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Minor Dissertation

ESTABLISHING LOCALLY DERIVED REFERENCE INTERVALS FOR FULL BLOOD COUNT PARAMETERS AND WHITE CELL DIFFERENTIAL COUNTS IN THE WESTERN CAPE REGION OF SOUTH AFRICA

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DECLARATION

I, Annemarie de Koker, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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ABSTRACT

Establishing locally derived reference intervals for full blood count parameters and white cell differential counts in the Western Cape region of South Africa.

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Background:

The recognised variation observed in normal haematological parameters in different populations and geographic locations, emphasizes the need to establish locally derived reference intervals (RIs) with appropriate representation of the various ethnic groups. Accurate RIs are essential to distinguish between health and disease.

Objective:

To establish locally derived RIs for full blood count (FBC) and white blood cell (WBC) differential count parameters in healthy adults in the Western Cape region of South Africa.

Methods:

A prospective, descriptive study was performed, utilizing blood samples of healthy first-time blood donors, presenting voluntarily for blood donation to the Western Cape Blood Service (WCBS) between November 2016 and October 2017. African, Coloured and Caucasian participants aged between 18 and 60 years of age were included based on convenience sampling. Participants testing positive for human immunodeficiency virus (HIV), hepatitis B and C viruses (HBV, HCV) and syphilis, and those with serum ferritin levels outside the reference range were excluded. Donors with an elevated serum ferritin were also excluded. Reference intervals were derived using non-parametric statistical methods and expressed to include the central 95% of the sample population (2.5th to 97.5th percentiles). Outliers for individual parameters were identified and excluded from the analysis.

Results:

A total of 376 females and 244 males were included for analysis; 31.61% were African, 39.68% Coloured and 28.71% Caucasian. For all race groups combined, gender-based differences were found in most FBC parameters, including the haemoglobin (Hb), WBC count, neutrophil count and platelet count. When comparing RIs for males and females in the three ethnic groups, statistically significant differences were found for parameters including the Hb, WBC count and red cell indices. There were no significant differences in the absolute eosinophil counts and mean cell volume (MCV) in females, and platelet counts in males. The ranges differed for a number of FBC variables compared to the National Health Laboratory Service (NHLS) coastal reference ranges in current use.

Conclusion:

Locally established and population-specific RIs are essential for accurate interpretation of blood counts. Implementation of separate RIs for the main ethnic groups in the Western Cape should be considered and would have implications for the diagnosis of anaemia and other blood count abnormalities as well as decision rules on haemoglobin levels for blood donor deferral.

ACKNOWLEDGEMENTS

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My family for making it possible for me to pursue this degree; A/Prof Jessica Opie and Dr Arthur Bird for their guidance and instruction as supervisors; and to my co-investigators at the Western Cape Blood Services (WCBS), Dr Caroline Hilton (WCBS)Transfusion Medicine Specialist), Dr Gregory Bellairs (CEO/Medical Director, WCBS) for facilitating and funding this project; Bev Mitchell (WCBS Technical Services Manager), Russell Cable (WCBS Laboratory supervisor), Charlotte Pistorius (WCBS Deputy Laboratory Supervisor). As well as to all the WCBS, clinic and laboratory staff for the recruitment of participants, sample collection and processing. Lastly to Raymond Nhapi for his assistance with the statistical analysis of the data.

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v ABBREVIATIONS

CLSI	Clinical and Laboratory Standards Institute
EDTA	Ethylene-diamine-tetraacetic acid
FBC	Full blood count
Hb	Haemoglobin
HBV	Hepatitis B Virus
Hct	Haematocrit
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
ICSH	International Committee of Standardization in Haematology
IFCC	International Federation of Clinical Chemistry
ISO	International Organisation for Standardization
LL	Lower limit
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volume
MPV	Mean platelet volume
NAT	Nucleic Acid Testing
NCHS	National Centre for Health Statistics
NHANES	National Health and Nutrition Examination Survey
NHLS	National Health Laboratory Service
RCC	Red cell count
RDW	Red cell distribution width
SANAS	South African National Accreditation System
SANBS	South African National Blood Service
SCRV	Standing Committee on Reference Values
UCT	University of Cape Town
UL	Upper limit
WCC	White cell count
WHO	World Health Organisation
WCBS	Western Cape Blood Service

1. OBJECTIVES

The objectives of this literature review are to obtain the following background information:

- Defining haematological reference intervals (RIs)
- Guidelines for establishing haematological RIs
- The relevance of locally derived RIs
- A review of international and local literature, focusing on gender and ethnic differences in haematological RIs
- Blood donor deferral for anaemia and the potential role of screening donors for iron deficiency

2. SEARCH STRATEGY AND QUALITY CRITERIA

Pubmed was used as the primary online search engine. Articles in languages other than English and those not available via the University of Cape Town library were not included. Referencing was performed using Mendeley.

Search parameters included:

- 'Reference' AND 'blood count' AND 'sea level'
- 'Africa' AND 'reference' AND 'hemoglobin/ haemoglobin'
- 'Defining reference ranges' AND 'reference intervals'
- Haematological reference ranges/intervals
- Establishing haematological reference ranges/intervals in South Africa
- 'Ferritin' AND 'diagnosis of iron deficiency'
- 'Anaemia' and 'blood donor deferral'

Limits included in the Pubmed search (title and abstract)

- Adults
- Human

3. LITERATURE REVIEW

3.1 INTRODUCTION

Accurate laboratory RIs are required for the appropriate interpretation of laboratory results, and thus the diagnosis of disease, monitoring of disease and/or health. Most African countries have relied on RIs obtained from international Caucasian populations.[1] However, studies on RIs in different regions of the African continent have demonstrated statistically significant differences compared to populations in developed countries for various haematological parameters. This emphasizes the importance of establishing locally derived population-specific RIs.

Although there have been efforts toward the standardization and harmonisation in the reporting of pathology results, progress has been especially slow in the field of haematology. A United Kingdom (UK) based initiative, the Pathology Harmony project (2007), was commissioned to investigate, and if appropriate, reduce the variations that exist in different clinical laboratories. This required critical appraisal of relevant literature to provide scientific evidence to justify these variations in RIs of analytes/parameters.[2] The lower limit (LL) of the RI for haemoglobin (Hb) is an example of how this inter-laboratory variation can lead to problems for clinicians in defining and diagnosing anaemia.[3] Regarding the Hb, it is noted that 'international standards have long been available for the calibration and acquisition of Hb measurement on automated analyzers, thus variation in Hb reference intervals between laboratories can no longer be blamed on different analytical methodologies'.[3,4] With the current technology available, the same is likely to be true for white blood cell (WBC) differentials and platelet counts.

The 'Pathology Harmony' expert panel, recognized that the well-described variation in RIs for extended full blood count (FBC) and other haematological parameters is a major limiting factor to the harmonization in the field of haematology, and explained that a huge amount of work and funding is required to fulfill their objectives.[5,6]

3.2 DEFINING AND DETERMINING REFERENCE INTERVALS

Ideally, RIs should be derived from a carefully selected and adequately-sized sample population, who represent the local population with regard to relevant demographic features.

The Clinical and Laboratory Standards Institute (CLSI) have a protocol for determining RIs, most recently updated in October 2010 which includes the basic requirements.[5] The CLSI defines reference values as 'values obtained by observation or measurement on reference individuals in the reference sample group. The reference interval is usually the central interval of values bounded by the reference limit values at certain designated percentiles'[5], usually 95% of the sample. Similarly, Gräsbeck states that the term 'reference interval' is more appropriate to use when statistical calculations and manipulations are applied to eliminate some of the observations.[7]

The term reference interval is therefore preferred, not only to avoid the ambiguity of the terms 'healthy' or 'normal' ranges, but also in recognition of the overlap between laboratory results in health and disease.[8] Statistically, values of some healthy individuals will lie outside the RI, while values of some diseased patients will fall within it. An added level of complexity results from population diversity in terms of gender, age, ethnicity and environmental factors, especially altitude.[8] As far as possible, these factors should be taken into account when determining RIs, and eventual stratification/partitioning into subgroups may have to be considered. Several additional confounding factors include subclinical infection, parasite infestation, autoimmune disease, genetic factors or nutritional deficiencies in the reference subjects which may remain hidden despite extensive efforts to identify truly healthy 'reference individuals'.[8]

If the purpose of a laboratory test is to determine health or diagnose disease, it follows that the first step in selecting reference individuals, is to establish criteria for excluding unhealthy individuals from the reference sample. When evaluating the health of an individual, the completion of a health questionnaire is considered the minimum.[5] The criteria used to define the reference individuals together with the statistical analysis of the data will influence the RI. In selecting reference individuals the working group of the CLSI endorses the use of direct sampling techniques as well

as using clearly defined specific criteria for selecting reference individuals from a reference population. Appropriate reference subject selection, adequate numbers of subjects and avoiding pre-analytical errors all require special consideration.[5] These factors are considered more important than the statistical methods used to estimate the RIs from the observed data. If the reference subjects are deemed representative and the testing of samples and analyses standardized, a relatively small sample (n=40) per subgroup may be considered acceptable.[9,10]

3.2.1 Statistical Analysis Required

Haematological laboratory parameters for a population group tend to be lognormal rather than Gaussian.[5] As explained by Gräsbeck, the frequent lognormal distribution is caused by the fact that values below zero are impossible but high values are possible (unless incompatible with life).[7] Homeostatically controlled analytes such as electrolytes, vary little and the Gaussian distribution thus applies to them. Many methods have been proposed for the purpose of transformation of data from lognormal to Gaussian, with the logarithmic function and specific elements of the Box-Cox power family being the most frequent choices.[11]

Most authors recommend the non-parametric statistical approach in the haematology setting for establishing RIs.[9] This obviates the need to make assumptions regarding the data distribution and the substantial proportion of statistical transformations towards normality, which is often required.[9,11] Moreover, if the sample size is adequate ($n \geq 120$) and outliers have been excluded appropriately, the non-parametric approach will provide the most accurate results and allow the calculation of confidence limits for upper and lower cut-offs.[9] However, though non-parametric methods are considered superior in most cases, they require a minimum of 120 participants to allow calculation of 90% confidence intervals for the upper and lower reference limits.[5,10] Confidence intervals are required for the calculated reference limits, as this provides a “confidence level” for the estimated upper and lower reference limit.[5]

The Expert Panel on Theory of Reference Values, part of the International Federation of Clinical Chemistry and Laboratory Medicine, states that either parametric or non-parametric methods can be used to estimate inter-percentile RIs

(bound by the 2.5th and 97.5th percentiles).[5,10,12] They described their preferred statistical parametric methods, in an attempt to prevent the use of other inferior methods, neither of which is further discussed here.[12] In view of the challenges in obtaining a large enough reference sample for non-parametric methods, a third method, the 'robust method' has also been described for RI estimations.[5,13] This allows for the use of reference samples with smaller numbers and does not require a minimum number of observations.[12,13] The robust method is a compromise between the parametric and non-parametric methods, as it does not require the large numbers of the non-parametric method, and the analytical values do not need to follow a Gaussian distribution.[14]

3.2.2 The effect of outliers on the reference interval estimation

The potential inclusion of unhealthy individuals in any calculation of RIs is well recognised.[15] Even in carefully defined healthy populations, outlier detection and removal is required to obtain a better estimate of the RIs. As noted by Horn et al. outliers will exist even in a physician-defined, supposedly healthy sample, and they propose combining traditional and robust statistical techniques to help identify outliers. The effect that outlier values can have on estimates when nonparametric methods are employed is important to recognize, however if the reference sample is large enough the effect will be diluted. Methods used for outlier detection in RI estimations include the 'ratio D/R rule', the 'Block procedure' and 'Tukey's method'.[16-18] The latter only uses the middle 50% of the values which reduces the potential effect that multiple outliers could have if concentrated on one side of the reference limit.[5,18]

3.3 IMPORTANCE OF LOCAL REFERENCE INTERVALS

It is of fundamental importance that the RIs for commonly performed laboratory tests such as the FBC, reflect the ethnic and geographical diversity of the region(s) served by the laboratory.

Both international and local research in this area, have demonstrated significant differences in the RIs between distinct population groups and within sub-populations in the same geographical area. Regarding hematological parameters, higher altitudes have a well recognised influence on red cell parameters, leading to an increased Hb

and haematocrit (Hct), and a lower mean cell volume (MCV). Authors postulated that the latter might be because of increased iron turnover secondary to increased erythropoiesis.[19]

The CLSI recommends that each laboratory determines its own RIs or at least validate the use of those obtained from a different setting since haematological RIs are specific to local analyser and reagent combinations, as well as pre-analytical variables.[20]

Current South African (SA) National Health Laboratory Services (NHLS) FBC and WBC differential count RIs (coastal and at altitude) are based on Gauteng data from a 2009 cohort of 719 participants.[21] In the absence of locally derived population based RIs, the NHLS sea level red cell count (RCC), Hb and Hct RIs are derived from Europe and North American sources.[8]

In accordance with the International Organization for Standardization (ISO) requirements for quality and competence, the NHLS should initially establish appropriate local RIs, periodically review them and update the intervals based on changed demographics in the target population or relevant laboratory/technological advances.[22]

3.4 INTERNATIONAL STUDIES

International studies on FBC RIs also show some variability. In the United States (US) the largest databases available are the Scripps-Kaiser and the third US National Health and Nutrition Examination Survey (NHANES III). The former includes data collected from adults attending a health appraisal clinic, from 1998 to 2002 in the San Diego area (n= 24 532).[23,24] The latter is a periodic survey conducted by the National Centre for Health Statistics (NCHS) which looked at data from 1988-1994 in the civilian non-institutionalised population of the US (n=7664). The limitation in interpreting the data of the NHANES III cohorts is that individuals were not screened for disease.[25]

The LL of the RIs for Hb for white males and females, from both of these US databases, were higher than those published by Lawrie et al. in Gauteng.[21] This is unexpected, since the US sample populations resided at sea level. The racial

differences in the LL for Hb RIs determined in males in the US reviews (Caucasian males = 13,7 g/dL and African American males = 12.9 g/dL), were not confirmed in the Gauteng study.[21] In the latter, ethnic differences were only demonstrated in females for various red cell parameters, however this could be due to the small sample size of the African males. When using the LL of the Hb RI to define anaemia, factors such as altitude and ethnicity need to be taken into account.

The term 'benign ethnic neutropenia' was coined in view of absolute neutrophil counts being found to be <1.5 x 10^9/L in up to 5% of African Americans.[26] Reich et al. proposed that a Duffy Null polymorphism may be responsible, resulting in altered chemokine signaling, which in turn could affect neutrophil production and marginalization.[27]

3.5 AFRICAN STUDIES

Two studies from Botswana, aimed to establish local RIs and determine if there were significant differences compared to Western population ranges.[28,29] Both Mine and Segolodi, noted that screening and enrolment of volunteers in African vaccine trials was based on RIs established from predominantly Western populations, which could lead to healthy African volunteers being excluded.[28] Subsequent comparisons of haematological parameters for different populations groups found that women from Botswana had Hb reference values comparable with regional consensus RIs defined for Eastern and Southern Africa, but were lower that the female Hb values found in the 2009.[21,28] Altitude differences of the various study locations would be the most likely explanation for this discrepancy. Similarly in 2014, Segolodi et al. demonstrated differences between their newly established Botswana reference ranges for the Hb and Hct and those of Western populations.[29]

In a 1996 observational study on hospital staff in London, it was shown that total white cell counts, neutrophil counts and platelet counts were lower in individuals of African ancestry compared to Caucasians.[30] This study included 115 participants of sub-Saharan African origin, but did not include South Africans. Furthermore, the study established that females had higher neutrophil and platelet counts than males across all the ethnic groups studied.[30] The latter was confirmed in Zimbabwean (n=769), Zambian (n=200), and Kenyan (n=1293) studies, but not in the adults from

the Central African Republic (n=150).[31-35] The latter study was limited to a small sample number and thus may not have been truly representative of the population.

3.6 SOUTH AFRICAN STUDIES

To date, there have been no SA studies describing regional RIs with equally represented numbers of participants from all ethnic groups. Current NHLS haematological RIs are largely based on data from the 2009 Gauteng cohort, which included 719 participants, the majority female with only 88 males (56% African and 44% Combined Asian, Coloured and Caucasian). A total of 631 participants were female (63% African and 37% Combined Asian, Coloured and Caucasian).[21]

Unpublished data on RIs for FBC and WBC differential counts in new blood donors (n= 973) in the Western Cape in 2008, showed significant differences in various haematological parameters compared to the NHLS RIs at that time. In that study the reference population was mainly Coloured and Caucasian. Unfortunately insufficient African males (n=33) were recruited to allow for meaningful interpretation on African male reference ranges. In addition, screening for iron deficiency was not performed.[36] See Table 1 for a comparison of results found in South African studies.

3.6.1 Red blood cell parameters

In view of the lack of sufficient data relating to red blood cell parameter RIs for SA regions at sea level, NHLS coastal RIs for red cell parameters have been derived from European and North American sources, i.e. primarily Caucasian populations.[8]

Haemoglobin concentrations in Africans were found to be lower than those of Caucasians both at higher altitudes and at sea level according to a study from the Witwatersrand (1760m) and East London.[37,38] The more recent cross-sectional study in Gauteng (2009) demonstrated gender-specific differences in red blood cell parameters including lower red cell counts (RCC), Hb and Hct values in African females. However, no significant ethnic differences in the red cell parameters were noted in the male participants.[21]

Other published SA studies have largely focussed on Africans and have found broadly similar results, with some notable differences. In 1995, Badenhorst et al. looked at haematological parameters in an urban African population in the Cape Peninsula (n=819) and compared their results to those of Cross and Heyns's study of healthy Basotho residents in the lowlands of Lesotho (n=259).[39,40] In the former study, the LL of normal for Hb was calculated at 10.3 g/dL and 9.0 g/dL for males and females respectively, which is much lower than any other published studies and also lower than the current NHLS coastal Hb RIs. This could be due to nutritional deficiencies and specifically iron deficiency in the reference population.[39] The fact that participants were screened for iron deficiency in the Lesotho study would explain the discrepancy in the Hb values between the two cohorts.[40] (Refer to Table 2)

In another study of healthy African adults in the Witwatersrand area, the Hb RIs for females were determined to be 12.4-15.5 g/dL, while for the Basotho population the range was 11.7-16.0 g/dL.[39,40] Considering the slightly lower altitude in Lesotho the lower RI is to be expected, however it should also be noted that the Witwatersrand reference sample was approximately half the size of that of the Basotho study population. Furthermore, the former did not exclude iron deficiency specifically based on serum ferritin levels, however participants were screened clinically and excluded if they reported 'pica', a symptom of iron deficiency. Similarly, ranges for the MCV are variable.

3.6.2 White blood cell counts

As with red cell parameters there are various factors that can cause variations and fluctuations in the RIs of the respective WBC subtypes, including but not limited to ethnicity, normal diurnal variation and individual habits such as smoking and exercise. Heavy smoking is known to increase Hb levels, due to chronic hypoxia. Similarly documented increases in WBCs are probably also related to the chronic hypoxia with chronic inflammation possibly contributing. A definite correlation between the degree of leukocytosis and the number of cigarettes smoked has been demonstrated.[41-43] Increases in the various WBC types have been shown, in

different combinations or even as a panleukocytosis, with neutrophilia being the most consistent finding.[43-45]

The neutropenia reported initially by Bain et al. in British Black Africans, has consistently been confirmed in SA studies and internationally. The neutropenia may be due to genetic factors, dietary and/or environmental influences.[39] Tikly et al. demonstrated a leucopenia with a reduction in all classes of white cells in the study population in the Witwatersand, as opposed to only a reduced neutrophil count.[37] With regards to monocyte counts, ethnic variations have also been reported over time, however this may reflect the advances in automated cell counters, where many of the earlier studies relied on manual differential counts.[21]

3.6.3 Platelet counts

In some of the older SA studies, platelet count RIs were not established. This includes research performed in Lesotho in 1983 and the Cape Peninsula in 1995 [39,40] when automated platelet counts were not widely available. Lawrie's 2009 finding that platelet counts were higher in females regardless of ethnicity, confirms Bain's London findings from 1996.[21,30] Hormonal influences, together with menstrual blood loss, may be contributing factors to this gender difference.[37]

3.7 RELEVANCE OF REFERENCE INTERVALS TO THE DIAGNOSIS OF ANAEMIA AND POLYCYTHEMIA

3.7.1 Anaemia and polycythemia

Anaemia, particularly iron deficiency anaemia, is a common global condition and requires accurate diagnosis and monitoring. The World Health Organisation (WHO) defines anaemia as an insufficient RBC mass in the circulation, or in public health terms, a Hb level below reference thresholds.[46,47] The LLs of the Hb concentration RIs, as defined by the WHO in 1968, are 13 g/dL for men and 12 g/dL for women.[23] The validity of these cut offs for diagnosing anaemia, have been questioned by many, particularly considering improved laboratory practice, techniques and available data in the last 40 years.[23,48,49] These Hb cut offs are thus considered to be outdated and are currently under review.[3]

Due to the increased Hb concentration found in individuals who reside at higher altitudes and in smokers, the possibility of underestimating the prevalence of anaemia in these groups is a concern. Recommendations have thus been made by the WHO (2011) for the adjustment of Hb concentrations for those living at altitudes of 1000 m or more above sea level as well as for smokers.[50] (Refer to Table 2)

The upper limit of the Hb interval is equally important to clarify and thus ensure that polycythaemia is correctly diagnosed. In addition to Hb, the Hct values are central to the diagnosis of polycythemia. The Hct provides a measure of the oxygen carrying capacity of blood, as it represents the percentage of blood volume that is filled by red blood cells. Depending on the analyser it can either be directly measured (centrifugation) or it is calculated. The International Council for Standardization in Haematology recommends using the term Hct when referring to the automated technique of measurement and PCV for the manual test method.[8] Most automated impedance counters estimate the Hct from the mean cell volume (MCV) and red blood cell count (RCC). Alternatively, the Hct is measured and then the MCV is derived from it.[8] As such, any factors that affect one of these three variables will influence the Hct results, including inadequate sample mixing, small sample volumes, agglutination or haemolysis. As with all haematological parameters, patient factors are important to consider for establishing RIs. Sex, age, body mass index, smoking and the presence of any chronic hypoxic disease state will influence results.[51] Further investigation of polycythaemia often requires costly laboratory tests. As stated by Fairbanks et al. "improperly defined normal ranges often lead to serious diagnostic errors, inappropriate and costly laboratory investigations, and therapeutic misadventures". [52]

3.7.2 Serum ferritin for the diagnosis of iron deficiency

The WHO and Disease Control Prevention Expert Committee have provided guidelines for determining the prevalence of iron deficiency in specific population groups.[53,54] They define iron depletion as "the state in which storage iron is absent or nearly absent but the tissues that need iron are able to maintain normal physiological functions".[54] Thus iron deficiency can exist without anaemia. The

WHO has identified the serum ferritin as one of the best indicators of iron stores, with levels below the RI being indicative of iron deficiency.[55]

The WHO RI for serum ferritin, ranges from 15-300 µg/L and is slightly lower in children and premenopausal women.[46,55] In the study done by Hallberg et al, they correlated serum ferritin levels with iron stores, by assessing the amount of stainable iron in the bone marrow, confirming that a serum ferritin level of <15 µg/L was the best predictor of iron deficiency. If a cut-off of <15 µg/L, is used the overall sensitivity and specificity for diagnosing iron deficiency is 82% and 95% respectively.[55] A low ferritin is thus diagnostic of iron deficiency, but a normal serum ferritin may occur in individuals with iron deficiency and co-existing inflammation resulting in the poorer sensitivity of the test.

The importance of considering iron deficiency, when establishing 'normal' RIs, was demonstrated by Cross and Heyns in their study of healthy adults living in Basotho.[40] They determined that if iron deficient females were not excluded from the reference sample, the estimated LL of Hb RI was 1 g/dL lower compared to when iron deficiency was screened for and used as an exclusion criterion.[40]

3.7.3 Interpretation of serum ferritin in an acute phase response

The interpretation of the serum ferritin has some potential pitfalls. Ferritin is an acute phase protein and thus will increase in the settings of infection and inflammation. Iron deficiency could therefore be underestimated in a given population without careful screening.[56]

3.7.4 Donor deferral based on haemoglobin thresholds

One of the main reasons for blood donor deferral is 'their inability to meet minimum haemoglobin thresholds'.[57] Blood donation intervals are thus carefully stipulated by blood banks to prevent anaemia in donors who donate frequently. Studies that have looked at identifying those groups of donors that are more at risk of having or developing iron deficiency have considered factors such as gender, age, weight, ethnicity and environmental factors.[58-60] The South African National Blood Transfusion services recently examined the prevalence of iron depletion and iron deficiency among blood donors in South Africa.[61] They determined that

young Asian (defined by the US Census Bureau as a panethnic group, including individuals from the Far East, South East Asia and India) and Black females were most at risk of iron deficiency, although the incidence of iron deficiency between the genders was similar.[61]

Donor deferral practices are primarily aimed at donor safety, however deferral for whatever reason has a negative impact on the motivation of donors and may have serious implications for blood collection services.[57] It is thus logical that the cut off values for anaemia should be established in the local setting.

4. JUSTIFICATION FOR THE CURRENT STUDY

Numerous studies have demonstrated significant differences in the RIs for various haematological parameters both between and within sub-populations.

This study aims to establish local RIs for FBC and WBC differential counts in the main ethnic groups in the Western Cape region of SA at sea level, including African, Coloured and Caucasian. Furthermore, the aim is to determine if there are statistically significant differences between these ethnic groups and between the newly established RIs and those currently used by the NHLS. These newly established RIs should then be implemented into the reporting and interpretation of local FBC, which is anticipated to have significant clinical impact.[30]

4.1 AIM

To establish local RIs for FBC and WBC differential counts in adult men and women across the three main ethnic groups in the Western Cape, including the African, Coloured and Caucasian groups.

4.2 OBJECTIVES

4.2.1 Primary Objectives:

a) To determine the means and 2.5th and 97.5th percentile RIs for FBC and WBC differential count parameters in healthy adults in the Western Cape region.
b) To compare the locally derived RIs with the current coastal NHLS ranges and other SA and internationally derived RIs.

c) To determine if there are significant ethnic differences in the FBC and WBC differential count RIs, which could support reporting of race-specific values.

4.2.2 *Secondary Objectives:*

a) To screen for iron deficiency using the serum ferritin concentrations in all participants thereby eliminating the confounding effect of iron-deficient erythropoiesis on Hb RIs.
b) To determine whether higher mean Hb values in males can be attributed to iron deficiency in the female population.
c) To consider the revision of Hb cut-offs for blood donors in the Western Cape.

4.3 CONCLUSION AND NEEDS FOR FUTURE RESEARCH

Any statistically significant differences between our results and the current NHLS RI would inform review of current reference values at coastal laboratories in SA. Furthermore, if significant racial differences are confirmed, race specific RI could be considered.

In addition, the blood transfusion service may consider revision of decision rules on cut-off Hb levels for blood donor deferral. If local LLs of the RI for Hb are established, it would allow the blood transfusion services to adjust their screening parameters and potentially access a larger donor pool.

Although iron deficiency is not the main focus of this project the data will also inform the Western Cape Blood Services (WCBS) whether it may be appropriate to implement an iron surveillance program for their donors.

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B: CHAPTER 2: Publication-ready Manuscript

This manuscript was compiled in accordance with the author guidelines of South African Medical Journal (Part C, Appendix K).

B.1 TITLE

Establishing locally derived reference intervals for full blood count parameters and white cell differential counts in the Western Cape region of South Africa.

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All authors contributed toward the study design and implementation, WCBS funded the study, facilitated the recruitment of subjects and analysed the samples. Raymond Nhapi assisted in data analysis and reference range computation. AD wrote the manuscript, which was edited by JO, AB and CH.

KEY WORDS

Reference intervals; Full blood count reference ranges; white blood cell count reference ranges; Western Cape

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B.2 BACKGROUND / INTRODUCTION

Accurate laboratory reference intervals (RIs) are essential to establish the distinction between health and disease. The Clinical and Laboratory Standards Institute (CLSI), which is responsible for setting laboratory standards internationally defines a RI as that interval set of values observed in the reference sample group or defined by a specific percentage, usually 95%.[1]

Many African countries rely on RIs obtained from European and North American populations**,** which is problematic in view of the recognised diversity within populations in terms of gender, age, ethnicity and environmental factors, particularly altitude.[2,3] Important considerations in developing reliable RIs are selecting appropriate reference subjects, testing adequate numbers of subjects and avoiding pre-analytical errors.[3] Previous South African (SA) studies have not reported RIs with equal representation from the main ethnic groups. Current SA haematological reference intervals at altitude are based on Gauteng data from a 2009 cohort of 719 participants, consisting of mainly females with only 49 African male participants.[4] The coastal RIs used by the National Health Laboratory Services (NHLS) in SA are the same as those used in non-coastal regions, with the exception of the RIs for red cell parameters. In the absence of locally-derived, population based RIs, international sources are currently used for our red cell count (RCC), haemoglobin (Hb) and haematocrit (Hct) values at sea level. This should be reviewed, considering the international CLSI recommendations that each laboratory should determine its own RIs or validate the use of those obtained from a different setting.[1]

The primary aim of this study was thus to establish locally derived RIs for full blood count (FBC) and white blood cell (WBC) differential count parameters, in healthy adults, across the three main ethnic groups in the Western Cape including African, Coloured and Caucasian. These newly established RIs could inform the revision of current RIs in use, with

the possibility of ethnic-specific RIs. A secondary aim was to investigate the possible confounding effect of iron-deficient erythropoiesis in haemoglobin (Hb) RI determination. This data could assist the Western Cape Blood Service (WCBS) in deciding if the implementation of an iron surveillance program for their donors would be beneficial and potentially increase the numbers of blood donors in the setting of high demands on the local blood supply.

B.3 METHODS

Study Design

A prospective, descriptive study design with direct convenience sampling was employed. Samples were collected from healthy adults presenting voluntarily for blood donation to WCBS. Ethnicity was self-identified by participants as Asian, Black, White or Coloured on the routine confidential blood WCBS donor questionnaire. Samples were collected between November 2016 and October 2017 at WCBS donor clinics.

Ethical Considerations

The University of Cape Town (UCT) Human Research and Ethics Committee approved the study. Voluntary informed consent was obtained from all participants prior to participation, in addition to their completion of the standard WCBS blood donor questionnaire.

Participants and Measurements

Participants were first time prospective blood donors between the ages of 18 and 60 years. The WCBS donor screening questionnaire was used as part of the screening procedure and provided relevant information about potential reference individuals.[1] Based on this, individuals were excluded if the screening procedure indicated a 'lack of good health'.

The following exclusion criteria were applied: testing positive for human immunodeficiency virus (HIV), hepatitis B and C viruses (HBV, HCV) and syphilis using current internationally accepted technology. Those with serum ferritin levels outside of the current NHLS reference range (females 13 - 150 μg/L and males 30 - 400 μg/L) were excluded, to eliminate the effect of iron deficiency, underlying inflammatory conditions, liver disease and/or haemochromatosis which could lead to raised serum ferritin.

Laboratory Methods

After consent was obtained, blood samples were collected with minimum stasis, in accordance with international recommendations.[1,5] Two venous samples were collected, labeled with the blood donation barcode and subsequently processed as per the standard WCBS procedures. For purposes of the study a full blood count (FBC), white blood cell (WBC) differential and serum ferritin testing were performed. Screening for HIV, HBV and HCV was performed using standard serology (Roche Prism c8000 & second line confirmatory testing with Architect i20000 HIV Ag/Ab Combo Assay: Abbott Diagnostics) and nucleic acid testing (NAT: Ultrio Elite Assay which is a multiplex assay). All FBC samples were processed in the WCBS laboratory, which is quality control accredited by South African National Accreditation System[6] and were analysed on the Sysmex Counter, XN 1000 PURE, serial number 16891 (Sysmex Corporation, Kobe, Japan), using internationally accepted modern technology for automated cell counting.

Samples for FBC testing were collected into 4 mL ethylene diamine tetra-acetic acid (EDTA BD Vacutainer Systems, Plymouth, UK) and stored at 20±2°C prior to preparation and analysis, which was performed within 24 hours of collection. The following FBC parameters were analysed: red cell count (RCC), haemoglobin (Hb), haematocrit (Hct), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC), platelet count, total white blood cell count (WBC) and 5-part automated WBC differential count. The serum ferritin specimens were collected in 4 mL, Z-Serum Clot Activator tubes (Greiner Bio-One, GBO, Vacuette, North America), and centrifuged, with the serum refrigerated at 2-6°C prior to analysis. These samples were processed at WCBS using a ferritin testing kit (Abbott AxSYM Ferritin).

Statistical Analysis

Data was analysed using R Core team 2018 (R foundation for Statistical computing, Vienna, Austria. URL https://www.R-project.org) and StataCorp 2015 (Stata Statistical Software: Release 14. Stata Corporation, College Station, TX, USA). Since outliers will exist in a physician-defined supposedly healthy sample, outliers for FBC and WBC parameters, were excluded from the RI calculations during statistical analysis as per published

recommendations (i.e. data less than 25th percentile – (1.5 x IQR) or greater than 75th percentile – (1.5 x IQR)).[7]

Reference intervals were derived using parametric and non-parametric methods. While both express the central 95% of the data, the parametric method calculates the 95% confidence interval around the mean, while the non-parametric method calculates the 2.5th and 97.5th percentiles. For both methods, the lower and upper limits of the RIs, are reported with their respective 90% confidence intervals. For secondary analyses, data were stratified according to gender and ethnicity. The Mann-Whitney U-test was employed for comparisons between male and female participants and the Kruskal-Wallis test was used to assess differences between data for ethnic groups. An alpha level < 0.05 was deemed statistically significant.

B.4 RESULTS

792 prospective blood donors were screened between November 2016 and October 2017. After exclusions, 620 (376 females and 244 males) were included for analysis (Figure 1). Five screened donors were excluded based on positive HIV serology testing, two were confirmed HIV positive on nucleic acid testing (NAT) and the remainder were regarded as having false positives results. Of the 620 included participants, 31.61% were African, 39.68% Coloured and 28.71% Caucasian (Figure 2). Approximately half of the participants (53.06%) were 20 to 29 years of age and 21.45% between 30 and 39 years (Figure 3). The derived RIs are summarised in Tables 1 & 2. For all race groups combined, gender-based differences were found in most FBC parameters. When comparing the RIs for males and females in the three ethnic groups (Table 2), statistically significant differences were found for many parameters including Hb and total WBC count. Figure 4 describes the ferritin results in the various ethnic groups and figure 5 illustrates the variation in serum ferritin between males and females. Sixty-six (13.12%) of all females had iron deficiency (serum ferritin <13 μg/L). Coloured and African females made up the majority of the iron deficient population, comprising 29 (43.94%) and 24 (36.36%) respectively (data not shown).

B.5 DISCUSSION & CONCLUSION

The process of establishing RIs for biological parameters is inherently challenging. In addition to the normal physiological variations within and between individuals (relating to

diurnal fluctuations and other habits such as smoking and exercise)[8,9], analytical variables such as obtaining adequate samples, variability of analysers, expense, population subgroup stratification and choice of statistical analysis also need to be considered. These factors all contribute to limiting the availability of quality data, especially in resource-poor settings. Selecting an appropriate reference sample is paramount in establishing valid, transferable and clinically relevant RIs. Strengths of this study include a healthy and ethnically diverse cohort, the use of standardized testing methodology, exclusion of iron deficiency and sample sizes that are regarded as adequate to make the required inferences.[3,10]

According to published guidelines, either parametric or non-parametric methods can be used to estimate inter-percentile RIs which are bound by the 2.5th and 97.5th percentiles.[11,12] Although the non-parametric methods are considered superior in most cases, they require a minimum of 120 participants to allow calculation of 90% confidence intervals for the upper and lower limits.[1,11,12] We thus used the parametric approach for all the groups with normal data distribution and lower participant numbers. While the non-parametric method was considered appropriate for the groups with >120 subjects i.e. combined male and female groups (Table 1), and the African and Coloured females (included in Table 2).

Gender differences

We stratified results according to gender (for the three ethnic groups combined) and found statistically significant differences between males and females in all parameters except absolute monocyte, eosinophil and basophil counts; and for all red cell parameters except MCH (Table 1). Similar observations were made by Bain and other studies in Zambia, Nigeria and Kenya.[13-16] In contrast, significant differences in WBC and absolute neutrophil count between men and women were not demonstrated by Lawrie et al.[4]

The mean Hb of female participants was significantly lower than that of the males, despite the exclusion of 66 females with iron deficiency. This suggests that additional factors, such as hormonal influences, particularly testosterone and total body muscle mass and body mass index contribute to this gender-based difference in Hb.[17,18] Our findings refute the use of the same Hb RI for males and females, which was suggested by Rushton et al, based on their observations of primates and their hypothesisis that the gender difference in Hb could largely be explained by unrecognized iron deficiency.[19]

Interestingly, when including iron deficient subjects in the analysis, the mean Hb for female participants was unchanged, as was the difference in mean Hb of females compared to males (data not shown). Our findings support those of Cross and Heyns in healthy adults living in Lesotho (n=259) where subjects who had serum ferritin levels <13µg/l were excluded from the study and the RI for Hb remained significantly lower in females.[20] Similar comparisons in a larger population are likely to demonstrate a lower mean Hb with the inclusion of iron-deficient participants. Of the 792 prospective donors initially screened for inclusion in this study, 18.48% had confirmed iron deficiency with serum ferritin values below the RIs (Figure 4). Females of Coloured and African ancestry showed the highest numbers of iron deficiency and these groups may benefit from screening programs for iron deficiency and iron supplementation as part of blood donation drives. The WHO defines anaemia as an insufficient RBC mass in the circulation, or in public health terms, a Hb level below reference thresholds.[21,22] The WHO lower reference limit of Hb concentrations, which have been in use since 1968 are 13 g/dL for men and 12 g/dL for women.[23] The validity of these cut offs for diagnosing anaemia, have been questioned by many, particularly considering improved laboratory practice, techniques and the volume of data acquired in the last 40 years.[18,23,24] The lower limit (LL) of our study RIs for Hb in women is 11.72 g/dL, which is also lower than the current NHLS RIs in use, implying that anaemia will be over-diagnosed if our local population-specific intervals are not used.

Similar to other sources, females across all ethnic groups had higher WCC than their male counterparts, due to the higher neutrophil and lymphocyte counts. Similarly, the well-recognised higher platelet counts in females, irrespective of ethnicity was also confirmed.

Ethnic differences

In calculating the means for the various full blood count parameters for the different ethnic groups, statistically significant differences were noted in most parameters, except for the eosinophil count and MCV in females, and platelet count in males (non-parametric Kruskal-Wallis tests were used).

Due to the limited number of relevant studies and several of these being outdated, direct comparison with historical similar studies is challenging. There have been huge technological advances made in the field of haematology analysers in recent decades, thus

comparison of recent studies using modern automated cell counters with those of the older studies where manual differential counts were used or platelet counts were omitted, is not possible.

Although there is a statistically significant difference when comparing the mean Hb of the three ethnic groups, the clinical significance is negligible. African females were found to have the lowest ranges for RCC, Hb and Hct, which mirrors the findings reported in other SA studies and internationally.[3,25,4] The RCC of Coloured males is the highest of the three ethnic groups, which may be due to the increased incidence of smoking and thalassaemia trait in this population group.[26-29] Since thalassaemic red cell indices (a combination of low MCV and raised RCC) did not constitute an exclusion criterion for our study, we conducted a post-analytical review of our data and outliers, identifying participants with such red cell indicess. Of the seven participants that were identified as having thalassaemic indices, only three male participants were included in the final calculation of the RIs, and thus the effect, if any is regarded as negligible.

We confirmed the previously reported leucopenia in African males, with a reduction in all types of WBC, not only the neutrophil count.[25] This well documented neutropenia in African males is likely due to genetic factors, in view of the good health and diet of our reference population. With regard to the WBC differential count, African males were noted to have the lowest monocyte count, which has also been found in other studies.[4,30]

Historically people of African ethnicity have been noted to have lower platelet counts.[30,31] Our results show a significant difference between the respective ethnic groups for platelet RIs in females, with African females having the lowest RI. However, a significant ethnic difference was not demonstrated for males. This is in contrast to Lawrie et al, who reported no significant difference in platelet RIs for either gender.[4]

Comparison with previous SA studies on FBC Reference Intervals

In our cohort, African females had the lowest RI for Hb, which is significantly lower than the Hb ranges currently used by the NHLS. However, the Hb RI for Coloured and Caucasian females correspond well with current NHLS ranges. Interestingly, in another Cape Peninsula study of urban Africans in 1995, the mean Hb concentrations, were found to be significantly lower for both genders when compared to our estimated intervals and those of other South

African reports before 1995.[32] This is probably because iron deficient individuals were not excluded in their cohort.

The upper limit for Hb for all males was found to be 0.5 g/dL higher than the NHLS upper limit reference, with Coloured males having the highest upper limit of 17.76 g/dL (refer to Table 1). Similarly, Coloured females and males have the highest RCC both of which are significantly higher than the current ranges in use. This is relevant and may lead to the over diagnosis of polycythaemia. Although the difference is more pronounced in males, the upper limit of MCV for both genders for all ethnic groups is significantly lower than the current NHLS ranges. The reason for this is unclear.

Neutrophil counts for males were found to be somewhat lower than the current NHLS ranges, however this was only significant in the African male group (Table2). The well-documented lower neutrophils counts in African males was confirmed in this study.[30,333,34] African patients may thus be incorrectly labeled as neutropenic. A possible explanation is genetic factors such as a Duffy null polymorphism.[34] In contrast, the lower limit of the neutrophil RI in females, was higher than the current NHLS range, with Coloured females having the highest LL values, followed by African females, while the female neutrophil RI for Caucasians are within the present NHLS range. This may be due to increased smoking contributing to the higher neutrophil counts in the Coloured group.[8,9,35] Overall, the males and females in our cohort show similar upper limits for absolute eosinophil counts, with the upper limit for all males being significantly lower than the current NHLS upper reference limit (0.48 x 10^9/L), and African males being the lowest (0.37 x 10^9/L). Arguably, subclinical allergic disease, seasonal factors and even parasite infections, may account for the higher eosinophil counts in males that were observed in the Gauteng study from which current NHLS RIs are obtained.[4]

Limitations

Due to the convenience sampling method we employed, sample size was limited to eligible first time blood donors consenting to study participation during the study recruitment period. We aimed for 120 subjects in each ethnic and gender group, however this target was only reached for African and Coloured females. The smaller sample size influenced our choice of statistical analyses.

A further limitation is the lack of data on participants of Indian/Asian descent, which comprises a small ethnic group in the Western Cape. This limits the transferability of our findings to regions such as KwaZulu Natal (KZN), which have a large Indian/Asian population. A similar study in KZN incorporating large numbers of Indian/Asian donors would provide clarity in this regard.

We did not include some population groups i.e. paediatric, pregnant, post-menopausal women or the elderly. Since more than half of our sample population was under the age of 30 years, a degree of bias in certain parameters is likely. For example a more equal age distribution may have yielded a lower mean Hb, considering the increased incidence of anaemia in the elderly. Some studies have looked at specific hospitalised patient populations, and compared results from inpatient populations with RIs derived from healthy populations. They concluded that, 'laboratory data interpretation may benefit from greater consideration of clinically contextual and outcomes-related factors'.[36]

Individuals with serum ferritin levels below the lower reference limit were excluded from our study in an attempt to remove the influence of iron deficiency on red cell parameters. Since ferritin is an acute phase protein and often raised in other disease states, there is a possibility that a proportion of subjects with iron deficiency, but 'normal' serum ferritin may have been included in our data set. However, considering the age, demographics and assumed good health of blood donors, the effect of this is regarded to have minimal impact on our calculated RIs. Additional work looking at the validity of using a ferritin level of 30 μg/L instead of 13 μg/L for a more accurate predictor of iron deficiency in females should be considered to determine whether or not this would have any noteworthy effect on calculated RIs.

We were able to confirm that no subjects with the combination of a low MCV and raised RCC to suggest a thalassaemia trait were included in our analysis. It is thus unlikely that the increased gene frequency for thalassaemia trait in the Coloured population would have contributed to their RIs for red cell parameters in this population group.[28,29]

Conclusion

Locally established and population-specific RIs are essential for accurate interpretation of blood counts. Our results confirm the importance of gender and ethnic-specific RIs in the local setting. Implementation of our RIs for use at sea level in the Western Cape (and

possibly the Eastern Cape in view of similar ethnic population profiles) should be considered and would have implications for the diagnosis of cytopenias, cytoses and other blood count abnormalities. There are also potential implications for blood donor Hb threshold values, used for blood donor deferral. The lower Hb RI is confirmed in females compared to males, after exclusion of iron deficiency, which is a particular strength of this study.

A further strength is the numbers of African male donors included, which is the highest in any similar recent South African study employing modern automated analysers.[4,37] Furthermore, considering that the majority of the current coastal RIs (excluding those for red cell parameters) employed by the NHLS are derived from the Gauteng study[4] where the male reference group was significantly smaller than ours (n=88 vs. n=244), our findings are regarded as more representative, particularly for the African male group.

The use of separate FBC RIs for the three main ethnic groups in the Western Cape would be clinically meaningful, however there are significant practical barriers to their implementation. Clinicians are unlikely to consistently provide sufficient details on sample request forms to allow for the accurate reporting of ethnic-specific RIs. In addition, reporting RIs for all three ethnic groups is cumbersome and would likely cause confusion with limited added value to improved patient management. Considering these challenges, we recommend employing the study RIs for the combined male and female population groups, using results of all three ethnic groups in the Western Cape. Efforts and resources should be focused on educating clinicians on the interpretation and limitations of these RIs. Although RIs provide comparison data for the interpretation of patients' laboratory results, these should always be interpreted in the particular clinical context with an emphasis on following trends, rather than individual values.[12,36]

A clear distinction between RIs and clinical decision limits is needed, since the latter describes thresholds of clinical significance, which are associated with a certain diagnosis and/or increased risk of adverse effects.[38,39]

Conflict of interest and sources of funding

No conflicts of interest to disclose, funding for the project was provided by the WCBS.

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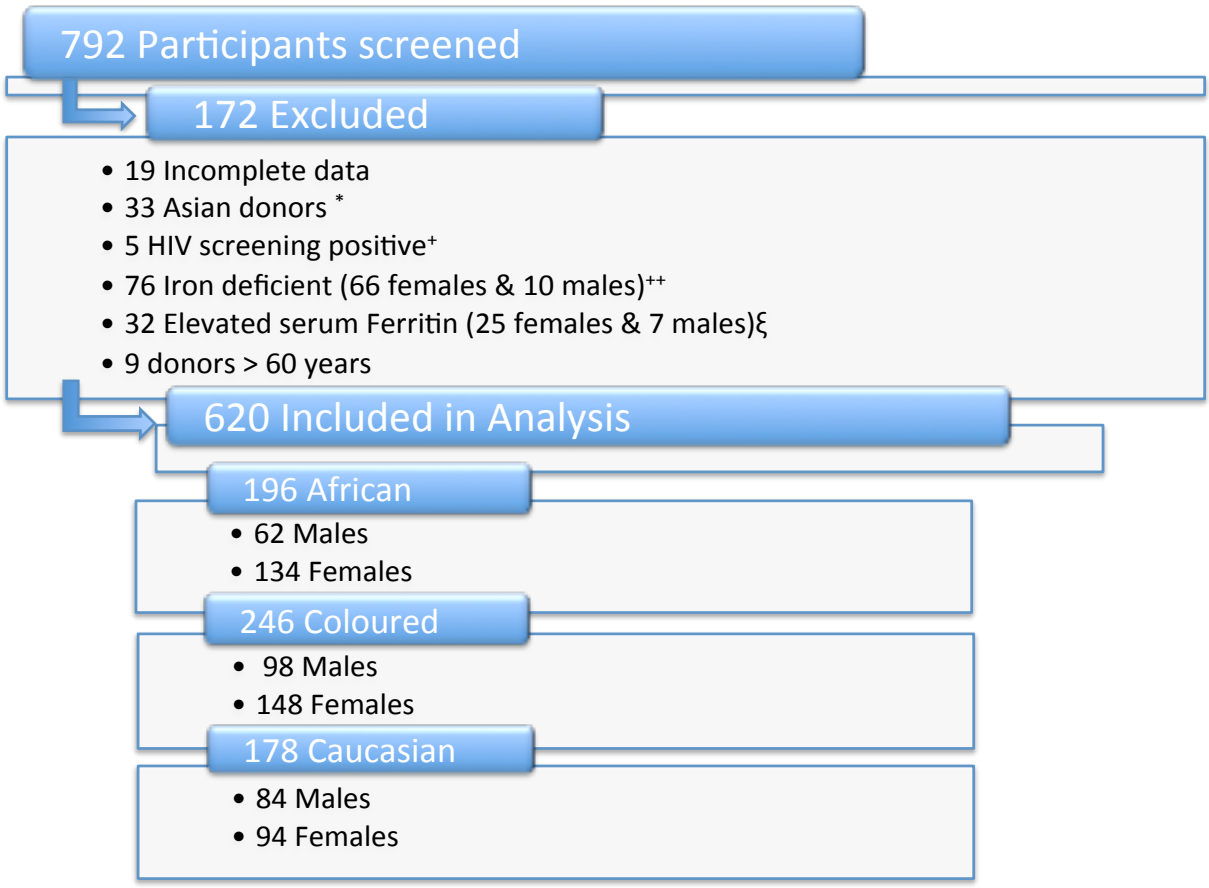
B.7 TABLES & FIGURES

Figure 1 Study cohort diagram



Note: Some participants met more than one exclusion criterion.

* Asians were excluded due to small numbers

\+ Five screened donors were excluded based on positive HIV serology testing. Two were confirmed HIV positive on nucleic acid testing (NAT)

++ Serum ferritin < 13 µg/L for females <30 µg/L for males

ξ Serum ferritin >150 µg/L for females and >400 µg/L for males

Figure 2 Demographics of study population

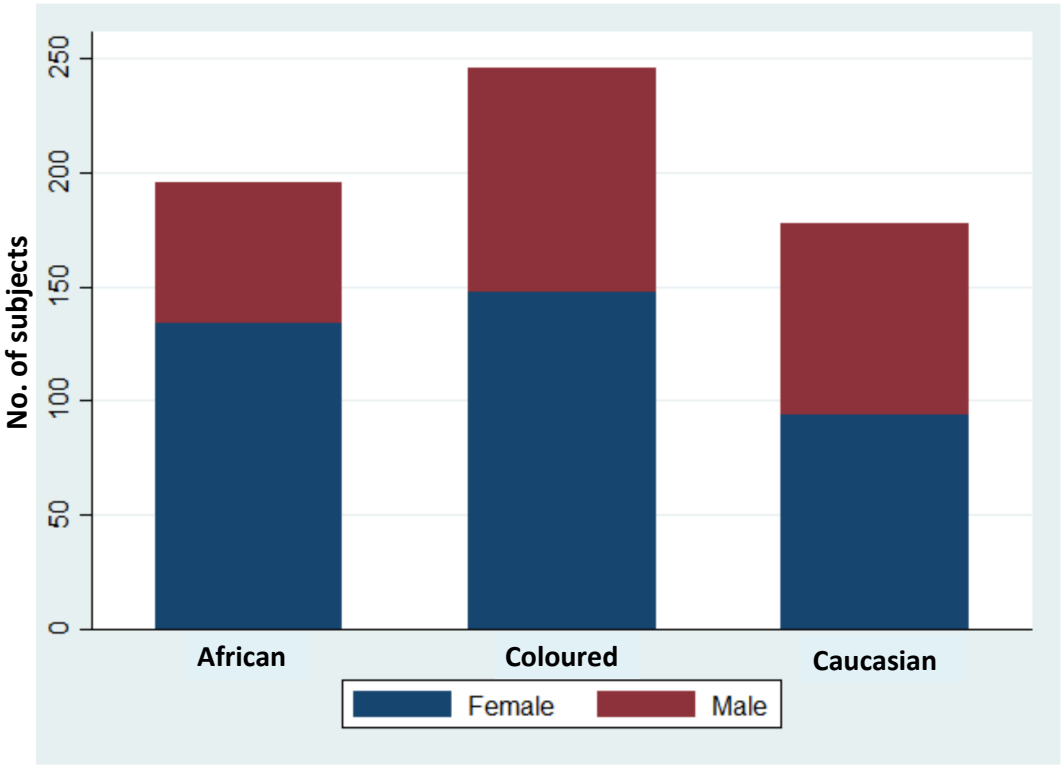


Figure 3 Age distribution of study participants

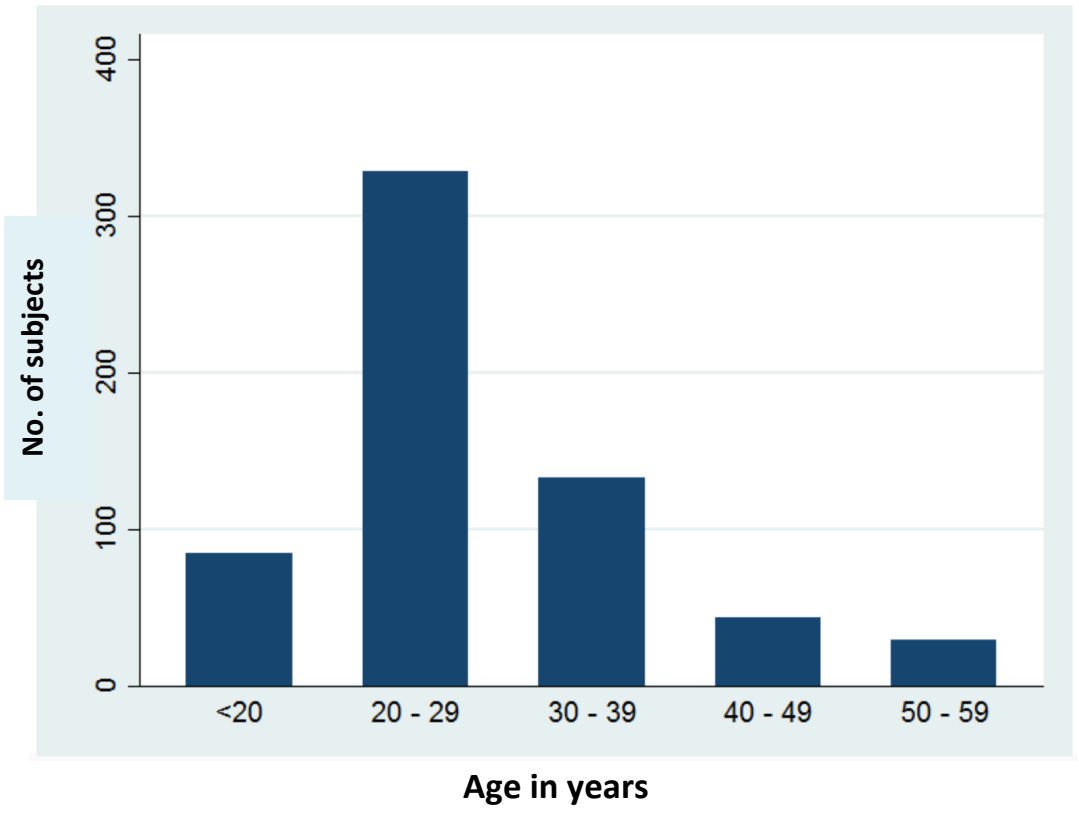
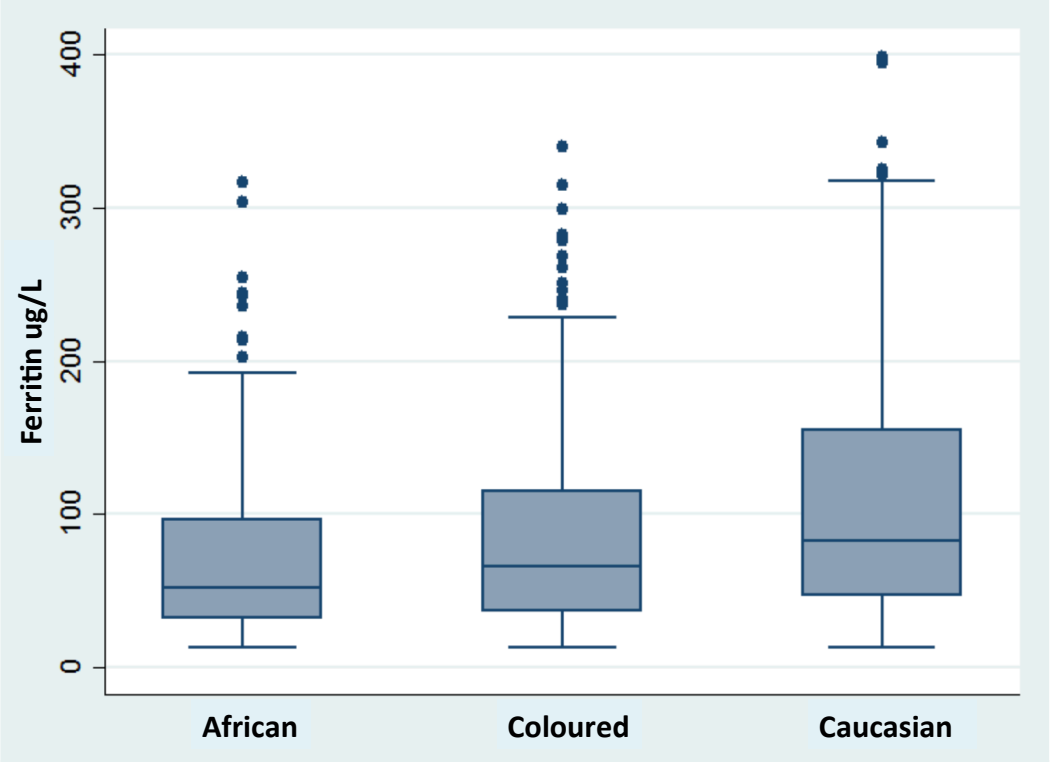
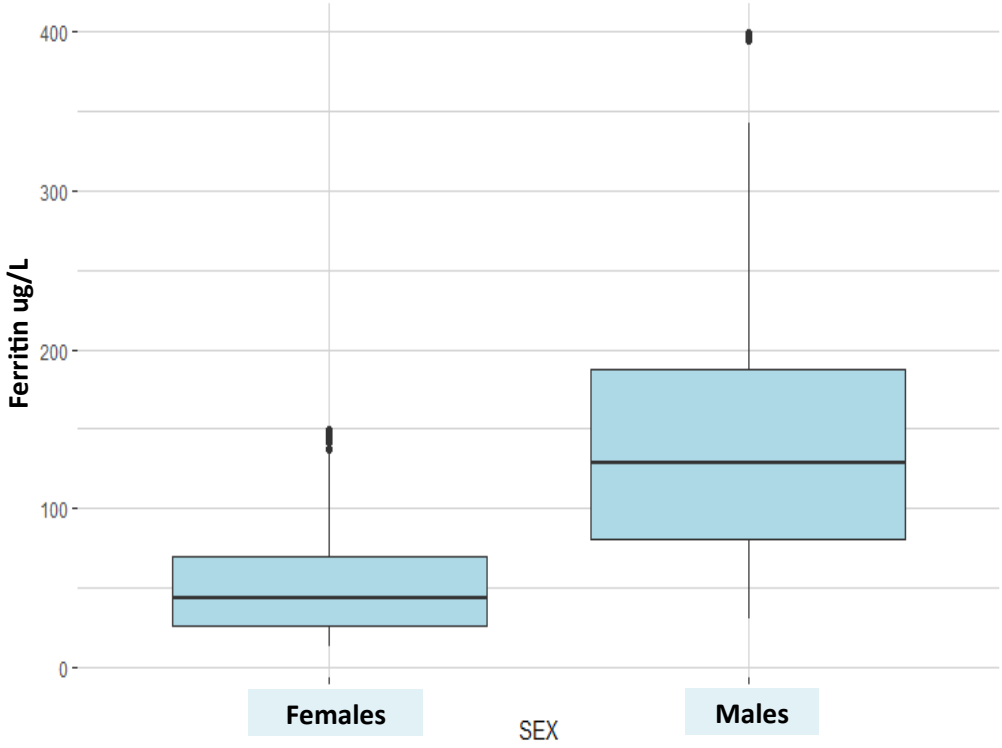


Figure 4 Serum ferritin distributions by race, including males and females



Box and whisker plot: The box represents the interquartile range (IQR) with the median as a horizontal line. Whiskers stretch to 1.5 times the IQR above and below the upper and lower bounds of the IQR and outliers beyond this, are represented with dots.

Figure 5 Serum ferritin for male and female participants



The difference in serum ferritin levels between males and females reached statistical significance with females having significantly lower levels.

Table 1 Comparison of study reference intervals with current coastal NHLS reference intervals

Parameter	New RI from this study	90% CI for upper and lower limits	p-value¶	NHLS current Coastal ranges[II]
Red Cell Count (10^{12}/L) Males Females	4.67 - 6.22 3.96 – 5.41	(4.6-4.71) (6.14-6.5) (3.89-4.08) (5.34-5.54)	<0.0001	4.5 – 5.5 3.8 – 4.8
Hb (g/dL) Males Females	13.6 -17.5 11.72 – 15.2	(13.3-13.9) (17.3-17.7) (11.5-12) (15-15.5)	<0.0001	13 -17 12 - 15
Hct (L/L) Males Females	0.41 – 0.53 0.36 – 0.46	(0.40-0.42) (0.52-0.54) (0.35-0.36) (0.46-0.48)	<0.0001	0.40 - 0.50 0.36 – 0.46
MCV (fL) Males Females	78.8989 – 94.0 81 – 96.01	(77.1-80.5) (93.6-95) (79.9-81.3) (95.6-96.9)	<0.0001	83.1 – 101.6 79.1 – 98.9
MCH (pg) Males Females	25.77 – 32.01 25.33 – 32.27	(25.2-26.7) (31.7-32.7) (25.2-25.9) (31.9-32.7)	0. 3093	27.8 – 34.8 26.1 – 33.5
MCHC (g/dL) Males Females	31 – 35.2 30.62 – 34.3	(30.8-31.7) (35.1-35.6) (30.4-31.0) (34.2-34.8)	<0.0001	33.0 – 35.0 32.7 – 34.9
Platelets (10^9/L) Males Females	164.35 – 367 184.5 -428	(119-182) (360-376) (176-195) (423-452)	<0.0001	171 – 388 186 - 454
WBC (10^9/L) Males Females	4.26 -10.52 4.52 – 12.41	(3.88-4.43) (10.09-11.63) (4.31-4.94) (12.24-13.18)	<0.0001	3.92 – 10.40 3.90 – 12.60
Neutrophils (10^9/L) Males Females	1.51 – 6.49 1.95 – 8.02	(1.29-1.75) (6.16-7.17) (1.87-2.12) (7.84-8.3)	<0.0001	1.60 – 6.98 1.60 - 8.30
Lymphocytes (10^9/L) Males Females	1.49 – 3.8 1.55 – 4.29	(1.32-1.54) (3.7-4.13) (1.37-1.69) (4.2-4.47)	<0.0001	1.4 - 4.2 1.4 – 4.5
Monocytes (10^9/L) Males Females	0.34 – 0.93 0.30 – 0.87	(0.28-0.35) (0.87-0.97) (0.28-0.32) (0.85-0.93)	0.5617	0.3 – 0.8 0.2 – 0.8
Eosinophils (10^9/L) Males Females	0.03 - 0.48 0.03 - 0.45	(0.01-0.04) (0.45-0.52) (0.02-0.04) (0.43-0.49)	0.2282	0 – 0.95 0 – 0.4
Basophils (10^9/L) Males Females	0.01 - 0.09 0.01 - 0.09	(0.01-0.02)(0.08-0.1) (0.01-0.02) (0.08-0.1)	0.7607	0 – 0.1 0 – 0.1

¶ *Mann-Whitney U test comparing ranges for men and women, with $p<0.05$ regarded as statistically significant*

II *The current coastal ranges for red cell parameters are derived from international sources,[3] all other ranges derived from the 2009 Gauteng study by Lawrie et al.[4]*

Table 2 Comparison of RIs for African, Coloured and Caucasian ethnic groups in this study

Parameter	**Africans** n=62 (M) n=134(F)		**Coloureds** n=98 (M) n=148 (F)		**Caucasians** n=84 (M) n=94 (F)		**Kruskal-Wallis p-value**
	RI	**Mean (SD)**	**RI**	**Mean (SD)**	**RI**	**Mean (SD)**	
Red Cell Count (10^{12}/L) *Males* *Females*	 4.55 - 5.89 3.91 - 5.18	 5.22 (0.34) 4.58 (0.33)	 4.66 – 6.34 4.1 - 5.54	 5.5 (0.43) 4.74 (0.36)	 4.65 - 6.03 3.95 – 5.29	 5.34 (0.35) 4.62 (0.34)	 0,0003 0,0031
Hb (g/dL) *Males* *Females*	13.61 - 16.79 11.5 - 14.85	15.2 (0.81) 13.25 (0.81)	13.72 – 17.76 12.11 - 15.2	15.74 (1.03) 13.56 (0.79)	14.14 - 17.52 11.96 – 15.56	15.83 (0.86) 13.76 (0.92)	0.0001 0.0002
Hct (L/L) *Males* *Females*	0.41 – 0.50. 0.35 – 0.45	45.73 (2.37) 40.5 (2.48)	0.41 – 0.54 0.37 – 0.48	47.26 (3.22) 41.57 (2.47)	0.42 – 0.52 0.36 – 0.47	46.8 (2.65) 41.47 (2.64)	0.0043 0.004
MCV (fL) *Males* *Females*	79.88 - 94.14 80.65 - 95.9	87.01 (3.64) 88.61 (4.06)	78.26 – 94.76 79.95 - 96.3	86.51 (4.21) 88.15 (3.76)	82.27 - 93.79 81.90 – 96.44	88.03 (2.94) 89.17 (3.71)	0.0297 0.0123*
MCH (pg) *Males* *Females*	25.71 - 32.45 25.33 - 31.91	29.08 (1.72) 29.03 (1.69)	25.88 – 31.88 25.16 - 31.54	28.88 (1.53) 28.74 (1.57)	27.60 - 32.02 26.89 – 32.65	29.81 (1.13) 29.77 (1.47)	0.0001 0.0001
MCHC (g/dL) *Males* *Females*	31.41 - 35.22 30.9 - 34.37	33.32 (0.97) 32.66 (0.94)	33.17 – 35.29 30.37 - 34.2	33.17 (1.08) 32.6 (0.99)	32.39 - 35.29 31.62 – 34.64	33.84 (0.74) 33.13 (0.77)	<0.0001 0.0001
Platelets (10^9/L) *Males* *Females*	 158.89 - 371.79 202.57 - 402.55	 265.34 (54.31) 296.43 (52.02)	 170.87 – 382.15 181.03 - 442.85	 276.51 (53.9) 311.08 (67.71)	 161.61 - 373.87 169.44 – 406.88	 267.74 (54.15) 288.16 (60.57)	 0.4415* 0.015
WBC (10^9/L) *Males* *Females*	2.94 - 9.8 4.2 - 12.09	6.37 (1.75) 7.64 (2.08)	4.80 – 10.88 5 - 12.88	7.84 (1.55) 8.82 (2.06)	4.12 – 10.16 4.52 - 11.46	7.14 (1.54) 7.99 (1.77)	<0.0001 0.0001

Neutrophils (10^9/L) *Males* *Females*	0.84 - 5.30 1.89 - 7.96	3.07 (1.14) 4.17 (1.66)	1.83 – 6.65 2.05 - 8.19	4.24 (1.23) 4.96 (1.56)	1.64 - 6.10 1.66 – 7.34	3.87 (1.14) 4.5 (1.45)	<0.0001 0.0001
Lymphocytes (10^9/L) *Males* *Females*	1.33 - 3.49 1.54 - 3.91	2.41 (0.55) 2.67 (0.6)	1.44 – 3.88 1.39 - 4.51	2.66 (0.62) 2.94 (0.83)	1.24 - 3.56 1.36 – 4.02	2.4 (0.59) 2.69 (0.68)	0.0075 0.0164
Monocytes (10^9/L) *Males* *Females*	0.24 - 0.74 0.29 - 0.86	0.49 (0.13) 0.54 (0.16)	0.29 – 0.87 0.3 - 0.89	0.58 (0.15) 0.58 (0.16)	0.31 - 0.89 0.32 – 0.86	0.6 (0.15) 0.59 (0.14)	<0.0001 0.011
Eosinophils (10^9/L) *Males* *Females*	0 - 0.37 0.03 - 0.47	0.15 (0.11) 0.17 (0.12)	0 – 0.46 0.03 - 0.46	0.21 (0.13) 0.17 (0.12)	0 - 0.40 0 – 0.39	0.18 (0.11) 0.17 (0.11)	0.0233 0.609*
Basophils (10^9/L) *Males* *Females*	0 - 0.07 0.01 - 0.07	0.03 (0.02) 0.04 (0.01)	0 – 0.47 0.02 - 0.09	0.04 (0.02) 0.04 (0.02)	0.01 - 0.09 0 – 0.11	0.05 (0.02) 0.05 (0.03)	<0.0001 0.0001

**NS indicates no significant difference*

Appendix A:

WPBTS Confidential Donor Questionnaire

WP Blood Transfusion Service
Do something remarkable

www.wpblood.org.za / info@wpbts.org.za

Head Office
Old Mill Road, Pinelands, 7405 • PO Box 79, Howard Place 7450
t:021 507 6300 / f:021 531 0322

CONFIDENTIAL DONOR QUESTIONNAIRE

Please make sure that you complete all required sections carefully and honestly.

SECTION 1

Lifestyle Questionnaire:

Though personal, these questions don't aim to offend, but rather to identify potential risk to the recipient.

SECTION 2

Health Questionnaire:

Your safety is as important to us as the safety of the recipient. Therefore, you might not be able to donate if you answer 'yes' to any of these questions. The qualified nurse will discuss your answers with you.

SECTION 3

Contact Details and Donor Enrolment Form:

New donors must complete all sections of the questionnaire.
Regular blood donors should only complete section 3 if any personal information has changed.

PLEASE DO NOT DONATE BLOOD IF YOU MAY HAVE BEEN EXPOSED TO HIV/AIDS.

You may be endangering someone's life.
Never donate blood for personal health screening purposes.

Thank you for donating blood today!

Your donation could save at least three lives. Remarkable, isn't it? As a Service, we provide safe blood and blood products to those who need them. Please continue to make a difference by remaining a regular blood donor.

DONOR LABEL

BAR CODE

Section 1 | # LIFESTYLE QUESTIONNAIRE

Please circle your answers.

Please read all questions carefully and answer honestly. Your answers will be treated confidentially.

1	Do you consider your blood safe to be transfused to a patient?	No	Yes	STAFF SECTION
2	**In the past 6 months have you:** Had a tattoo, body piercing, ear piercing or permanent make-up applied?	No	Yes	
	Had Raatib, ritual scarring, ritual piercing, ritual circumcision, blood sharing or been stabbed?	No	Yes	
	Taken antiretroviral (ARV's) medication, including Truvada?	No	Yes	
3	**For Health Care Workers and their partners only:** **In the past 6 months:** Have you or your sexual partner had a needle stick or skin penetrating injury; or had skin, eye or mouth contact with another person's blood?	No	Yes	
The following questions are of a sexual nature. We ask these questions as sexual contact may cause infectious diseases like HIV/AIDS. "Sexual contact" refers to vaginal sex (contact between penis and vagina); oral sex (mouth or tongue contact with vagina, penis or anus) and anal sex (contact between penis and anus). Where applicable, please answer "Yes" to the following questions even if a condom was used:				
4	Do you have AIDS or are you HIV positive?	No	Yes	
	Have you ever had sexual contact with anyone who has AIDS or is HIV positive?	No	Yes	
	Are you only giving blood for an HIV test?	No	Yes	
5	**In the past 6 months (with or without a condom):** - Have you started having sexual contact with a new sexual partner?	No	Yes	
	- Have you had sexual contact with more than one person?	No	Yes	
	- To the best of your knowledge has your sexual partner had sexual contact with more than one person?	No	Yes	
	- Have you had sexual contact with someone whose sexual history you do not know?	No	Yes	
	- Have you had sexual contact with anyone who takes money, drugs or other favours for sex?	No	Yes	
	- Have you received money, drugs or other payment for sex?	No	Yes	
	- Are you a sex worker?	No	Yes	
	- Have you been sexually assaulted?	No	Yes	
6	**In the past 6 months:** Have you or your sexual partner had any sexually transmitted disease (STD) including genital herpes, syphilis, gonorrhoea (drop) or human papilloma virus?	No	Yes	
7	Have you or your sexual partner ever used recreational/street drugs by nose, mouth or injection needle?	No	Yes	

DECLARATION : Please read and sign before donating blood.

1. I have read and understood the pamphlet "Important Information for Blood Donors".
2. To the best of my knowledge all the information supplied is the truth.
3. I understand that if I have not answered these questions truthfully this could endanger the patient and lead to legal proceedings against me. I undertake that should I for any reason deem my blood not safe for use, I will immediately inform WPBTS.
4. I consent to my blood being tested for Syphilis, Hepatitis B, Hepatitis C and HIV.
5. I understand that I will be informed of any test results that are important to my health or affect my ability to donate blood.
6. I accept that samples of my blood and / or donation data may be used on occasion for scientific research, the objective of which is to improve the safety of the blood supply to patient and donor health and well-being. On occasion the Service may permit researchers to request additional samples from me with my consent.
7. I confirm that I am 16 years of age or older.
8. I understand that the information on this form will be kept in a secure facility indefinitely under my donor code, not my name.
9. I understand the donation process and the possible risks involved as explained.
10. I consent to the administration of such fluids and medications as deemed necessary in the management of an untoward donor reaction.
11. I consent to the infusion of fluids, medications and re-infusion of my own blood components during apheresis collection procedures.
12. I consent to being offered information on the Service's Iron Replacement Programme and that any decision to take the iron replacement tablets rests with me.

Please do not sign until you have answered all the questions and read the declaration.

Cell number: Tel number:	
Name and surname:	
Date of birth:	
Donor's signature:	

FOR OFFICE USE:				
Interview done	No		Yes	
Signature: Phlebotomist				
Signature: Interviewer (only if interview was done)				

Section 2 | HEALTH QUESTIONNAIRE

Please circle your answers.

Please read all questions carefully and answer honestly. Your answers will be treated confidentially.

1	Are you feeling well today?	No	Yes	STAFF SECTION
	In the last 4 hours have you had something to eat and drink?	No	Yes	
2	**Are you involved in any of the following:** Driving a public or heavy-duty vehicle, flying an aeroplane, working on scaffolding or using power tools?	No	Yes	
	Sky diving, deep-sea diving or mountaineering?	No	Yes	
3	**In the past 3 days:** Have you been to the dentist?	No	Yes	
	Have you taken any painkillers, anti-inflammatories or aspirin (Ecotrin)?	No	Yes	
	In the past 7 days: Have you had a cold, flu, sore throat, fever, infection or allergies?	No	Yes	
	In the past 30 days: Have you had diarrhoea or vomiting?	No	Yes	
	Have you taken Androcur, Proscar, Propecia, Roaccutane, Warfarin or Dabigtran Etexilate (Pradaxa)?	No	Yes	
4	**In the past 3 months:** Have you taken any medication (including traditional medication), injections or tablets?	No	Yes	
5	**In the past 6 months:** Have you or your sexual partner had a blood transfusion or received blood products or clotting factors?	No	Yes	
	Have you had acupuncture, botox or dry-needling?	No	Yes	
	Have you had a vaccination or immunization (inoculation)?	No	Yes	
	Have you taken part in a drug trial, vaccine trial, or clinical research?	No	Yes	
6	**In the past 6 months:** Have you had a surgical procedure or been admitted to hospital?	No	Yes	
	Are you scheduled to have surgery **in the next 6 weeks?**	No	Yes	
7	**In the past 2 years:** Have you taken (Neo) Tigason for skin problems?	No	Yes	
8	**Have you ever had:** High blood pressure?	No	Yes	
	Heart, lung, circulatory problems or a bleeding disorder?	No	Yes	
	Epilepsy, convulsions or strokes?	No	Yes	
	Cancer, skin cancer or leukaemia?	No	Yes	
	Diabetes, asthma, TB or kidney disease?	No	Yes	
9	Has your doctor ever advised you to donate blood for medical reasons (high iron, 'thick blood', polycythaemia or haemochromatosis)?	No	Yes	
10	**HEPATITIS:** Have you ever had yellow jaundice, hepatitis, liver disease or a positive test for hepatitis?	No	Yes	
	In the past 6 months have you been in contact or lived with anyone who has hepatitis (jaundice)?	No	Yes	
11	**MALARIA:** Have you been in a malaria area **in the last 3 months**?	No	Yes	
	Have you had malaria **in the last 3 years**?	No	Yes	
	Did you grow up in a malaria prevalent area?	No	Yes	
	If "yes", have you been in any malaria area in the last 3 years?	No	Yes	
12	**VARIANT CREUTZFELD-JAKOB DISEASE: (also known as Mad Cow disease)** Have you ever had neuro surgery, received a dura mater (brain covering) graft or taken pituitary growth hormone?	No	Yes	
	Have you or your sexual partner ever received a tissue, cornea or organ transplant?	No	Yes	
	Were you residing in the United Kingdom for a total period of 12 months or longer between Jan. 1980 and Dec. 1996?	No	Yes	
13	Have you ever had any other serious illnesses, severe allergic reactions, tropical diseases or medication not mentioned above?	No	Yes	
14	Are you participating in a regular training or athletic programme?	No	Yes	
15	Have you ever injected yourself or been injected with illegal steroids (body building drugs)?	No	Yes	
16	**FOR WOMEN ONLY:** Are you pregnant or undergoing fertility treatment?	No	Yes	
	In the last 3 months have you had a baby, miscarriage or abortion?	No	Yes	
	Are you breastfeeding?	No	Yes	

FOR OFFICE USE:		
Blood pressure:	Pre	Post
Donor's pulse:	Pre	Post
Hb: g/dL		Sign

Donor set-up by:	BARCODE
Sample taken by:	
Needle removed by:	

Section 3 | # DONOR ENROLMENT FORM

First time donors: Complete fully

Regular donors: Only complete this page if your personal information has changed.

Surname:															Age:			Title:							
First name:											Second name initials:							Female:		Male:					
Date of birth:	D	D	M	M	Y	Y	Y	Y		ID no:															
Home address:																									
																		Postal code:							
Postal address:																									
																		Postal code:							
Telephone no's:	H:	C	O	D	E								W:	C	O	D	E								
Cellphone no:																									
Workplace name & address:																									
																		Postal code:							
E-mail address:																									

Please circle your answers:

Language:	Eng	Afr	Other							Ethnic Group:	Asian			Black				Coloured				White			
Have you ever attended a blood donor clinic or donated blood before?											Yes			No				No. of previous donations:							
If you have donated blood before, where:																		When:	Y	Y	Y	Y			
Have you ever used a different name to donate blood?										Yes			No					If yes, state name used:							
Preferred place of donation:																									
I hereby agree to receive notifications and reminders from WPBTS via:											E-mail			SMS				Written material				Phone calls			

I understand that all calls received from WPBTS will be recorded for quality purposes.

I hereby declare that I would like to enrol as a blood donor. Donor Signature: _____ Date: _____

FOR OFFICE USE ONLY:

STATS CODE:
PANEL CODE:
RESIGNATION CODE:
COMMENTS:

BARCODE

DONOR LABEL

DEFERRAL REASON:											
RECEPTIONIST SIGNATURE:										DATE:	
MEDICAL COMMENTS:											
SPECIAL INSTRUCTION:											
ATTACH MALARIA STICKER TO BLOOD PACK UNTIL (DATE):											
DONOR CODE:											

Iron replacement tablets taken:	YES		NO		Gifts received: _____
Signature: _____					Donor signature: _____

Appendix B:

General Consent Form

WP Blood Transfusion Service
Do something remarkable

NATIONAL HEALTH LABORATORY SERVICE

UNIVERSITY OF CAPE TOWN
IYUNIVESITHI YASEKAPA • UNIVERSITEIT VAN KAAPSTAD

NORMAL REFERENCE RANGE STUDY

Who is conducting the study?

The study entitled "*Establishing locally derived reference intervals for full blood count parameters and white cell differential counts in the Western Cape region of South Africa*" is being conducted by Dr A de Koker. Dr De Koker is an employee of the National Health Laboratory Services (NHLS) and this research project will be submitted as part of the requirements for specialist training, degree of Master of Medicine in Pathology (Haematology) at the faculty of Health Sciences at the University of Cape Town. Dr J Opie, Senior Haematology Pathologist, at the NHLS (Groote Schuur Hospital) is the primary supervisor.

We will be conducting the study in collaboration with the Western Province Blood Transfusion Services (WPBTS) and the results of the study may be used to guide their policies on screening, deferring and managing donors.

This study may be reported in medical journals and scientific congresses. Information obtained from this study will likely be used to adjust the 'normal' values that are currently used by the NHLS at sea level in South Africa. The main benefit of the study is that these 'normal ranges' obtained will be representative of the local population that our laboratory serves, rather than adapted from another population or country.

What is the purpose of the study?

1. The main purpose is **to establish local normal ranges for full blood count parameters,** which includes haemoglobin levels as well as the numbers of different cell types in the blood i.e. white blood cells (and

subtypes), red cells and platelets. We aim to get this information across the three main ethnic groups in the Western Cape.

2. The results obtained may provide some information on how many people have **iron deficiency** among donors in the Western Cape in various ethnic groups.

What will happen if you participate in the study?

If you agree to participate, a qualified WPBTS employee will collect study-related **blood specimens (*two to three teaspoons of blood)*** *(point no. 9)* from you. These will be used to perform more sensitive tests of donor's blood counts and iron levels (serum ferritin). In accordance with the study protocol, the WPBTS will notify you if any of your blood results are abnormal. **There are no follow-up appointments or other blood tests required for the purpose of this study.**

Are there risks to you participating in the study?

Other than the small risk of some bruising and mild discomfort from collecting the specimens, there is no risk involved in participating in the study.

Are there benefits to you participating in the study?

There are no personal benefits for you from participating in this study, but you will assist in medical research and indirectly aid both the NHLS and WPBTS to provide a better service.

Will I be paid and are there any costs to the research?

No, there is no payment or compensation for participating in the study.

Will my personal information be shared? (How will confidentiality be maintained?) *(Point no. 6)*

By agreeing to be a blood donor you will not be anonymous initially, as the WPBTS follows routine blood donor policies and screening tests. If any abnormal results are found, the information will immediately be relayed to you. The WPBTS have

established processes in place for notifying and counselling their donors when abnormal test results are confirmed.

All reasonable measures, including keeping all paper records locked away and making use of password protected computer systems will be done to maintain confidentiality as a WPBTS donor.

The data collected will be treated confidentially. Confidentiality will be ensured through the design of the data collection i.e. participant names and identifying data will be removed. Only identifiers such as sex, age, race and blood results will be used for the purpose of the study. *By signing the consent form you give your permission for the researchers to use this information and to group the data to give normal ranges for specific gender, race and age groups. (Point no. 7)*

What if I don't want to participate after I have given the blood specimen?

You are not forced to participate in this study and you may retract your consent at any time, by contacting the investigator listed at the bottom of the consent form, who will then delete your results from the study database. Your decision to remove yourself form the study will not affect your relationship with the WPBTS in any way or influence future donations.

If, however, the data has already been analysed and reported in medical journals, we will not be able to remove this data from the study, but you can be reassured that the data is in no way traceable back to you, as all personal identifiers would have been removed from your results before analysis.

What happens if you do not want to take part in the study?

You are under no obligation to participate in the study, and are completely within your rights to decline. Your participation is voluntary, and you will not be penalized if you refuse to participate or decide to terminate participation.

What if you have additional questions?

You can contact the responsible investigator listed at the bottom of the consent form with any questions or concerns.

Contact details of Researchers:

At any time, if you have questions about the research or if you are injured as a result of the research you may contact:

Dr Caroline Hilton (WPBTS Transfusion Medical Specialist and co-investigator)

021-507-6329 / 082-372-3133

Or

Dr Annemarie de Koker (The principle investigator): 072-060-2781

Contact details for the Human Research Ethics Committee (HREC) in the Faculty of Health Sciences at the University of Cape Town:

The UCT's Faculty of Health Sciences Human Research Ethics Committee can be contacted on 021 406 6338 in case you have any ethical concerns or questions about your rights or welfare as a participant on this research study. The HREC is situated in the Old Main building of Groote Schuur Hospital, Floor E52, Room 23, Observatory 7925. (Point 8)

Consent:

I DECLARE THAT I HAVE READ AND UNDERSTOOD THIS CONSENT FORM AND I AGREE TO PARTICIPATE IN THE ABOVEMENTIONED RESEARCH STUDY. I AM FREE TO RETRACT MY CONSENT AT ANY STAGE OF THE PROJECT

Donor/Participant name: _____

Signature: _____

Date: _____

Donor Number:	
Blood Pack Label:	
Barcode:	
Consent taken by: **Staff Number and Signature**	

Appendix C:

Information pamphlet for donor

NHLS/WPBTS NORMAL REFERENCE RANGE STUDY

FULL TITLE: *Establishing locally derived reference intervals for full blood count parameters and white cell differential counts in the Western Cape region of South Africa.*

1. Who is conducting the study?

This study is run by Dr A De Koker, a doctor in Haematology at Groote Schuur Hospital. The project will partly fulfil criteria for her to obtain her Pathology (Haematology) degree from the University of Cape Town (UCT). She is helped by Dr C Hilton from the Western Province Blood Transfusion Services, as well as Dr J Opie, a senior specialist, who will supervise the project. Also, the National Health Laboratory Services (NHLS) and the Western Province Blood Transfusion Services (WPBTS) will be collaborating on the project. (Point no.3)

2. Purpose of the study

Our goal is to better define the blood counts of normal people (i.e. the amount of different cells in the blood, such as white cells, haemoglobin and platelets). We want to see if there are important differences between men and women, as well as for the different ethnic groups in the Western Cape. We then want to use what we learn in the day-to-day work of the NHLS and WPBTS, since these laboratories need to know which blood counts are normal and which are high or low.
Another goal of the study is seeing how many people have low iron levels in their blood. If the results are meaningful, this will help the WPBTS to know which blood donors to test for low iron levels in future. Also, our results may be published in medical journals and presented at congresses to inform and assist other laboratories – but no participant names or identifiers will be mentioned.

3. When / Where / How will the study be done?

All first time donors coming to donate blood at certain WPBTS centres in the Western Cape will be invited to participate in the study. Being a first time donor, if you agree, the process to donate blood continues normally. However, the nurse will draw ***two extra tubes of blood (equal to two teaspoons)*** *(point no.9)* for the project, to perform more sensitive tests to count your different blood cells and measure iron level. **No other tests are done.** Recruiting for the study will happen on Mondays to Thursdays from May to December 2016. If needed, we may extend this period, if more participants are required.

4. Will every first time blood donor in the Western Cape participate?

No. We only need 720 participants in total to work out the normal blood counts using medical statistics. As soon as we have these 720 participants, we will not recruit any more.

5. Who will not be included in the study?

If you test positive for HIV, hepatitis or syphilis on the routine tests done for blood donors, you cannot be included in the study. These diseases could influence the blood counts we are investigating and give a false impression of healthy people's counts. Otherwise, all people eligible for blood donation will be eligible for the study.

6. Will I know if I am included in the study?

Yes. The study will be explained and you will sign a consent form if you agree to participate. You are free to refuse to participate in the study or to change your mind after you signed the consent form – your blood donation will then proceed normally and no extra tubes of blood will be drawn.

7. What happens if any of my tests are abnormal?

If any abnormal results are seen which may impact your health, you will be informed by the WPBTS. Your results will be discussed with you and if required, you may be referred to your own doctor for treatment. If you test positive for HIV, it is routine for the WPBTS to then run a second test to confirm the results. If confirmed positive, you will be notified by the WPBTS. They will provide you with counselling and direct you to your own doctor or nearest clinic for treatment.

8. Other questions/concerns?

Please discuss any questions/concerns with the staff at the clinic.

You can also contact:
Dr Annemarie De Koker (Principle Investigator): 072-060-2781
Dr Caroline Hilton (WPBTS Transfusion Medicine Specialist): 021-507-6329 or 082-372-3133

The UCT's Faculty of Health Sciences Human Research Ethics Committee can be contacted on 021 406 6338 in case you have any ethical concerns or questions about your rights or welfare as a participant on this research study. The HREC is situated in the Old Main building of Groote Schuur Hospital, Floor E52, Room 23, Observatory 7925. (Point 8)

Thank you for assisting in this study and for your help in the provision of safe and sufficient blood for patients in the Western Cape.

Appendix D:

Information pamphlet for deferred donors

NHLS/WPBTS NORMAL REFERENCE RANGE STUDY

FULL TITLE: *Establishing locally derived reference intervals for full blood count parameters and white cell differential counts in the Western Cape region of South Africa.*

1. Who is conducting the study?

This study is run by Dr A De Koker, a doctor in Haematology at Groote Schuur Hospital. The project will partly fulfil criteria for her to obtain her Pathology (Haematology) degree from the University of Cape Town (UCT). She is helped by Dr C Hilton from the Western Province Blood Transfusion Services, as well as Dr J Opie, a senior specialist, who will supervise the project. The National Health Laboratory Services (NHLS) and the Western Province Blood Transfusion Services (WPBTS) will be collaborating on the project. (Point no.3)

2. Purpose of the study

Our goal is to better define the blood counts of normal people (i.e. the amount of different cells in the blood, such as white cells, haemoglobin and platelets). We want to see if there are important differences between men and women, as well as for the different ethnic groups in the Western Cape. We then want to use what we learn in the day-to-day work of the NHLS and WPBTS, since these laboratories need to know which blood counts are normal and which are high or low.

Another goal of the study is seeing how many people have low iron levels in their blood. If the results are meaningful, this will help the WPBTS to know which blood donors to test for low iron levels in future. Also, our results may be published in medical journals and presented at congresses to inform and assist other laboratories – but no participant names or identifiers will be mentioned.

3. Why are we inviting you to participate?

Although your haemoglobin count is too low to be eligible to donate blood, the level might still fall within the normal range for your gender and ethnic group. We want to include all the healthy people we can, without bias, to best determine normal blood counts and amount of iron in the blood. (Point no. 10)

4. When / Where / How will the study be done?

All first time donors coming to donate blood at certain WPBTS centres in the Western Cape will be invited to participate in the study. If they agree, the process to donate blood continues normally. However since no blood will be taken from you for donation, if you agree to participate, the nurse will draw ***three tubes of blood (equal to 3 teaspoons)***

(point no. 9) for the project. This will be used to count your different blood cells, measure iron level and test for HIV. **No other tests are done.**
Recruiting for the study will happen on Mondays to Thursdays from May to December 2016. If needed, we may extend this period, if more participants are required.

5. Will every first time blood donor in the Western Cape participate?

No. We only need 720 participants in total to work out the normal blood counts using medical statistics. As soon as we have these 720 participants, we will not recruit any more.

6. Who will not be included in the study?

If you test positive for HIV you cannot be included in the study. This is because it could influence the blood counts we are investigating and give a false impression of healthy people's counts. Otherwise, all people eligible for blood donation will be eligible for the study.

7. Will I know if I am included in the study?

Yes. The study will be explained and you will sign a consent form if you agree to participate. You are free to refuse to participate in the study or to change your mind after you have signed the consent form.

8. What happens if any of my tests are abnormal?

If any abnormal results are seen which may impact your health, you will be informed by the WPBTS. Your results will be discussed with you and if required, you may be referred to your own doctor for treatment. If you test positive for HIV, we will run a second test to confirm the result, if confirmed positive, you will be notified by the WPBTS. They will provide you with counselling and direct you to your own doctor or nearest clinic for treatment.

9. Other questions/concerns?

Please discuss any questions/concerns with the staff at the clinic.
You can also contact:
Dr Annemarie De Koker (Principle Investigator): 072-060-2781
Dr Caroline Hilton (WPBTS Transfusion Medicine Specialist): 021-507-6329 or 082-372-3133

The UCT's Faculty of Health Sciences Human Research Ethics Committee can be contacted on 021 406 6338 in case you have any ethical concerns or questions about your rights or welfare as a participant on this research study. The HREC is situated in the Old Main building of Groote Schuur Hospital, Floor E52, Room 23, Observatory 7925. (Point 8)

Thank you for assisting in this study and for your help in the provision of safe and sufficient blood for patients in the Western Cape.

Appendix E:

Budget Summary

Tests	Number of units	Unit Price	Total Price
FBC & Differential counts (WPBTS cost price)	720	R12.00	R8 640.00
Ferritin levels: (WPBTS cost price)	720	R5.33	R3 837.60
Statistician fees	approx. 17hrs	R150/hr	R2 550.00
HIV serology for donors deferred for failing Hb screening (WPBTS cost price)	60*	HIV serology: R11,56	R693.60
TOTAL			**R15 721.20**

* Based on the analysis from WPBTS data of 2015 approximately 18.3% of first time new donors are deferred and of these 44% are due to low haemoglobin levels. If this data is extrapolated to our study, we anticipate that approximately 58 donors of the 720 subjects will be deferred for low Hb levels.

- The prevalence of HIV in first time new donors at the WPBTS in 2014 was 0.31%. This would equate to be less than one, in our 58 deferred donors. With the possibility of one subject that tests positive, a confirmatory serology test will be done. Also note that even if the 2% false positive rate is taken into account, only 1.34 individuals should require repeat confirmatory testing. Following from this, we estimate that HIV serology testing will be required for approximately 60 samples.

Appendix F:

Letter of authorization from the WPBTS

WP Blood Transfusion Service
Do something remarkable

www.wpblood.org.za / info@wpbts.org.za

Head Office
Old Mill Road, Pinelands, 7405 • PO Box 79, Howard Place, 7450
T: 021 507 6300 / F: 021 531 0322

4th December 2015

University of Cape Town Ethics Committee

Groote Schuur Hospital

Observatory

7925

To Whom It May Concern,

This letter is to confirm that Dr Annemarie de Koker has been given permission to conduct a study for her Masters in Medicine thesis using samples and information from first-time blood donors enrolled by the Western Province Blood Transfusion Service (WPBTS). The results of the study will be beneficial to both the University of Cape Town haematological pathology department and WPBTS in determining accurate reference ranges for patients and blood donors within the province.

Study Title: **Establishing local reference intervals for full blood counts and differential counts in the Western Cape region of South Africa.**

Principle Investigator: Dr Annemarie de Koker

Supervisor: Dr Jessica Opie (NHLS, Groote Schuur)

Co-Supervisor: Dr Arthur Bird (WPBTS)

Co-Investigators: Dr Gregory Bellairs (WPBTS), Dr Caroline Hilton (WPBTS) & Ms Beverley Mitchell (WPBTS)

Please direct any queries to myself or Dr Caroline Hilton (caroline@wpbts.org.za).

Kind regards,

Dr Gregory Bellairs

CEO/Medical Director WPBTS

greg@wpbts.org.za | (021) 507 6319

Appendix G:

WPBTS SOP for sample handling (Oct 2015)

WORK INSTRUCTION: TIM-W15	Page: 1 of 5
SAMPLE HANDLING	Issue date: 23 October 2015
AUTHORISED: S Valensky R Cable B Mitchell	APPROVED: L Bust G Bellairs

PRINCIPLE

Various types of blood samples are required for testing by the respective laboratories eg. anticoagulated and coagulated. The proper collection, receipt, handling and storage of samples is important in validation of test results. Blood samples are required to be packed, distributed and transported in such a manner that leakage does not occur and the risk of contamination of sample, staff and/or the public is reduced.

All samples must be correctly labelled as to the identification of the donor/ patient. All samples are to be treated as potentially infectious and precautions are to be taken as stipulated in Work Instruction SAF-W04 (Safety Regulations). Unsuitable samples which have been received and captured on the computer system or where paperwork will be retained in the laboratory, but which cannot be tested, must have the reason documented. In specific circumstances, where it may be necessary to use a sample which is not ideal, the reason for use and the problem with the sample must be documented eg. baby's sample, slightly haemolysed.

Samples for testing are obtained from various sources:

- Donor samples – collected at donor clinics per Work Instruction CLN-W10 (Blood Collection) and tested in WPBTS laboratories.
- Patient samples – collected by hospital staff into labelled tube and submitted to Blood Bank for testing.
- Miscellaneous samples – submitted by pathologists, doctors or collected by trained WPBTS staff as indicated below.

COLLECTION OF SAMPLES

1. Label all sample tubes with name/ code, serial number, as applicable immediately prior to taking the sample. If the tube is found to be defective, replace, collect sample and then label immediately.
2. Prepare relevant documentation as indicated by the requesting laboratory.
3. Request donor to complete Health and Lifestyle Questionnaire, HIV Testing Consent Form or other relevant documents.
4. Make person comfortable.
5. Check that details on sample and documentation correlate with donor by requesting donor identification.
6. Insert venoject multisampling needle into vacutainer barrel.
7. Apply cuff/ tourniquet to upper arm. (If BP cuff is used, inflate to ± 50 mmHg.)
8. Select vein and clean surrounding area in accordance with Work Instruction CLN-W10 (Blood Collection).
9. Insert needle into vein.
10. Insert sample tube into vacutainer device and allow to fill to maximum level.
11. Remove the tube and, if anticoagulated sample, invert a number of times to mix the anticoagulant adequately.
12. Remove needle when all samples have been collected.
13. Leave needle and vacutainer intact and dispose of into sharps container. **Do not recap needle.**
14. Dispose of any tubes found defective or not meeting specifications as biohazardous risk waste.
15. Transport samples, at correct temperatures, to applicable laboratory with relevant documentation.

SAMPLE TYPES

LABORATORY	TEST	SAMPLE TYPE (Colour of top)
Haematology	Full blood counts on donors. Product QC.	4 ml EDTA anticoagulated (purple). 4 ml Coagulated (red).
Blood Grouping	Blood group test and TPHA test on donors. Blood group test of donation unit. Control samples eg. aliquoted plasma.	6 ml EDTA anticoagulated (purple). Labelled segment. 6 ml EDTA anticoagulated (purple).
Virology	Infectious disease screening.	10 ml EDTA anticoagulated (purple). 10 ml EDTA anticoagulated (gold).
Paternity	Paternity testing - venous sample. - heelprick sample.	4 ml EDTA anticoagulated (purple). Capillary tubes emptied into 6 ml ACD tube (yellow).
Reference Laboratory	Rare donor samples. Routine work. Postnatal testing.	6 ml ACD anticoagulated (yellow). Above/ 4 ml EDTA anticoagulated (purple). Umbilical cord sample collected into 6 ml EDTA anticoagulated (purple). Venous or heelprick sample.
Reagents	Various.	As requested.
Blood Banks	Crossmatch procedures. Transfusion Reaction investigations Postnatal testing.	4/ 6 ml EDTA anticoagulated (purple/ pink) for automated testing. Umbilical cord sample collected into 6 ml EDTA anticoagulated (purple/ pink).
Miscellaneous	Pathology/ other testing.	As requested.

RECEIPT OF SAMPLES

Clerical Check

On receipt of samples, Laboratory/ Blood Bank to:

1. Check clerical details of patient/ donor on samples and related forms in accordance with departmental Work Instruction.
2. Take appropriate action if discrepancies are found.
3. Record sample details/ date and time of receipt and sign as required.

Visual Check

On receipt of samples, Laboratory/ Blood Bank to:

1. Visually check condition of sample.

1.1 If certain abnormalities exist and have been confirmed by the doctor, the sample may be used if the doctor assumes responsibility for permitting use of the sample.

2. Identify and investigate any non-conformances – refer to table below.
3. Discard any broken/ damaged/ unsuitable samples in biohazardous waste container. Wipe up any spills in accordance with Work Instruction SAF-W04. Seal forms, contaminated with body fluids, in plastic sleeve.
4. Record insufficient/ unsuitable samples on request form/ computer record etc. if applicable.
5. Request new samples for testing if possible.
6. Notify Central Sorting/ Components to discard products as biohazardous waste, where applicable.

RECEIPT OF SAMPLES (continued)

Problem solving of non-conforming samples

PROBLEM	POSSIBLE CAUSE	CHECK/ ACTION TO TAKE
Clots present in anticoagulated sample	Inadequate mixing with anticoagulant Disease condition eg. DIC	Request new sample. Remove clot(s) and test accordingly. (Use BioVue in Blood Banks.)
Yellow (icteric) sample	Hyperbilirubinaemia due to AIHA, HDN, cirrhosis or **transfusion reaction**. Dietary causes	Diagnosis. Previous blood groups transfused. Use sample for testing.
Red serum/ plasma	Haemolysis due to intravascular haemolysis of incompatible blood, burns, administration of non-physiological intravenous solutions etc. Incorrect storage and handling of samples.	Diagnosis. Previous blood groups transfused. Age of sample. Other solutions given. Request new sample. Samples showing a slight pink/orange discolouration may be used if no new sample can be obtained. Use BioVue method in the Blood Banks.
Brown serum/ plasma	May indicate a significant level of methaemalbumin resulting from an acute haemolytic incident, such as incompatible transfusion or excessive RBC destruction due to inherited RBC membrane abnormalities or haemoglobin defects. Transfusion of Hemapure (blood replacement).	Diagnosis. Previous blood groups transfused. Use sample for testing provided that interpretation of results is possible.
Brown red blood cells	Indicates methaemoglobinaemia, which may be a signal of uremia or inhalation/ ingestion of certain toxic substances.	Diagnosis (eg. drug overdose). Patient history. Use sample for testing.
Haemolysed (where results may be affected), insufficient, pooled, heat inactivated serum/ plasma	Incorrect storage or handling.	Check other samples taken at donation and if unaffected, use for test. If all samples are affected, reject unit on computer. Central Sorting/ Components to discard products into a labelled biohazardous waste container.
Spontaneous agglutination of umbilical cord sample	May be contaminated with Wharton's Jelly.	Request venous or heelprick sample from baby.

OBTAINING CELLS/ SERUM FOR TESTING

PROBLEM	ACTION REQUIRED
Fibrin clots	1. Incubate sample at 37 °C for 10 – 15 minutes. 2. Spin at high speed for 5 minutes.
Difficult to obtain serum eg. polycythaemia	1. Incubate samples in waterbath at 37 °C for 1 hour. 2. Spin at high speed for 5 minutes. 3. Then incubate at 4 °C for 1 hour and spin again for 10 minutes.

Check on Sample Age

1. Laboratory/ Blood Bank to check age of sample.
1.1 Samples accepted by Blood Bank must be less than 48 hours old.
1.2 Compatibility test can be performed on the same sample up to 48 hours after the sample was taken. In emergency cases, samples up to 1 week old may be used for testing at the discretion of the CEO/ Medical Director, who assumes responsibility. CEO/ Medical Director to be contacted telephonically.
1.3 Thawed serum can be used up to 24 hours after thawing providing that 48 hour period has not elapsed since issue of first unit. Any testing required on a thawed sample after 24 hours must be at the discretion of the CEO/ Medical Director, who must be contacted telephonically.
1.4 Blood Grouping to test samples within 7 days of collection.
1.5 Haematology to test LHB samples within 72 hours of collection. Refer to Work Instruction HEM-W03 (Full Blood Count (FBC) Testing of Low Hb Donors and Testing for other Departments).
1.6 Virology samples to be centrifuged within 72 hours of collection and tested within 7 days of collection, if stored at 2 – 6 °C.
1.7 Samples for Reference Laboratory can be received up to 72 after collection and may be used for testing up to 2 weeks after collection if separated.
1.8 In Paternity Laboratory, red cell testing to be performed within 72 hours of collection. For DNA testing, age of sample is irrelevant.

TEMPERATURE AND STORAGE OF SAMPLES

Keep all samples refrigerated or, if sample is freshly bled, transport as soon as possible to be refrigerated.
If required, separate samples and freeze serum. Store frozen serum at < -18 °C.

LABORATORY	PRIOR TO TESTING	AFTER TESTING
Haematology	Donor samples are read onto the computer system by Central Sorting staff, placed in the coldroom overnight and delivered to Blood Grouping/ Haematology the following day. Apheresis samples are delivered to Haematology during working hours by Apheresis staff. All samples are kept at room temperature.	Store samples in labelled containers in cold room for 1 week at 2 – 6 °C.
Blood Grouping	Centrifuged samples awaiting testing are stored in unlabelled blocks in coldroom at 2 – 6 °C. Aliquots of plasma stored at 2 – 6 °C in coldroom.	Store in coldroom in appropriately labelled blocks, by date, for 1 week at 2 – 6 °C. Aliquots of plasma stored in freezer at -20 °C for at least 1 year.
Virology	Centrifuged samples awaiting testing are stored in unlabelled blocks in coldroom at 2 – 6 °C.	Store in coldroom in appropriately labelled blocks, by date, for 1 week at 2 – 6 °C.
Paternity	Samples awaiting testing are stored in demarcated area in the lab fridge at 2 – 6 °C. For DNA testing extracted aliquots are stored frozen in eppendorf tubes at –25 °C.	Depending on test required, store samples at 2 – 6 °C for ± 1 month or until results are issued. A small aliquot of the original DNA extract is stored at –25 °C for 5 years.

TEMPERATURE AND STORAGE OF SAMPLES (continued)

LABORATORY	PRIOR TO TESTING	AFTER TESTING
Reference Laboratory	Samples are to be placed in a demarcated area in laboratory fridge.	Store current tested samples in containers in laboratory fridge for 1 week, thereafter in coldroom for another week at 2 – 6 °C.
Blood Banks	All samples not in use to be refrigerated at 2 – 6 °C.	Store samples for 1 week in daily blocks at 2 – 6 °C and thereafter for another week at 2 – 6 °C in labelled bags. Store frozen plasma for 2 weeks. If there is an expected delay in transport to another laboratory, separate plasma from red cells and place in correctly labelled tubes on the day they are received. Regional Blood Banks to routinely separate samples before dispatch to Reference Laboratory.

DISCARD OF SAMPLES

1. Store samples as above.
2. At end of storage period, discard as biohazardous waste per Work Instruction TIM-W20 (Biohazardous (Health Care Risk) Waste).
3. Blood Bank/ Laboratory staff to check freezer weekly and discard samples which have reached the end of their storage period.

TRANSPORT OF SAMPLES

Internal transport:

1. After collection, samples for internal delivery within the building are kept at room temperature.
2. Donor samples/ segments for testing are placed in trays and transported on a trolley.
3. Apheresis samples are placed in a rack and carried to Haematology.
4. Antenatal samples are carried in Pathcare boxes.
5. Samples used by Training departments are carried in trays.

Local transport:

1. Package samples in watertight or leakproof containers eg. sealed plastic bag.
2. Label the container with contents and storage temperature eg. human blood; 2 – 6 °C where critical.
3. Send the containers with identifying forms showing contents of containers.

EXPORT OF SAMPLES/ BLOOD COMPONENTS

1. Infectious products to be released only on CEO/ Medical Director's consent and identified accordingly.
2. Blood components may be exported to other countries in time of need.
3. Refer to Work Instruction VIR-W29 (Virology Testing on Organ Donor Samples) for details.

REFERENCES: Work Instructions: BBK-W37 (Sample Receipt and Registration)
CLN-W10 (Blood Collection)
HEM-W03 (Full Blood Count (FBC) Testing of Low Hb Donors and Testing for other Departments)
SAF-W04 (Safety Regulations)
TIM-W20 (Biohazardous (Health Care Risk) Waste)
VIR-W29 (Virology Testing on Organ Donor Samples)

Appendix H:

List of Abbreviations

CDC	US Centres for Disease Control
CLSI	Clinical and Laboratory Standards Institute
CMIA	Chemiluminescent Microparticle Immunoassay
EDTA	Ethylene-diamine-tetraacetic acid
EPTR	Expert Panel on Theory of Reference Values
FBC	Full blood count
Hb	Haemoglobin
HBV	Hepatitis B Virus
Hct	Haematocrit
HCV	Hepatitis C Virus
HIV	Human Immunodeficiency Virus
HREC	Human Research Ethics Committee
ICSH	Internation Committee of Standardization in Haematology
IFCC	International Federation of Clinical Chemistry
ISO	Internation Organisation for Standardization
LL	Lower limit
MCH	Mean cell haemoglobin
MCHC	Mean cell haemoglobin concentration
MCV	Mean cell volume
MPV	Mean platelet volume
NAT	Nucleic Acid Testing
NCHS	National Centre for Health Statistics
NHANES	National Health and Nutrition Examination Survey
NHLS	National Health Laboratory Service
RCC	Red cell count
RDW	Red cell distribution width
SANAS	South African National Accreditation System
SANBS	South African National Blood Service
SCRV	Standing Committee on Reference Values
UCT	University of Cape Town
UL	Upper limit
WCC	White cell count
WHO	World Health Organisation
WCBS	Western Cape Blood Service

Appendix I:

Official Ethics approval letter from the Faculty Research Ethics Committee

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

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

21 April 2016

HREC REF: 132/2016

Dr J Opie
Division of Haematology
NHLS C-17
NGSH

Dear Dr Opie

PROJECT TITLE: ESTABLISHING LOCALLY DERIVED REFERENCE INTERVALS FOR FULL BLOOD COUNT PARAMETERS AND WHITE CELL DIFFERENTIAL COUNTS IN THE WESTERN CAPE REGION OF SOUTH AFRICA (MMed-candidate A de Koker)

Thank you for your response letter to the Faculty of Health Sciences Human Research Ethics Committee dated 08 April 2016.

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

Approval is granted for one year until 30 April 2017.

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

Please quote the HREC REF in all your correspondence.

We acknowledge that the student Dr A de Koker will also be involved in this study.

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

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

Yours sincerely

pp T Burgess

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

This serves to confirm that the University of Cape Town Human Research Ethics Committee complies to the Ethics Standards for Clinical Research with a new drug in patients, based on the Medical Research Council (MRC-SA), Food and Drug Administration (FDA-USA), International Convention on Harmonisation Good Clinical Practice (ICH GCP), South African Good Clinical Practice Guidelines (DoH 2006), based on the Association of the British Pharmaceutical Industry Guidelines (ABPI),and Declaration of Helsinki (2013) guidelines.
The Human Research Ethics Committee granting this approval is in compliance with the ICH Harmonised Tripartite Guidelines E6: Note for Guidance on Good Clinical Practice (CPMP/ICH/135/95) and FDA Code Federal Regulation Part 50, 56 and 312.

Appendix J:

Cover letter for renewal of Ethics approval with amendement of Research Protocol

FHS016: Annual Progress Report / Renewal

HREC office use only (FWA00001637; IRB00001938) **This serves as notification of annual approval, including any documentation described below.**			
☐ Approved	Annual progress report	Approved until/next renewal date	30/4/18
☐ Not approved	See attached comments		
Signature Chairperson of the HREC		Date Signed	12/5/2014

Comments to PI from the HREC

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	12 May 2017		
HREC REF Number	132 /2016	Current Ethics Approval was granted until	30/04/2017
Protocol title	Establishing locally derived reference intervals for full blood count parameters and white cell differential counts in the Western Cape region of South Africa		
Protocol number (if applicable)			
Are there any sub-studies linked to this study?	☐ Yes ☑ No		
If yes, could you please provide the HREC Ref's for all sub-studies? **Note:** A separate FHS016 must be submitted for each sub-study.			
Principal Investigator	Dr Jessica Opie (Mmed candidate Dr A de Koker)		
Department / Office Internal Mail Address	Pathology: Division of Haematology Groot Schuur Hospital, NHLS Pathology division – Haematology, New Main Building C17, Observatory, 7935		

1.1 Does this protocol receive US Federal funding?	☐ Yes	☑ No
1.2 If the study receives US Federal Funding, does the annual report require full committee approval?	☐ Yes	☑ No
1.3 Has sponsorship of this study changed? If yes, please attach a revised summary of the budget.	☐ Yes	☑ No

(Note: Please complete the Closure form (FHS010) if the study is completed within the approval period)

Form FHS006: Protocol Amendment

HREC office use only (FWA00001637; IRB00001938)			
☐ Approved	☐ Type of review: Expedited	☐ Full committee	
This serves as notification that all changes and documentation described below are approved.			
Signature Chairperson of the HREC		Date	12/5/2017

Note: All major amendments must include a local **PI Synopsis** justifying the changes for the amendment. Please note that incomplete amendment submissions will not be reviewed.

Comments from the HREC to the Principal Investigator:
Plese also submit a staff amendment form for MR Gable; Thank you
Note: The approval of this protocol amendment does not grant annual approval. Please complete the FHS016 / FHS017 form for annual approval at least one month before study expiration.

Principal Investigator to complete the following:

1. Protocol information

Date (when submitting this form)	12 May 2017
HREC REF Number	132 / 2016
Protocol title	Establishing locally derived reference intervals for full blood count parameters and white cell differential counts in the Western Cape region of South Africa
Protocol number (if applicable)	
Principal Investigator	Dr Jessica Opie
Department / Office Internal Mail Address	Pathology: Division Haematology, Groote Schuur Hospital, NHLS Pathology-Haematology, New Main Building C17, Observatory, 7935

1.1 Is this a major or a minor amendment? (see FHS006hlp) Major (tick box) Minor (tick box)	☐ Major	☑ Minor
1.2 Does this protocol receive US Federal funding?	☐ Yes	☑ No
1.3 If the amendment is a major amendment and receives US Federal Funding, does the amendment require full committee approval?	☐ Yes	☑ No

Appendix K:

Supplementary tables for literature review – Chapter 1

Table 1 Comparison of South African studies (published after 1980) for African males and females

Parameter	**Gauteng**[1]* **(2009)** n=49(M) n=398(F)	**Cape Peninsula**[2] **(1995)** n=364(M) n=455(F)		**East London**[3]ξ **(1989)** n=100(M) n=110(F)	**Witwatersrand**[4] **(1987)** n=99(M) n=101(F)	**Lesotho**[5] **(1983)** n=122(M) n=137(F)
	Reference Interval	Reference Interval	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
Red Cell Count (10^{12}/L)						
Males		3.2 – 5.8	4.6 (0.6)	4.54 (0.37)	5.23 (0.41)	5.2 (0.35)
Females	3.89 – 5.32	3.0 – 5.3	4.2 (0.5)	4.18 (0.33)	4.60 (0.27)	4.6 (0.35)
Hb (g/dL)						
Males		10.3 -16.7	14.0 (1.6)	14.3 (0.97)	15.82 (1.05)	15.7 (1.0)
Females	11.6 – 16.1	9.0 – 15.2	12.4 (1.4)	12.7 (1.04)	13.95 (0.80)	13.8 (1.0)
Hct (L/L)						
Males		0.31 – 0.53	42.3 (5.2)	41 (0.2)		0.47 (0.03)
Females	0.34 – 0.47	0.27 – 0.47	37.6 (4.4)	37 (0.2)		0.41 (0.29)
MCV (fL)						
Males		82.0 – 110.4	91.8 (6.0)	91.3 (3.8)	85.07 (4.33)¶	89.9 (4.5)¶
Females		76.2 – 106.7	89.8 (6.2)	88.7 (4.4)		
MCH (pg)						
Males		25.3 -34.9	30.3 (2.1)	31.6 (1.7)	30.43 (1.47)¶	30.4 (1.5)¶
Females		24.8 – 33.8	29.6 (2.1)	30.3 (1.7)		
MCHC (g/dL)						
Males		29.9 – 36.0	33.0 (1.4)		35.2 (0.92)¶	33.8 (1.1)¶
Females		30.3 – 35.4	32.9 (1.2)			
Platelets (10^9/L)						
Males				245 (49)	280 (59.4)	
Females				302 (69)	317 (64.0)	
WBC (10^9/L)						
Males	3.93 – 10.04	3.7 – 12.6¶	7.2 (2.2)	6.62 (2.14)¶	5.60 (1.51)¶	4.9 (1.4)
Females	3.80 – 11.40		7.1 (1.9)			5.5 (1.5)
Neutrophils (10^9/L)						
Males	1.46 – 6.40	2.9 – 7.1¶	3.8 (0.4)	3.79 (1.77)¶	3.54 (1.17)¶	2.6 (1.2)
Females	1.53 – 7.27		3.4 (0.4)			2.9 (1.3)
Lymphocytes (10^9/L)						1.87 (0.4)
Males	21 – 58%⁺	2.1-6.0¶	2.8 (0.7)	2.338 (0.685)¶	1.79 (0.568)¶	2.05 (0.6)
Females	23 – 57%⁺		2.8 (0.6)			
Monocytes (10^9/L)						
Males	0.2 – 0.7	0.17 – 0.75¶	0.32 (0.11)	0.279 (0.20)¶	0.185 (0.135)¶	0.32 (0.11)
Females	0.2 – 0.8		0.32 (0.10)			0.30 (0.14)
Eosinophils (10^9/L)						
Males		0.14 – 1.56¶	0.38 (0.23)	0.23 (0.20)¶	0.090 (0.105)¶	0.09 (0.09)
Females	0 – 0.4		0.37 (0.23)			0.09 (0.09)

* *Lawrie et al. only published RIs with significant differences*

¶ *No gender differences reported for these Rs*

ξ *Manual WBC differential counts performed*

[1] Lawrie D, Coetzee LM, Becker P, Mahlangu J, Stevens W, Glencross DK. Local reference ranges for full blood count and CD4 lymphocyte count testing. S Afr Med J. 2009;99(4):243–8
[2] Badenhorst CJ, Fourie J, Steyn K, Jooste PL, Lombard CJ, Bourne L, et al. The haematological profile of urban black Africans aged 15-64 years in the Cape Peninsula. East Afr Med J 1995;72(1):19–24.
[3] East London Morris CD, Dickson D. Haematological reference values in adult blacks at sea level. S Afr Med J. 1989;75(1):35–6.
[4] Tikly M, Blumsohn D, Solomons HD, Govender Y, Atkinson PM. Normal haematological reference values in the adult black population of the Witwatersrand. South African Med J. 1987;72(2):135–6.
[5] Cross Cross JP, Heyns AD. Haematological reference values for the Basotho. S Afr Med J [Internet]. 1983;63(13):480–3.

Table 2 Altitude adjustments to measured Hb concentrations and adjustment for measured Hb concentrations based on smoking intensity (adapted from VMNIS/WHO 2011 document)

Altitude (metres above sea level)	Measured haemoglobin adjustment (g/L)
< 1 000	0
1 000	-2
1 500	-5
2 000	-8
2 500	-13
3 000	-19
3 500	-27
4 000	-35
4 500	-45

Table 3 Adjustment for measured Hb concentrations based on smoking intensity (adapted from VMNIS/WHO 2011 document)

Smoking Status	Measured haemoglobin adjustment (g/L)
Non-smoker	0
Smoker (all)	-0.3
½ - 1 packet/day	-0.3
1 – 2 packets/day	-0.5
≥ 2 packets/day	-0.7

** WHO. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. Miner Nutr Inf Syst World Heal Organ. 2011NS indicates no significant difference*

Appendix L:

Instructions to authors of the SAMJ

JOURNAL AUTHOR INSTRUCTIONS

SOUTH AFRICAN MEDICAL JOURNAL AUTHOR INSTRUCTIONS

All manuscripts and correspondence to:

The Editor
South African Medical Journal
Private Bag X1
Pinelands 7430 (CT)

COPYRIGHT

Material submitted for publication in the South African Medical Journal (SAMJ) is accepted provided it has not been published elsewhere. Copyright forms will be sent with acknowledgement of receipt and the SAMJ reserves copyright of the material published.

The SAMJ does not hold itself responsible for statements made by the authors.

AUTHORSHIP

All named authors must give consent to publication. Authorship should be based only on substantial contribution to: (i) conception, design, analysis and interpretation of data; (ii) drafting the article or revising it critically for important intellectual content; (iii) final approval of the version to be published. All three of these conditions must be met (Uniform requirements for manuscripts submitted to biomedical journals; www.icmje.org/index.html).

CONFLICT OF INTEREST

Authors must declare all sources of support for the research and any association with the product or subject that may constitute conflict of interest.

PROTECTION OF PATIENTÃ¢â‚¬â„¢S RIGHTS TO PRIVACY

Identifying information should not be published in written descriptions, photographs, and pedigrees unless the information is essential for scientific purposes and the patient (or parent or guardian) gives informed written consent for publication. Informed consent for this purpose requires that the patient be shown the manuscript to be published. (www.icmje.org)

ETHNIC CLASSIFICATION

Work that is based on or contains reference to ethnic classification must indicate the rationale for this.

MANUSCRIPTS

Short items are more likely to appeal to our readers and therefore to be accepted for publication. Please provide a word count for all submissions.

Original articles of 3 000 words or less, with up to 6 tables or illustrations, should normally report observations or research of relevance to clinical medicine. References should preferably be limited to no more than 15.

Short reports or scientific letters, which include case reports, side effects of drugs and brief or negative research findings should be 1000 words or less, with 1 table or illustration and no more than 6 references.

Editorials, Opinions, Issues in Medicine, etc. should be about 800 words and are welcome, but unless invited, will be subjected to the SAMJ peer review process.

Review articles are rarely accepted unless invited.

Letters to the editor, if intended for the correspondence column, should be marked 'for publication', signed by all authors and presented in triple spacing. Letters should be no longer than 400 words with only one illustration or table.

Obituaries should not exceed 400 words and may be accompanied by a photograph.

MANUSCRIPT PREPARATION

- Please send your manuscript on disc accompanied by three printouts, in triple spacing, with wide margins and paginated.
- Research articles should have a structured abstract not exceeding 250 words (50 for short reports) comprising: Objectives, Design, Setting, Subjects, Outcome measures, Results and Conclusions.
- Refer to articles in recent issues for guidance on the presentation of headings and subheadings.
- Abbreviations should be spelt out when first used in the text and thereafter used consistently.
- Scientific measurements should be expressed in SI units except: blood pressure should be given in mmHg and haemoglobin values in g/dl.
- If in doubt, refer to Ã¢â‚¬Ëœuniform requirementsÃ¢â‚¬â„¢ above.

ILLUSTRATIONS

1. Figures consist of all material that cannot be set in type, such as photographs and line drawings.
2. Tables and legends for illustrations should appear on separate sheets and should be clearly identified.
3. Line drawings should be arranged to conserve vertical space. Note that reduction to 80 mm for a single column or 170 mm for double columns should not render lettering illegible. Explanations should be included in the legend and not on the figure itself.
4. Figure numbers should be clearly marked on the back of prints and the top of illustrations should be indicated.

5. If any tables or illustrations submitted have been published elsewhere, written consent to republication should be obtained by the author from the copyright holder and the author(s).
6. A limited number of illustrations are free at the discretion of the editor. Colour illustrations are encouraged but are charged to the author.
   A quote will be provided on request. Consider sponsorship.

REFERENCES

References should be inserted in the text as superior numbers and should be listed at the end of the article in numerical and not in alphabetical order.

Authors are responsible for verification of references from the original sources.

References should be set out in the Vancouver style and approved abbreviations of journal titles used; consult the List of Journals in Index Medicus for these details.

Names and initials of all authors should be given unless there are more than six, in which case the first three names should be given followed by et al. First and last page numbers should be given.

Journal references should appear thus:

- Price NC . Importance of asking about glaucoma. BMJ 1983; 286: 349-350.

Book references should be set out as follows:

- Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975: 96-101.
- Weinstein L, Swartz MN. Pathogenic properties of invading microorganisms. In: Sodeman WA jun, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974: 457-472.

Manuscripts accepted but not yet published can be included as references followed by (in press).

Unpublished observations and personal communications may be cited in the text, but not in the reference list

GALLEY PROOFS

Galley proofs will be forwarded to the author before publication and if not returned within 2 weeks will be regarded as approved. Pleas note that alterations to typeset articles are costly and will be charged to the authors.

CHANGES OF ADDRESS

Please notify the Editorial Department of any address changes so that proofs and invoices may be mailed without delay.

REPRINTS

An order form for reprints, with a price list, will be sent to the author as soon as an article has been placed.

CPD POINTS

Authors can earn up to 15 CPD points for published **articles**. Certificates will be provided on request after the article has been published.

General article format/layout

Accepted manuscripts that are not in the correct format specified in these guidelines will be returned to the author(s) for correction, which will delay publication.

General:

- Manuscripts must be written in UK English.
- The manuscript must be in Microsoft Word or RTF document format. Text must be single-spaced, in 12-point Times New Roman font, and contain no unnecessary formatting (such as text in boxes).
- Please make your article concise, even if it is below the word limit.
- Qualifications, ***full*** affiliation (department, school/faculty, institution, city, country) and contact details of ALL authors must be provided in the manuscript and in the online submission process.
- Abbreviations should be spelt out when first used and thereafter used consistently, e.g. 'intravenous (IV)' or 'Department of Health (DoH)'.
- Scientific measurements must be expressed in SI units except: blood pressure (mmHg) and haemoglobin (g/dL).
- Litres is denoted with an uppercase L e.g. 'mL' for millilitres).
- Units should be preceded by a space (except for % and ºC), e.g. '40 kg' and '20 cm' but '50%' and '19ºC'.
- Please be sure to insert proper symbols e.g. µ not u for micro, a not a for alpha, b not B for beta, etc.
- Numbers should be written as grouped per thousand-units, i.e. 4 000, 22 160.
- Quotes should be placed in single quotation marks: i.e. The respondent stated: '...'
- Round brackets (parentheses) should be used, as opposed to square brackets, which are reserved for denoting concentrations or insertions in direct quotes.
- If you wish material to be in a box, simply indicate this in the text. You may use the table format –this is the *only* exception. Please DO NOT use fill, format lines and so on.

SAMJ is a generalist medical journal, therefore for articles covering genetics, it is the responsibility of authors to apply the following:

- Please ensure that all genes are in italics, and proteins/enzymes/hormones are not.

- Ensure that all genes are presented in the correct case e.g. TP53 not Tp53.

**NB: Copyeditors cannot be expected to pick up and correct errors wrt the above, although they will raise queries where concerned.

- Define all genes, proteins and related shorthand terms at first mention, e.g. '188del11' can be glossed as 'an 11 bp deletion at nucleotide 188.'

- Use the latest approved gene or protein symbol as appropriate:

- Human Gene Mapping Workshop (HGMW): genetic notations and symbols
- HUGO Gene Nomenclature Committee: approved gene symbols and nomenclature
- OMIM: Online Mendelian Inheritance in Man (MIM) nomenclature and instructions
- Bennet et al. Standardized human pedigree nomenclature: Update and assessment of the recommendations of the National Society of Genetic Counselors. J Genet Counsel 2008;17:424-433: standard human pedigree nomenclature.

Research

Guideline word limit: 4 000 words

Research articles describe the background, methods, results and conclusions of an original research study. The article should contain the following sections: introduction, methods, results, discussion and conclusion, and should include a structured abstract (see below). The introduction should be concise – no more than three paragraphs – on the background to the research question, and must include references to other relevant published studies that clearly lay out the rationale for conducting the study. Some common reasons for conducting a study are: to fill a gap in the literature, a logical extension of previous work, or to answer an important clinical question. If other papers related to the same study have been published previously, please make sure to refer to them specifically. Describe the study methods in as much detail as possible so that others would be able to replicate the study should they need to. Results should describe the study sample as well as the findings from the study itself, but all interpretation of findings must be kept in the discussion section, which should consider primary outcomes first before any secondary or tertiary findings or post-hoc analyses. The conclusion should briefly summarise the main message of the paper and provide recommendations for further study.

Select figures and tables for your paper carefully and sparingly. Use only those figures that provided added value to the paper, over and above what is written in the text.

Do not replicate data in tables and in text .

Structured abstract

- This should be 250-400 words, with the following recommended headings:
  - **Background:** why the study is being done and how it relates to other published work.
  - **Objectives:** what the study intends to find out
  - **Methods:** must include study design, number of participants, description of the intervention, primary and secondary outcomes, any specific analyses that were done on the data.
  - **Results:** first sentence must be brief population and sample description; outline the results according to the methods described. Primary outcomes must be described first, even if they are not the most significant findings of the study.
  - **Conclusion:** must be supported by the data, include recommendations for further study/actions.
- Please ensure that the structured abstract is complete, accurate and clear and has been approved by all authors.
- Do not include any references in the abstracts.

Here is an example of a good abstract.

Main article

All articles are to include the following main sections: Introduction/Background, Methods, Results, Discussion, Conclusions.

The following are additional heading or section options that may appear within these:

- Objectives (within Introduction/Background): a clear statement of the main aim of the study and the major hypothesis tested or research question posed
- Design (within Methods): including factors such as prospective, randomisation, blinding, placebo control, case control, crossover, criterion standards for diagnostic tests, etc.
- Setting (within Methods): level of care, e.g. primary, secondary, number of participating centres.
- Participants (instead of patients or subjects; within Methods): numbers entering and completing the study, sex, age and any other biological, behavioural, social or cultural factors (e.g. smoking status, socioeconomic group, educational attainment, co-existing disease indicators, etc)that may have an impact on the study results. Clearly define how participants were enrolled, and describe selection and exclusion criteria.
- Interventions (within Methods): what, how, when and for how long. Typically for randomised controlled trials, crossover trials, and before and after studies.
- Main outcome measures (within Methods): those as planned in the protocol, and those ultimately measured. Explain differences, if any.

Results

- Start with description of the population and sample. Include key characteristics of comparison groups.
- Main results with (for quantitative studies) 95% confidence intervals and, where appropriate, the exact level of statistical significance and the number need to treat/harm. Whenever possible, state absolute rather than relative risks.
- Do not replicate data in tables and in text.
- If presenting mean and standard deviations, specify this clearly. Our house style is to present this as follows:
- E.g.: The mean (SD) birth weight was 2 500 (1 210) g. Do not use the ± symbol for mean (SD).
- Leave interpretation to the Discussion section. The Results section should just report the findings as per the Methods section.

Discussion

Please ensure that the discussion is concise and follows this overall structure – sub-headings are not needed:

- Statement of principal findings
- Strengths and weaknesses of the study
- Contribution to the body of knowledge
- Strengths and weaknesses in relation to other studies
- The meaning of the study – e.g. what this study means to clinicians and policymakers
- Unanswered questions and recommendations for future research

Conclusions

This may be the only section readers look at, therefore write it carefully. Include primary conclusions and their implications, suggesting areas for further research if appropriate. Do not go beyond the data in the article.

Tables

- Tables should be constructed carefully and simply for intelligible data representation. Unnecessarily complicated tables are strongly discouraged.
- Large tables will generally not be accepted for publication in their entirety. Please consider shortening and using the text to highlight specific important sections, or offer a large table as an addendum to the publication, but available in full on request from the author
- Embed/include each table in the manuscript Word file - do not provide separately as supplementary files.
- Number each table in Arabic numerals (Table 1, Table 2, etc.) and refer to consecutively in the text.
- Tables must be cell-based (i.e. not constructed with text boxes or tabs) and editable.
- Ensure each table has a concise title and column headings, and include units where necessary.
- Footnotes must be indicated with consecutive use of the following symbols: * † ‡ § ¶ || then ** †† ‡‡ etc.

Do not: Use [Enter] within a row to make 'new rows':

Rather:

Each row of data must have its own proper row:

Do not: use separate columns for *n* and %:

Rather:

Combine into one column, *n* (%):

Do not: have overlapping categories, e.g.:

Rather:

Use <> symbols or numbers that don't overlap:

References

NB: *Only complete, correctly formatted reference lists in Vancouver style will be accepted. Reference lists must be generated manually and not with the use of reference manager software. Endnotes must* ***not*** *be used.*

- Authors must verify references from original sources.
- Citations should be inserted in the text as superscript numbers between square brackets, e.g. These regulations are endorsed by the World Health Organization,[2] and others.[3,4-6]
- All references should be listed at the end of the article in numerical order of appearance in the Vancouver style (not alphabetical order).
- Approved abbreviations of journal titles must be used; see the List of Journals in Index Medicus.
- Names and initials of all authors should be given; if there are more than six authors, the first three names should be given followed by et al.
- Volume and issue numbers should be given.
- First and last page, in full, should be given e.g.: 1215-1217 **not** 1215-17.
- Wherever possible, references must be accompanied by a digital object identifier (DOI) link). Authors are encouraged to use the DOI lookup service offered by CrossRef:
    - On the Crossref homepage, paste the article title into the 'Metadata search' box.
    - Look for the correct, matching article in the list of results.
    - Click Actions > Cite
    - Alongside 'url =' copy the URL between { }.
    - Provide as follows, e.g.: https://doi.org/10.7196/07294.937.98x

Some examples:

- *Journal references:* Price NC, Jacobs NN, Roberts DA, et al. Importance of asking about glaucoma. Stat Med 1998;289(1):350-355. http://dx.doi.org/10.1000/hgjr.182
- *Book references:* Jeffcoate N. Principles of Gynaecology. 4th ed. London: Butterworth, 1975:96-101.
- *Chapter/section in a book:* Weinstein L, Swartz MN. Pathogenic Properties of Invading Microorganisms. In: Sodeman WA, Sodeman WA, eds. Pathologic Physiology: Mechanisms of Disease. Philadelphia: WB Saunders, 1974:457-472.
- *Internet references:* World Health Organization. The World Health Report 2002 - Reducing Risks, Promoting Healthy Life. Geneva: WHO, 2002. http://www.who.int/whr/2002 (accessed 16 January 2010).
- Legal references

- Government Gazettes:

National Department of Health, South Africa. National Policy for Health Act, 1990 (Act No. 116 of 1990). Free primary health care services. Government Gazette No. 17507:1514. 1996.

In this example, 17507 is the Gazette Number. This is followed by :1514 - this is the notice number in this Gazette.

- Provincial Gazettes:

Gauteng Province, South Africa; Department of Agriculture, Conservation, Environment and Land Affairs. Publication of the Gauteng health care waste management draft regulations. Gauteng Provincial Gazette No. 373:3003, 2003.

- Acts:

South Africa. National Health Act No. 61 of 2003.

- Regulations to an Act:

South Africa. National Health Act of 2003. Regulations: Rendering of clinical forensic medicine services. Government Gazette No. 35099, 2012. (Published under Government Notice R176).

- Bills:

South Africa. Traditional Health Practitioners Bill, No. B66B-2003, 2006.

- Green/white papers:

South Africa. Department of Health Green Paper: National Health Insurance in South Africa. 2011.

- Case law:

Rex v Jopp and Another 1949 (4) SA 11 (N)

Rex v Jopp and Another: Name of the parties concerned

1949: Date of decision (or when the case was heard)

(4): Volume number

SA: SA Law Reports

11: Page or section number

(N): In this case Natal - where the case was heard. Similarly, (C) woud indicate Cape, (G) Gauteng, and so on.

NOTE: no . after the v

- *Other references (e.g. reports) should follow the same format:* Author(s). Title. Publisher place: Publisher name, year; pages.
- Cited manuscripts that have been accepted but not yet published can be included as references followed by '(in press)'.
- Unpublished observations and personal communications in the text must **not** appear in the reference list. The full name of the source person must be provided for personal communications e.g. '...(Prof. Michael Jones, personal communication)'.

From submission to acceptance

Submission and peer-review

To submit an article:

- Please ensure that you have prepared your manuscript in line with the SAMJ requirements.
- All submissions should be submitted via Editorial Manager
- The following are required for your submission to be complete:
  - Anonymous manuscript (unless otherwise stated)
  - **Author Agreement form**
  - Manuscript
  - Any supplementary files: figures, datasets, patient consent form, permissions for published images, etc.
- Once the submission has been successfully processed on Editorial Manager, it will undergo a technical check by the Editorial Office before it will be assigned to an editor who will handle the review process. If the author guidelines have not been appropriately followed, the manuscript may be sent back to the author for correcting.

Submission Preparation Checklist

As part of the submission process, authors are required to check off their submission's compliance with all of the following items, and submissions may be returned to authors that do not adhere to these guidelines.

1. Named authors consent to publication and meet the requirements of authorship as set out by the journal.
2. The submission has not been previously published, nor is it before another journal for consideration.
3. The text complies with the stylistic and bibliographic requirements in **Author Guidelines**.
4. The manuscript is in Microsoft Word or RTF document format. The text is single-spaced, in 12-point Times New Roman font, and contains no unnecessary formatting.
5. Illustrations/figures are high resolution/quality (not compressed) and in an acceptable format (preferably TIFF or PNG). These must be submitted individually as 'supplementary files' (not solely embedded in the manuscript).
6. For illustrations/figures or tables that have been published elsewhere, the author has obtained written consent to republication from the copyright holder.
7. Where possible, references are accompanied by a digital object identifier (DOI) and PubMed ID (PMID)/PubMed Central ID (PMCID).
8. An abstract has been included where applicable.
9. The research was approved by a Research Ethics Committee (if applicable)
10. Any conflict of interest (or competing interests) is indicated by the author(s).

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Antenatal screening for hepatitis B virus in HIV-infected and uninfected pregnant women in the Tshwane district of South Africa

Q Diale, R Pattinson, R Chokoe, L Masenyetse, S Mayaphi

Abstract

Background. Despite enormous strides in preventing hepatitis B virus (HBV) infection, perinatal transmission still contributes significantly to HBV epidemiology worldwide; this could account for approximately 50% of chronically infected individuals.

Objective. To assess the need for HBV screening in antenatal clinics in the HIV/AIDS era.

Methods. This was a retrospective study conducted at the antenatal clinic of 1 Military Hospital, Tshwane, South Africa. Laboratory data for HBV, HIV and CD4 count were obtained and analysed for the period January 2008 December 2013.

Results. A total of 2 513 patients' results were retrieved and 2 368 patients were enrolled as both their HBV and HIV serology results were available. The mean age of participants was 29 years (range 14 46). HIV prevalence in this study was 20.5% (95% confidence interval (CI) 0.189 0.222). The median CD4 count in HIVinfected patients was 522 cells/μL (interquartile range 370 711). There was an overall HBV prevalence of 0.8% (95% CI 0.005 0.011). The hepatitis B surface antigen (HBsAg) prevalence was significantly higher (2.1%) among HIV coinfected compared with HIVuninfected patients (0.4%) (p=0.0001). Hepatitis e antigen (HBeAg) positivity was 30% in the HIV coinfected compared with 37.6% in the HIVuninfected individuals (p=0.7400).

Conclusion. This study showed a significantly higher HBV prevalence in HIVinfected compared with HIVuninfected patients. The comparable HBeAg prevalence between the two groups indicates that both were at an increased risk of vertical transmission, therefore demonstrating a need for antenatal screening for HBV. Since antenatal screening is often not affordable in lowincome countries, administration of HBV vaccine at birth is needed for prevention of vertical transmission.

Appendix M:

University of Cape Town Dissertation Guidelines

UNIVERSITY OF CAPE TOWN

FACULTY OF HEALTH SCIENCES

MMed Part III (minor dissertation)
Guidelines for candidates, supervisors and examiners

The MMed minor dissertation is one of three examination components of the MMed degree. This minor dissertation carries one third of the weight of a full master's dissertation in terms of it's credit weighting, i.e. 60 credits (nominally 600 hours of work). In order to register as a specialist in South Africa, the Health Professions Council of South Africa (HPCSA) requires all specialist trainees who register for training after 1 January 2011 to have completed a relevant research study. The MMed Part III fulfils HPCSA research requirements as well as research requirements by the specialties who include a research project as part of their examination process by the Colleges of Medicine of South Africa (CMSA).

Educational aims

The research project should demonstrate that the student:

- can work independently and ethically under supervision (contributions/assistance must be acknowledged);
- is sufficiently acquainted with the relevant literature to provide appropriate motivation for the research question;
- can plan research or clinical audit (write a protocol), which is approved by an assessor group (delegated by the head of department) and ethics committee where relevant, that contributes new or additional data to the collective knowledge base (the specific data has not been presented as part of other research), but need not produce a unique contribution to the scientific literature;
- uses an appropriate method/design/technique and analysis;
- can adequately present and discuss the significance of the results of the study;
- can present the study in an academically acceptable manner.

Type and scope of the research

The following types of studies are acceptable:

- A clinical audit with or without a repeat data collection cycle;
- A systematic review of the literature on its own **with** extraction and extrapolation of data **OR** a meta-analysis using recognised research methods (eg Cochrane, PRISMA);
- A research study – pro-/retrospective lab or clinical or database review;
- Description and analysis of a case series or cohort, deemed sufficient to supply new knowledge/data, even if only contextual or exploratory;
- Epidemiological research;
- Health service/systems/education research;
- Qualitative research;

Noting:

- *The sample size* can be limited by time (Registrars have limited time allocated/available to collect data and write it up concurrently with their clinical training) - data collection and write up should be possible to complete within two consecutive or cumulative months.

- *Data analysis* may use simple descriptive statistics alone – more advanced analysis can be used, but the student must demonstrate (in the write up) insight into the choice of analysis.

- The above limitations may be associated with the use of descriptive cohort studies based on medical record review; exploratory or pilot studies with small convenience samples; or audits without a repeat data collection cycle to prove quality improvement (QI). Despite limitations, these studies can provide an adequate basis for learning research methodology and can add new data to the collective knowledge base – they may also provide the basis for further publishable work such as a second audit to complete a full QI cycle. As long as these limitations are appropriately acknowledged, these studies should still be acceptable.

- The topic, study design and scope of research may depend on the particular discipline and must be agreed on in consultation with the supervisor(s). The topic must be approved as being suitable for MMed dissertation by the Departmental Research Committee (DRC) and/or a group appointed for this purpose by the head of department.

Submission formats

The dissertation may be presented in one of three formats:

I: Publication-ready format;
II: Published (or accepted for publication) Paper format
III: Monograph format.

As disciplines differ in their requirements, it is important that the format chosen is acceptable to the discipline and appropriate College within the CMSA.

Research protocol

NOTE: All communication from UCT regarding the MMed and the examination process will occur via student UCT e-mailaddress – [student number]@myuct.ac.za. Students must also make sure they have username and password and are able to access the PeopleSoft Student Administration Self Service.

Candidates intending to register for the MMed Part III are required to submit a research protocol for approval to their respective Departmental Research Committees (DRC). The research protocol should briefly summarise the existing knowledge on the topic and justify the research question; it should clearly describe the objectives and methodology and should be structured according to the guidelines in Form FHS015. Write a synopsis according to Form FHS014. Complete a new protocol application form FHS 013. All FHS forms are available at http://www.health.uct.ac.za/fhs/research/humanethics/forms.

The candidate must then obtain approval from the UCT Faculty of Health Sciences Research Ethics Committee (HREC) prior to conducting their research. Studies that involve the audit of clinical records or services also require formal HREC approval. Any primary research that is taking place in a provincial or local authority health facility, such as public sector hospitals or clinics, must also be submitted to the provincial government for approval, after the UCT Research Ethics Committee approval has been obtained. **Approval to access public sector facilities for research is needed for all provincial and local authority facilities.** There are five points where approval for research can be applied for; Groote Schuur Hospital, Red Cross War Memorial Children's Hospital, Tygerberg Hospital), the local authorities and "all other province". Teaching hospitals and the local authorities approve research projects in-house. "All other province" approvals are done via the Directorate: Health Impact Assessment (Sub-directorate: Research) at provincial head office. If research crosses these boundaries, up to five approvals may be needed. Further details can be found at https://www.westerncape.gov.za/general-publication/health-research-approval-process
The Provincial Health Research Committee does not approve research proposals itself, but oversees this approval process by reviewing difficult applications on referral.

The proposal contents should comply with requirements stipulated in Form D1a. This full research protocol together with FHS 013, a copy of the HREC approval letter and completed Forms D1 (Protocol approval), D3 (supervisor appointment form), and D1a must be submitted to the postgraduate administration office, for approval by the Professional Masters Committee (PMC) Chair

and the Board of the Faculty of Health Sciences, prior to commencement of the research. If the title, aims, objectives or any other aspect of the research change following initial submission, an ammendment must be submitted to HREC. **All D-forms are available from the post graduate faculty office or on the UCT Vula Mmed/Mphil site (All registrars and supervisors must be added to this site – your departmental programme manager must send names and email addresses to gregory.doyle@uct.ac.za in order to be added to the site).**

Timelines

Submission of the research protocol for approval should generally be made within the first 12 - 24 months of the registrar programme (this varies between disciplines). Heads of Departments or Divisions should meet with their registrars at least biannually to review progress towards their research project. Unless otherwise stipulated by your Division / Department, the research project should generally be completed by the end of Year 3. For a number of specialties, a dissertation must be submitted before writing the Part II examination. Often the research component of specialist training is only initiated after successful completion of the Part I examination.

Supervisors

The supervisor must: have research experience, ideally a Master's degree, equivalent (eg appropriate publications), or higher; be able relate to the candidate's research project; be available for regular discussion and advice; and be someone with whom the candidate can develop a good working relationship. If the primary supervisor does not have adequate experience, then a secondary supervisor who has appropriate experience will need to be appointed in addition. **Supervisors who have not had extensive experience supervising are required to attend a supervisor training course.** Where specialised equipment and/or laboratory work is required for the study, the supervisor should assist in facilitating access to appropriate facilities.

The primary supervisor may be based outside the candidate's home department, faculty or university. In such a case, a member of UCT staff will also be required as co-supervisor in addition to the primary supervisor, to serve as a guide and link to UCT faculty and discipline-specific procedures. Primary supervisors retain responsibilities to the candidate and the university until the dissertation process is complete. In addition to the forms mentioned above, the supervisor and student must complete D2a which describes the contractual memorandum of agreement (MOU) between supervisor and student regarding the minor dissertation.

The dissertation

Submission of all formats of the dissertation should include the following:

The title page should contain the candidate's name, dissertation title and the name of the university. It must also state the degree, e.g. Master of Medicine (MMed) in, Medicine, Paediatrics, etc.

The Table of contents

The declaration page should include a statement to the effect that the research reported is based on independent work performed by the candidate and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree to any other university. It must also state that this work has not been reported or published *prior to registration* for the abovementioned degree.

The abstract should summarise the study rationale, methods, results, discussion and conclusion in fewer than 500 words.

Acknowledgements and contributions. This section should acknowledge and describe the support or input from supervisors and other co-author(s) if applicable. In a dissertation derived from work started by others, e.g. analysis of data collected for another project, the origin of the data and the candidate's contribution must be clearly stated. The candidate must complete the dissertation after his/her registration for the degree and therefore under supervision. In a published manuscript from a

multi-authored project, the candidate must be first author.

List of Tables
List of Figures
Abbreviations

The remainder of the dissertation must be presented in one of three formats:

I: Publication-ready format;
II: Published (or accepted for publication) paper format
II: Monograph format.

I: Publication format

The dissertation must include a manuscript in publication-ready format. The body of the dissertation must be structured as follows:

Chapter 1: Introduction and Literature review

This section must give the background and context of the research question and must include a review of the literature relevant to the subject matter and methods of the study. The review should summarise and interpret the existing knowledge in the field with relevance to the research setting and should identify knowledge gaps and hence the rationale for the dissertation. This chapter should end with a clear statement reflecting the aims and objectives of the research reported in the publication-ready manuscript. References quoted in this chapter should appear at the end of the chapter, not at the end of the thesis. This chapter should be between 2 000 and 5 000 words.

Chapter 2: Publication-ready Manuscript

This chapter must be presented in the form of a manuscript of an article for a named peer reviewed journal, meeting all the requirements of the "Instructions for Authors" of that journal, including the word count and referencing style. Unless specially motivated, the journal chosen should allow for at least 2000 words (not more than 5000 words) excluding abstract, tables, figures and references. The "Instructions to Authors" of the journal must be appended. The co-authors should be listed in the appropriate order, and each of their contributions to the manuscript stated. The journal chosen for publication must be appropriate to the subject matter of the dissertation and listed in the citation index of the Institute for Scientific Information (ISI) or accredited by the Department of Education http://www.lib.uct.ac.za/medical/index.php?html=/libs/accredjnls.htm&libid=24); *other journals with similar review processes, particularly South African journals may be acceptable if permission is obtained from the PMC Chair after appropriate motivation is provided.*

Note 1: In this format, the candidate need not have submitted the article for publication, nor is the acceptance of the article for publication a requirement for passing the degree. However, the norm is to publish the study with the supervisor(s) as co-author(s), and candidates are strongly encouraged to submit their manuscript for publication after examination of the minor dissertation.

NOTE 2: IF THE RESEARCH IS A FULL SYSTEMATIC REVIEW, THERE IS NO NEED FOR A SEPARATE CHAPTER 1 – THE REVIEW SHULD BE SUBMITTED AS ONE CHAPTER.

Appendices

Append all supporting documents including:

- Questionnaire/data capture instrument(s)
- Consent forms and any related participant information sheets
- Technical appendices, including, if considered necessary, any additional tables not included in the main manuscript for the examiner to have available. These should be accompanied by a brief narrative.
- Official Ethics approval letter from the Faculty Research Ethics Committee (except for a full systematic review) and any other approvals required (e.g. Provincial Government).
- Instructions to Authors of the chosen journal

II: Published (or accepted for publication) paper format

A manuscript that has already been published or accepted for publication in a journal that is listed in the citation index of the Institute for Scientific Information (ISI) or accredited by the Department of Education (*other journals with similar review processes, particularly South African journals may be acceptable if permission is obtained from the PMC Chair after appropriate motivation is provided),* may be submitted **if the candidate was the first author, the candidate's contribution was completed under supervision during his/her registration for the degree, and the paper is in line with the educational aims and scope of research described in the first part of this document.**

The dissertation must be submitted in similar format to the publication-ready format – the only differences being: a separate literature review is not required; the accepted publication is submitted as a single chapter following the same format as described above under "Chapter 2"; and the reviewer comments from the journal should be attached as an appendix. *When this format is used, the contributions of all the authors must be very clearly stated under a sub-heading in the "Acknowledgments and contributions" section in the first part of the thesis.*

III: Standard monograph format

Some disciplines and constituent Colleges of the Colleges of Medicine of South Africa require a standard monograph presented in a comprehensive and scholarly style to be submitted as part of the examination. The length is typically 16 000 to 20 000 words in length, but may vary. If the length is not stipulated, the monograph should be 6000 – 16000 words, excluding references and tables.

A recommended structure for the body of the dissertation is as follows;

Chapter 1: Introduction and Literature review
(see guidelines above)

Chapter 2: Methods
Material and methods of the study must be fully described and factually presented.

Chapter 3: Results

Chapter 4: Discussion and conclusions

Appendices

(see guidelines above - omit the instructions to authors)

Language and writing

Clear, grammatically correct English is essential.

Supervisors may assist candidates in developing scientific communication skills but they are not required to do detailed editing or correction of spelling, grammar, or style. Training in scientific writing is available at the Health sciences Writing Centre. Registrars need to make an appointment via the website: http://www.writingcentre.uct.ac.za/about/healthsciences

Candidates should refer to Form D4, Guidelines on the Layout and Style of the Dissertation or Thesis. As long as the dissertation is readable and internally consistent, any of a number of styles are acceptable. For a publication-ready manuscript, references should be formatted according to the instructions to authors for the journal selected, and candidates should use the same style throughout their dissertation. For a monograph format manuscript, the Harvard style for referencing is

recommended, but not compulsory. For reference management, Refworks or Endnote can be downloaded from the ICTS or UCT library website.

Candidates should look at previous examples of Master's dissertations in the library. Master's dissertations are available in the Health Sciences Library. A search will need to be done to obtain a list of titles and authors. This search can be done using search words (e.g. dissertation, health, health sciences, etc.). The librarian can be asked for assistance. Some of these dissertations are available via: http://www.medical.lib.uct.ac.za/hsl/theses-dissertations

Annual approval

After 1 year, apply to HREC for continuing approval Form FHS016 (for intervention study) or FHS017 (for record review) or submit a study closure form, FHS010, if the study is complete. If registration in MMED III is required for more than one year then complete form D2(b) and submit to Post Grad Office when re-registering.

Submission of dissertations

On completion, the dissertation and a Turn-it-in originality report must be submitted to the Faculty Postgraduate Office. The candidate should inform the Faculty Officer one month in advance of the intention to submit, using Form D8 (Intention to submit) online with PeopleSoft system and should subsequently submit their dissertation using the same system **– guidelines for this process and the use of Turn-it-in are on the Mmed/Mphil Vula Website and detailed guidelines are also available in the UCT student help document: " Digital submission of a thesis/dissertation for examination and library access"**. This document is available online at http://www.uct.ac.za/usr/current_students/postgrad/digital_upload_dissertations_theses.pdf

Supervisors will be requested by the Faculty Postgraduate Officer to submit a letter supporting submission, and clearly specifying whether the format of submission, so that the appropriate instructions are sent to the examiners. This letter should be supplied by the primary supervisor. If this supervisor is external, the internal supervisor must be kept informed at every stage of the process.

Please note: In the event that any of your external examiners request a hard copy of your dissertation/ thesis, you will be required to supply this. The Faculty office will inform you should this be necessary.

Specific submission requirements may be set by individual disciplines or constituent Colleges of the CMSA, and registrars are obliged to ensure that their research projects and dissertations meet these specific requirements. UCT Dissertation submission deadlines:

1. March 15th for June graduation
2. August 15th for December graduation

Note on fees: To avoid attracting fees, dissertations need to be submitted before the beginning of the first quarter (first day of academic year), and before the start of the second semester (mid July) to qualify for a 50% fee rebate.

Examiners

The full dissertation will be submitted for examination through the Postgraduate Office to two examiners (nominated by the supervisors and HOD) – at least one examiner must be external to UCT. An internal examiner must not be involved in the research.

It is the supervisors' responsibility to submit names of three potential examiners (or two examiners who have already agreed to examine pending approval of the Post Graduate Office) to the Faculty Officer when the candidate is ready to submit. Appointment of examiners from outside South Africa is encouraged. These nominations need to be approved by the Deputy Dean: Postgraduate Affairs on behalf of the Faculty Board and submitted to the Faculty Board for ratification via a Dean's Circular.

Details required for each examiner are: academic qualifications, postal and/or physical address, telephone and fax numbers and e-mail address, and one paragraph description of their standing in the relevant field (drawn from their CV if need be). The examiners will be sent a copy of these guidelines as well as a guideline for marking. *The candidate may not be informed of the identity of the examiners*. After the outcome of the minor dissertation has been finalised, the examiners' identities are made known if the examiners have indicated that they do not object to this.

Publication agreement

The university has a moral responsibilty to publish all research undertaken when publication is stated as an anticipated output. A candidate who fails to submit a manuscript to a journal for publication within 1 year of submission of their thesis, must accept that their supervisor(s) are entitled to publish their data on their behalf, with the student as co-author - this should be stated in the memorandum of understanding.