensive, requiring a lot of space, manpower, large number of freezers and back-up generators and if biospecimens need to be transported frozen, it may be difficult to maintain them in that state (Howlett *et al.* 2014).

If these factors are still maintained and, contingency plans and disaster recovery are not in place in a laboratory and/or biobank set up then in the event of disasters, there is significant loss of biospecimen integrity which aids to biospecimen loss with horrible downstream effects. One such example that highlighted the need for research institutions to have business continuity and disaster recovery plans in place is Hurricane Sandy, which devastated large parts of New York City and the surrounding area. From a laboratory and biobank perspective, hundreds of thousands of refrigerated and frozen biological samples (decades of research) were at risk of being lost or destroyed due to electrical power outages in these areas (Hager 2014).

Due to these abovementioned challenges, an alternative, more innovative, cost-effective and green alternative logistic as well as storage strategy needs to be investigated as part of a transportation logistics and storage solution in comparison to CCM. Thus, RTS technologies were explored as a low energy option compared to CCM and logistics as it would play an essential role in low resource settings which have infrastructural challenges due to numerous complications with regards to power failure as well as the unavailability of dry ice and LN2 in certain parts of SA and Africa and the cost associated with CCM. In addition, CCM of biospecimens is very expensive with regards to power, availability of dry ice and LN2, the need for heating, ventilation, and air conditioning (HVAC) and the usage of CO2 tanks for backup purposes. RTS requires low space, no need for HVAC, no associated cost for CO2 usage and electricity's, except for the cost of a dehumidifier to maintain low humidity and remove moisture from air as part of the storage solution.

Likewise shipping at ambient temperatures would also be ideal and more cost effective. While DNA is very robust and stable, and transportation within 24-48 hours is allowed, we have to anticipate the challenges associated with transportation within SA and Africa, where ambient temperatures can rise to over 40 degrees Celsius. The risk of exposure to such extreme conditions in addition to possible delays at customs will increase the risk of nucleic acid (NA) degradation if it's not kept in a temperature controlled enclosed environment. These will have downstream effect on diagnostic assays and skew results resulting in increased pricing for the laboratory to repeat assays and prolonged turnaround time. In anticipation of these potential scenarios, we propose that extracted DNA/RNA or buffy coats from blood or whole blood as a whole as well as cells can be stored in a stabilizing solution at room temperature, which allows batching and subsequent transportation at ambient temperature as well as provide an alternative backup storage adding to built-in risk management plans.

## **1.8. Room temperature storage technologies**

RTS technology enables safe storage of biological material at room temperature (RT) preventing the degradation of biological materials at RT and thus eliminating the need for CCM and frozen shipping thus providing a cost-effective alternative (Howlett et al. 2014). Various products involved in RTS will be considered and evaluated.

### **1.8.1. Biomatrixa ®**

One such product included is the synthetic chemistry-based stabilization science called the BiomatrixaSampleMatrix® technology. This science is based on the principles of extremophile biologics of long time surviving multicellular organisms in dry environments via a process called anhydrobiosis (meaning "life without water"), or the tolerance of these organisms to dessication that enables their survival in a dry state for up to 120 years. Anhydrobiotic organisms (such as tardigrades and brine shrimp) can protect their complex cellular systems in a dried arrested state, and can be revived by simple rehydration. The basic molecular stabilization technology has been successfully applied to the stabilization of DNA, RNA, bacterial clones, proteins and complex biospecimens such as blood, buffy coat, cells and tissue. The stabilization technology is widely used in to prevent degradation of bio-molecules during transport and long term storage at ambient and elevated

temperatures. It was shown that this storage media forms a thermostable barrier during the drying process and “shrink wraps” and protects against degradation (Wan *et al.*, 2009). These products can be used from sample collection, through transport, access and storage and on various samples types that includes DNA, RNA, proteins blood, buffy coat, saliva or cells and tissue. It has two types of technologies, the Liquid GARD-technology, more appropriate for collection and transportation and the Dry Stable technology, which is more appropriate for the transportation, analysis and storage purposes. The process is simple and straightforward as the archiving aspect of this technology only requires a dry-down step and storage in a low humidity environment which along with the stabilizer protect samples from hydrolysis, oxidation and microbial growth. Various Biomatrixa® products and their uses include:

- A. DNA**Gard**®Blood for the collection and stabilization of DNA in whole blood for a period of 14months.
- B. RNA**Gard**®Blood system technology, which is designed for the collection, preservation and purification of RNA from whole blood biospecimens. Preservation is effective for 14 days at RT and 1 month at 4°C.
- C. DNA**Gard**®Saliva is also designed for collection, preservation and shipping of saliva biospecimens for DNA isolation and analysis. The preservation period is 2 years at RT.
- D. DNA**Stable**® and/or **Plus** is specifically for the protection of purified DNA from degradation for storage at RT. Storage can be either in liquid or dried down state. This long term storage of DNA has been demonstrated for 30years accelerated aging and approx. 4years real time.
- E. RNA **Stable**® protects purified RNA from degradation for storage at RT for 12years accelerated aging and 2.5years real time.
- F. DNA **Stable Blood** protect DNA in whole blood and buffy coat from degradation for storage at RT. DNA is preserved at ambient temperature for at least 12 years.
- G. DNA**Gard**® **tissues and cells** also stabilize DNA in human cells and tissues at RT for 6 months.

Most of these products have been tested via accelerated aging which is based on the Arrhenius equation which states that ‘reaction rates double with every 10°C increase in temperature. For example, a biospecimen left at ambient RT (15-25°C) for 5 years would have the same level of degradation as a biospecimen placed at 50°C for 37.5 weeks. The



application of the Arrhenius equation enables different industries to accurately accelerate product aging and support their shelf life claims (biomatrica. Inc n.d.)

### 1.8.2. GenVault's GenTegra

Another RT storage alternative is GenVault's GenTegra DNA® which is an inorganic mineral matrix with oxidation protection and antimicrobial activity for storage of purified DNA at RT. GenTegra DNA® is supplied as a transparent coating at the bottom of each GenTegra DNA tube. Purified DNA can be added to the GenTegra tube and dried down in a laminar flow hood or GenVault's FastDryer, a boxed enclosure with built-in fans. Recovery of DNA also occurs with the addition of water (Wan *et al.* 2010). GenTegra RNA is also available and stabilises RNA biospecimens at ambient temperatures in a dry state.

A study done by Wan *et al.* (2010) compared the integrity and quality of DNA stored at RT using both the Biomatrix's DNA Biospecimen Matrix and GenVault's GenTegra DNA technologies against DNA stored in a  $-20^{\circ}\text{C}$  freezer by performing downstream testing with short range PCR, long range PCR, DNA sequencing, and SNP microarrays. They also tested Biomatrix's RNASTable product for its ability to preserve RNA at RT for use in a quantitative reverse transcription PCR assay. Human genomic DNA from 8 different whole blood biospecimens was extracted according to the manufacturer's instructions for a commercial extraction kit. To assess DNA quality, biospecimens were respectively stored for 3 weeks at RT with Biospecimen Matrix and GenTegra and at  $-20^{\circ}\text{C}$ . Subsequently the integrity was checked on a 2% agarose gel to compare band intensity and size. The results showed that RT stored DNA did not degrade and remained in good condition compared to the frozen controls. In addition, the DNA yield and DNA concentration was also measured before and after RT storage. The median percent DNA recovery of BiospecimenMatrix and GenTegra stored biospecimens was excellent at 103% and 116%, respectively. The authors observed that some biospecimens had greater than 100% recovery rate and ascribed this to variances in Nanodrop measurement. They also noted that RT storage did not alter the A260/A280 ratio, but lowered the A260/A230 ratio which most likely could be explained by the fact that both Biomatrix's and GenVault's DNA-preserving compounds show strong absorbance at the 230 nm wavelength, but minimal absorbance at 260 nm and 280 nm. Therefore, the decreased A260/A230 reflects a spectrophotometric property of the inorganic preservative compounds rather than unknown contaminants. Likewise short range and long range PCR for RT storage showed

comparable band intensity and size with the frozen controls. In addition, DNA sequences obtained from short range PCR using BiospecimenMatrix and GenTegra stored DNA were compared to frozen control DNA and the traces of the RT stored biospecimens showed clear peaks with very low background noise. For the RNAstable, total RNA was extracted from skin tissue biospecimens using a ProScientific homogenizer and QiagenRNeasy extraction kit. RNA was quantified and 500 ng of RNA was aliquoted into the Biomatrix RNAstable tubes and the remaining RNA was stored at  $-80^{\circ}\text{C}$ . After 11 days of Biomatrix-based RT storage, RNA quality and yield of 3 biospecimens was compared to  $-80^{\circ}\text{C}$  frozen controls using an Agilent 2100 bioanalyzer. As compared to pre-stored RNA biospecimens, frozen and Biomatrix stored biospecimens had similar RNA Integrity Number (RIN) values, indicative of high quality RNA. This study showed that NA integrity can be maintained at RT for 3weeks. However, it failed to show long term stability of biospecimens as mentioned by manufacturers because biospecimens were only preserved for 3weeks. Thus, to determine long term storage potential, stability studies with long stabilization time should be explored.

### **1.8.3. DNAGenotek HemaGene buffy coat DNA**

HemaGene buffy coat DNA (HG-BCD) by DNAGenotek is another RT stabilizer solution that stabilizes high molecular weight DNA in buffy coat at ambient temperature. This technology offers reliable, RT preservation of DNA in buffy coat biospecimens for the recovery of high molecular weight DNA. A 0.5 mL buffy coat biospecimen stored in HG-BCD can withstand multiple freeze-thaw cycles with minimal DNA loss and no degradation compared to the substantial DNA loss incurred after multiple freeze-thaw cycles of an unprotected buffy coat biospecimens (Bouevitch *et al.* 2013). Other products by DNAGenotek are the Oragene DNA and RNA self-collection saliva kits. Numerous studies confirms that DNA extracted from Oragene saliva biospecimens result in DNA of high integrity suitable for performing downstream analysis such as PCR, SNP, genotyping and next generation sequencing (NGS). A study conducted in 2010 by Bahlo, M. *et al.* stated “...saliva collected using the Oragene kit provides good-quality genomic DNA ... comparable to blood as a template for SNP genotyping on the Illumina platform (Bahlo *et al.* 2010) Similarly, another study by Nunes *et al.* showed that an 8month saliva biospecimen stored at RT in Oragene solution does not affect DNA quantity or quality (Nunes *et al.* 2012). Saliva biospecimens for this study were collected with an Oragene™ DNA Self-Collection Kit from 4,110 subjects aged 14–15 years. The biospecimens were

processed in two aliquots with an 8-month interval between them. Quantitative and qualitative evaluations were carried out in 20% of the biospecimens by spectrophotometry and genotyping and descriptive analyses and paired t-tests were performed. The mean volume of saliva collected was 2.2 mL per subject, yielding on average 184.8 µg DNA per kit. Most biospecimen showed a Ratio of OD differences (RAT) between 1.6 and 1.8 in the qualitative evaluation. The evaluation of DNA quality by TaqMan®, High Resolution Melting (HRM), and restriction fragment length polymorphism-PCR (RFLP-PCR) showed a rate of success of up to 98% of the biospecimens. The biospecimen store time did not reduce either the quantity or quality of DNA extracted with the Oragene kit.

#### **1.8.4. Imagene DNAsell® and RNAsell®**

DNAsell® and RNAsell® by Imagene are minicapsules that preserve DNA and RNA from their main degradation factors (water, oxygen, and light) by maintaining an anhydrous and anoxic atmosphere in a hermetic manner. The minicapsules consists of a small glass vials fitted in stainless-steel, laser-sealed capsules. A study done which included analysis of the effect of accelerated aging by using a high temperature (76°C) at 50% relative humidity with biospecimens stored in DNAsells® showed no detectable DNA degradation in biospecimens stored at RT for 18 months. PCR experiments, pulsed field gel-electrophoresis, and RFLP-PCR analyses also demonstrated that the protective properties of DNAsells® are not affected by storage under extreme conditions (76°C, 50% humidity) for 30hours, guaranteeing 100 years without DNA biospecimen degradation(Clermont *et al.* 2014).

#### **1.8.5. Urine stabilization via Norgen Biotek Corp**

Urine collection and processing brings its own challenges as it has a high degree of variability with regards to volume, protein concentration, total protein excreted, pH (ranges from 4 to 8), as well as variability in urine components due to age, health, diet, or other factors such as proteolysis and degradation of collected urine biospecimens upon storage(Thomas *et al.* 2010).With regards to urine stabilization, the Norgen Biotek Corp urine preservative tubes are designed for the rapid preservation of DNA, RNA, microRNA and proteins from fresh urine biospecimens. The urine preservative prevents the growth of Gram-negative and Gram-positive bacteria and fungi, and also inactivates viruses allowing

the resulting non-infectious biospecimens to be handled and shipped safely. Moreover, the buffer preserves exfoliated cells, bacterial cells, and viruses without lysing them. In addition, the urine preservative eliminates the need to immediately process or freeze biospecimens and allows the biospecimens to be shipped to centralized testing facilities at ambient temperatures thus reducing the challenges associated with CCM. The components of the urine preservative allow biospecimens to be stored for over 2 years at RT with no detected degradation of urine DNA, RNA or proteins (Abdalla et al. n.d.). Norgen Biotek also provides kits for DNA extraction from urine. In a study done to isolate DNA from urine using the DNA Isolation Kit from Norgen Biotek, the authors demonstrated that the kit was able to isolate high quality DNA that was free from contaminants using small volumes of urine (Abdalla et al. n.d.). The kit provides a fast and simple procedure for isolating both species of DNA from 2 mL of urine. The kit is based on spin column chromatography, using Norgen's proprietary resin as the separation matrix. Preparation time for a single biospecimen is stated as less than 90 minutes, and purified biospecimens can be used in various downstream applications including PCR. The kit was evaluated based on these claims, in order to see if it provides a good alternative to the traditional methods of DNA isolation from urine. DNA was isolated from two different biospecimens of human male urine using Norgen's Urine DNA Isolation Kit as per the provided protocol. The procedure was also performed in order to isolate DNA from smaller volumes of urine. Using a human male urine biospecimen, 25  $\mu$ L, 50  $\mu$ L, 100  $\mu$ L, 250  $\mu$ L, 500  $\mu$ L, 750  $\mu$ L and 1 mL of urine were used for the input. Forty percent of each elution was then run on a 2% agarose gel in TAE buffer. Three different pictures of the gel were taken, corresponding to a running time of 5 minutes, 10 minutes and 15 minutes which showed that the kit is indeed isolating both the higher MW DNA (greater than 1 kb in size) and the lower MW DNA (150 – 250 bp). To determine DNA quality, the DNA was used as a template for PCR reactions. Y-chromosome-specific sequences were targeted using a nested-PCR procedure. The result indicates that the isolated DNA is of a high quality, and can be used in downstream applications involved in diagnostics, including PCR. Furthermore, these results indicate that sufficient amounts of DNA are isolated from 2 mL of urine for downstream applications.

#### **1.8.6. Qiagen PreAnalytix PaxGene products**

The PaxGene DNA and RNA tubes by PreAnalytix a Qiagen/BD company is one of the most used RT solutions for the isolation of genomic DNA and RNA from whole blood. DNA

in blood tubes are stabilised for 14days and 3days for RNA. Regarding RTS technologies for tissue specimens the range of products is limited. Interestingly, the PAXgene® Tissue Systems (PreAnalytix, Qiagen) provide a formalin-free alternative for the simultaneous preservation of histomorphology and stabilization of biomolecules and allow for the isolation of high-quality DNA, RNA, miRNA, proteins and phosphoproteins from the same sample (Ergin et al. 2010) Traditional tissue fixation via formalin has limited use for molecular analysis as it normally preserves histomorphology but does not stabilize biomolecules. Likewise RNAlater® stabilizes biomolecules in tissue but does not preserve the histomorphology. Likewise the DNAGard® tissues and cells mentioned above (section 1.7.1) stabilize DNA within cells and tissue but do not preserve the histomorphology. Therefore, the option of having the PAXgene® Tissue Systems would allow for the collection, fixation, and stabilization of the tissue which can then be processed and embedded in paraffin similarly to formalin-fixed tissue without destructive cross-linking and degradation normally found in formalin-fixed tissues. This method does not introduce molecular modifications that can inhibit sensitive downstream applications such as quantitative PCR or qPCR. Using this system, tissue samples are fixed at RT (15–25°C) within 2 hours depending on tissue type and size and can be embedded and processed in paraffin. The tissue also remains stable for days at RT, for weeks when refrigerated (2–8°C), and for years when frozen at –20°C or –80°C (Loibner et al. 2016). The biospecimens fixed and preserved using this system has been tested and validated by the manufacturer to be suitable for a range of analysis, including histochemical staining, immunohistochemistry (IHC), in situ hybridization, gene expression analysis, genetic analysis, sequencing, and protein and phosphoprotein analysis. While this product has been used mostly in a research based capacity, literature does exist for the use and testing in a diagnostic setting as well (Loibner et al. 2016). Belloni et al. 2013 performed a morphological and molecular comparative study using this system; their data suggests advantages however, they still were cautious justifying the substitution of formalin fixation in a routine pathology laboratory (Belloni et al., 2013).

### **1.8.7. Dried Blood Spots (DBS)**

Paper cards are also a popular method and gold standard of storing blood due to their ease of use and long term stability at RT. Examples used in the field, include the Whatman FTA/FTA Elute cards by GE Healthcare life sciences which provides simple solutions to collect, preserve, and purify biological biospecimens at RT for downstream DNA analysis.

FTA cards requires only a small amount of biospecimen and can be used even in the most remote settings as it does not require much expertise and biospecimens can be transported via normal postal services once dried as this are classified as an exempt category for shipment purposes. Genomic DNA stored on FTA Cards at RT for more than 17.5 years has been successfully amplified by PCR(Healthcare n.d.).Furthermore, an assessment of DNA extracted from FTA gene cards for use in the illumina Select beadchip which requires unbound, relatively intact (fragment sizes  $\geq 2$  kb), and high-quality DNA was assessed in a study by McClure et al which indicated that DNA extracted from FTA cards produce results comparable to those obtained from DNA extracted from whole blood(McClure et al. 2009).

Based on the brief summary on the various RTS products available it is clear that RT storage would offer an alternative to low temperature biospecimens preservation for blood and NA that can be utilized by biobanks and or diagnostic/research laboratories to reduce freezer storage costs while maintaining the quality of the biospecimens.

## **1.9 The present study**

For the present study, the aim is to investigate sample collection and storage approaches that are low cost or/and evaluate ambient temperature stabilization products for nucleic acids (DNA and RNA), blood and tissue and urine. For the purpose of this study and due to time restraints only Biomatrix solutions (DNAGard, RNAGard,) along with novel technology, DNAGenotek Hemagene BuffyCoat solution (DNA in buffy coats) and Norgen's Biotek Corp's Urine Preservative tubes would be compared and validated to known established and implemented ambient storage products such as the PaxGene range for nucleic acids in whole blood and BD blood collection tubes for collection, transport and storage of human samples at room temperature.

Thus to achieve the aim of this study, the objectives were:

- 1). Courier companies namely, Marken, DHL and world courier were evaluated to determine the supply chain cost analysis and ascertain the most cost effective transport solution between RT and cold chain logistics.
- 2). To test for genomic DNA/RNA Stabilization with and without a stabilizer under different temperature conditions over different time periods and to confirm genomic integrity of

stored DNA/RNA at RT by further downstream applications by polymerase chain reaction (PCR), Q-PCR and sequencing analysis.

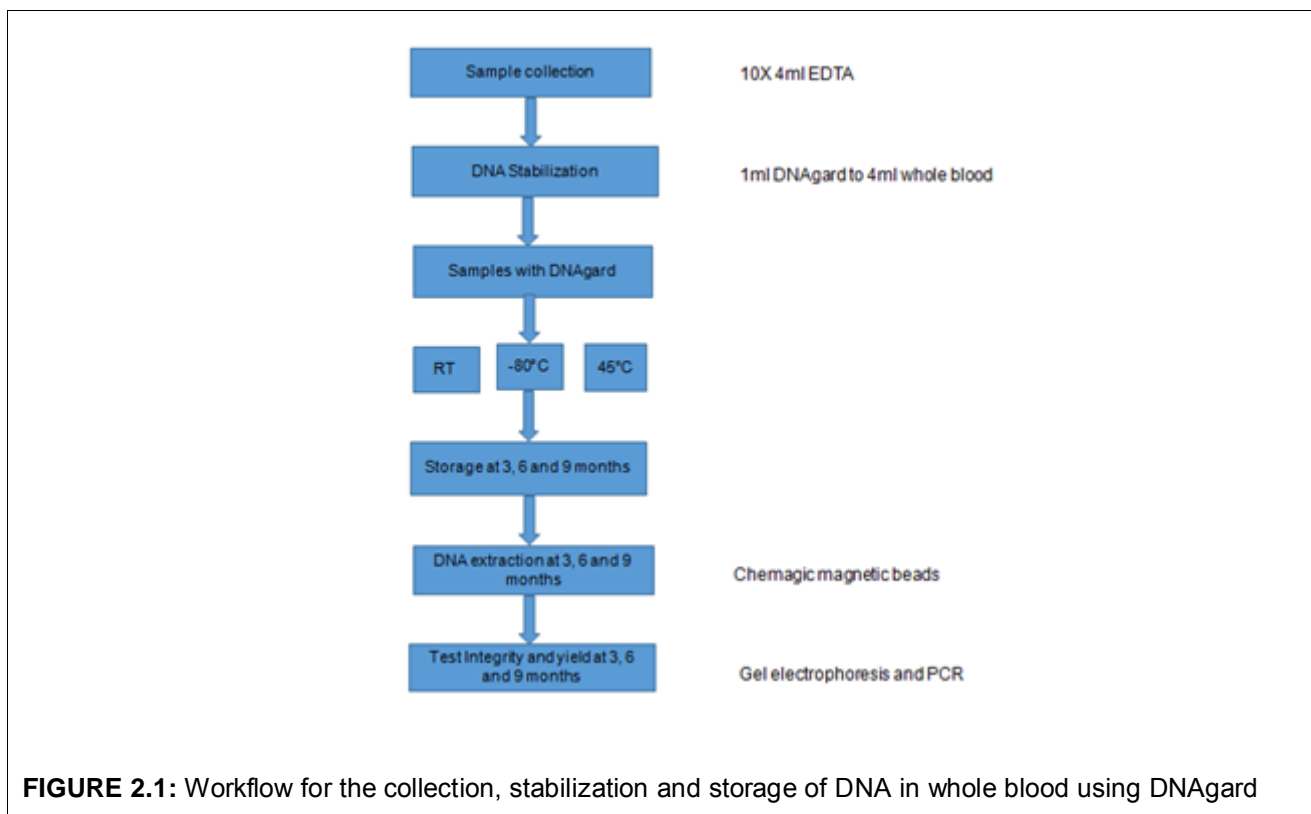
## **Chapter 2 Materials and Methods**

The present study forms part of a larger study of which ethical approval has been given by the Health Research Ethics Committee (HREC), Faculty of Medicine and Health Sciences (FMHS) at Stellenbosch University Ethics Reference S16/02/017. All participants who took part were advised on the study and informed consent was given willingly to allow for sample collection, processing, storage and transportation if required.

### **2.1 Sample stabilization and nucleic acid isolation**

#### **2.1.1 Stabilization of DNA in whole blood (DNAgard) and Buffy coat (HEMAGene BUFFY COAT)**

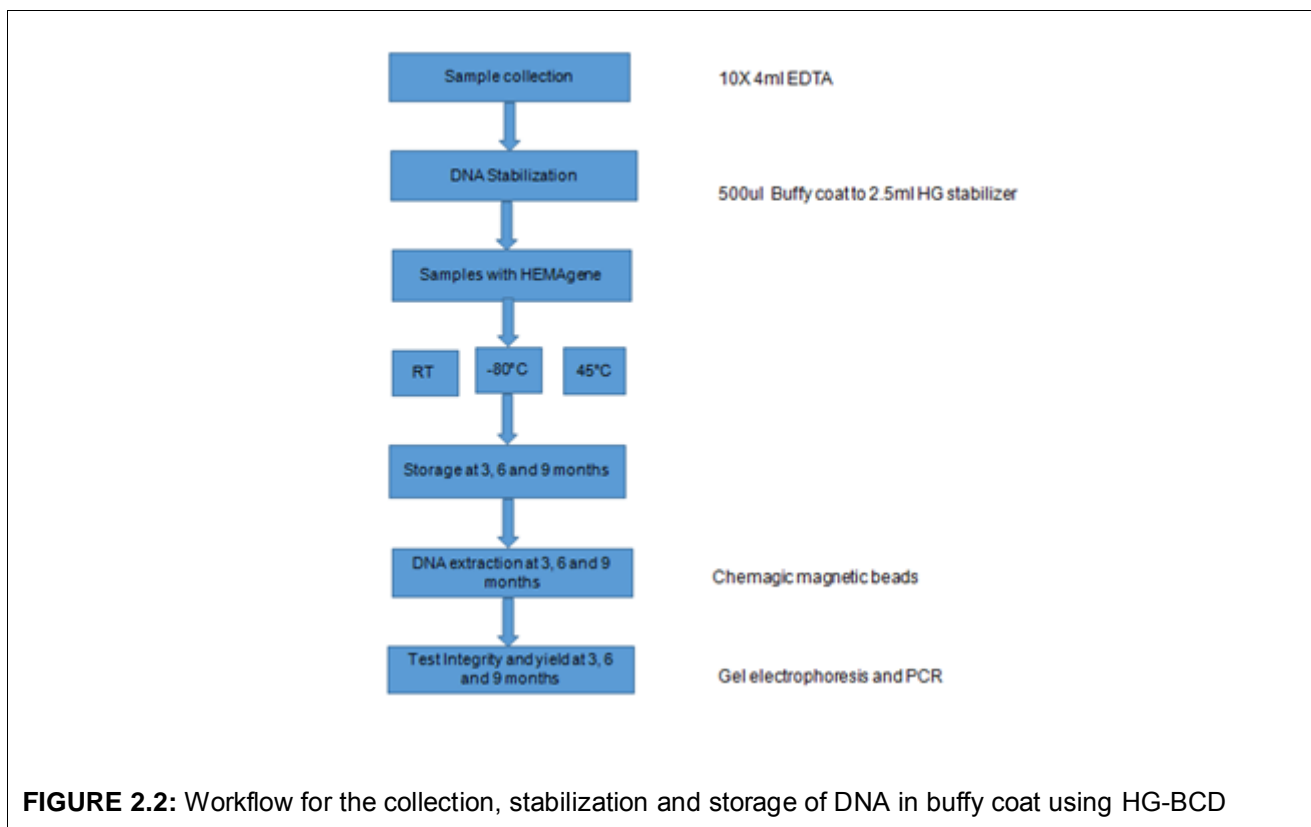
For the stabilization of DNA in whole blood, 4ml of whole blood samples was drawn from each participant in ethylene-diamine-tetra-acetic acid (EDTA) tubes (Vacutainer, RSA). See Figure 2.1 for the workflow for the collection, stabilization and storage of DNA in whole blood using DNAgard. The 4ml of collected blood sample was transferred into a 15ml Greiner tube containing 1ml of DNAgard stabilizer (Biomatrix, San Diego, CA) and mixed well by vortexing (Rotamixer deluxe Hook and Tucker 220-240v). Each tube was subsequently aliquoted into 500 $\mu$ l in nine 1.5ml sterile and labelled tubes. Three aliquots were stored at room temperature (RT), 3 aliquots at 45°C and the last 3 aliquots at -80°C for storage at 3, 6 and 9 months respectively. This was done for 10 whole blood samples.



For the stabilization of DNA in buffy coat, whole blood EDTA samples were obtained from 12 voluntary participants in 4ml tubes (Vacutainer, RSA). Samples were rocked gently to mix and centrifuged at 2500 x g for 15 minutes at room temperature (MSE MISTRAL 1000, MSE Scientific, Leicestershire, England). After centrifugation, 3 different fractions were distinguishable: the upper clear layer is plasma, the intermediate layer is buffy coat containing leukocytes and the bottom layer contains concentrated red cells. The top layer of plasma was removed with a Pasteur pipette and discarded leaving 1ml of plasma above the buffy coat; 500µl of the buffy coat was transferred to a 15ml Greiner tube containing 2.5ml of HEMAgene BUFFY COAT stabilizer (DNA Genotek, Ontario, Canada). This was mixed gently and aliquots of 300µl was made into nine 1.5ml labelled tubes for each sample. Three tubes were stored at room temperature, another 3 tubes at 45°C and the last 3 tubes at -80° C for 3, 6 and 9months respectively. This was done for 10 samples.

Two samples were set up as control for both whole blood and buffy coat without stabilizers at the same temperature and time frame as those with stabilizers. See figure 2.2 for the workflow for the collection, stabilization and storage of DNA in buffycoat using HEMAgene BUFFY COAT stabilizer.





At the end of each time frame i.e. 3, 6 and 9 months DNA was extracted using 200µl of the stored samples for both whole blood and buffy using the Chemagic DNA Blood Kit(10k), a magnetic beads based extraction method according to the manufacturer's instructions (Perkin Elmer, Baesweiler, Germany). Briefly, a heating block (ACCUBLOCK™ MINI, labnet Inc.) was heated to 55°C. A 200µl aliquot of whole blood was transferred into a sterile 1.5ml eppendorf centrifuge tube and lysis buffer 1 (125µl) was added to it. The mixture was incubated for 5 minutes at room temperature. Thereafter, 14µl of Magnetic beads, premixed with 360µl of binding buffer 2 was added and mixed well and incubated at room temperature for 5 minutes. Magnetic beads/DNA complex mixture was separated by placing it on a 2x12 Chemagic stand for 2 minutes. The supernatant was removed with the aid of a P1000 pipette and discarded. The magnetic beads pellet was then thoroughly resuspended by adding 600µl of wash buffer 3. The magnetic beads/DNA complex was separated by placing it on the magnetic stand for 1 minute and the supernatant discarded. A repeat washing step using wash buffer 4 was done and the tube left in the stand after discarding the supernatant. With the beads attracted to the magnetic stand, 1ml of wash buffer 5 was gently added without resuspending the pellet for 90 seconds and the supernatant discarded. The tube was then removed from the stand and 100µl of elution buffer 6 was added and the magnetic beads/DNA complex resuspended by gentle

pipetting. The tube was incubated at 55°C for 8 minutes with gentle agitation. The tube was then placed in the Chemagic stand for 2 minutes until all the magnetic beads had separated from the eluate. The eluate was then transferred into a sterile 1.5ml tube.

For the extraction of DNA from buffy coat, the same protocol was used as above using 200µl of buffy coat.

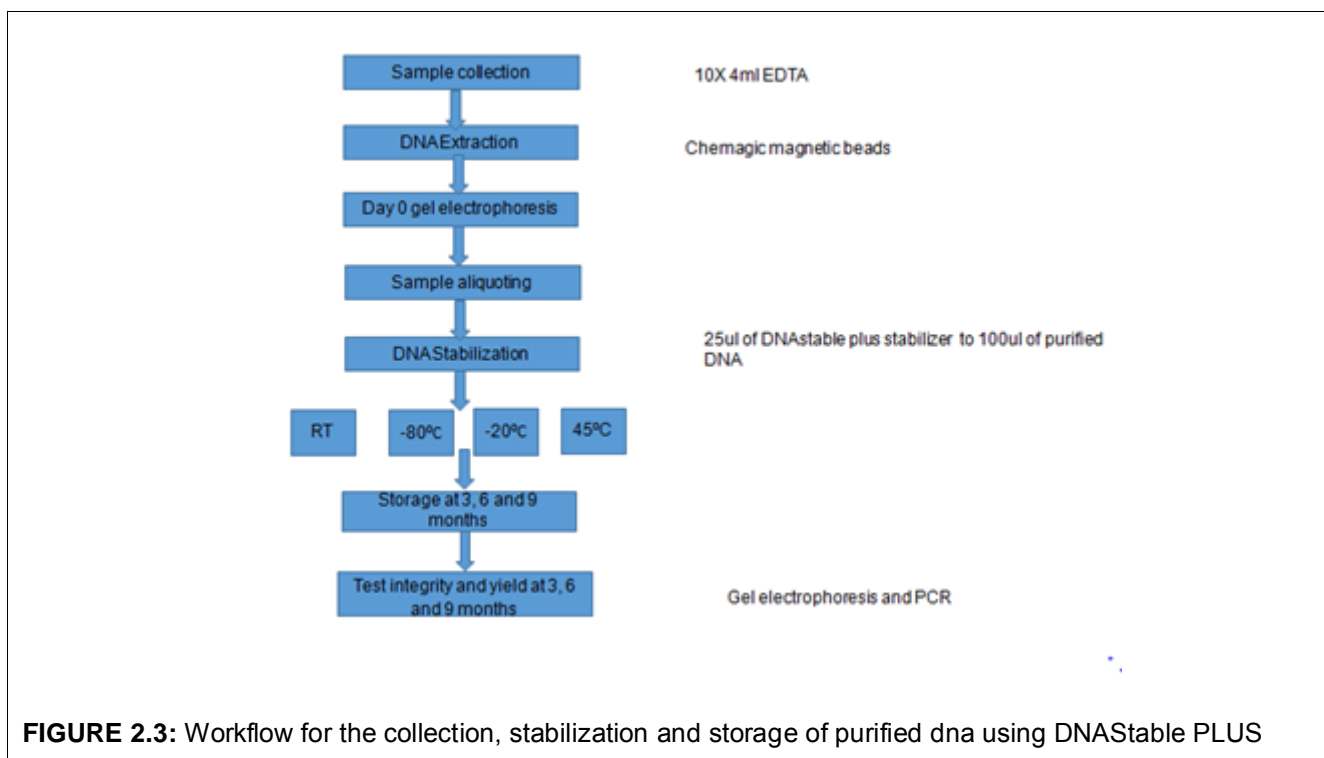
### **2.1.2 Stabilization of purified DNA (DNAstable Plus Stabilizer)**

Whole blood EDTA samples were drawn from 12 voluntary participants in 8ml EDTA tubes. See figure 2.3 for the workflow for the collection, stabilization and storage of purified DNA in DNAstablePLUS.

Each sample was transferred into a labelled 50ml Greiner tube. Human genomic DNA was extracted from all the 12 samples using Chemagic DNA Blood Kit (10k) a magnetic beads extraction method (Perkin Elmer, Baesweiler, Germany) according to the manufacturer's instructions. Briefly, 8ml of whole blood sample was vortexed (Rotamixer deluxe, Hook and Tucker 220-240V) and transferred into a 50ml Greiner tube. Thereafter, 50µl of protease and 9ml of lysis buffer were added to the tube and vortexed for 30 seconds. The mixture was then incubated at room temperature for 5 minutes. Following the incubation step, 1.2ml of well resuspended magnetic beads and 29ml of binding buffer 2 was added. This was mixed thoroughly by pipetting and then incubated for 5 minutes. The tube was subsequently placed in the left position of a chemagic stand 50k for 4 minutes to separate all the beads. The supernatant was discarded and the tube removed from the stand. Thereafter, 15ml of wash buffer 3 was added to the tube and vortexed vigorously for 1 minute and incubated for 2 minutes, the tube was then placed in the chemagic stand to separate all the beads and the supernatant discarded. The wash, incubation and separation steps were repeated using 15ml of wash buffer 4 and 5 respectively. The tube was left in the stand after removing all traces of wash buffer 5. While in the stand, 40ml of wash buffer 6 was added gently without resuspending the pellet and incubated for 1 minute. The supernatant was discarded and the tube removed from the stand. With the tube off the magnetic stand, 1ml of elution buffer 7 was added and vortexed vigorously for 20 seconds. The suspension was then incubated at room temperature with gentle agitation for 10 minutes. After the incubation period the tube was placed in the right position of the

chemagic stand for 3 minutes to separate the beads. The eluate containing purified DNA was then carefully removed.

The DNA yield and purity was determined using the BioDrop  $\mu$ LITE (ISOGEN Life Sciences, Netherlands) and a 0.8% Agarose gel was ran in 1x TBE to obtain a baseline indication of DNA quality prior to stabilization and storage. Aliquots of 100 $\mu$ l of the extracted DNA was made into twelve 1.5ml sterile tubes with 3 tubes each stored at RT, -20°C, -80°C and 45°C at 3, 6 and 9 months respectively. Twenty five microliters (25 $\mu$ l) of DNASTable Plus Stabilizer (Biomatrix, San Diego, CA) was added to each tube as the ratio of DNA to stabilizer is 1:4 except for aliquots made from sample 11 and 12 which were control sample stored without stabilizers. Samples were mixed thoroughly by gentle pipetting while avoiding forming air bubbles and stored at the respective temperatures and time frame.



### 2.1.3 Stabilization of DNA in urine (Norgen Biotek Corp)

Morning midstream urine samples were collected from 8 healthy male and female voluntary participants in a sterile urine cup. Thirty millilitres (30ml) of urine from each volunteer was transferred into a 50ml Norgen urine tube (Norgen Biotek Corp, Ontario,

Canada) containing an orange dried preservative. The tube was inverted several times until the orange pellet had completely dissolved. This was done for 6 samples the remaining 2 samples were left without preservative to serve as control. Aliquots of 3ml were made for each sample into six 15ml Greiner tubes. Three were labelled for RT storage at 3, 6, and 9 months and the remaining 3 for storage at -80°C for the same time frame. At the end of each time frame, urine DNA was extracted using the Norgen urine DNA isolation kit #18100 (Norgen Biotek Corp, Ontario, Canada) according to the manufacturer's instructions. Briefly, a heating block (ACCUBLOCK™ MINI, labnet Inc.) was heated to 60°C. In a 15ml Greiner tube, 1.75ml of urine and 250µl of binding solution K was added and mixed well by inverting a few times. Using a P1000 pipette, 650µl of the urine sample was transferred onto a spin column and centrifuged for 1 minute at 6700 x g, the flow through was discarded and the spin column and its collection tube reassembled. This step was repeated until all the urine sample had passed through the column. Thereafter, 35µl of proteinase K and 35µl of Pronase was added to the column and centrifuged for 1 minute at 6700 x g. The collection tube and the column were then incubated on the preheated heating block for 20 minutes at 60°C. After the 20 minutes incubation, 450µl of Solution WN was added to the lysate in the collection tube, mixed well by pipetting and the entire contents transferred onto the spin column. This was centrifuged for 1 minute at 6700 x g and the flow through discarded. To the column, 450µl of Wash Solution D was added and centrifuged for 1 minute at 14,000 x g the flow through was discarded and 450µl of Wash Solution B was added to the column and centrifuged for 1 minute also at 14,000 x g and the flow through discarded. To the reassembled column, 450µl of 99% ethanol was added and centrifuged for 1 minute at 14,000 x g the flow through was discarded and the wash step with ethanol repeated. The spin column was then spun empty for 2 minutes at 14,000 x g and the collection tube discarded. The spin column was transferred to a fresh elution tube and 50µl of Elution buffer B added to the column. This was centrifuged for 200 x g for 2 minutes followed by 2 minutes at 5,800 x g. The spin column was transferred to a second elution tube to which 50µl of Elution Buffer B was added and centrifuged for 2 minutes at 5,800 x g as a final elution step.

#### **2.1.4 Stabilization of DNA in cultured cells (DNAgard Tissue)**

Cultured HEK293T cells were assessed for nutrient utilization and bacterial/fungal contamination prior to tryptinization. Media was clear showing nutrients being used up and

cells ready for sub culturing and lack of turbidity ruled out contamination. After addition of trypsin cells detached easily indicating healthy cells. A cell count was performed using TC20™ Automated cell count (BIO RAD) to obtain a cell count of  $1 \times 10^6$  cells/ml for 100µl of DNAgard Tissue and cells stabilizer per aliquot required. Based on the cell count 10 aliquots were made five for room temperature storage and 5 for storage in liquid nitrogen. For each storage location 2 were protected with 100µl of DNAgard stabilizer, 2 with cryopreserving DMSO and 1 unprotected with water. All samples were stored for a period of 1 month. At the end of the storage period, DNA was isolated using the QIAGEN QIAamp blood mini kit according to the manufacturer's instructions. Briefly, all samples were brought to room temperature. A heating block was set at a temperature of 56°C. Samples were resuspended in phosphate buffered saline (PBS) to bring the total volume to 200µl prior to extraction. Twenty microliters of QIAGEN protease was added into a 1.5ml tube. To the tube, 200µl of cells in PBS and 200µl of Buffer AL were added and pulse vortexed for 15 seconds. The tubes were then incubated at 56°C on a heating block (ACCUBLOCK™ MINI, labnet Inc.) for 10minutes. Thereafter, 200µl of ethanol (96-100%) was added and vortexed for 15seconds. The mixture was carefully added to a QIAamp Mini spin column and centrifuged at 8000rpm for 1 minute. The spin column was transferred to a new 2ml tube and 500µl of Buffer AW1 was added and centrifuged for 1 minute at 8000rpm a second wash with buffer AW2 using same volume but at 14,000rpm for 3min. A full speed spin was done using an empty 2ml tube prior to sample elution in a clean 1.5ml microcentrifuge tube using 200µl of Buffer AE.

### **2.1.5 Stabilization of RNA in whole blood**

Whole blood samples from 5 voluntary participants were drawn in RNAgard (Biomatrix, San Diego, CA) and PAXgene RNA tubes (PreAnalytiX, Hombrechtikon, Switzerland) prefilled with stabilizers. To each RNA tube 2.5ml of blood was added. It was mixed by inverting the tubes 5-7 times. Aliquots of 1.5ml for each sample were made into 6 labelled 2ml sterile tubes. Three tubes were stored at RT and 3 at -80°C for 3, 7 and 14 days. At the end of each time frame RNA was extracted from PAXgene and RNAgard tubes using the QIAGEN RNA blood mini kit according to the manufacturer's instructions. Briefly, 5ml of buffer AL was added to 1ml of blood in a 15ml Greiner tube and incubated on ice for 15minutes. Samples were vortexed twice briefly during the incubation period. It was spun at 400 x g (MISTRAL 1000, MSE Scientific, Leicestershire, England) and supernatant discarded. The cell pellet was resuspended in 10ml of buffer EL and centrifuged for 10min

at 400 x g. The supernatant was discarded and 600µl of buffer RLT was added and the lysate transferred to a QIAshredder spin column and centrifuged for 3 min at maximum speed (Labnet PRISM microcentrifuge). To the homogenised lysate 600µl of 70% ethanol was added and mixed by pipetting and added onto a QIAamp spin column and centrifuged for 15seconds at 8000 x g. The flow through was discarded and the spin column transferred into a new 2ml collection tube and 700µl of buffer RW1 added and centrifuged for 15seconds at 8000 x g. The spin column was transferred to a new 2ml tube and 500µl of buffer RPE added and centrifuged for 3min at full speed. A full spin in an empty collection tube was done before adding 50ul of RNase-free water for elution in a clean 1.5ml tube.

## **2.2 Nucleic acid quality assessment**

### **2.2.1 DNA quantification using BioDrop µLITE**

All samples were assessed at room temperature. Frozen samples were thawed before analysis. The concentration of the eluted genomic DNA was determined by spectrophotometry with the BioDrop µLITE spectrophotometer (ISOGEN Life Science, Netherlands). The DNA, yield (µg/ml) and purity (absorbance ratio A<sub>260</sub>/A<sub>280</sub>) was determined for all samples, where pure DNA had an A<sub>260</sub>/280 ratio ranging between 1.7-1.9. A ratio of <1.7 is indicative of residual protein, phenol, or other reagent associated with the extraction protocol, where a ratio of >2.0 indicates RNA contamination.

### **2.2.2 DNA and RNA quantification using the Qubit 2.0 fluorometer**

The Qubit 2.0 fluorometer (Invitrogen Life Technologies, Carlsbad, California) was also used to quantify double stranded DNA using the dsDNA BR assay (broad range) and RNA at room temperature for all samples.

### **2.2.3 Agarose gel electrophoresis**

The integrity of all extracted DNA samples were assessed by standard TBE gel electrophoresis on a 0.8% agarose gel (Seakem LE Agarose) in 1x TBE. A 2kb molecular marker was used for DNA gel analysis.

## 2.3 Polymerase chain reaction (PCR)

### 2.3.1 PCR amplification primers and parameters

A standard PCR reaction was performed with different primer sets for the  $\beta$ -globin gene to assess whether the purified DNA were suitable for downstream applications. These primer sets were different PCR fragments of  $\beta$ -globin as shown in Table 2.1. The rationale for these experiments were to determine whether short and longer fragments of the gene amplified successfully to indicate that isolated DNA was still stable and not degrading.

**TABLE 2.1** Primer sequences and annealing temperatures used for the amplification of  $\beta$ -globin gene

Primer set name	Sequence 5'-3'	Product size (bp)	T <sub>m</sub> °C
GH20+PCO4	F- GAAGAGCCAAGGACAGGTAC R -CAACTTCATCCACGTTCCACC	268	55
RS42+KM29	F- GCTCACTCAGTGTGGCAAAG R- GGTTGGCCAATCTACTCCCAGG	536	58
RS40+RS80	F- ATTTTCCCACCCTTAGGCTG R- TGGTAGCTGGATTGTAGCTG	989	55
KM29+RS80	F- GGTTGGCCAATCTACTCCCAGG R- TGGTAGCTGGATTGTAGCTG	1327	57

For the amplification of each DNA sample, 2.5 $\mu$ L of extracted DNA was used as a template in a 25 $\mu$ L reaction mix, which contains the following reagents: 1 $\mu$ L of each primer (1x KAPA Taq Ready Mix PCR Kit (12.5 $\mu$ L) and dH<sub>2</sub>O (8 $\mu$ L) to a total volume of 25 $\mu$ L. Amplification was done in a thermal cycler (Bio-Rad T100 Thermal Cycler, California, USA ) using the following thermal cycle profile: a denaturing step of 95°C for 3 minutes followed by 40 cycles of 95°C for 1 minute, annealing temperature at 55°C for 2 minutes and 72°C for 1 minute. A final extension step was performed for 5 minutes at 72°C.

### 2.3.2 Gel electrophoresis for amplified PCR product

PCR-amplified fragments were separated on a 2% agarose gel for verification of successful amplification.

## 2.4 Quantitative real time Polymerase chain reaction (RT-PCR)

### 2.4.1 cDNA synthesis

Selected RNA samples extracted from whole blood storage at 3, 7 and 14 days were converted to cDNA using the ImProm- II™ Reverse Transcription System (Promega, Madison USA.) according to the manufacturer's instructions. Briefly, target RNA and primer combination and denaturation was done by combining 2µl of target RNA (~200 ng), 2µl of nuclease-free water and 0.5µg of Primer (oligodT) giving a total volume of 5µl. A negative template control of 5µl consisting of 1µl of primer and 4µl of water was also made up. For the reverse transcription, a 15µl mix consisting of 3.7µl of nuclease-free water, 1 x ImProm II, 1X Reaction buffer, 6 mM of magnesium chloride, 2 mM DNTP mix, 10 U of ribonuclease inhibitor and 1U of ImProm II™ Reverse transcriptase. All reactions were performed on ice. The RNA and cDNA 5µl mix was incubated on a heating block set at 70°C for 5minutes and then chilled on ice for another 5minutes. Thereafter, the 15µl reverse transcription mix was added to the RNA mix and annealed at 25°C for 5 minutes. Finally, first-strand synthesis reaction was done at 42°C for 60minutes. Samples were stored at -20°C prior to RT-PCR reaction.

### 2.4.2 Quantitative RT-PCR (qRT-PCR)

The integrity of all isolated RNA was determined by assessing the expression of the housekeeping gene,  $\beta$ -globin on a few stored RNA samples from both PAXgene and RNAgard blood tubes after cDNA synthesis. A standard curve was done on cDNA from both PAXgene and RNAgard ranging in concentrations from 200ng to 0.02 ng of input RNA. The PCR efficiency for each sample was determined using the QuantStudio™ software. For the qRT-PCR, a 20µl reaction was set up in duplicate using the KAPA SYBR FAST qPCR Master Mix (KAPA Biosystems) according to manufacturer's instructions. Briefly, 200 nM of each primer was mixed with 2X KAPA SYBR® FAST qPCR Master Mix (Final concentration 1X). To this mixture, ROX High (final concentration 1X) and 2µl of the input DNA at different concentrations were added. This mixture was made up to 20µl with Nuclease-free water.

Only the shorter fragment of the  $\beta$ -globin (primer set GH20+PCO4) was analysed, as the other primer combinations used produced too large a product for Q-PCR analysis (Table 2.1) The qPCR reactions were performed on the QuantStudio™ 3 systems (Applied



Biosystems) and analysed with the QuantStudio™ Design and Analysis software (Applied Biosystems) using the following parameters: Initial denaturation at 95°C for 10 minutes followed by 40 repeat cycles of 95°C for 15 seconds, 60°C for 15 seconds, and 72°C for 30 seconds. An additional melt curve was added with the following parameters: 95°C for 15 seconds, 60°C for 1 minute followed by a slow incremental increase of 0.1°C until 95°C.

## **2.5 DNA Sequencing**

### **2.5.1 PCR Product purification for sequencing.**

An enzymatic clean up using Exonuclease 1 and Calf Intestinal Alkaline phosphatase (New England Biolabs) was used. A 1 µl mix of the two enzymes was added to 3 µl of PCR product and the reaction was incubated at 37°C for 15 minutes and 80°C for another 15 minutes in a thermal cycler (Bio-Rad T100 Thermal Cycler, California, USA). The shortest (PCO4+GH20) and the longest fragments (KM29+RS80) for the *β-globin* gene was sent for sequencing reaction at the Centre for Analytical Facility (CAF) at Stellenbosch University.

### **2.5.2 Analysis of sequencing reactions:**

Chromatograms of sequenced products were analysed using BioEdit (Tom Hall Ibis Biosciences Carlsbad, CA) to assess the quality of the sequences traces. Sequences were extracted in FASTA format and these were queried against the BLASTn (<https://blast.ncbi.nlm.nih.gov/Blast.cgi?>) database at the National Center for Biotechnology Info (NCBI) (<https://www.ncbi.nlm.nih.gov/>).

## **2.6. Transportation cost analysis**

Briefly, we approached and evaluated various courier companies operating on the continent, including World Courier, Marken and DHL. Companies were evaluated based on their familiarity with the African landscape, their service record, reputation, GxP compliance and whether they provide a fully integrated clinical supply chain service. Both World Courier and Marken are cold chain logistical companies that specifically focus on controlled temperature transportation and are the major courier services used by local clinical trial laboratories and private biobank facilities. These courier services have agents that represent their companies in the majority of the African countries and their staffs are specifically trained in temperature controlled shipping from and to these countries with the

specified, temperature requirements. DHL Express also has a good footprint within Africa and has a Medical Express division that specifically focuses on the transport of biological materials on the continent. The transportation cost analysis was initially done as part of our lab's H3Africa consortium initiative contribution comparing the cost for the movement of samples from 8 African satellite sites to NSB at normal ambient, validated ambient, refrigerated and at normal ambient including the additional costing of a Stabilizer. The cost was based on a 1kg shipment and assuming that it would fit  $\pm 500$  (0.5 and/or 0.75ml) vials and at an exchange rate of R14 to the USA dollar. The data generated would help to inform us on the cost and logistics associated with the movement of biospecimens on a larger scale over a period of 5 years.

## Chapter 3-Results

### 3.1 Stabilization of DNA in whole blood, Buffy coat and Purified DNA

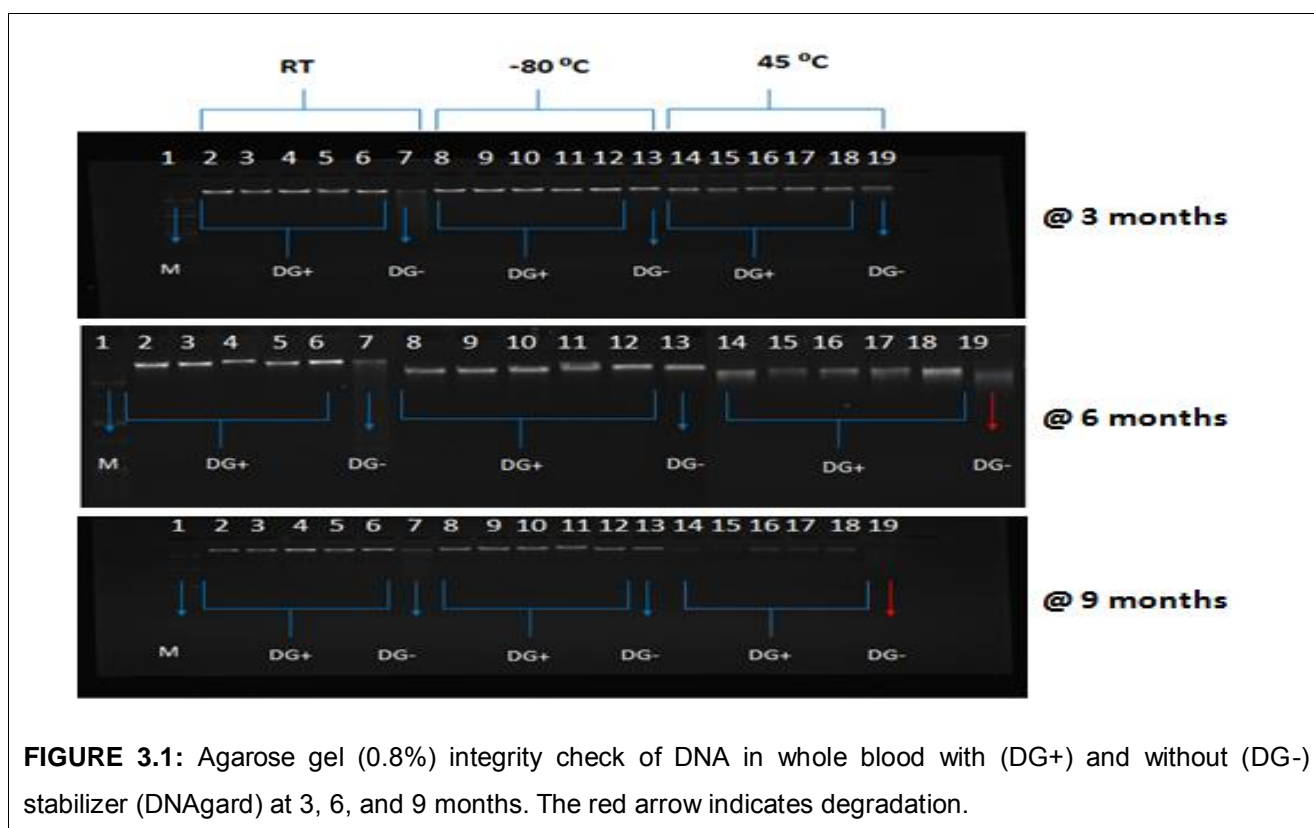
#### 3.1.1 DNA Concentrations of isolated samples

Concentrations of DNA isolated and purified from whole blood (DNAgard) and buffy coat (HEMAgene BUFFY COAT) were determined by the Biodrop and Qubit 2.0 fluorometer after 3, 6 and 9 month storage at different temperatures. Concentrations for all DNA samples isolated and purified are seen in Appendix 1

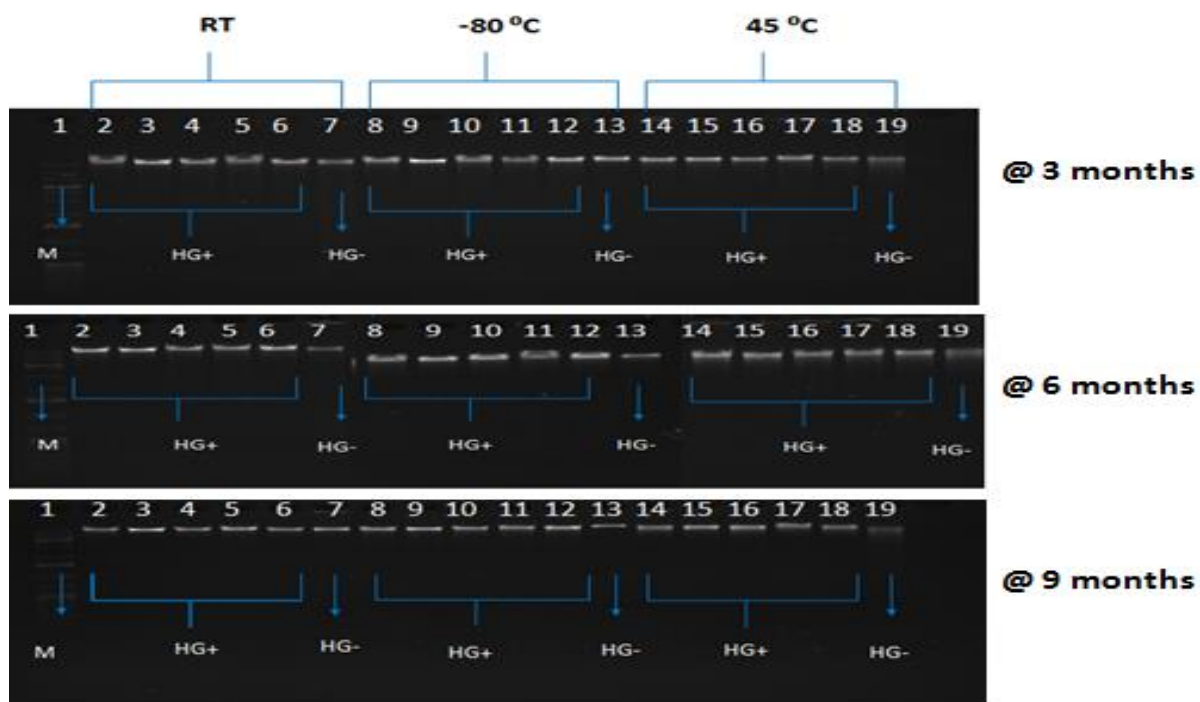
In addition, samples that have been stored for 3 years in HEMAgene Buffy coat stabilizers at RT,  $-80^{\circ}\text{C}$  and  $45^{\circ}\text{C}$  were also processed.

#### 3.1.2 Determination of DNA integrity by agarose gel electrophoresis

To assess DNA integrity, all samples were run on a 0.8% Agarose gel in TBE to compare the band intensity and size. The gel images for whole blood, buffy coat and purified DNA is shown below in Figure 3.1-3.5



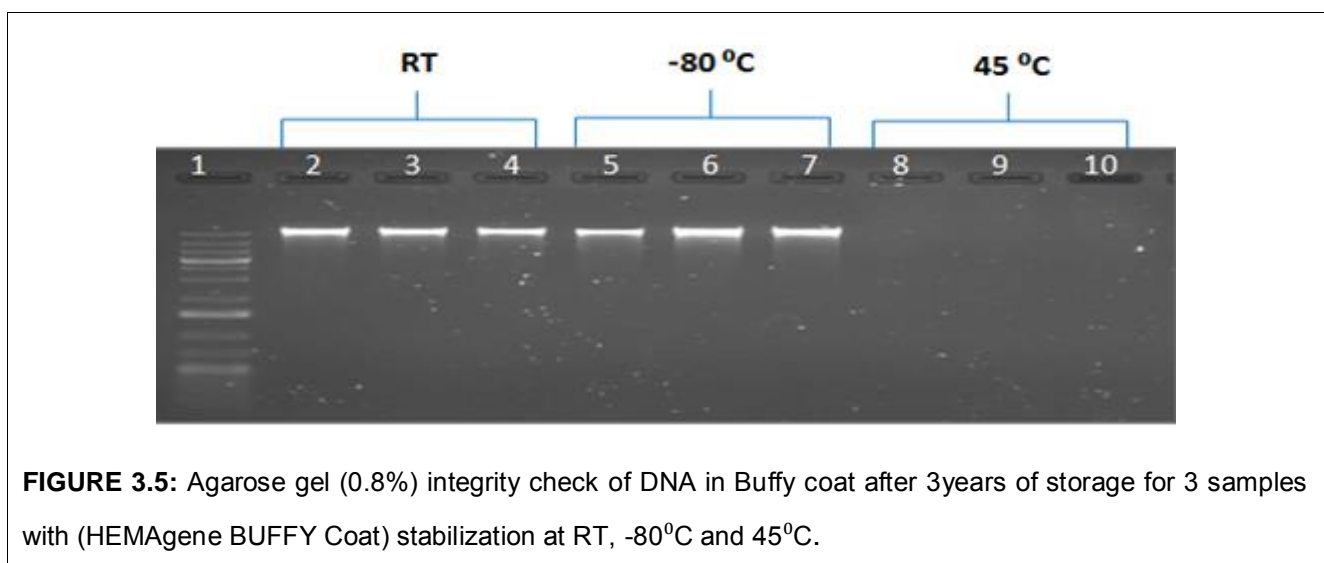
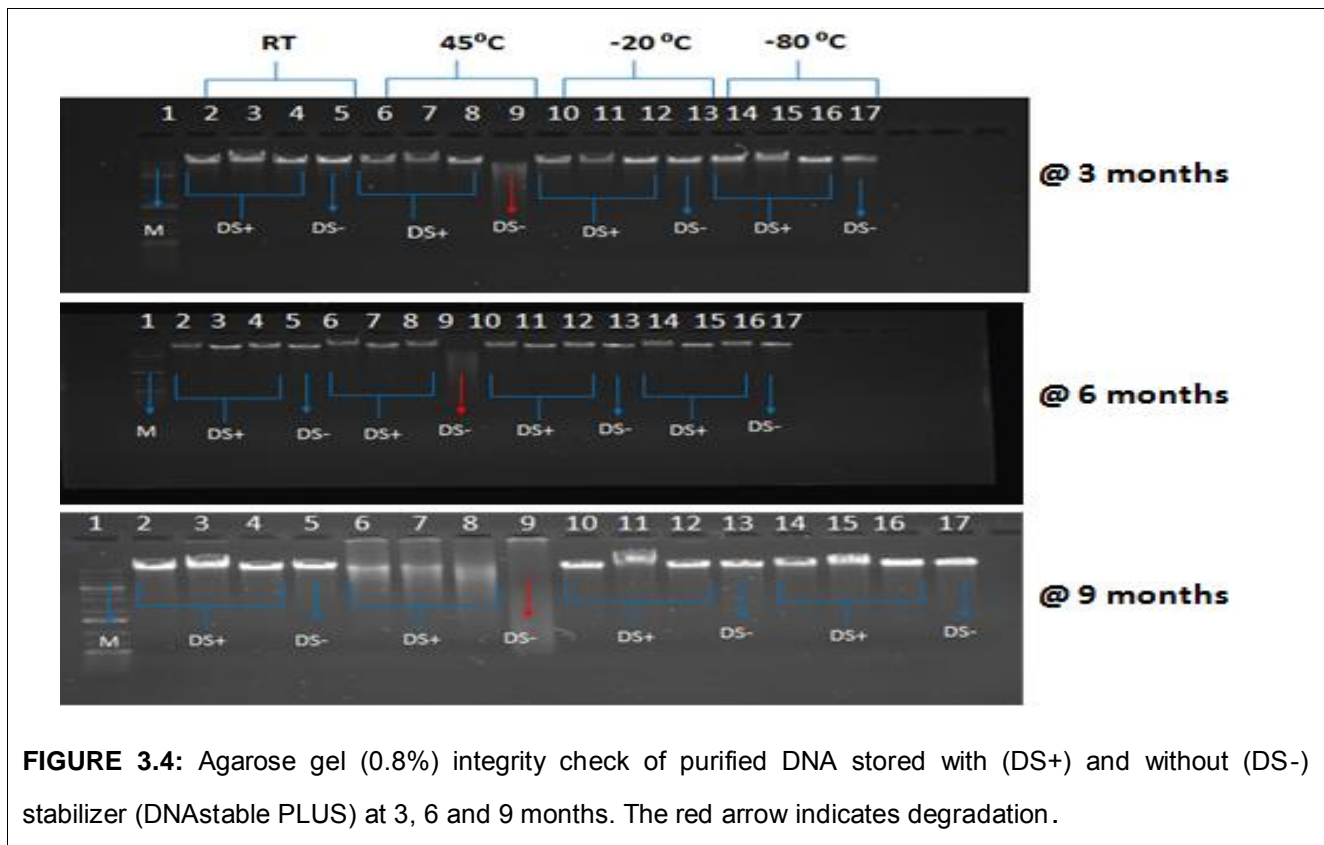
**FIGURE 3.1:** Agarose gel (0.8%) integrity check of DNA in whole blood with (DG+) and without (DG-) stabilizer (DNAgard) at 3, 6, and 9 months. The red arrow indicates degradation.



**FIGURE 3.2:** Agarose gel (0.8%) integrity check of DNA in Buffy coat with (HG+) and without (HG-) stabilizer (HEMAGene BUFFY Coat) at 3, 6, and 9 months.



**FIGURE 3.3:** Agarose gel (0.8%) integrity check of purified DNA samples prior to stabilization and storage of 12 samples from lane 2-13.



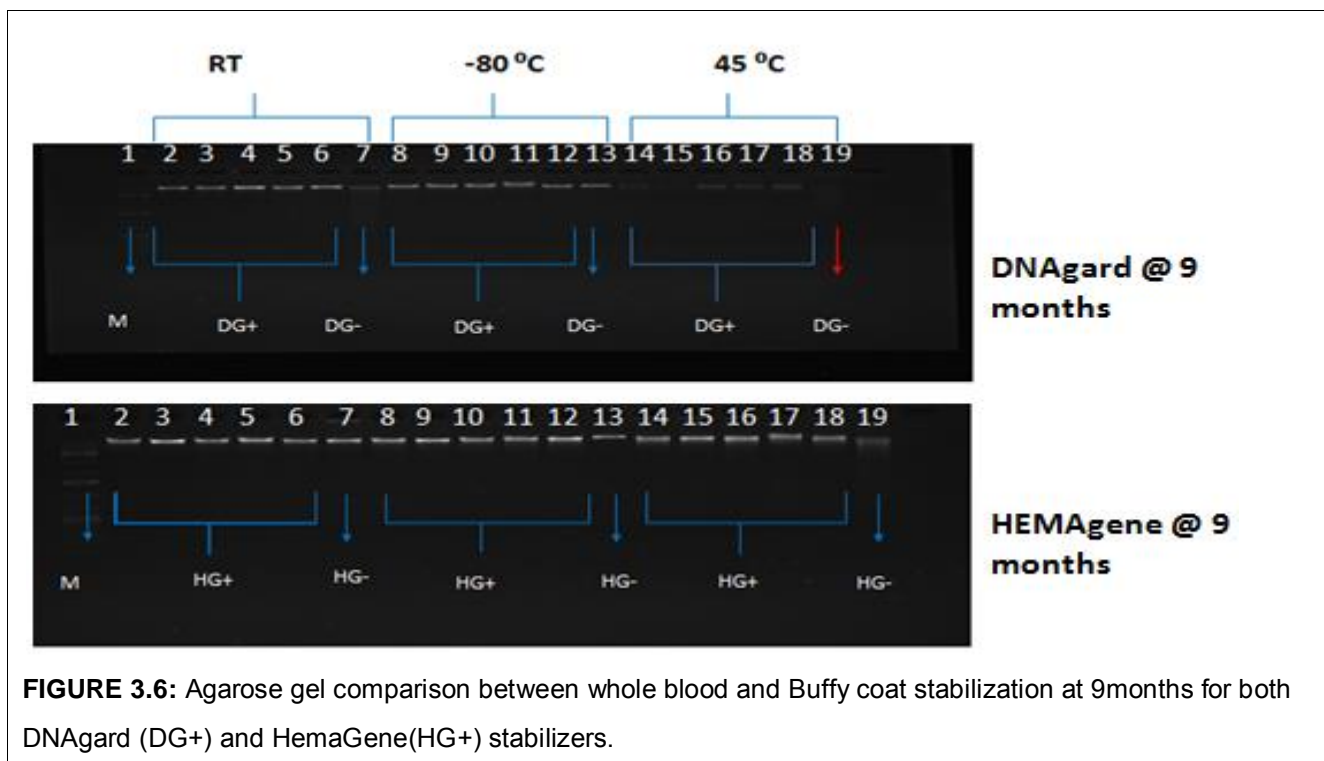
Figures 3.1-3.5 shows the integrity of all DNA samples isolated and purified using a chemagic magnetic beads extraction kit. In Figure 3.1 it can be seen that all DNA samples stabilized in DNAGard were intact after isolation and purification. These were for samples stored at RT, -80°C and 45°C for 3, 6 and 9 months respectively. Samples without stabilizer were still intact, with slight degradation.

DNA isolated from Buffy coats stored in HEMAGENE Buffy Coat were all stable at RT, -80°C and 45°C for 3, 6 and 9 months respectively (Figure 3.2). Samples that were not stored in HEMAGENE were also stable. For DNA samples stored in DNASTable all samples were stable RT, -80°C, and -20°C for 3, 6 and 9 months (Figure 3.4). At 45°C a slight degradation of DNA is observed after 9 months of storage. The samples that were not stored in DNASTable were also intact, except for at 45°C for 3, 6 and 9 months.

In addition to the above experiments sample integrity was also checked after 3 year storage in HEMAGENE Buffy coat. All samples were intact, except for those stores at 45°C (Figure 3.5).

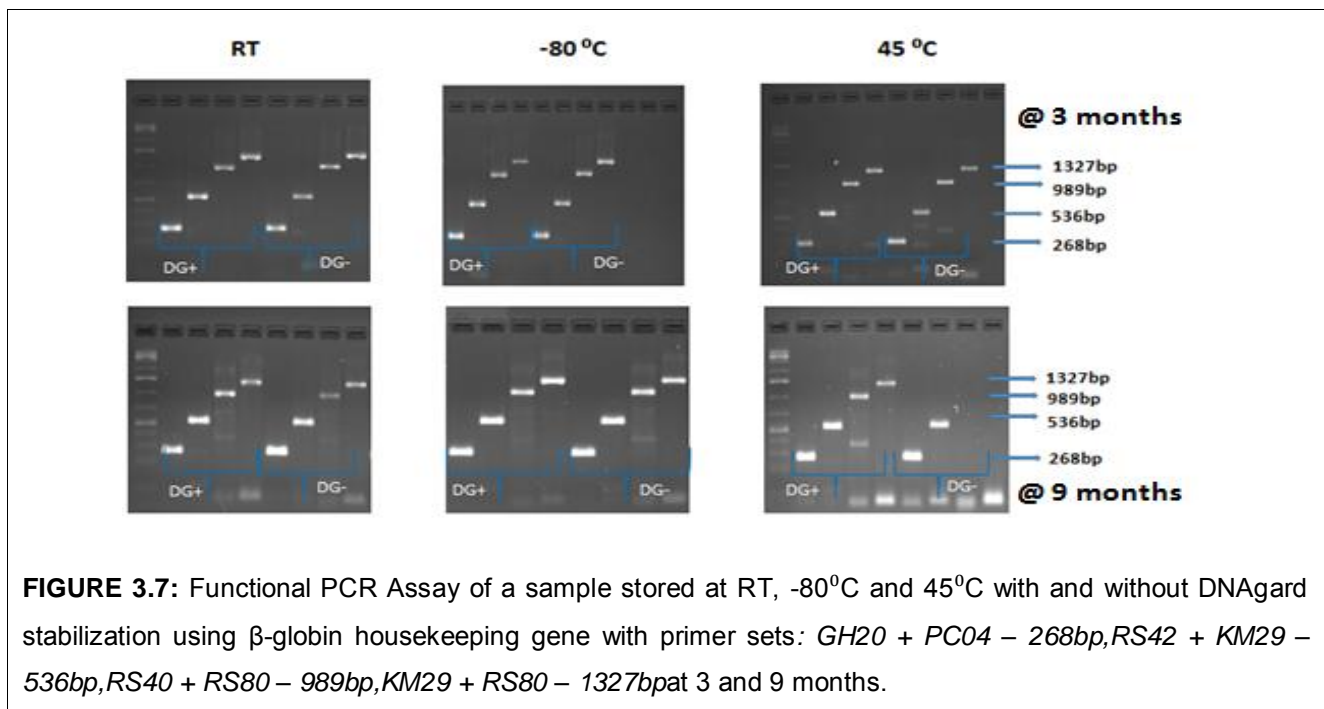
### 3.1.3 Comparison between Whole blood and Buffy coat samples

The band intensity and size of whole blood extracted DNA (DNAgard) and buffy coat extracted DNA (HEMAGene) gel images were compared at 9 months storage (Figure 3.6). Results indicate that stabilizer of buffy coat provides a better protection to DNA integrity compared to whole blood stabilization. As samples stored at 45°C in whole blood showed some degradation while that stored in buffy coat were intact.



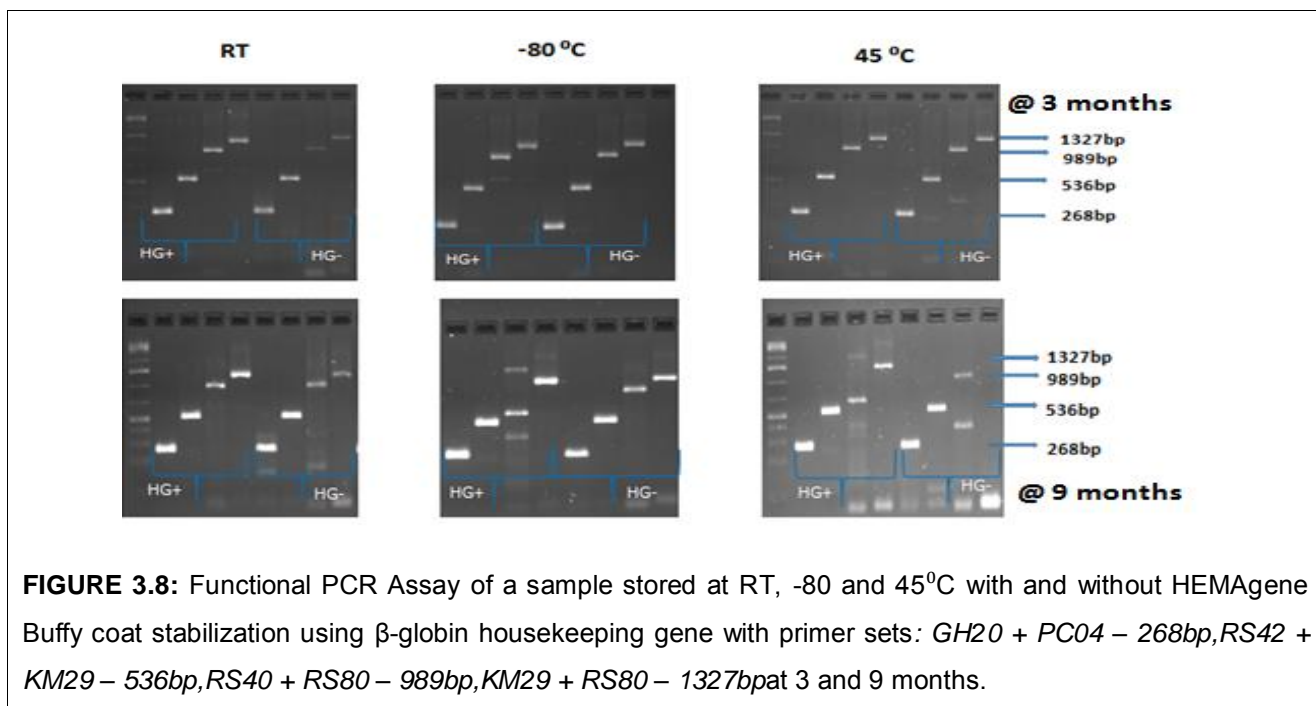
### 3.1.4 Polymerase chain reaction (PCR)

The housekeeping gene  $\beta$ -globin was amplified using a standard PCR procedure as detailed in Section 3.3. Four different primer sets were used (Table 2.1) with amplicon sizes 268bp, 536bp, 989bp and 1327bp. PCR was performed for samples stored at room temperature, 45 and corresponding frozen samples with and without stabilizer (Figure 3.7-3.10).



**FIGURE 3.7:** Functional PCR Assay of a sample stored at RT, -80°C and 45°C with and without DNAgard stabilization using  $\beta$ -globin housekeeping gene with primer sets: *GH20 + PC04 – 268bp*, *RS42 + KM29 – 536bp*, *RS40 + RS80 – 989bp*, *KM29 + RS80 – 1327bp* at 3 and 9 months.

Figure 3.7 represent the PCR amplification of samples extracted after storage in stabilizer DNAgard at RT, -80°C and 45°C, it is shown that all PCR fragments for  $\beta$ -globin were successfully amplified by PCR using the respective primer sets. The only exception being at 45°C, where the larger fragments of 989 and 1327 bp were not amplified in the 9 months samples without stabilizer.



**FIGURE 3.8:** Functional PCR Assay of a sample stored at RT, -80 and 45<sup>0</sup>C with and without HEMAgene Buffy coat stabilization using  $\beta$ -globin housekeeping gene with primer sets: *GH20 + PC04 – 268bp*, *RS42 + KM29 – 536bp*, *RS40 + RS80 – 989bp*, *KM29 + RS80 – 1327bp* at 3 and 9 months.

Figure 3.8 represents the PCR amplification of samples extracted after storage in stabilizer HEMAgene Buffy coat stabilization at RT, -80<sup>0</sup>C and 45<sup>0</sup>C; it is shown that all PCR fragments for  $\beta$ -globin were successfully amplified by PCR using the respective primer sets. The only exception being at 45<sup>0</sup>C, where the larger fragments and 1327 bp was not amplified in the 9 months samples without stabilizer. Some additional PCR products are observed which could be due to non-specific amplication.



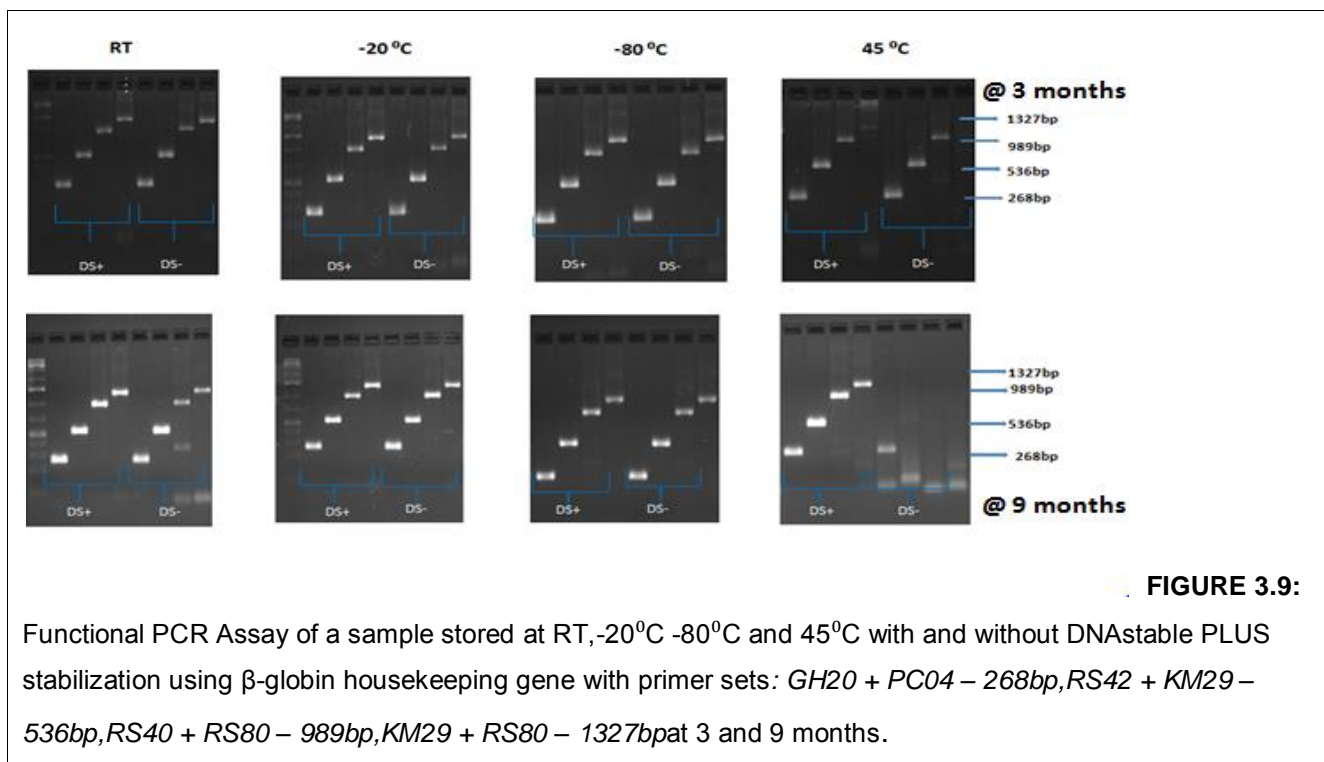
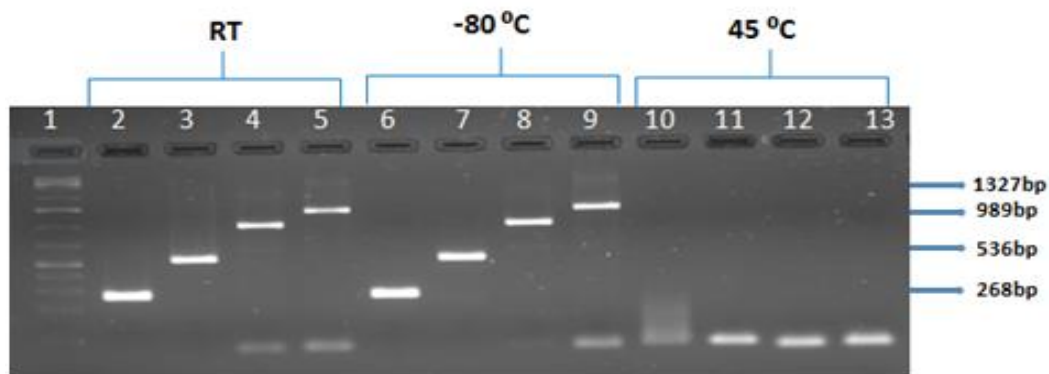


Figure 3.9 represent the PCR amplification of samples extracted after storage in stabilizer DNASTABLEPLUS at RT, -20°C -80°C and 45°C, it is shown that all PCR fragments for  $\beta$ -globin were successfully amplified by PCR using the respective primer sets. The only exception being at 45°C, where the larger fragment 1327 bp was not amplified in the 3 months samples without stabilizer. No amplification was observed in the 9 month sample without stabilizer at 45°C.

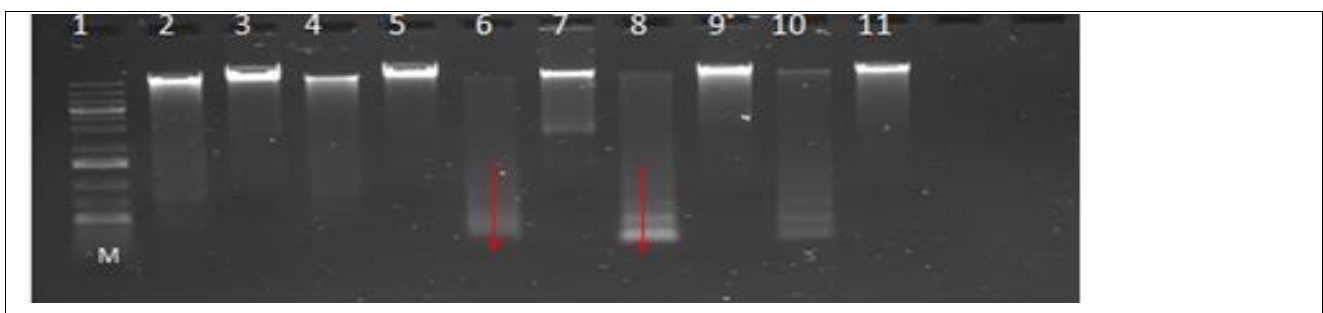


**FIGURE 3.10:** Functional PCR Assay of a sample stored for 3 years at RT,  $-80^{\circ}\text{C}$  and  $45^{\circ}\text{C}$  with HEMAgene Buffy coat stabilization using  $\beta$ -globin housekeeping gene with primer sets: *GH20 + PC04* – 268bp, *RS42 + KM29* – 536bp, *RS40 + RS80* – 989bp, *KM29 + RS80* – 1327bp

Figure 3.10 represent the PCR amplification of samples extracted after a 3 year storage in stabilizer HEMAgene Buffy coat stabilization at RT,  $-80^{\circ}\text{C}$  and  $45^{\circ}\text{C}$ , it is shown that all PCR fragments for  $\beta$ -globin were successfully amplified by PCR using the respective primer sets in samples kept at RT and  $-80^{\circ}\text{C}$ . No amplification was observed at  $45^{\circ}\text{C}$ .

### 3.2 Stabilization of DNA in cells

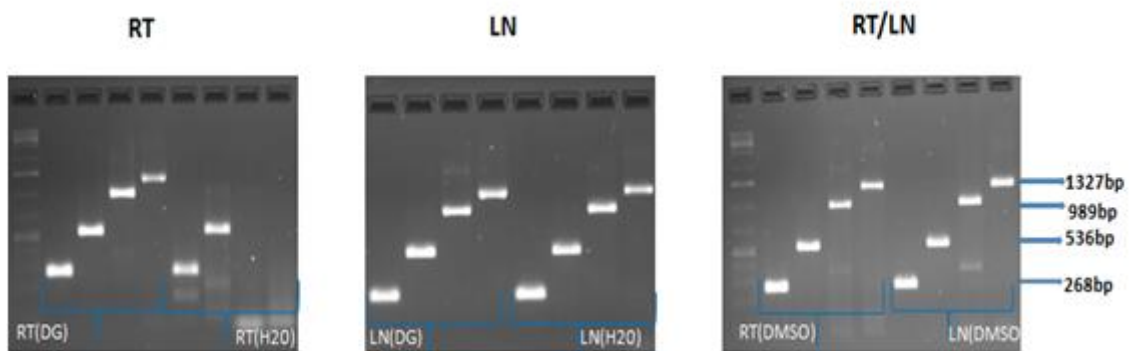
DNA was also extracted from HEK 293 cultured cells after 1 month of storage in liquid nitrogen (LN) and room temperature (RT). The concentration was determined using both the Biodrop and Qubit fluorometer. Extracted DNA from cells was separated on a 0.8% agarose gel in TBE to assess DNA integrity (Figure 3.11). DNA samples stored in DNAgard was at RT were intact. Lanes 2 and 4 show samples that was intact, whereas samples in lanes 6, 8 and 10 were degraded. These samples were stored in water (Lane 6) and DMSO (Lanes 8 and 10). Lanes 3, 5, 7, 9 and 11 represents samples stored in LN. Lanes 3 and 5 were protected with DNAgard, Lane 7 in water and Lane 9 and 11 in LN. All samples were intact.



**FIGURE 3.11:** Agarose gel electrophoresis for DNA extracted from cultured cells with and without stabilization (DNAgard) after 1 month of storage. Lane 1 Molecular marker, 2, 4, 6, 8 and 10 are samples stored at RT. Lanes 2 and 4 protected, 6 NP with water and 8 and 10 in DMSO. Lanes 3, 5, 7, 9 and 11 are samples stored in LN. Lanes 3 and 5 protected 7 NP in water and 9 and 11 cryopreserved in DMSO.

To determine the ability of the extracted DNA from cells to undergo downstream analysis, a PCR using *β-globin* was done for a sample at all storage conditions.

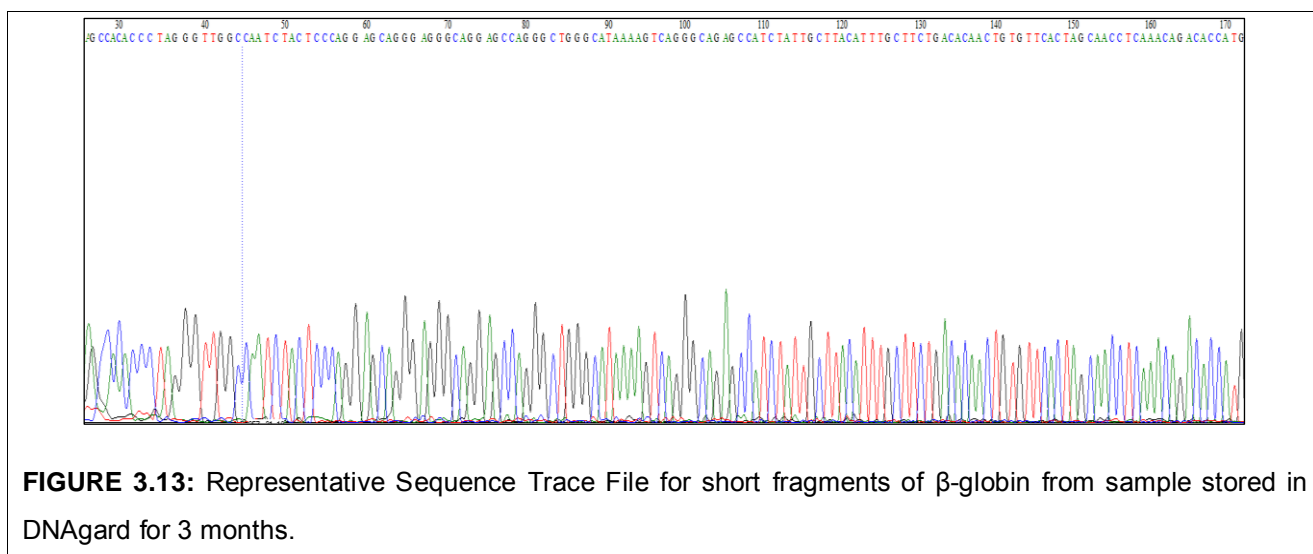
All PCR fragments for *β-globin* were successfully amplified by PCR using the respective primer sets. The only exception being at RT stored in water, where the larger fragments of 989 and 1327 bp were not successfully amplified.



**Figure 3.12:** Functional PCR for DNA from cultured cells stored for 1 month at RT and liquid nitrogen (LN) with and without DNAgard using  $\beta$ -Globin (housekeeping gene) with *Primer Sets: GH20 + PC04 – 268bp, RS42 + KM29 – 536bp, RS40 + RS80 – 989bp, KM29 + RS80 – 1327bp*

### 3.3 DNA Sequencing of $\beta$ -globin gene

All samples that were successfully amplified by PCR were considered for sequencing. Samples that were selected for sequencing are shown in Appendix II. Sample selection criteria included: samples that were stored in different stabilizers; samples at different temperatures; and samples stored over different time periods. Samples were selected to cover all the different parameters. Figure 3.13 shows the Sequence Trace File for DNA samples stored in DNAgard at RT for 3 months.



Figures 3.14 A and B, represent the BLAST results for DNA samples stored in DNAgard at RT for 3 months. Figure 4.14 A indicates a 100 % identity to the reference  $\beta$ -globin gene from Homo sapiens for the shorter PCR fragment that was amplified, whereas Figure 4.14 B indicates a 99 % identity to the  $\beta$ -globin gene for the larger fragment that was amplified. The quality of the sequence trace and the accuracy of the BLAST results suggest that the integrity of the DNA samples was of a high standard after isolation. All the DNA samples sent for sequencing produced the same quality and accuracy, as shown in Figures, 4.13. 4.14 A and 4.14 B.

Homo sapiens beta-globin gene, complete cds  
 Sequence ID: [AH001475.2](#) Length: 4355 Number of Matches: 1

Range 1: 1461 to 1684 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
414 bits(224)	2e-113	224/224(100%)	0/224(0%)	Plus/Plus
Query 19	CTGTGGAGCCACACCCCTAGGGTTGGCCAATCTACTCCAGGAGCAGGGAGGGCAGGAGCC			78
Sbjct 1461	CTGTGGAGCCACACCCCTAGGGTTGGCCAATCTACTCCAGGAGCAGGGAGGGCAGGAGCC			1520
Query 79	AGGGCTGGGCATAAAAAGTCAGGGCAGAGCCATCTATTGCTTACATTTGCTTCTGACACAA			138
Sbjct 1521	AGGGCTGGGCATAAAAAGTCAGGGCAGAGCCATCTATTGCTTACATTTGCTTCTGACACAA			1580
Query 139	CTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGCACCTGACTCCTGAGGAGAAGTC			198
Sbjct 1581	CTGTGTTCACTAGCAACCTCAAACAGACACCATGGTGCACCTGACTCCTGAGGAGAAGTC			1640
Query 199	TGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTG			242
Sbjct 1641	TGCCGTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTG			1684

**FIGURE 3.14 A:** Representative Blast result of short fragment of B-globin stored in DNAgard for 3 months.

Homo sapiens beta-globin gene, complete cds  
 Sequence ID: [AH001475.2](#) Length: 4355 Number of Matches: 1

Range 1: 1525 to 2013 [GenBank](#) [Graphics](#) ▼ Next Match ▲ Previous Match

Score	Expect	Identities	Gaps	Strand
872 bits(472)	0.0	482/489(99%)	2/489(0%)	Plus/Plus
Query 16	CTGGGCAT--AAGTCAGGGMARAGCCATCTATTGCTTACATTTGCTTCTGACACAACCTGT			73
Sbjct 1525	CTGGGCATAAAAAGTCAGGGCAGAGCCATCTATTGCTTACATTTGCTTCTGACACAACCTGT			1584
Query 74	GTTCACTAGCAACCTCAAACAGACACCATGGTGCACCTGACTCCTGAGGAGAAGTCTGCC			133
Sbjct 1585	GTTCACTAGCAACCTCAAACAGACACCATGGTGCACCTGACTCCTGAGGAGAAGTCTGCC			1644
Query 134	GTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTGGTGGTGAAGCCCTGGGCAGG			193
Sbjct 1645	GTTACTGCCCTGTGGGGCAAGGTGAACGTGGATGAAGTTGGTGGTGAAGCCCTGGGCAGG			1704
Query 194	TTGGTATCAAGGTTACAAGACAGGTTTAAAGGAGACCAATAGAAACTGGGCATGTGGAGAC			253
Sbjct 1705	TTGGTATCAAGGTTACAAGACAGGTTTAAAGGAGACCAATAGAAACTGGGCATGTGGAGAC			1764
Query 254	AGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTCTGCTATTGGTCTATTTTCC			313
Sbjct 1765	AGAGAAGACTCTTGGGTTTCTGATAGGCACTGACTCTCTCTGCTATTGGTCTATTTTCC			1824
Query 314	CACCCCTTAGGCTGCTGGTGGTCTACCCCTGGACCCAGAGGTTCTTTGAGTCTTTGGGGA			373
Sbjct 1825	CACCCCTTAGGCTGCTGGTGGTCTACCCCTGGACCCAGAGGTTCTTTGAGTCTTTGGGGA			1884
Query 374	TCTGTCCACTCCTGATGCTGTTATGGGCAACCTAAGGTGAAGGCTCATGGCAAGAAAAGT			433
Sbjct 1885	TCTGTCCACTCCTGATGCTGTTATGGGCAACCTAAGGTGAAGGCTCATGGCAAGAAAAGT			1944
Query 434	GCTGGTGCCTTTWGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTTKYCAC			493
Sbjct 1945	GCTGGTGCCTTTAGTGATGGCCTGGCTCACCTGGACAACCTCAAGGGCACCTTTGCCAC			2004
Query 494	ACTGAGTGA 502			
Sbjct 2005	ACTGAGTGA 2013			

**FIGURE 3.14 B:** Representative Blast result of larger fragment of β-globin stored in DNAgard for 3 months.

### **3.4 Stabilization of RNA in whole blood**

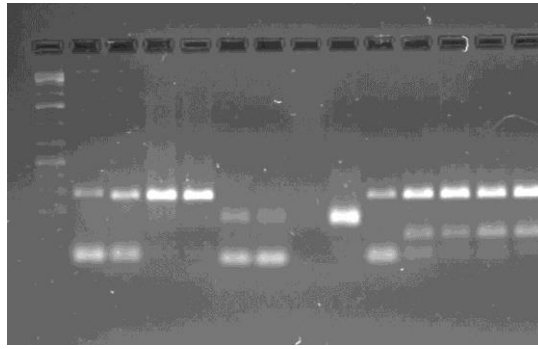
A further aim of the present study was to test the effect of two stabilizers, PAXgene and RNAgard, on the quality of RNA extracted from whole blood.

#### **3.4.1 Determination of RNA concentration**

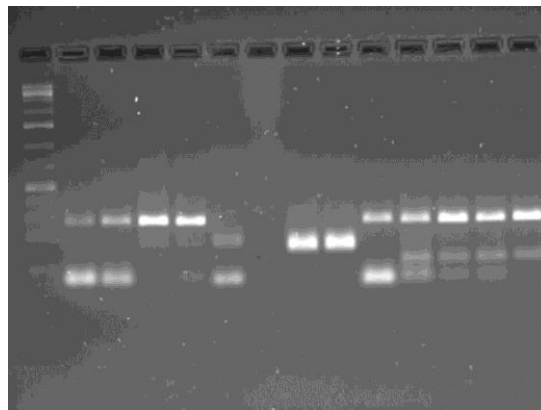
After 3, 7 and 14 days of storage of RNA in whole blood using PAXgene and RNAgard blood tubes. RNA was extracted and concentrations determined by Biodrop and Qubit flourometer prior to cDNA synthesis (Appendix I).

#### **3.4.2 Standard PCR on isolated RNA from whole blood cells**

In Figures 3.15 and 3.16 it was observed that RT-PCR amplification of the short fragment of  $\beta$ -globin was successful on RNA samples stored in PAXgene and RNAgard.



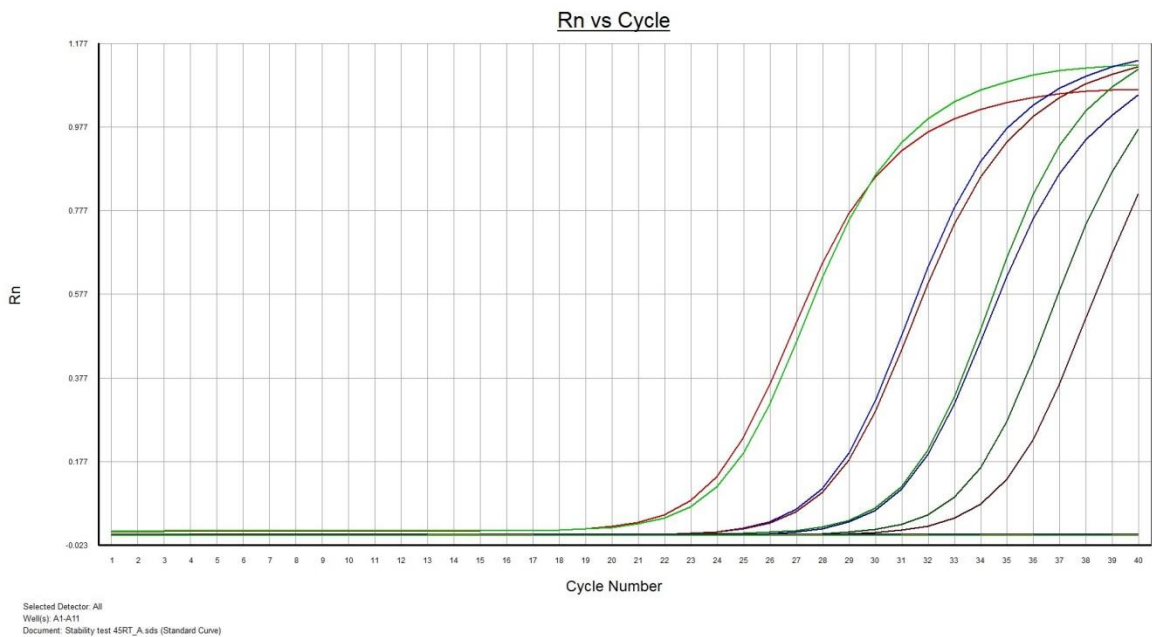
**FIGURE 3.15:** Representative 0.8 % gel of  $\beta$ -globin PCR amplification with Primer Set GH20+PCO4 from cDNA synthesized after isolation with PAXgene.



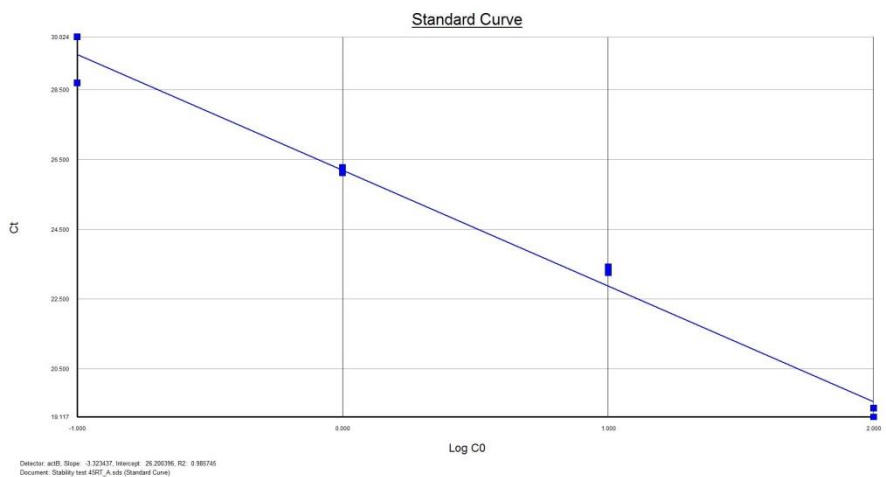
**FIGURE 3.16:** Representative 0.8 % gel of  $\beta$ -globin PCR amplification with Primer Set GH20+PCO4 from cDNA synthesized after isolation with RNAgard.

### 3.4.3 Quantitative Real Time PCR to assess suitability of isolated DNA and RNA in downstream qRT-PCR applications

QRT-PCR reactions were done on all samples as detailed in section 2.4. Primer Set GH20+PCO4 was used for the  $\beta$ -globin gene to determine the integrity and performance of the isolated DNA and RNA in downstream PCR applications. From Figure 3.17 and 3.18 it was concluded that DNA samples stored in DNAgard for 3 months were still highly intact, and produced excellent amplification during standard qRT-PCR. The PCR efficiency of the reaction was 98 % with a slope of -3.32 and  $r^2 = 0.99$ . In addition the dissociation curve/melting peak analysis (Figure 3.19) showed the presence of only one PCR product as a temperature of 84.8°C. The negative control (straight line at level 0.000 on the y-axis) indicated no contamination with DNA or primer dimers (usually peaks between 60-70°C)



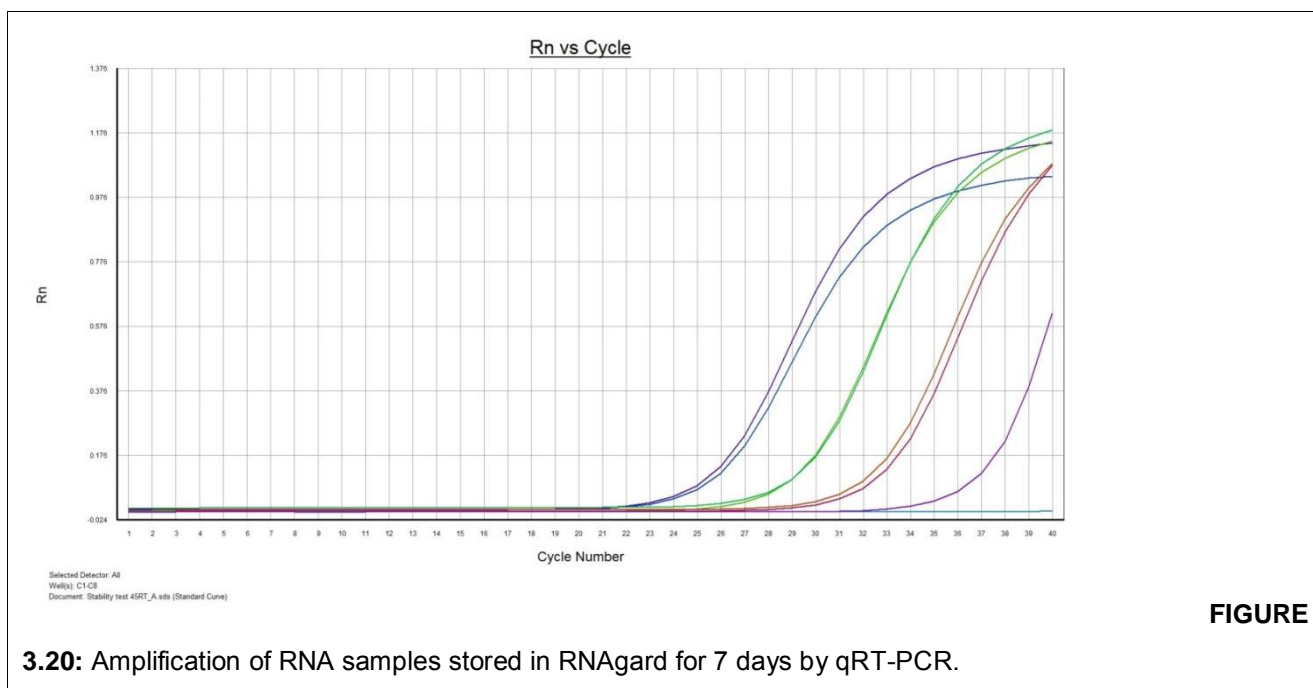
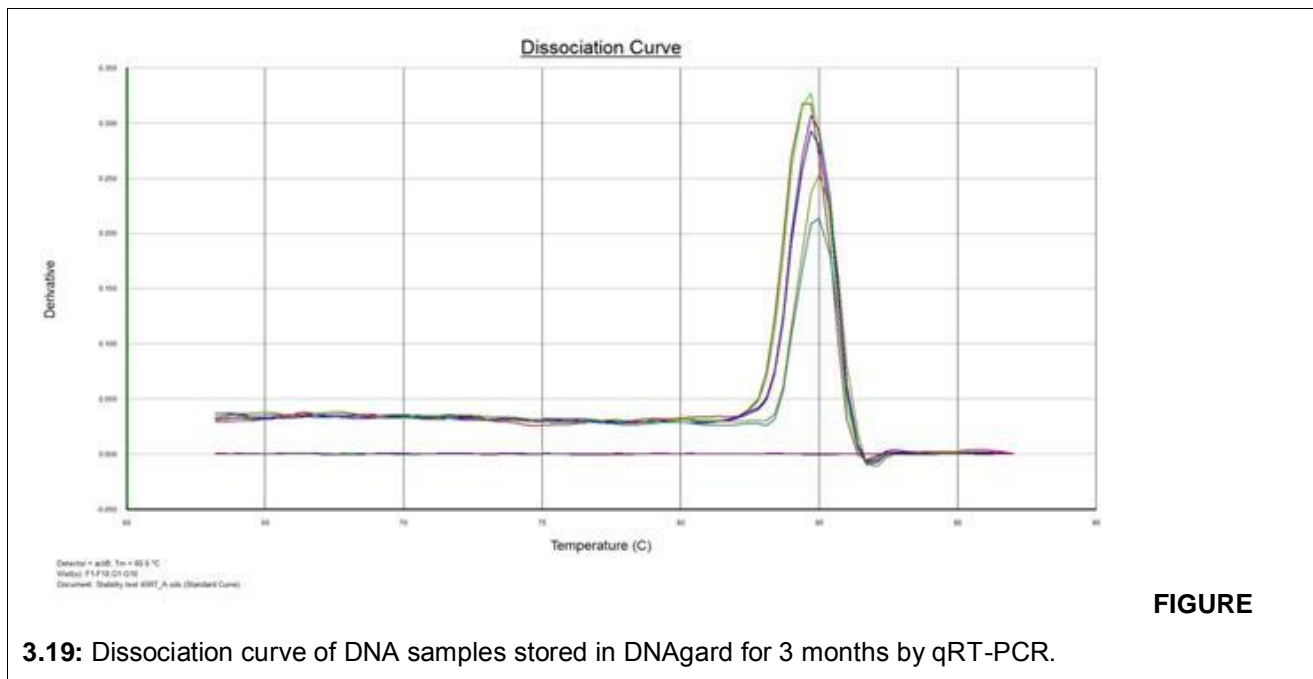
**FIGURE 3.17:** Amplification of DNA samples stored in DNAgard for 3 months by qRT-PCR.

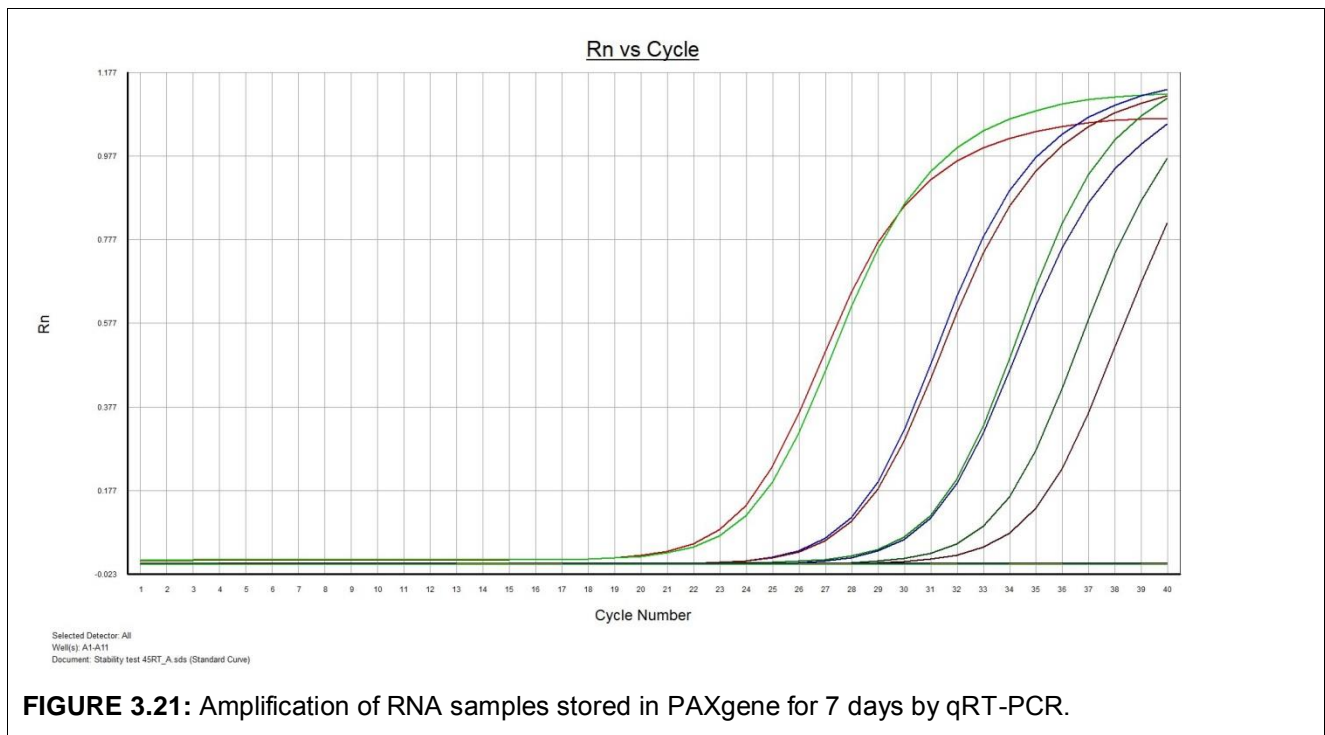


**FIGURE 3.18:** Standard curve of DNA samples stored in DNAgard for 3 months by qRT-PCR.

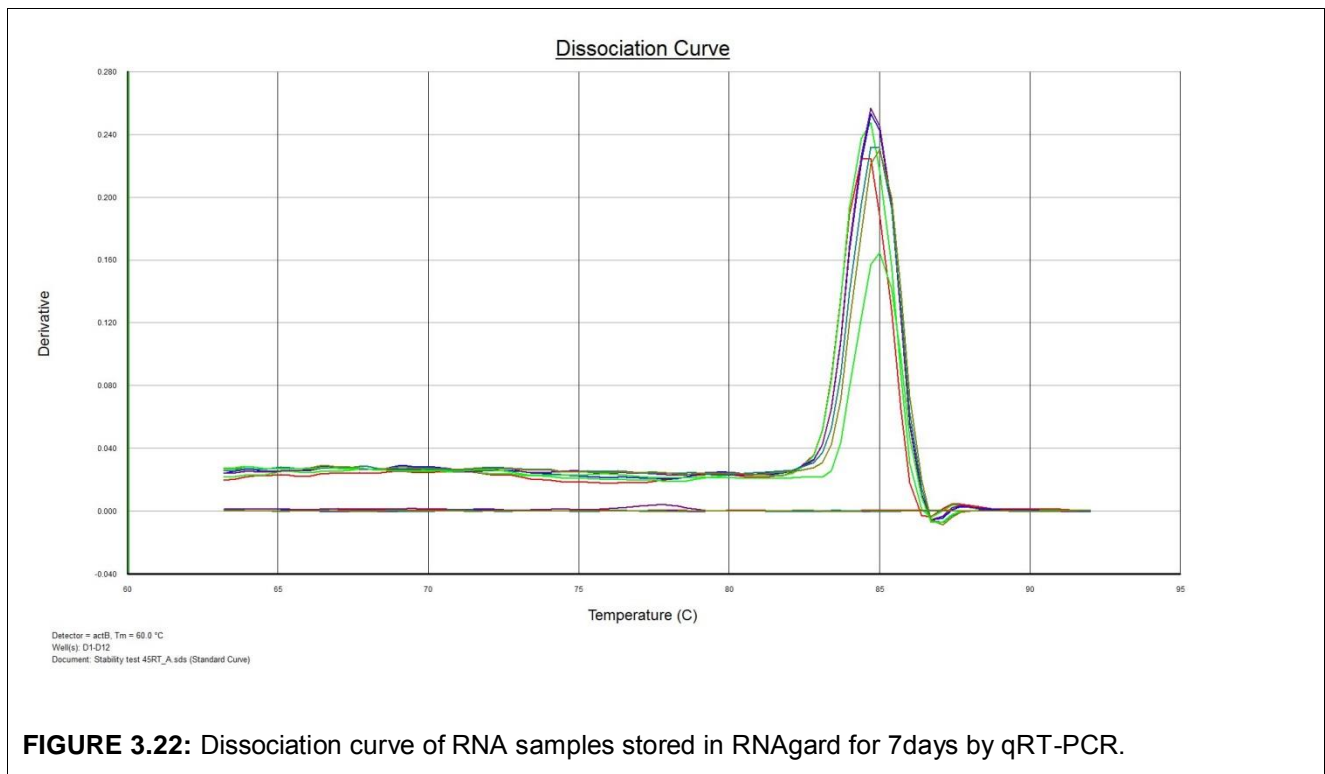


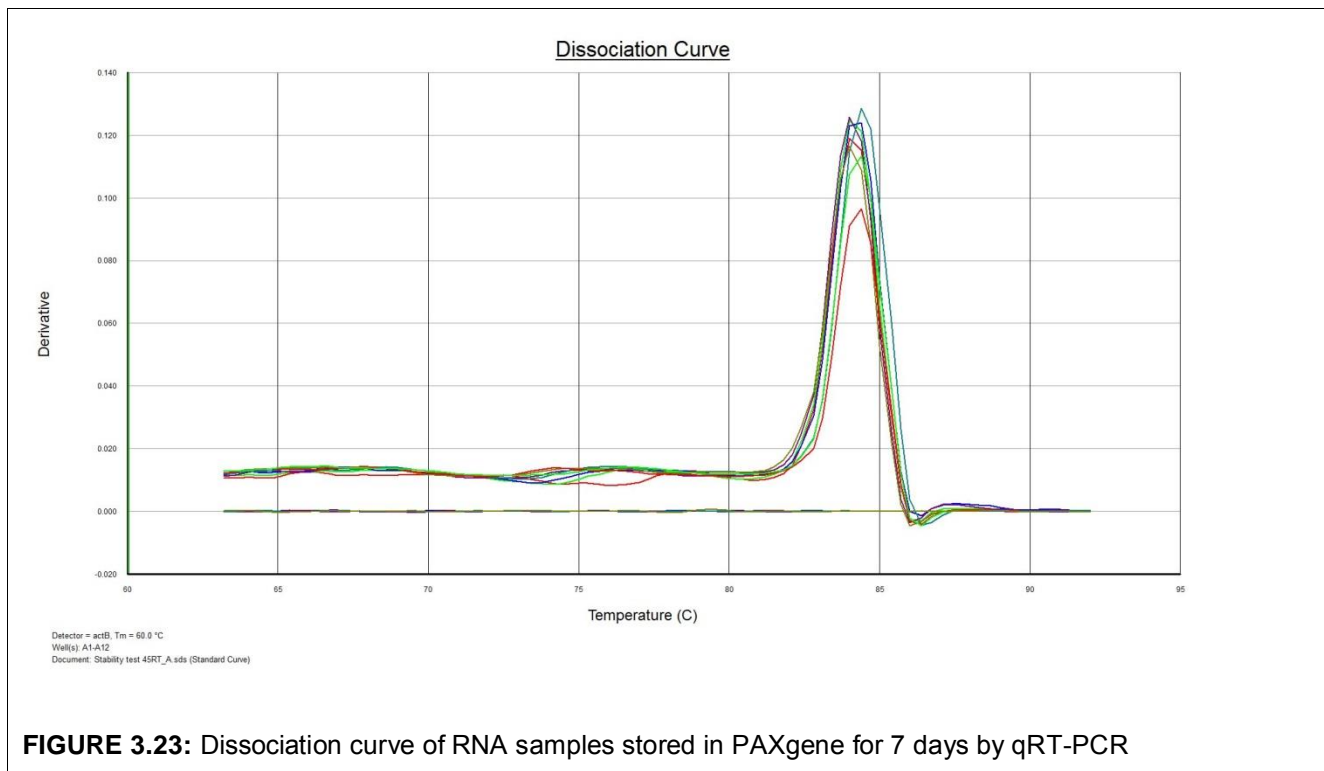
In Figure 3.20 and 3.21 the amplification curves for the short fragment of B-globin are shown for both RNA that was stored in RNAgard and PAXgene respectively. The curve showed excellent amplification in both instances, with a PCR efficiency of 96 % for RNAgard with  $R^2=0.99$ , and a PCR efficiency of 97 % for PAXgene with and  $R^2=0.99$ .





The dissociation curve/melting peak analysis (Figures 3.22 and 3.23) showed the presence of only one PCR product as a temperature of 85°C for both the RNAgard and PAXgene samples. The negative control (straight line at level 0.000 on the y-axis) indicated no contamination with cDNA or primer dimers (usually peaks between 60-70°C).

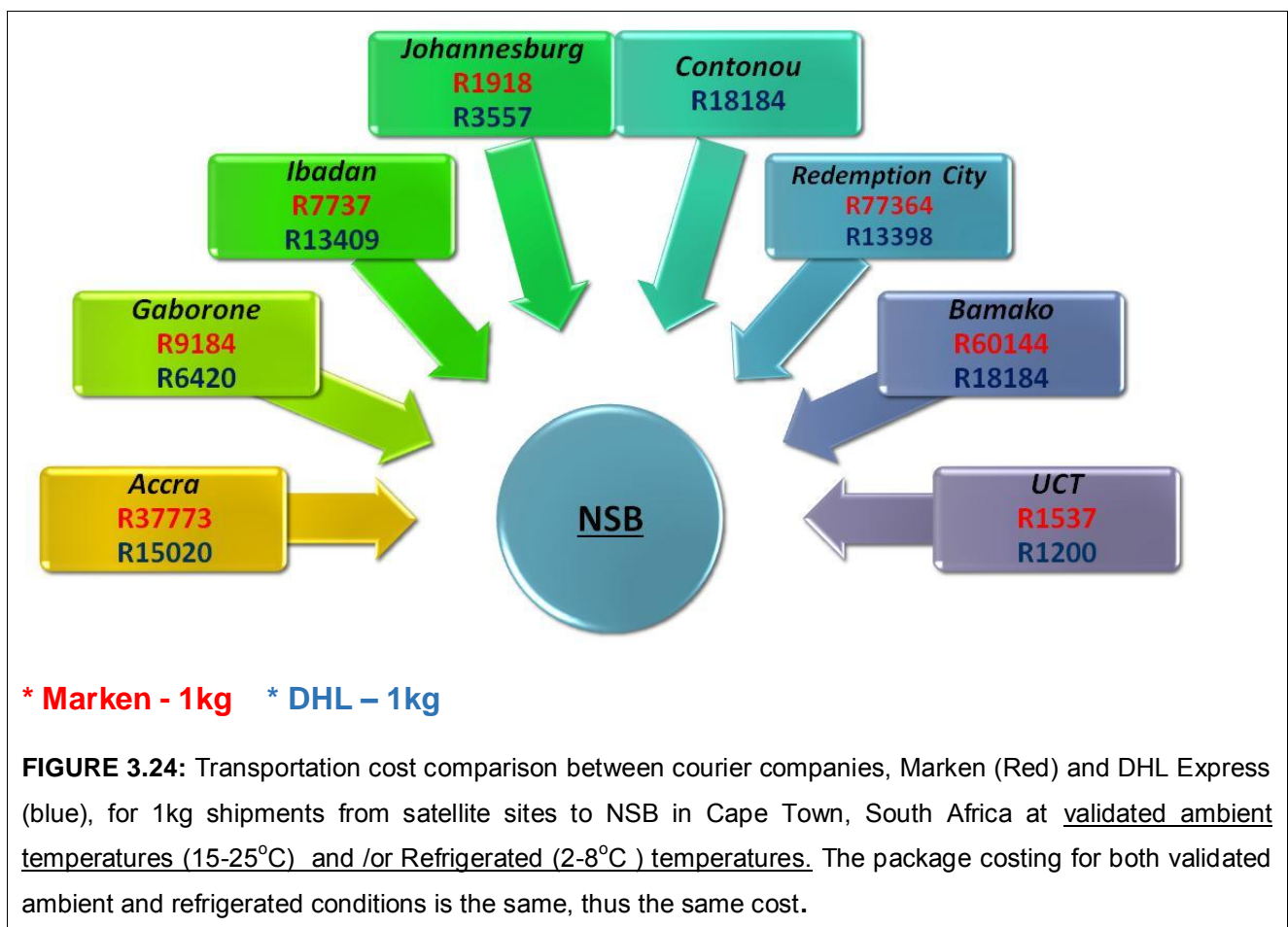


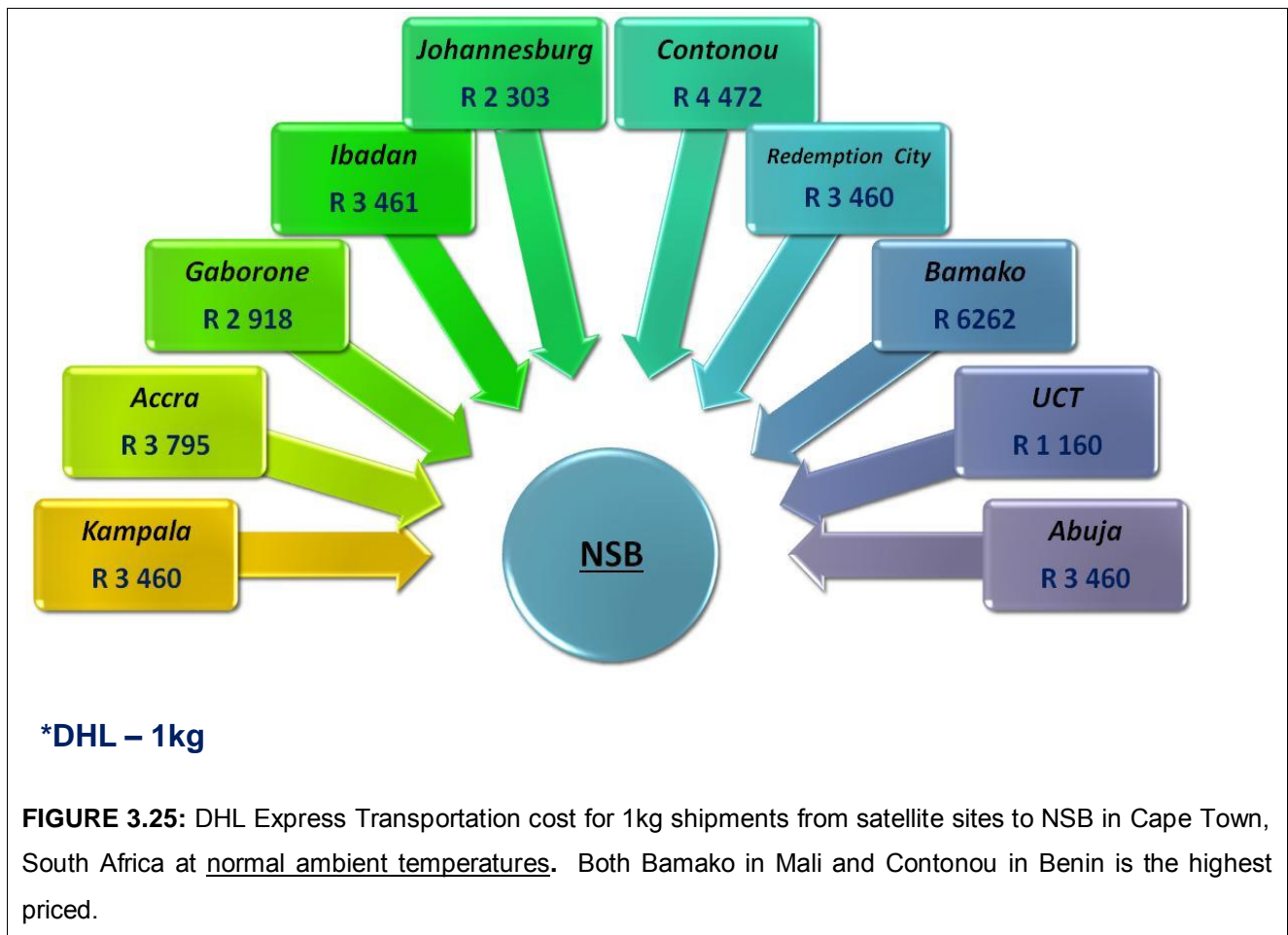


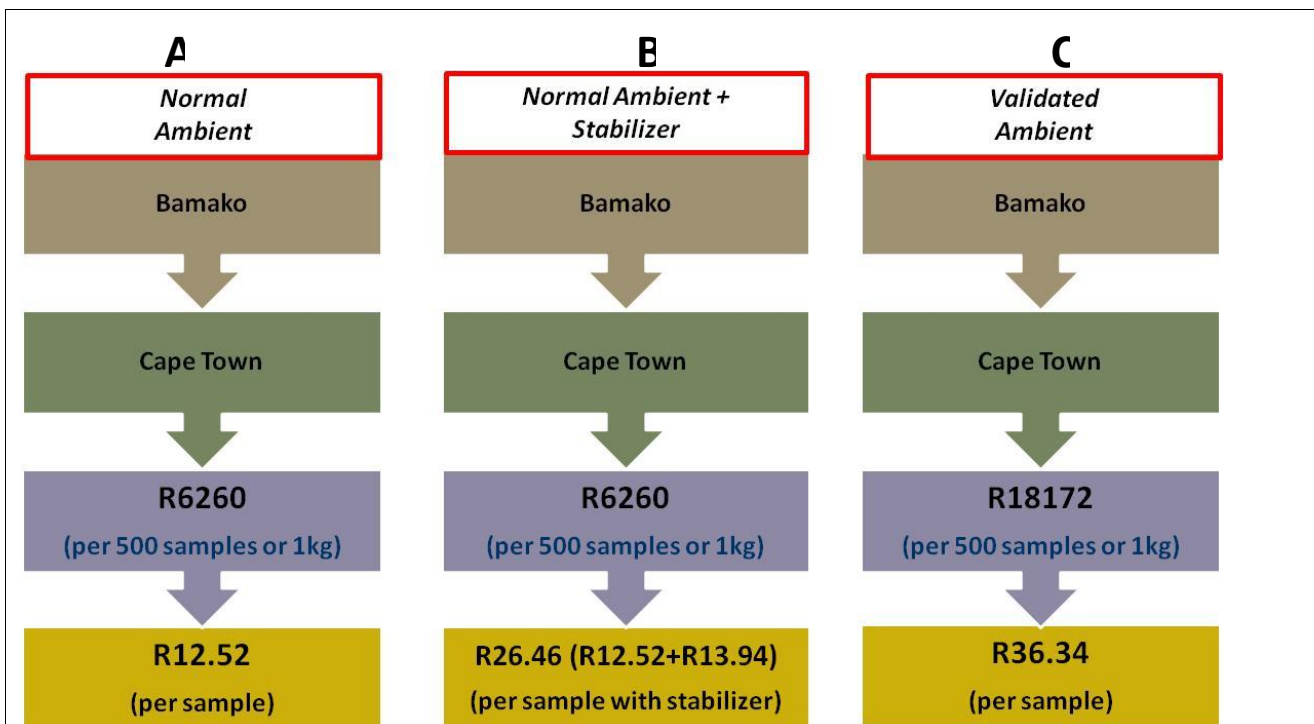
### 3.5: Sample transportation cost analysis for African biobanking/laboratory operations:

A transportation cost analysis comparing the cost for the movement samples from the 8 African satellite sites to NSB at normal ambient, validated ambient, refrigerated and at normal ambient including the additional costing of a stabilizer such as DNASTable was done. The cost was based on a 1kg shipment and assuming that it would fit  $\pm 500$  (0.5 and/or 0.75ml) vials and at an exchange rate of R14 to the dollar. The data generated helped to inform us on the cost and logistics associated with the movement of biospecimens on a larger scale as part of a biobank or laboratory sustainability plan. From the 8 sites evaluated, Bamako in Mali was the most expensive. Figure 3.24 highlights the cost comparison between Marken (Red) and DHL (Blue) at validated and/or refrigerated temperatures whereas figure 3.25 shows normal ambient shipment costs for DHL only from the 8 satellites sites. As Marken only does shipment with specialized packaging, no costs for normal ambient shipment was possible. In order to determine the specific cost comparison between the different temperatures and the addition of a stabilizer, the pricing estimates at normal ambient conditions for 1kg shipment from Bamako in Mali to CT was used as it was the highest priced at R6262. Assuming that 1 kg shipments would fit  $\pm 500$  (0.5 and/or 0.75ml) vials, the cost per sample at normal ambient would be R12.52 per sample. In addition validated and/or refrigerated 1kg shipments from Bamako in Mali to

CT, amounts to R18172 at R36.34 per sample. Knowing the risk of sample degradation at ambient temperature without DNA stabilization, we calculated the cost of a DNA stabilizer (R13.94 per sample) in addition to the normal ambient pricing for 1kg shipment from Bamako in Mali to CT. This additional cost amounts to R26.46 per sample, for normal ambient conditions with the DNA stabilizer, which compared to validated ambient and refrigerated conditions is much more cost effective. We therefore propose transportation at ambient conditions with the addition of a stabilizer to the DNA as a more cost effective transportation solution for South Africa and Africa in general.







**FIGURE 3.26:** Cost comparison for the shipment of 500 samples to NSB in Cape Town, South Africa at normal ambient (A), normal ambient with the addition of a DNA stabilizer (B) and validated ambient/refrigerated conditions (C). The highest priced sites of the 8 satellite sites were chosen to calculate shipping cost per sample, assuming a 1kg shipment can fit ± 500 vials. These prices are based on the **DHL Express estimates**

## **Chapter 4- Discussion**

This study was performed in the National Health Laboratory – Stellenbosch University Biobank (NSB) to evaluate and validate room temperature storage technologies for sample storage not only in South Africa, but in the whole of Africa. It is a well-known fact that the infrastructure in most African countries is not conducive to patient sample transportation and storage, and this was identified as one of the challenges that faces sample management logistics in Africa. The lack of equipment to store samples and the high temperatures that are experienced in Africa necessitates the identification of appropriate mechanisms to ensure the effective storage of biological biospecimens. This study thus seeks to address some of the sample management logistical issues faced in Africa, and attempts to make recommendations on appropriate protocols that could be put in place to ensure a cost-effective means to maintain viability and integrity of valuable biospecimens that are crucial for biomedical research on the African continent.

In our study, we evaluated various room temperature storage technologies/stabilizers namely, the Biomatrix Sample Matrix technology products (DNAgard, DNASTable, RNAgard and DNAgard Tissue), the HEMAgene BUFFY Coat by DNAGENOTEK as well as PAXgene RNA and Norgen Urine stabilizers. The aims of this study were essentially two-fold.

Firstly, the effect of the different stabilizers was evaluated on DNA and RNA samples stored at different temperatures over time periods. For DNA, the temperatures were RT, -80°C and 45°C, and in some instances -20°C (DNASTable PLUS). Stabilization of DNA samples in the cultured cell line, HEK293, was also compared to samples stored in LN while RNA stabilizers were tested at RT and -80°C only.

Secondly, a sample transportation cost analysis for African biobanking/laboratory operations was conducted comparing room temperature transportation versus cold chain logistics costs to ensure a more cost-effective solution that produced high quality biological samples for possible downstream molecular and/or diagnostic applications.

### **4.1 Stabilization of DNA in whole blood, Buffy coat, purified DNA, cells and urine**

Whole blood was stored in Biomatrix's DNAGard stabilizer at RT, -80°C and 45°C up till 9 months and integrity checks were conducted at time points of 3, 6 and 9 months following DNA isolation and purification of stored aliquots. Likewise control samples were also stored without the addition of a stabilizer as observed in figure 3.1. The agarose gel integrity checks at 3, 6 and 9 months at room temperature was comparable to frozen controls at -80°C for samples with and without DNAGard stabilization. In addition, the stabilizing effect of DNAGard was further confirmed with *β-globin* functional PCR analysis (Figure 3.7) and Sanger sequencing (Figure 3.13). However, samples that was stored at extremes of temperature (45°C) to mimic temperatures in certain parts of Africa showed slight degradation with longer duration of storage when integrity was evaluated by gel electrophoresis (Figure 3.1). Nonetheless, these samples amplified well with PCR even with larger fragments of *β-globin* housekeeping gene (1327bp) as shown in Figure 3.7 at 3 months. However at the 9 months' time point amplification of the larger products wasn't possible except for the smaller fragments which further confirm degradation at 45°C, which is to be expected. Sanger sequencing experiments also indicated that the PCR fragments isolated and purified from these samples stored at 45°C were from *β-globin* as confirmed by sequence comparison using BLAST.

Similarly, purified DNA stored in DNASTable and DNA isolated from Buffy coat stored in HEMAgene BUFFY COAT at room temperature did not degrade and remained in good condition compared to the frozen control samples stored at -80°C after a period of 9 months as observed in figures 3.2 and 3.4 . However, slight degradation was observed for those samples stored at 45°C without HEMAgene BUFFY COAT (Figure 3.2) at the 6 and 9 month time points whereas more degradation was observed for purified DNA without DNASTable at time points 3, 6 and 9 months as to be expected (Figure 3.4). Furthermore, from observations of figure 3.4, despite protection of purified DNA with DNASTable, samples at 45°C still showed slight degradation (lane 6, 7, 8) at 9 months compared to no degradation at month 3 and 6. From this we can deduce that it is better to batched purified DNA in stabilizer for 3-6 months than longer at this temperature. These observations are contradictory to what the manufactures stated for DNASTable as mentioned in Chapter 1. The manufacturers indicate that long term storage of DNA in DNASTablePLUS has been demonstrated for 30years accelerated aging and approx. 4years real time. However one must consider that these results might have been observed for samples that have been dried down before storage rather that storage in the liquid phase, thus explaining our observations.



We also observed that DNA isolated from buffycoats stabilized with HEMAgene BUFFY COAT and stored at 45°C performed better than purified stabilized DNA after 9 months of storage with DNASTable PLUS. From this we can deduce that storage and batching of whole blood and/or buffy coats for 3-6 months for future DNA isolation and purification would be more ideal than storage of purified DNA. For satellite sites in far parts of SA and Africa, this would be ideal as not all sites have the capabilities and infrastructure in place to do NA isolations.

As we are interested in long term storage of specifically whole blood and/or buffy coat for batching, we also stored buffy coats for a period of 3 years rather than doing an accelerated aging study. From our observations as shown in figure 3.5, DNA from buffy coat samples stored for 3 years at room temperature and protected/stabilized with HEMAgene Buffy coat stabilizer did not degrade and retained good integrity when compared to the frozen controls at -80°C, which was also protected. Unfortunately, samples stored at 45°C were dried up and the isolation and purification of DNA was not as successful which were to be expected as manufacturers indicate stabilizer protection of 2 years for this product. Literature further support our observations for room temperature storage even though accelerated aging studies were demonstrated rather than in real time (Bouevitch et al.2014).For the accelerated aging studies performed by Bouevitch et al.2014 samples was stored with HG-BCD at 50°C for 35 weeks which would correspond to samples stored for at least 36 months (3 years) at room temperature calculated using the Arrhenius equation.(Bouevitch et al. 2014). The Arrhenius equation is based upon the assumption that the rate of a chemical reaction typically decreases by half for every 10°C decrease in temperature. Therefore the rate of chemical degradation of DNA at +24°C is expected to be 5-fold slower than the rate of degradation at +50°C(Iwasiow et al. 2011). However, even though samples were stored at a higher temperature, the storage time was still less thus the result matches our observations seen in figure 3.2 rather than observations in figure 3.5. From this we can conclude that despite the accelerated aging studies, one need to test/validate long term storage in real time as our observations is contradictory. On the other hand, variable such as lack of desiccation at 45°C for the 3 year period could have also contributed to lack of isolated DNA from the 45°C storage samples.

As a small pilot study, cultured HEKS93 cells were also stored with and without DNAgard tissue over a 1 month period at room temperature and -196°C (control). The results as observed in figure 3.12 show that sample integrity of extracted DNA from cultured HEK293 cells are being maintained at room temperature compared to samples stored in liquid nitrogen. Only samples that were not protected (water) (lane 6 in figure 3.11) showed degradation on agarose gel electrophoresis.

We also evaluated the preservation of DNA at room temperature in urine. However due to a lot of experimental issues, the results for this will not be presented in this thesis. Analysis of urine DNA isolated using Norgen Biotek Corp system could not be completed as most samples degraded after 3 months of storage at RT and frozen which is contradictory to what manufacturer suggested. Nonetheless the few samples that didn't degrade amplified with the shorter fragments of *β-globin* namely, the 268 and 538 bp (results not included).

#### **4.1.1. Concentrations of DNA isolated**

The concentrations and purity for all DNA isolated at the various temperatures and time points were also measured via spectrophotometry and flourometry, however these results wasn't shown in the results section but are added in the addendum as the focus was more on the integrity of the NA. From these analyses, the A260/280 ratios on the spectrophotometer were mostly within limits compared to the lowered A260/230 ratio. According to similar studies done by Wan et al (Wan et al. 2010) DNA preserving compounds in stabilizers by Biomatrix show strong absorbance at the 230nm wavelength but minimal absorption at the 260nm and 280nm. Therefore, a lower A260/230 wavelength reflects a spectrophotometric property of the inorganic preservative compounds rather than unknown contaminants. In addition DNA was isolated and purified using a magnetic bead based process and per manufacturer's observations, these magnetic beads also influence absorbance readings to a slight extend. However agarose gel electrophoresis done on these samples confirmed the integrity of the DNA that was isolated as mentioned above and shown in the integrity check figures 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.11, 3.15 and 3.16.

## **4.2 Comparison between DNA in whole blood and Buffy coat**

As both HEMAgene BUFFY COAT and DNAGard provide stabilization for buffycoats and whole blood respectively, we also compared the integrity of isolated DNA for these two products. Results as shown in figure 3.6, at 9 months of storage shows that while protection with both stabilizers have been observed; samples that were protected with DNAGard stabilizers at 45°C had some degradation compared to samples protected with Hemagene buffy coat stabilizers. These results indicate that HEMAgene offers better protection with respect to the band intensity than DNAGard whole blood stabilizer. HEMAgene also had less interference with spectrophotometric wavelengths than DNAGard as shown in tables of concentrations in Appendix I.

### 4.3 Polymerase chain reaction

Polymerase chain reaction (PCR) performed on whole blood DNA, purified DNA and DNA in buffy coat using  $\beta$ -globin housekeeping gene with fragments between 268bp to 1327bp showed comparable band intensity and size with the frozen controls as shown in the figures 3.7, 3.8, 3.9, 3.10 and 3.12 for the various stabilizers. Amplification of all fragments of  $\beta$ -globin from smallest to largest as indicated by the appearance of all four bands on gel electrophoresis indicated DNA with good integrity. As observed, amplification of all four bands was achieved for preserved samples. These results indicate that the DNA samples that were kept in stabilizers and then isolated at a later stage could still perform well in downstream molecular biology application such as PCR. There were some exceptions where samples stored without stabilizer, such as the case with DNAGard at 45°C for 9 months did not produce a band for the larger fragments of the  $\beta$ -globin gene after PCR amplification (Figure 3.7) which is indicative of sample degradation. The fact that smaller fragments still amplified confirms this as well. The same result was also observed in samples stored without HEMAgene Buffy coat after storage 45°C for 9 months (Figure 3.8). This could be either due to the fact that these samples needed to be reconstituted in water after it evaporated after 9 months. However, the fact that smaller fragments still amplified confirm that samples were degraded. For samples stored for 9 months without DNASTable in figure 3.9, only amplification of the smallest fragment was observed. In the case of those samples stored in HEMAgene Buffy coat for 3 years, none of the fragments were able to amplify for the 45°C condition in comparison to those stored at room temperature and -80°C (Figure 3.10) The inability of the larger fragments to amplify could be due to various reasons, whereby PCR component concentrations and/or cycling conditions may not be sufficient for longer target sequences. In this instance, since

amplification occurred in other DNA samples isolated, the problem would most probably be due to a degree of DNA degradation that occurred in these samples.

#### **4.4 DNA sequencing**

Sequencing was also done on all DNA samples that were stored in stabilizers and some without stabilizer to determine the quality of sequences produced after short and long term storage. See figure 3.13 and 3.14 for representative sequence traces of the short and long fragment of the *β-globin* gene for one of the samples stored in DNAgard. These DNA sequences obtained from the *β-globin* PCR for short fragment and long fragment of the gene produced results that indicated high similarity to the known human *β-globin* gene after BLAST searches were done. These results clearly suggest that even after long term storage in chemical stabilizers used in this study, the quality of DNA isolated still produced high quality of sequence data that can be used in downstream sequencing application to detect SNPs or mutations, if any, in individuals whose blood or DNA has been stored for extended periods of time.

#### **4.5 RNA Quality, Yield and Quantitative Real Time PCR**

RNA was extracted and concentrations determined prior to cDNA synthesis for samples stored at 3, 7 and 14 days at RT and -80°C in RNAgard and PAXgene (Appendix I). Approximately, 200 ng of starting RNA was converted to cDNA and used in subsequent qRT-PCR experiments. The purpose of this part of this study was not to determine the expression ratio differences between samples for the *β-globin* gene, but rather to assess the amplification efficiency and the performance of the isolated RNA in downstream qRT-PCR applications. Data generated (figure 3.17-figure 3.23) during this study showed that the RNA used from all samples produced good PCR efficiencies and melting peaks. These results suggest that the quality of RNA isolated during this study was of a high standard, and could confidently be used in downstream applications for both molecular and diagnostic purposes.

#### **4.6 Quantitative PCR with *β-globin* gene on DNA samples.**

DNA isolated from samples stored in stabilizers was also subjected to Q-PCR analysis. For all experiments 200 ng of DNA was used as starting material and used to construct

standard curve using the  $\beta$ -globin gene for the short fragment of the gene (268 bp). The Q-PCR analysis showed that PCR efficiencies ranged between 95-98 % with no contamination as indicated in melting peak analysis (figure 3.17-figure 3.23). These findings suggest that samples isolated after storage in the DNA stabilizers used in this study could generate a good quality of DNA that can be used for downstream quantitative measurements for copy numbers of DNA in both a research and diagnostic environment. However, this study did not evaluate the diagnostic applications of samples stored in the different stabilizers. Despite the fact that these samples were not used in diagnostic tests, the technologies mentioned could contribute to an effective means to store valuable biological specimens in any laboratory which does not have sufficient equipment for appropriate storage. This study showed that DNA and RNA stabilizers could be used to maintain sample integrity under various different temperature conditions, and that these samples could still be used for downstream molecular and diagnostic applications.

#### **4.7 Transportation cost analysis**

Based on the cost analysis done, pricing comparison of Marken and DHL shipments at room temperature refrigerated and controlled ambient shows big price differences from the 8 sites that were evaluated. See figure 3.24 and figure 3.25 for cost comparisons between these couriers. Further cost comparison for the shipment of 500 samples with and without stabilizer was also performed for the highest priced sites. Based on cost comparison as shown in figure 3.26, we demonstrate that transportation at ambient conditions with the addition of a stabilizer to the DNA as a more cost effective transportation solution for SA and Africa in general. However, we must also take into consideration that the cost could vary for different sites. Other factors that also influence cost include:

- a. Whether the final location is in a main hub and receives regular shipments.
- b. Whether either courier company operates regular large volume services to such hubs
- c. Whether significant additional packaging is required
- d. Temperature of sample being transported room temperature( ambient) ambient validated( with a thermometer) or the requirement for frozen and or refrigerated

- e. The capabilities of the receiving hub to re-freeze and re-transport the specimen maintaining the integrity of the shipment and sample.
- f. Cost are greatly affected by the minimum weight limitations set by courier companies
- g. The lock in of rates over a 12month period in terms of contractual negotiations
- h. Meeting the minimum number of samples to be transported
- i. Dollar Rand exchange rate which has worsened significantly since the start of these projects
- j. Both operators set a minimum of one (1) kilogram. One kilogram equates to setting a much higher base rate. All courier companies operate on a base minimum rate per destination offering a door to door delivery service
- k. Based rate variables per transport hub and route
- l. Inward and outward destination rates vary
- m. Fuel surcharges which are dependent on currency fluctuations and currently stand at between 15% and 16% charged over and above the rate.
- n. Bulk group purchasing discounts such as DHL enjoy through being part the Universities purchasing consortium.

#### **4.8 Limitations of the study**

Blood and buffy coat stored at 45°C clotted after prolonged storage but was more in samples without stabilization than samples that had stabilizers added. This could be due to evaporation as a result of the constant heat. The fact that the lids were not air tight is also a possibility as 1.5ml centrifuge tubes were used for storage of samples. But this was not assessed in the study. Purified DNA stored at 45°C also evaporated leading to smaller volumes or completely evaporated especially without stabilization.

The Urine DNA using the Norgen Biotek Corp was not completed as most samples degraded after 3 months of storage at RT and frozen. Nonetheless, the few samples that didn't degrade amplified with the shorter fragments of  $\beta$ -globin namely, the 268 and 538 bp (results not included) but because of the non-specificity of the results it was decided that it will be further explored in subsequent studies.

The Biomatrix dried down technology of the sample matrix was not explored as purified DNA was stored in liquid format which could attribute to some of the contradictory results observed for some of the stabilizers.

## 4.9 Future directions

The study though aimed at room temperature storage of biospecimens, did not explore all biospecimens stored in biobanks for diagnostic and clinical research. Thus, future studies are aimed to evaluate various cells and tissues for storage at room temperature.

Furthermore, due to time constraints, the use of dried blood spots wasn't evaluated and will be explored in a separate study. As they offer a quick and easier technique of collection, storage and transportation of biospecimen especially from rural communities.

Next generation sequencing (NGS) validation is also currently being explored however for the purpose of this study it was excluded because of cost and time constraints. However, results from qPCR and Sanger sequencing show that samples stored are suitable for NGS studies. Nonetheless, it needs to be evaluated and validated to determine if proprietary ingredients in stabilizers have an effect on NGS.

And finally, the application of the use of stabilizers would be determined in a diagnostic setting.

## 4.10 Conclusion

The present study aimed at stabilization and storage of biospecimens at room temperature and transport cost logistics. Results from the various stabilizers explored show that RTS provides products that are able to maintain the integrity of nucleic acids at room temperature with no loss of sample integrity. The transport cost analysis also shows that biospecimen shipping at room temperature provides a cheaper alternative to frozen shipment. This further confirms that room temperature storage provides a cheaper and greener alternative to cold chain management and would be a better and simpler solution especially for the challenges in our SA and/or African setting.



**REFERENCES**

- Abayomi, A. et al., 2013. Challenges of biobanking in South Africa to facilitate indigenous research in an environment burdened with human immunodeficiency virus, tuberculosis, and emerging noncommunicable diseases. *Biopreservation and biobanking*, 11(6), pp.347–354. Available at: <http://ovidsp.ovid.com/ovidweb.cgi?T=JS&PAGE=reference&D=prem&NEWS=N&AN=24835364>.
- Abdalla, M. et al., Isolation of DNA from as Little as 25 µL of Urine Using Norgen's Urine DNA Isolation Kit. Available at: [http://www.researchgate.net/publication/255647345\\_Isolation\\_of\\_DNA\\_from\\_as\\_Little\\_as\\_25\\_L\\_of\\_Urine\\_Using\\_Norgen's\\_Urine\\_DNA\\_Isolation\\_Kit](http://www.researchgate.net/publication/255647345_Isolation_of_DNA_from_as_Little_as_25_L_of_Urine_Using_Norgen's_Urine_DNA_Isolation_Kit) [Accessed November 3, 2015].
- Anon, 2011. NCI Best Practices for Biospecimen Resources Office of Biorepositories and Biospecimen Research National Cancer Institute National Institutes of Health U . S . Department of Health and Human Services Table of Contents.
- Bahlo, M. et al., 2010. Saliva-derived DNA performs well in large-scale, high-density single-nucleotide polymorphism microarray studies. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*, 19(3), pp.794–8. Available at: <http://cebp.aacrjournals.org/content/19/3/794.long> [Accessed October 30, 2015].
- Belloni, B. et al., 2013. Will PAXgene substitute formalin? A morphological and molecular comparative study using a new fixative system. *Journal of Clinical Pathology*, 66(2), pp.124–135. Available at: <http://jcp.bmj.com/lookup/doi/10.1136/jclinpath-2012-200983> [Accessed November 29, 2016].
- biomatrica. Inc, *Sustainable Biological Sample Management Storage Solution Proposal Example Ambient Temperature Bio-banking System for DNA & RNA Storage Prepared for : Your Important Biorepository By :*,
- Biopreservation And, 2012. 2012 Best Practices for Repositories. , 10(2).
- Bouevitch, A. et al., 2013. HEMAgene™ • BUFFY COAT DNA stabilizing reagent protects DNA in buffy coat samples through multiple freeze-thaw cycles. , (2), pp.1–4.
- Clermont, D. et al., 2014. Assessment of DNA encapsulation, a new room-temperature DNA storage method. *Biopreserv.Biobank.*, 12(1947–5543 (Electronic)), pp.176–183.
- Ergin, B. et al., 2010. Proteomic Analysis of PAXgene-Fixed Tissues. *Journal of Proteome Research*, 9(10), pp.5188–5196. Available at: <http://pubs.acs.org/doi/abs/10.1021/pr100664e> [Accessed November 28, 2016].
- Fleige, S. & Pfaffl, M.W., 2006. RNA integrity and the effect on the real-time qRT-PCR performance. *Molecular Aspects of Medicine*, 27(2–3), pp.126–139. Available at: <http://linkinghub.elsevier.com/retrieve/pii/S0098299705000944>.
- H3 Africa Working Group, 2011. Harnessing Genomic Technologies Toward Improving Health in Africa : Opportunities and Challenges. [Http://H3Africa.Org/About/White-Paper](http://H3Africa.Org/About/White-Paper), (January).
- Hager, R., 2014. Biobanking Operations: Contingency Planning and Disaster Recovery of Research Samples. *BioProcessing Journal*, 13(1), pp.56–58. Available at: <http://www.bioprocessingjournal.com/index.php/article-downloads/577-j131-biobanking-operations-contingency-planning-and-disaster-recovery-of-research-samples> [Accessed November 28, 2016].
- Harris et al., 2012. Toward a roadmap in global biobanking for health. , pp.1105–1111.
- Healthcare, G.E., FTA™ Cards.
- Howlett, S.E. et al., 2014. Evaluation of DNASTable™ for DNA storage at ambient

- temperature. *Forensic Science International: Genetics*, 8(1), pp.170–178. Available at: <http://dx.doi.org/10.1016/j.fsigen.2013.09.003>.
- Loibner, M. et al., 2016. Pathogen Inactivating Properties and Increased Sensitivity in Molecular Diagnostics by PAXgene, a Novel Non-Crosslinking Tissue Fixative A. Sapino, ed. *PLOS ONE*, 11(3), p.e0151383. Available at: <http://dx.plos.org/10.1371/journal.pone.0151383> [Accessed November 28, 2016].
- Lu, Y.-F. et al., 2014. Personalized medicine and human genetic diversity. *Cold Spring Harbor perspectives in medicine*, 4(9), p.a008581. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/25059740> [Accessed November 29, 2016].
- Massett, H.A. et al., 2011. Assessing the need for a standardized cancer HUman Biobank (caHUB): findings from a national survey with cancer researchers. *Journal of the National Cancer Institute. Monographs*, 2011(42), pp.8–15. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/21672890> [Accessed November 29, 2016].
- Matimba, A. et al., 2008. Establishment of a biobank and pharmacogenetics database of African populations. *European journal of human genetics : EJHG*, 16(7), pp.780–783.
- McClure, M.C. et al., 2009. Assessment of DNA extracted from FTA cards for use on the Illumina iSelect BeadChip. *BMC research notes*, 2, p.107. Available at: <http://www.ncbi.nlm.nih.gov/pubmed/19531223> [Accessed November 29, 2016].
- Nunes, A.P. et al., 2012. Quality of DNA extracted from saliva samples collected with the Oragene™ DNA self-collection kit. *BMC medical research methodology*, 12, p.65. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3422993&tool=pmcentrez&rendertype=abstract> [Accessed October 30, 2015].
- Rotimi, C. et al., 2014. Research capacity. Enabling the genomic revolution in Africa. *Science (New York, N.Y.)*, 344(6190), pp.1346–8. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4138491&tool=pmcentrez&rendertype=abstract> [Accessed September 9, 2015].
- Siefers, H., 2014. The ISBER Strategic Planning Process and 2014 Operating Plan. *Biopreserv.Biobank.*, 12(1947–5543 (Electronic)), pp.220–221.
- Staunton, C. & Moodley, K., 2016. Data mining and biological sample exportation from South Africa: A new wave of bioexploitation under the guise of clinical care? *South African Medical Journal*, 106(2), p.136. Available at: <http://www.samj.org.za/index.php/samj/article/view/10248> [Accessed November 26, 2016].
- Sudmant, P.H. et al., 2015. An integrated map of structural variation in 2,504 human genomes. *Nature*, 526(7571), pp.75–81. Available at: <http://www.nature.com/doi/10.1038/nature15394> [Accessed November 26, 2016].
- Thomas, C.E. et al., 2010. Urine collection and processing for protein biomarker discovery and quantification. *Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology*, 19(4), pp.953–9. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2852495&tool=pmcentrez&rendertype=abstract> [Accessed October 30, 2015].
- de Vries, J. et al., 2015. The H3Africa policy framework: negotiating fairness in genomics. *Trends in genetics : TIG*, 31(3), pp.117–9. Available at: <http://www.cell.com/article/S0168952514001966/fulltext> [Accessed September 23, 2015].
- Wan, E. et al., 2010. Green technologies for room temperature nucleic acid storage. *Current issues in molecular biology*, 12(3), pp.135–42. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=4099058&tool=pmcentrez&rendertype=abstract> [Accessed September 8, 2015].

- Warnich, L. et al., 2011. Pharmacogenomic Research in South Africa: Lessons Learned and Future Opportunities in the Rainbow Nation. *Current pharmacogenomics and personalized medicine*, 9(3), pp.191–207. Available at: <http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3228231&tool=pmcentrez&rendertype=abstract>.
- Watson, P.H., 2014. Biobank Classification : Communicating Biorepository Diversity. , 12(3), pp.163–165.
- Isber.org. (2016). *ISBER*. [online] Available at: <http://isber.org> [Accessed 25 Nov. 2016].
- Iata.org. (2016). *IATA - Dangerous Goods*. [online] Available at: <http://iata.org/whatwedo/cargo/dgr/Pages/index.aspx> [Accessed 27 Nov. 2016].

**Appendices****Appendix I: DNA AND RNA Concentrations of isolated samples**

DNAGard Biodrop readings for samples stored at room temperature at 3month

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
1	12.36	1.94	0.86
2	13.32	1.82	1.43
3	10.20	1.96	0.91
4	10.28	1.95	0.39
5	7.15	2.27	2.27
6	12.52	1.92	4.97
7	17.47	2.06	1.67
8	18.84	1.91	1.47
9	36.51	1.97	2.35
10	4.63	2.83	-1.95
11	5.67	2.12	0.30
12	10.26	1.95	1.41

DNAGard Biodrop readings for samples stored at -80°C at 3months

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
1	15.65	1.81	1.47
2	35.36	1.66	1.00
3	16.16	1.98	0.94
4	18.92	1.91	0.73
5	24.70	1.95	1.25
6	21.51	2.05	1.71
7	23.97	2.00	0.96
8	24.24	1.98	1.04
9	8.91	1.51	0.45
10	17.38	2.08	1.29
11	16.32	1.96	0.77
12	17.84	2.02	0.56

## DNAGard Biodrop readings for samples stored at 45°C at 3months

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
1	11.75	1.74	0.66
2	14.65	1.69	0.84
3	5.74	2.09	0.42
4	10.29	1.95	0.63
5	8.36	1.92	0.36
6	11.80	1.74	0.31
7	12.92	1.87	0.49
8	12.69	1.65	0.22
9	15.31	1.84	0.61
10	10.43	1.92	0.51
11	9.04	1.49	0.19
12	4.00	1.99	0.40

## HEMAGene BUFFY COAT Biodrop readings for samples at room temperature at 3months

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
1	26.73	1.81	1.69
2	49.31	1.81	1.81
3	20.71	1.77	2.38
4	17.44	1.85	2.07
5	29.37	1.91	2.05
6	47.66	1.79	1.79
7	16.65	1.93	2.95
8	25.09	1.92	1.92
9	39.32	1.84	2.15
10	22.09	1.83	2.43
11	23.49	1.74	0.68
12	17.13	1.88	0.77

## HEMAGene BUFFY COAT Biodrop readings for samples at -80°C at 3months

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
1	15.43	1.83	1.35
2	37.51	1.83	1.53
3	18.15	1.79	1.38
4	8.94	1.81	1.29
5	15.55	1.81	1.35
6	27.17	1.91	1.92
7	11.12	1.81	1.82
8	36.44	1.78	1.43
9	35.55	1.82	1.58
10	22.46	1.96	1.29
11	9.78	2.05	1.00
12	4.41	3.14	1.00

## HEMAGene BUFFY COAT Biodrop readings for samples at 45°C at 3months

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
1	16.58	1.73	0.89
2	24.50	1.69	0.78
3	12.54	1.91	0.72
4	10.05	1.99	1.25
5	11.61	2.07	1.35
6	20.33	1.79	0.91
7	7.58	2.12	1.00
8	13.28	1.82	0.87
9	33.15	1.73	0.65
10	17.83	2.02	0.95
11	3.95	2.02	1.00
12	13.04	1.85	1.18

DNAgard Qubit 2.0 flourometer readings RT at 3months. A dilution factor of 1:19 was used for Qubit DNA Assays

Sample	DNA Concentration $\mu\text{g/ml}$	Stock Concentration $\mu\text{g/ml}$
1	0.367	7.34
2	0.335	6.70
3	0.231	4.62
4	0.271	5.42
5	0.221	4.42
6	0.582	11.6
7	0.552	11.0
8	0.518	10.4
9	1.52	30.4
10	0.209	4.18
11	0.133	2.66
12	0.425	8.50

DNAgard Qubit 2.0 flourometer readings  $-80^{\circ}\text{C}$  at 3months

Sample	DNA Concentration $\mu\text{g/ml}$	Stock Concentration $\mu\text{g/ml}$
1	0.673	13.5
2	0.894	17.9
3	0.487	9.74
4	0.564	11.3
5	0.584	11.7
6	0.527	10.5
7	0.485	9.70
8	0.605	12.1
9	0.141	2.82
10	0.545	10.9
11	0.309	6.18
12	0.152	3.04

## DNAgard Qubit 2.0 flourometer readings 45°C at 3months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
1	0.275	5.50
2	0.311	6.22
3	0.0931	1.86
4	0.196	3.92
5	0.113	2.26
6	0.132	2.64
7	0.283	5.66
8	0.0830	1.66
9	0.262	5.24
10	0.174	3.48
11	0.0408	0.81
12	0.0399	0.79

## HEMAGene Qubit 2.0 flourometer readings RT at 3months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
1	0.444	8.88
2	1.22	24.4
3	0.377	7.54
4	0.425	8.50
5	0.690	13.8
6	1.16	23.2
7	0.318	6.36
8	0.498	9.96
9	0.922	18.4
10	0.0611	1.22
11	0.275	5.50
12	0.20	4.10



## HEMAGene Qubit 2.0 flourometer readings -80°C at 3months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
1	0.365	7.30
2	0.882	17.6
3	0.393	7.86
4	0.225	4.50
5	0.295	5.90
6	0.557	11.1
7	0.414	8.28
8	0.543	10.9
9	0.764	15.3
10	0.483	9.66
11	0.213	4.26
12	0.366	7.32

## HEMAGene Qubit 2.0 flourometer readings 45°C at 3months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
1	0.309	6.18
2	0.656	13.1
3	0.265	5.30
4	0.278	5.56
5	0.283	5.66
6	0.455	9.10
7	0.162	3.24
8	0.312	6.24
9	0.416	8.32
10	0.331	6.62
11	0.095	1.90
12	0.320	6.40

## DNASTable PLUS Day 0 Concentrations

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
1	268.4	1.86	2.19
2	171.2	1.88	2.06
3	129.4	1.89	2.07
4	82.93	1.93	1.89
5	104.2	1.92	1.92
6	146.7	1.84	1.86
7	95.90	1.92	2.00
8	137.9	1.89	2.09
9	69.26	1.96	1.86
10	94.10	1.92	2.04
11	65.80	1.95	1.95
12	137.3	1.89	1.89

## DNASTable PLUS samples stored at room temperature at 3month

Sample ID	DNA Concentration µg/ml	A260/A280	A260/A230
DS2	172.3	1.789	0.087
DS3	118.3	1.659	0.051
DS4	81.90	1.641	0.036
DS5	99.95	1.725	0.045
DS6	132.7	1.686	0.056
DS7	89.97	1.667	0.039
DS8	128.1	1.909	0.059
DS9	69.01	1.971	0.031
DS10	92.89	1.940	0.042
DSC11	88.27	1.908	1.332
DSC12	176.5	1.887	2.227

## DNASTable PLUS samples stored at 45°C at 3month

Sample ID	DNA Concentration µg/ml	A260/A280	A260/A230
DS2	295.5	1.807	0.113
DS3	186.4	1.736	0.072
DS4	15.72	1.469	0.208
DS5	284.8	1.840	0.109
DS6	269.9	1.813	0.104
DS7	150.0	1.785	0.060
DS8	209.9	1.910	0.082
DS9	100.7	1.968	0.043
DS10	129.9	1.942	0.053
DSC11	169.0	1.920	0.503
DSC12	385.8	1.848	2.022

## DNASTable PLUS samples stored at -80°C at 3month

Sample ID	DNA Concentration µg/ml	A260/A280	A260/A230
DS2	154.3	1.809	0.087
DS3	143.4	1.829	0.110
DS4	73.06	1.971	0.033
DS5	91.80	1.739	0.042
DS6	112.9	1.687	0.053
DS7	80.01	1.778	0.037
DS8	129.9	1.942	0.063
DS9	63.07	2.030	0.029
DS10	79.98	1.482	0.034
DSC11	67.73	2.008	1.896
DSC12	136.8	1.905	2.287

## DNASTable PLUS samples stored at -20°C at 3month

Sample ID	DNA Concentration µg/ml	A260/A280	A260/A230
DS2	132.0	1.737	0.065
DS3	102.9	1.662	0.046
DS4	57.00	1.629	0.026
DS5	83.86	1.682	0.038
DS6	110.5	1.662	0.049
DS7	78.32	1.691	0.039
DS8	129.4	1.919	0.058
DS9	51.13	1.959	0.024
DS10	75.89	1.951	0.035
DSC11	62.69	2.043	0.277
DSC12	140.2	1.896	2.608

## DNASTable PLUS Qubit 2.0 flourometer readings RT at 3months

Sample ID	DNA Concentration µg/ml	Stock Concentration µg/ml
DS2	7.4	148
DS3	2.57	51.4
DS4	1.77	35.4
DS5	2.20	44.0
DS6	2.90	58.0
DS7	2.14	42.8
DS8	3.15	63.0
DS9	1.66	33.2
DS10	3.01	60.2
DSC11	1.21	24.2
DSC12	4.19	83.8

## DNASTable PLUS Qubit 2.0 flourometer readings 45°C at 3months

Sample ID	DNA Concentration µg/ml	Stock Concentration µg/ml
DS2	6.5	130
DS3	3.60	72.0
DS4	3.32	66.4
DS5	6.7	134
DS6	6.2	124
DS7	3.18	63.6
DS8	3.06	61.2
DS9	1.92	38.4
DS10	3.57	71.4
DSC11	2.66	53.2
DSC12	9.5	190

## DNASTable PLUS Qubit 2.0 flourometer readings -80°C at 3months

Sample ID	DNA Concentration µg/ml	Stock Concentration µg/ml
DS2	3.49	69.8
DS3	3.18	63.6
DS4	2.05	41.0
DS5	1.03	20.6
DS6	3.96	79.2
DS7	1.66	33.2

DS8	3.93	78.6
DS9	1.39	27.8
DS10	1.80	36.0
DSC11	1.79	35.8
DSC12	3.39	67.8

DNASTable PLUS Qubit 2.0 flourometer readings -20°C at 3months

Sample ID	DNA Concentration µg/ml	Stock Concentration µg/ml
DS2	4.95	99.0
DS3	3.19	63.8
DS4	1.23	24.6
DS5	1.98	39.6
DS6	2.03	40.6
DS7	1.49	29.8
DS8	3.22	64.4
DS9	1.34	26.8
DS10	1.75	35.0
DSC11	1.49	29.8
DSC12	2.98	59.6

## DNAGard Biodrop readings for samples stored at room temperature at 6 month

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
DG1	10.13	1.947	1.421
DG2	8.332	2.501	1.136
DG3	7.933	2.017	0.726
DG4	11.70	2.053	1.520
DG5	10.16	2.443	0.910
DG6	13.32	2.108	1.820
DG7	7.868	2.034	0.529
DG8	3.922	2.041	0.197
DG9	9.221	2.863	1.122
DG10	8.274	1.569	0.152
DGC11	10.11	2.461	0.771
DGC12	9.778	2.588	1.114

## DNAGard Biodrop readings for samples stored at room 45°C at 6month

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
DG1	2.956	1.511	0.425
DG2	3.925	2.039	0.495
DG3	3.933	2.035	0.360
DG4	2.442	1.000	0.213
DG5	4.094	1.955	0.290
DG6	5.177	1.630	0.270
DG7	3.697	1.371	0.163
DG8	3.315	1.432	0.293
DG9	3.771	2.169	0.382
DG10	4.416	1.828	0.424
DGC11	4.880	1.258	0.148
DGC12	8.183	1.584	0.619

## DNAGard Biodrop readings for samples stored at -80°C at 6 month

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
DG1	20.25	1.957	1.800
DG2	23.32	1.751	0.795
DG3	21.68	1.856	1.161
DG4	14.44	1.941	2.243
DG5	18.46	1.952	1.952
DG6	22.45	1.961	1.803
DG7	15.85	2.019	2.710
DG8	23.54	1.877	1.619
DG9	33.17	1.932	2.051
DG10	13.26	1.826	1.432
DGC11	8.246	1.942	2.540
DGC12	7.058	2.309	0.876

## DNAGard Qubit 2.0 flourometer readings for RT samples at 6 months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
DG1	0.259	5.18
DG2	0.364	7.28
DG3	0.272	5.44
DG4	0.368	7.36
DG5	0.302	6.04
DG6	0.506	10.1
DG8	0.215	4.30
DG8	0.0382	0.764
DG9	0.400	8.00
DG10	0.0816	1.63
DGC11	0.298	5.96
DGC12	0.385	7.70

## DNAgard Qubit 2.0 flourometer readings for 45°C samples at 6 months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
DG1	0.169	3.34
DG2	0.114	2.28
DG3	0.0957	1.91
DG4	0.0831	1.66
DG5	0.0809	1.62
DG6	0.102	2.04
DG7	0.136	2.72
DG8	0.0796	1.59
DG9	0.0753	1.51
DG10	0.0968	1.94
DGC11	0.0706	1.41
DGC12	0.388	7.76

## DNAgard Qubit 2.0 flourometer readings for -80°C samples at 6 months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
DG1	1.22	24.4
DG2	1.00	20.0
DG3	1.19	23.8
DG4	1.00	20.0
DG5	0.700	14.0
DG6	1.05	21.0
DG7	0.720	14.4
DG8	1.26	25.2
DG9	2.21	44.2
DG10	0.197	3.94
DGC11	0.654	13.1
DGC12	0.288	5.76



## HEMAGene Biodrop readings for samples stored at room temperature (RT) at 6month

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
HG1	34.16	1.881	1.614
HG2	51.01	1.821	1.700
HG3	26.69	1.950	1.599
HG4	18.53	1.760	1.479
HG5	29.00	1.706	1.115
HG6	29.60	2.028	2.028
HG7	16.66	2.502	1.563
HG8	31.40	2.039	1.467
HG9	33.82	1.898	1.261
HG10	22.84	2.107	2.107
HGC11	22.45	2.148	1.365
HGC12	23.60	1.873	0.596

## HEMAGene Biodrop readings for samples stored at 45°C at 6month

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
HG1	44.26	1.686	0.816
HG2	61.78	1.776	1.321
HG3	33.88	1.895	1.623
HG4	20.49	1.783	1.243
HG5	19.16	1.717	0.657
HG6	37.89	1.655	0.927
HG7	16.83	1.906	1.312
HG8	29.30	1.915	1.601
HG9	40.38	1.804	1.052
HG10	18.33	1.774	1.486
HGC11	4.113	1.947	0.804
HGC12	3.702	2.175	0.552

## HEMAGene Biodrop readings for samples stored at -80°C at 6month

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
HG1	27.21	1.789	1.494
HG2	45.76	1.848	1.776
HG3	23.04	1.914	1.641
HG4	17.44	1.848	1.525
HG5	21.92	1.839	1.697
HG6	39.66	1.920	2.017
HG7	16.97	1.892	1.308
HG8	28.60	1.959	1.723
HG9	41.57	1.842	1.842
HG10	26.30	1.839	1.613
HGC11	3.342	2.491	0.626
HGC12	6.397	2.669	0.865

## HEMAGene Qubit 2.0 flourometer readings for RT samples at 6 months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
HG1	1.57	31.4
HG2	3.25	65.0
HG3	3.01	60.2
HG4	1.26	25.2
HG5	1.20	24.0
HG6	2.24	44.8
HG7	0.857	17.1
HG8	3.39	67.8
HG9	2.15	43.0
HG10	1.52	30.4
HGC11	1.48	29.6
HGC12	0.488	9.76

## HEMAGene Qubit 2.0 flourometer readings for 45°C samples at 6 months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
HG1	1.51	30.2
HG2	1.78	35.6
HG3	1.07	21.4
HG4	0.875	17.5
HG5	0.580	11.6
HG6	1.11	22.2
HG7	0.860	17.2
HG8	0.866	17.3
HG9	1.04	20.8
HG10	0.466	9.32
DGC11	0.0638	1.28
DGC12	0.116	2.32

## HEMAGene Qubit 2.0 flourometer readings for -80°C samples at 6 months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
HG1	3.28	65.6
HG2	2.58	51.6
HG3	1.12	22.4
HG4	0.809	16.2
HG5	0.993	19.9
HG6	2.27	45.4
HG7	0.669	13.3
HG8	2.44	48.8
HG9	1.35	27.0
HG10	1.82	36.4
HGC11	0.0460	0.920
HGC12	0.150	3.00

## DNAGard Biodrop readings for samples stored at room temperature at 9 month

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
DG1	5.804	1.526	0.392
DG2	4.637	1.275	0.225
DG3	5.377	1.228	0.196
DG4	6.780	1.794	0.629
DG5	2.192	1.523	0.493
DG6	7.271	1.159	0.121
DG7	15.70	2.039	0.887
DG8	8.570	1.875	0.682
DG9	20.80	1.625	0.402
DG10	3.535	2.303	0.371
DGC11	13.80	1.770	0.775
DGC12	25.29	1.770	0.783

## DNAGard Biodrop readings for samples stored at -80 at 9 month

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
DG1	21.80	1.847	1.160
DG2	30.74	1.836	1.000
DG3	24.03	1.599	0.445
DG4	20.41	1.645	1.000
DG5	26.81	1.696	1.288
DG6	26.28	1.840	1.438
DG7	37.19	1.842	1.604
DG8	38.30	1.887	1.576
DG9	66.28	1.827	1.244
DG10	21.75	2.023	1.048
DGC11	17.24	1.866	0.519
DGC12	8.972	2.259	0.562

## DNAGard Biodrop readings for samples stored at 45 at 9 month

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
DG1	3.076	1.482	0.170
DG2	14.79	1.371	0.232
DG3	5.206	1.238	0.215
DG4	4.057	1.972	0.311
DG5	15.04	1.498	0.235
DG6	8.535	1.306	0.211
DG7	1.525	2.906	0.203
DG8	5.887	1.515	0.246
DG9	5.208	1.624	0.178
DG10	3.195	2.674	0.225
DGC11	2.630	4.175	0.305
DGC12	3.177	1.459	0.209

## DNAGard Qubit 2.0 flourometer readings for RT samples at 9 months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
DG1	0.346	6.92
DG2	0.102	2.04
DG3	0.0445	0.890
DG4	0.403	8.06
DG5	0.149	2.98
DG6	0.166	3.32
DG7	0.787	15.7
DG8	0.341	6.82
DG9	0.930	18.6
DG10	0.159	3.18
DGC11	0.489	9.78
DGC12	0.920	18.4

## DNAgard Qubit 2.0 flourometer readings for -80 samples at 9 months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
DG1	0.933	18.7
DG2	1.04	20.8
DG3	0.664	13.3
DG4	0.804	16.1
DG5	1.05	21.0
DG6	1.07	21.4
DG7	1.27	25.4
DG8	1.25	25.0
DG9	2.45	49.0
DG10	0.682	13.6
DGC11	0.460	9.20
DGC12	0.199	3.98

## DNAgard Qubit 2.0 flourometer readings for 45 samples at 9 months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
DG1	0.0537	1.07
DG2	0.107	2.14
DG3	0.0723	1.45
DG4	0.0474	0.948
DG5	0.136	2.72
DG6	0.0658	1.32
DG7	0.0552	1.10
DG8	0.0698	1.40
DG9	0.0622	1.24
DG10	0.0520	1.04
DGC11	0.0488	0.976
DGC12	0.0701	1.40

## HEMAgene Biodrop readings for samples stored at room temperature at 9 month

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
HG1	29.93	1.768	1.581
HG2	35.71	1.812	1.445
HG3	14.98	1.877	1.250
HG4	15.03	1.872	1.363
HG5	32.06	1.775	1.552
HG6	41.90	1.830	1.558
HG7	15.11	1.863	1.248
HG8	37.09	1.759	1.275
HG9	25.49	1.890	1.244
HG10	17.58	2.049	1.128
HGC11	23.88	1.854	0.666
HGC12	10.69	1.879	0.543

## HEMAgene Biodrop readings for samples stored at -80 at 9 month

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
HG1	22.42	1.805	1.287
HG2	31.78	1.894	1.787
HG3	18.12	1.986	1.283
HG4	14.05	1.993	0.737
HG5	22.34	1.811	1.155
HG6	28.63	1.832	1.212
HG7	11.69	2.055	0.854
HG8	24.36	1.971	0.961
HG9	31.29	1.810	0.817
HG10	19.86	2.015	1.337
HGC11	16.39	1.953	0.804
HGC12	4.494	5.213	0.497

## HEMAGene Biodrop readings for samples stored at 45 at 9 month

Sample	DNA Concentration µg/ml	A260/A280	A260/A230
HG1	36.23	1.884	1.791
HG2	43.23	1.861	1.945
HG3	13.66	2.051	1.281
HG4	15.54	2.062	1.475
HG5	24.22	1.982	1.982
HG6	23.54	2.040	2.234
HG7	10.92	2.220	1.379
HG8	19.20	2.087	1.882
HG9	35.25	1.931	1.396
HG10	17.73	2.031	1.653
HGC11	9.616	2.659	0.546
HGC12	18.17	1.982	1.124

## HEMAGene Qubit 2.0 flourometer readings for RT samples at 9 months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
HG1	1.34	26.8
HG2	1.91	38.2
HG3	0.696	13.9
HG4	0.745	14.9
HG5	1.12	22.4
HG6	1.25	25.0
HG7	0.701	14.0
HG8	0.181	3.62
HG9	1.23	24.6
HG10	0.824	16.5
HGC11	1.34	26.8
HGC12	0.417	8.34



## HEMAGene Qubit 2.0 flourometer readings for -80 samples at 9 months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
HG1	1.13	22.6
HG2	0.937	18.7
HG3	0.665	13.3
HG4	0.541	10.8
HG5	0.727	14.5
HG6	1.40	28.0
HG7	0.313	6.26
HG8	0.773	15.5
HG9	1.01	20.2
HG10	0.731	14.6
HGC11	0.448	8.96
HGC12	0.0371	0.742

## HEMAGene Qubit 2.0 flourometer readings for 45 samples at 9 months

Sample	DNA Concentration µg/ml	Stock Concentration µg/ml
HG1	1.50	30.0
HG2	1.20	24.0
HG3	0.416	8.32
HG4	0.524	10.5
HG5	0.668	13.4
HG6	0.712	14.2
HG7	0.288	5.76
HG8	0.574	11.5
HG9	1.07	21.4
HG10	0.384	7.68
HGC11	0.0407	0.814
HGC12	0.437	8.74

## DNASTable PLUS Biodrop readings for samples stored at RT at 6month

Sample ID	DNA Concentration µg/ml	A260/A280	A260/A230
DS2	158.4	1.772	0.074
DS3	115.0	1.692	0.050
DS4	91.51	1.710	0.039
DS5	111.5	1.756	0.049
DS6	152.9	1.780	0.074
DS7	102.1	1.644	0.043
DS8	167.9	1.889	0.075
DS9	76.43	1.989	0.039
DS10	125.9	1.910	0.053
DSC11	78.42	1.989	1.256
DSC12	223.6	1.870	20.78

## DNASTable PLUS Biodrop readings for samples stored at -80°C at 6 month

Sample ID	DNA Concentration µg/ml	A260/A280	A260/A230
DS2	142.8	1.724	0.061
DS3	137.4	1.822	0.079
DS4	84.87	2.027	0.103
DS5	100.5	1.844	0.052
DS6	121.3	1.726	0.052
DS7	84.21	1.863	0.041
DS8	121.5	1.976	0.053
DS9	65.56	2.145	0.029
DS10	96.11	1.998	0.043
DSC11	144.4	1.890	1.498
DSC12	179.2	1.902	2.103

## DNASTable PLUS Biodrop readings for samples stored at -20°C at 6 month

Sample ID	DNA Concentration µg/ml	A260/A280	A260/A230
DS2	176.8	1.846	0.101
DS3	117.6	1.739	0.053
DS4	141.7	1.823	0.065
DS5	219.7	1.851	0.098
DS6	167.6	1.810	0.080
DS7	65.06	1.756	0.029
DS8	211.0	1.936	0.090
DS9	75.73	2.062	0.035
DS10	74.60	2.038	0.033
DSC11	67.62	2.073	1.625
DSC12	150.6	1.941	2.047

## DNASTable PLUS Biodrop readings for samples stored at 45°C at 6 month

Sample ID	DNA Concentration µg/ml	A260/A280	A260/A230
DS2	882.2	1.853	0.286
DS3	359.2	1.840	0.133
DS4	339.1	1.812	0.125
DS5	293.2	1.889	0.116
DS6	523.9	1.839	0.184
DS7	450.7	1.820	0.159
DS8	673.5	1.879	0.233
DS9	196.5	1.955	0.078
DS10	333.5	1.889	0.124
DSC11	129.5	1.918	1.762
DSC12	427.7	1.799	2.069

## DNASTable PLUS Qubit 2.0 flourometer readings RT at 6 months

Sample ID	DNA Concentration µg/ml	Stock Concentration µg/ml
DS2	4.12	82.4
DS3	2.50	50.0
DS4	1.84	36.8
DS5	1.76	35.2
DS6	3.27	65.4
DS7	1.80	36.0
DS8	2.99	59.8
DS9	1.10	22.0
DS10	1.54	30.8
DSC11	1.28	25.6
DSC12	4.53	90.6

## DNASTable PLUS Qubit 2.0 flourometer readings -80°C at 6 months

Sample ID	DNA Concentration µg/ml	Stock Concentration µg/ml
DS2	5.8	116
DS3	7.3	146
DS4	4.85	97.0
DS5	4.32	86.4
DS6	6.4	128
DS7	3.97	79.4
DS8	7.1	142
DS9	2.74	54.8
DS10	5.1	102
DSC11	3.28	65.6
DSC12	9.9	198

## DNASTable PLUS Qubit 2.0 flourometer readings -20°C at 6 months

Sample ID	DNA Concentration µg/ml	Stock Concentration µg/ml
DS2	8.3	166
DS3	3.76	75.2
DS4	3.83	76.6
DS5	2.93	58.6
DS6	6.7	134
DS7	3.50	70.0
DS8	5.00	100
DS9	2.18	43.6
DS10	3.73	74.6
DSC11	2.17	43.4
DSC12	6.1	122

## DNASTable PLUS Qubit 2.0 flourometer readings 45°C at 6 months

Sample ID	DNA Concentration µg/ml	Stock Concentration µg/ml
DS2	0.950	19.0
DS3	3.77	75.4
DS4	1.77	35.4
DS5	2.48	49.6
DS6	1.16	23.2

DS7	3.51	70.2
DS8	5.3	106
DS9	1.39	27.8
DS10	2.00	40.0
DSC11	2.20	44.0
DSC12	0.465	9.30

RNAgard (RG) and PAXgene (PG) biodrop readings for room temperature samples (RT) at 3 days

SAMPLE ID	RNA CONC ug/ml	A260/280	A260/230
RG1	0.800	1.000	0.014
RG2	1.264	2.275	0.020
RG3	1.711	1.878	0.014
RG4	1.294	2.620	0.023
RG5	0.830	1.000	0.015
EDTA 6	51.93	1.914	0.783
PG1	4.550	1.542	0.198
PG2	4.068	1.648	0.039
PG3	11.15	1.756	0.127
PG4	41.48	1.681	0.318
PG5	4.662	2.061	0.089

## RNAgard (RG) AND PAXgene (PG) biodrop readings for -80 at 3 days

SAMPLE ID	RNA CONC ug/ml	A260/280	A260/230
RG1	2.364	3.094	0.011
RG2	1.808	1.793	0.013
RG3	1.351	2.452	0.108
RG4	1.384	2.374	0.008
RG5	1.296	2.614	0.008
EDTA 6	36.40	1.857	0.394
PG1	15.42	2.078	0.155
PG2	14.00	2.059	0.156
PG3	8.036	1.991	0.251
PG4	23.76	1.892	0.284
PG5	30.31	1.814	0.527

## RNAgard (RG) AND PAXgene (PG) biodrop readings for room temperature samples (rt) at 7 days

SAMPLE ID	RNA CONC ug/ml	A260/280	A260/230
RG1	2.156	3.878	0.022
RG2	1.075	3.908	0.019
RG3	2.488	2.802	0.023
RG4	1.992	1.671	0.026
RG5	1.552	2.064	0.115
EDTA 6	46.71	1.803	0.333
PG1	9.844	1.684	0.132
PG2	27.68	1.766	0.446
PG3	8.171	1.959	0.120
PG4	14.69	1.772	0.223
PG5	3.488	1.847	0.214

## RNAgard (RG) AND PAXgene (PG) biodrop readings for -80 at 7 days

SAMPLE ID	RNA CONC ug/ml	A260/280	A260/230
RG1	0.644	-4.114	0.023
RG2	0.160	0.251	0.039
RG3	0.228	0.339	0.023
RG4	0.0	0.273	0.032
RG5	0.564	-2.392	0.036
EDTA 6	24.19	1.985	1.754
PG1	14.12	2.041	0.069
PG2	15.56	2.058	0.069
PG3	10.42	2.592	0.086
PG4	30.64	1.798	0.456
PG5	25.25	2.028	0.191

## RNAgard (RG) AND PAXgene (PG) biodrop readings for room temperature samples (rt) at 14 days

SAMPLE ID	RNA CONC ug/ml	A260/280	A260/230
RG1	2.833	2.298	0.172
RG2	2.413	184.40	0.167

RG3	2.499	25.141	0.102
RG4	4.269	3.992	0.025
RG5	3.683	7.618	0.047
EDTA 6	50.13	1.861	0.309
PG1	5.839	3.175	0.201
PG2	6.710	2.476	0.035
PG3	9.061	2.618	0.053
PG4	14.26	2.020	0.448
PG5	6.586	2.547	0.057

RNAgard (RG) AND PAXgene (PG) biodrop readings for -80 at 14 days

SAMPLE ID	RNA CONC ug/ml	A260/280	A260/230
RG1	5.754	3.281	0.020
RG2	5.007	4.937	0.028
RG3	3.362	3.494	0.057
RG4	6.321	2.723	0.030
RG5	3.989	5.057	0.030
EDTA 6	26.85	2.026	0.045
PG1	23.47	2.046	0.311
PG2	11.10	2.361	0.164
PG3	10.54	2.135	0.397
PG4	13.48	2.147	0.498
PG5	10.52	2.554	0.687

## RNAgard (RG) AND PAXgene (PG) qubit readings for room temperature (RT) at 3 days

SAMPLE ID	RNA CONC ng/ml	STOCK CONC ng/ml
RG1	20	400
RG2	↓	↓
RG3	↓	↓
RG4	28.5	570
RG5	28.2	564
EDTA 6	770	1.54E+04
PG1	196	3.92E+03
PG2	130	2.60E+03
PG3	223	4.46E+03
PG4	39.1	782
PG5	184	3.68E+03

## RNAgard (RG) AND PAXgene (PG) qubit readings for -80 at 3 days

SAMPLE ID	RNA CONC ng/ml	STOCK CONC ng/ml
RG1	↓	↓
RG2	26.7	534
RG3	20	400
RG4	↓	↓
RG5	36.9	738
EDTA 6	252	5.04E+03
PG1	396	7.92E+03
PG2	245	4.90E+03
PG3	306	6.12E+03
PG4	115	2.30E+03
PG5	361	7.22E+03



## RNAgard (RG) AND PAXgene (PG) qubit readings for room temperature (RT) at 7 days

SAMPLE ID	RNA CONC ng/ml	STOCK CONC ng/ml
RG1	97.6	1.95E+03
RG2	42.0	840
RG3	75.1	1.50E+03
RG4	29.7	594
RG5	82.4	1.65E+03
EDTA 6	241	4.82E+03
PG1	223	4.46E+03
PG2	197	3.94E+03
PG3	172	3.44E+03
PG4	40.8	816
PG5	184	3.68E+03

## RNAgard (RG) AND PAXgene (PG) qubit readings for -80 at 7 days

SAMPLE ID	RNA CONC ng/ml	STOCK CONC ng/ml
RG1	32.4	648
RG2	↓	↓
RG3	↓	↓
RG4	↓	↓
RG5	↓	↓
EDTA 6	249	4.98E+03
PG1	388	7.76E+03
PG2	249	4.98E+03
PG3	341	6.82E+03
PG4	155	3.10E+03
PG5	279	5.58E+03

## RNAgard (RG) AND PAXgene (PG) qubit readings for room temperature (rt) at 14 days

SAMPLE ID	RNA CONC ng/ml	STOCK CONC ng/ml
RG1	173	3.46E+03
RG2	138	2.76E+03
RG3	151	3.02E+03
RG4	53.5	1.07E+03
RG5	92.9	1.86E+03
EDTA 6	105	2.10E+03
PG1	83.5	1.67E+03
PG2	93.8	1.88E+03
PG3	27.3	546
PG4	21	420
PG5	81.7	1.63E+03

## RNAgard (RG) AND PAXgene (PG) qubit readings for -80 at 14 days

SAMPLE ID	RNA CONC ng/ml	STOCK CONC ng/ml
RG1	↓	↓
RG2	↓	↓
RG3	↓	↓
RG4	↓	↓
RG5	↓	↓
EDTA 6	199	3.98E+03
PG1	331	6.62E+03
PG2	184	3.68E+03
PG3	183	3.66E+03
PG4	130	2.60E+03
PG5	291	5.82E+03

## Biodrop readings for cultured cell stored at room temperature (RT) at 1 month

SAMPLE ID	DNA CONC ug/ml	A260/280	A260/230
P1	62.30	1.929	1.169
P2	45.53	1.935	0.938
NP(H <sub>2</sub> O)	19.89	2.012	0.511
NP(DMSO 1)	16.52	1.735	0.560
NP(DMSO 2)	29.53	1.907	0.936

## Biodrop readings for cultured cell stored at liquid nitrogen (LN) at 1 month

SAMPLE ID	DNA CONC ug/ml	A260/280	A260/230
P1	68.50	1.929	1.212
P2	64.10	1.882	1.123
NP(H <sub>2</sub> O)	58.79	1.973	1.158
NP(DMSO 1)	38.60	1.874	1.027
NP(DMSO 2)	47.40	1.866	1.145

## Qubit readings for cultured cells stored at room temperature (RT) at 1 month

SAMPLE ID	DNA CONC ug/ml	STOCK CONC ug/ml
P1	4.03	80.6
P2	1.79	35.8
NP(H <sub>2</sub> O)	0.297	5.94
NP(DMSO 1)	0.702	14.0
NP(DMSO 2)	1.17	23.4

## Qubit readings for cultured cells stored at liquid nitrogen (LN) at 1 month

SAMPLE ID	DNA CONC ug/ml	STOCK CONC ug/ml
P1	2.06	41.2
P2	2.02	40.4
NP(H <sub>2</sub> O)	0.531	10.6
NP(DMSO 1)	0.988	19.8
NP(DMSO 2)	1.36	27.2

↓- RNA concentration too low to be detected by Qubit flourometer.

## **Appendix II: Samples selected for DNA sequencing**

For DNA in buffy coat stored for 3 months with or without HEMAgene buffy coat stabilizers, one sample at RT, -80°C and 45°C was sent for sequencing. Each sample had a forward and a reverse reaction for both small (268 bp and large (1327 bp) fragment of the Beta globin gene.

The same selection criteria were done for samples stored in DNAgard stabilizer for 3 months.