Congenital Hypothyroidism: Cord Blood Sample Reference Intervals for Thyroid Stimulating Hormone and Free Thyroxine on Roche Cobas® Analyser

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DECLARATION PAGE

PROJECT DECLARATION

I, Mariam Mahomed, hereby declare that the work on which this dissertation/thesis 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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This thesis/dissertation has been submitted to the Turnitin module (or equivalent similarity and originality checking software) and I confirm that my supervisor has seen my report and any concerns revealed by such have been resolved with my supervisor.

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Format

The format of the manuscript will be in the publication-ready format according to the manuscript submission guidelines and policies for Thyroid®, the Official Journal of the American Thyroid Association.

The STROBE (Strengthening the Reporting of Observational Studies in Epidemiology Statement: guidelines for reporting observational studies) and STARD reporting guidelines (STARD 2015: An Updated List of Essential Items for Reporting Diagnostic Accuracy Studies) developed by the Equator Network, were also used to guide the structure, content, and key elements included in this report.

LIST OF ABBREVIATIONS

CCC	Concordance correlation coefficient
CH	Congenital hypothyroidism
CLIA	Chemiluminescent immunoassay
CLSI	Clinical & Laboratory Standards Institute
CMIA	Chemiluminescent microparticle immunoassay
ECLIA	Electrochemiluminescent immunoassay
EFLM	European Federation of Laboratory Medicine
ELISA	Enzyme-linked immunosorbent assay
FIA	Fluorescence immunoassay
FT4	Free thyroxine
HREC	Human Research Ethics Committee
IFCC	International Federation of Clinical Chemistry
IRMA	Immunoradiometric assay
MOU	Midwife obstetric unit
NBS	Newborn screening
NHLS	National Health Laboratory Service
PMNS	The Peninsula Maternity and Neonatal Service
RIA	Radioimmunoassay
RMSE	Root Mean Squared Error
SANAS	South African National Accreditation System
T4	Thyroxine
TAE	Total allowable error
TSH	Thyroid-stimulating hormone
UCT	University of Cape Town

GLOSSARY OF TERMS

^{125}Iodine-Labelled	A substance labelled with Iodine-125 (^{125}I25) a radioisotope of iodine
25th Percentile	The 25th percentile is the value at which 25% of data points are below that value, and 75% of data points are above that value. It is also known as the first, or lower, quartile
75th Percentile	The 75th percentile is the value at which 25% of data points are above it and 75% are below it. It's also known as the third, or upper, quartile.
Absolute Difference	The absolute difference refers to the magnitude of the difference between two values
Adverse Effects	Adverse effects are undesirable or harmful outcomes resulting from a medical treatment, procedure, or intervention.
Antigen	An antigen is a substance that induces an immune response in the body, specifically by triggering the production of antibodies
Automated Platform	A system or device that performs tasks or processes automatically, often without the need for direct human intervention
Bias	Bias refers to systematic errors or inaccuracies in measurement or analysis that consistently skew results in a particular direction
Biotin Capture Antibody	An antibody that is conjugated or linked to biotin, a small molecule. Biotin capture antibodies are often used in immunoassays to capture and detect specific target molecules
Bland-Altman Difference Plots	Graphical tools used to assess the agreement between two quantitative measurement methods or instruments
Clinical Assessment	The systematic evaluation of a patient's medical history, symptoms, physical examination findings, and diagnostic test results to reach a diagnosis, monitor disease progression, or evaluate treatment efficacy
Clinical Decision Limits	Predefined thresholds used to interpret laboratory test results and make clinical decisions
Clinical and Laboratory Standards Institute	The Clinical and Laboratory Standards Institute is a non-profit organization that develops and publishes standards, guidelines, and best practices for clinical laboratory testing, quality assurance, and laboratory management
CLSI EP09-A3	A guideline published by the Clinical and Laboratory Standards Institute covering the design of measurement procedure comparison experiments using patient samples and subsequent data analysis
CLSI EP28-A3c	A guideline published by the Clinical and Laboratory Standards Institute for determining reference values and reference intervals for quantitative clinical laboratory tests
Congenital Hypothyroidism	Congenital hypothyroidism is defined as thyroid hormone deficiency present at birth.
Congenital Hypothyroidism Screening Program	A public health initiative aimed at early detection of congenital hypothyroidism in new-borns through systematic screening methods
Competitive Immunoassay	An immunoassay technique where a labelled antigen competes with an unlabelled analyte (present in the sample) for binding sites on an immobilized antibody.
Concordance	Agreement or consistency between two or more observations, measurements, or diagnostic tests
Confidence Intervals	A range of values calculated from sample data that is likely to contain the true population parameter with a specified level of confidence
Confirmatory Test	A diagnostic test performed to validate or confirm the results of an initial screening or diagnostic test
Cord Blood	Blood collected from the umbilical cord and placenta immediately after childbirth

Correlation	A statistical measure of the degree to which two variables are related or vary together
Data Analysis	The process of inspecting, cleaning, transforming, and interpreting data to extract meaningful insights, identify patterns, and make informed decisions
Declaration Of Helsinki	A set of ethical principles and guidelines for medical research involving human subjects
Deming Regression	A statistical method used to estimate the relationship between two variables while accounting for measurement errors in both the independent and dependent variables
Developed Nations	Countries with relatively high levels of economic development, industrialization, and technological advancement, typically characterized by high standards of living, advanced infrastructure, and access to healthcare and education
Diagnostic Laboratory	A facility equipped with specialized equipment, instruments, and personnel for conducting medical tests and analyses to aid in the diagnosis, monitoring, and treatment of diseases and health conditions
District Hospital	A healthcare facility that serves as a primary point of care for residents within a specific geographic area or district
Electrochemiluminescence Immunoassay	An immunoassay technique that uses electrical stimulation to induce luminescence in a reaction involving labelled molecules and electrochemical detection methods
Epitope	The specific region or site on an antigen molecule that is recognized and bound by an antibody or immune cell receptor
European Federation Of Clinical Chemistry And Laboratory Medicine	A professional organization representing clinical chemists, laboratory medicine specialists, and related professionals in Europe, promoting scientific excellence, education, and quality assurance in laboratory medicine across Europe
Exclusion Criteria	Predefined characteristics or conditions that disqualify individuals from participating in a research study or clinical trial
Fit For Purpose	A quality assurance concept indicating that a product, process, or service is suitable and effective for its intended use or application
Gamma Counter	A specialized instrument used to measure the radioactive decay of isotopes emitting gamma radiation
Heel-Prick Blood	Blood obtained by pricking the heel is often used for new-born screening tests due to its accessibility and ease of collection.
Hormone Replacement Therapy	Medical treatment involving the administration of hormones to supplement or replace those normally produced by the body, often used to alleviate symptoms of hormonal deficiency or imbalance.
Human Research Ethics Committee	A committee responsible for reviewing and approving research involving human participants to ensure that ethical standards and regulations are upheld, including the protection of participants' rights and welfare.
Imaging Studies	Medical procedures that produce visual images of the body's internal structures or functions, such as X-rays, ultrasounds, CT scans, MRI scans, thyroid scintigraphy and PET scans.
Imprecision	Lack of precision in measurement, resulting in variability or inconsistency in test results.
Incubated	Placement in a controlled environment for a specific duration, typically to stimulate growth, development, or biochemical reactions
Intellectual Disability	A developmental disorder characterized by limitations in intellectual functioning and adaptive behaviour
International Federation Of Clinical Chemistry	A global professional organization dedicated to advancing the science and practice of clinical chemistry and laboratory medicine worldwide
Iodine Deficiency	A condition resulting from insufficient intake of iodine, which can lead to impaired thyroid function

Lin's Concordance Correlation Coefficient	A statistical measure used to assess the agreement between two continuous variables, accounting for both accuracy and precision
Linear Regression	A statistical method used to model the relationship between two or more variables by fitting a linear equation to the observed data points
Log-transformed	Data that has been transformed using the logarithm function, is often done to stabilize variance, reduce skewness, or meet the assumptions of certain statistical tests
Lower Limits	The minimum or lowest acceptable value or threshold for a particular parameter or measurement
Median	The middle value in a set of ordered data points, dividing the distribution into two halves
Method Comparison Study	A type of study designed to evaluate the agreement or correlation between two or more measurement methods or instruments for the same analyte or variable
Midwife Obstetric Units	Healthcare facilities staffed by midwives that provide prenatal care, childbirth assistance, and postnatal care for women with uncomplicated pregnancies
Monoclonal Antibodies	Antibodies produced by identical immune cells that are clones of a single parent cell used in various diagnostic and therapeutic applications due to their specificity and consistency
Newborn Screening	A public health program that involves testing new-born babies for certain genetic, metabolic, or congenital disorders shortly after birth to enable early detection and intervention
Non-Competitive ("Sandwich") Immunoassay	An immunoassay format where the analyte of interest is captured between two antibodies without competition, typically resulting in high sensitivity and specificity
Non-Parametric	Statistical methods that do not rely on specific assumptions about the distribution of the data or the parameters of the population
Normality	In statistics, normality refers to the distribution of data following a normal or Gaussian distribution
Normally Distributed	Data that follows a normal distribution, characterized by a symmetrical bell-shaped curve when plotted
Operational Costs	The expenses incurred in the day-to-day operations of an organization or business, including salaries, utilities, supplies, and maintenance
Outliers	Data points that deviate significantly from the rest of the dataset, are often considered to be abnormal or erroneous observations
Parametric	Statistical methods that make assumptions about the distribution of the data or the parameters of the population, such as normality and homogeneity of variance
Passing-Bablok Regression	A non-parametric method used to compare two measurement methods or instruments, particularly when the assumption of linearity is not met
Pearson Correlation Co-efficient	A statistical measure of the linear relationship between two continuous variables, ranging from -1 (perfect negative correlation) to +1 (perfect positive correlation)
Polyclonal Antibodies	Antibodies produced by multiple immune cells in response to a specific antigen, characterized by a heterogeneous mixture of antibodies with varying specificities and affinities
Preventive Medicine	Medical practices and interventions aimed at preventing the occurrence or progression of diseases, injuries, and other health-related conditions
Prospective Study	A research study that follows participants forward in time, starting from the present or a defined point in the past, to observe outcomes and events as they occur
Quality Control	Procedures and measures implemented to ensure the reliability, accuracy, and consistency of laboratory test results, including the monitoring of analytical performance and the use of control materials

R Studio	An integrated development environment for the R programming language, commonly used for statistical computing, data analysis, and visualization
R-Squared Value	A statistical measure that represents the proportion of variance in the dependent variable that is explained by the independent variable(s) in a regression model
Radioactive Materials	Substances that emit ionizing radiation as a result of radioactive decay, commonly used in medical imaging, research, and therapy
Radioimmunoassay	An immunoassay technique that uses radioactive isotopes as labels to quantify the presence or concentration of antigens or antibodies in biological samples
Random Selection	The process of selecting individuals or samples from a population in such a way that each member of the population has an equal chance of being chosen
Recall	The action of contacting patients to return for further evaluation or treatment.
Reference Individual	A hypothetical person representing the standard or average characteristics of a population, often used in pharmacokinetic modelling and dose optimization studies
Reference Intervals	Also known as reference ranges, these are ranges of values obtained from healthy individuals that represent the normal variation of a particular laboratory test within a population
Relative Difference	The percentage or proportional difference between two values often expressed as a percentage of the average or reference value
Residual Sample	A portion of a sample that remains after initial testing or analysis and is retained for further investigation or backup purposes
Right-Skewed	A distribution of data where the majority of observations cluster toward the left side of the distribution, with a long tail extending to the right, indicating that the mean is greater than the median
Robust Method	A statistical or analytical method that is resistant to outliers, violations of assumptions, or deviations from normality, producing reliable results even under adverse conditions
Root Mean Squared Error	A measure of the average deviation between observed values and predicted values in a regression model, calculated by taking the square root of the average of the squared differences
Sample Number	The total number of individual units or observations included in a sample
Sample Size	The number of individual units or observations included in a sample is often determined based on statistical considerations to ensure the reliability and validity of study results
Screening	The process of identifying individuals at risk of a particular disease, condition, or trait through testing, examination, or other methods, often followed by confirmatory diagnostic tests or interventions
Serum Separator Tubes	Blood collection tubes containing a gel separator that separates serum from clotting blood cells during centrifugation, facilitating the collection of serum for laboratory analysis
Shapiro-Wilk Test	A statistical test used to assess the normality of a distribution by testing the null hypothesis that the data are drawn from a normally distributed population
Signal-Antibody	An antibody labelled with a detectable marker, such as a fluorescent dye or enzyme, used to detect the presence or concentration of a specific antigen in an immunoassay
South African National Accreditation System	The national accreditation body responsible for accrediting laboratories and certification bodies in South Africa, ensuring compliance with international standards and regulations
Standardised	When test results are uniform across routine measurement procedures and traceable to a recognized standard reference material defined by the

	International System of Units (SI) through a high-order primary reference material and/or a reference measurement procedure.
Systematic Bias	Consistent errors or inaccuracies in measurement or analysis that systematically skew results in a particular direction, often caused by flaws in instrumentation, methodology, or calibration
Term Infant	A new-born baby born at or after 37 weeks of gestation, considered to be at full-term
Thyroid Dysgenesis	A congenital condition characterized by abnormal development or malformation of the thyroid gland, leading to thyroid dysfunction and potential health problems
Total Allowable Error	The maximum acceptable deviation or discrepancy between a measured value and its true value that is considered acceptable for a particular laboratory test or measurement
Trendlines	Lines or curves fitted to a set of data points to visually represent trends or patterns in the data, often used in data visualization and analysis to identify relationships or make predictions
TSH Isotope Method	A method for measuring thyroid-stimulating hormone (TSH) levels in blood using isotopic labelling techniques, commonly used in thyroid function tests
Tukey's Fences	Statistical boundaries used to identify outliers in a dataset, calculated based on the interquartile range (IQR) and the first and third quartiles of the data distribution
Uncomplicated Pregnancies	Pregnancies that progress without significant medical or obstetric complications, typically resulting in favourable outcomes for both the mother and baby
Upper Limits	The maximum or highest acceptable value or threshold for a particular parameter or measurement

PUBLICATION READY FORMAT MANUSCRIPT

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ABSTRACT

Background: Congenital hypothyroidism (CH) is a significant health concern globally, with severe consequences if left untreated. Newborn screening (NBS) programs play a pivotal role in early detection and intervention of CH. However, due to resource constraints, South Africa lacks a national NBS program. This study aimed to establish reference intervals for thyroid stimulating hormone (TSH) and free thyroxine (FT4) in cord blood, as well as to compare the previous TSH radioimmunoassay with the current electrochemiluminescence immunoassay.

Methods: Utilizing residual samples from the Peninsula Maternal and Neonatal Services (PMNS) CH Screening Program, this prospective study collected samples from uncomplicated pregnancies, resulting in 121 samples for reference interval analysis. Additionally, 14 samples within pathological ranges were selected, bringing the total for the method comparison study to 135. TSH and FT4 levels were determined by automated immunoassay on the Roche Cobas® 6000 analyser (Elecsys TSH and Elecsys FT4 III assays). The data analysis was performed following relevant CLSI guidelines (CLSI EP28-A3c and CLSI EP09-A3).

Results: In the reference interval study, the mean birth weight was 3,211g (+/-387g). Non-parametric methodology yielded a TSH reference interval of 1.85 to 15.35 mIU/L and a free T4 reference interval of 13.0 to 20.4 pmol/L. The TSH method comparison study demonstrated strong agreement between the radioimmunoassay and electrochemiluminescence immunoassay (R-squared=0.98; Lin's CCC=0.97). Bland-Altman analysis revealed most points within the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) total allowable error goals for TSH, with RIA results showing a small positive bias.

Conclusion: This study establishes reference intervals for cord blood TSH and FT4 in a South African context. Cord blood presents advantages for CH screening in resource-constrained settings, integrating with existing labour and delivery protocols while minimizing logistical challenges. The established intervals align with existing literature and methodologies, supporting their validity and applicability. Continued monitoring of the CH program and clinical outcomes is crucial for validating the study results in a clinical context, ensuring ongoing relevance and accuracy.

(321 words, limit = 350)

INTRODUCTION

Congenital Hypothyroidism (CH) affects in 1 in 4,000 newborns globally and is the leading preventable cause of intellectual disability (1). CH is typically sporadic and can be either transient or permanent. The transient form is often linked to maternal factors such as iodine deficiency, thyroid autoantibodies, or the use of anti-thyroid medications (2). Permanent CH can result from various causes, many of which are related to either the development or function of the thyroid gland, while thyroid dysgenesis is most common (85-95%) (3).

Infants with CH often do not show immediate signs or symptoms, possibly due to maternal thyroid hormone or residual neonatal thyroid function (4). Early diagnosis and treatment are crucial to prevent irreversible intellectual and growth impairments. Advances in radioimmunoassay (RIA) techniques for thyroid stimulating hormone (TSH) and thyroxine (T4) in the 1970s enabled many countries to include CH in their newborn screening (NBS) programs (5). These programs have significantly improved outcomes by facilitating early diagnosis and timely hormone replacement therapy (6) (7). Successful CH prevention has been achieved in many parts of North America, Europe, Asia, Latin America, and a few African countries (4). Newborn screening for CH is considered one of preventive medicine's major achievements.

In developed nations, the initial TSH test is typically performed 24 to 48 hours after birth using heel prick blood placed on filter paper and sent to the laboratory for analysis (2). If the initial screening indicates a potential problem, confirmatory tests are done. These usually involve both TSH and free T4 testing and may also include imaging studies. Elevated TSH and low T4 levels are indicative of CH.

In contrast, South Africa, along with other resource-limited countries, lacks a national NBS program. Screening for CH is limited to small programs within the private and public sectors (8). One such public entity, the Peninsula Maternal and Neonatal Services (PMNS) Congenital Hypothyroidism Screening Program, was initiated in Cape Town in 1982 (8). Due to early post-delivery discharge and the risk of follow-up loss, the PMNS program has adopted a pragmatic approach to congenital hypothyroidism screening. Cord blood TSH testing using RIA technology has proven more practical in this context (9).

Recently, the production of specific RIA kits has been phased out globally due to technological advancements, automation, market trends, operational costs, and safety concerns regarding radioactive materials (10). When notified that both the TSH and T4 kits would be discontinued, we initiated reference interval studies for cord blood TSH and free T4 using the automated Cobas® 6000 platform (Roche Diagnostics, Basel, Switzerland), together with a method comparison study of the

Roche Elecsys TSH assay with the original IRMA TSH assay, in line with Clinical Laboratory Standards Institute (CLSI) recommendations. Due to lack of assay standardisation (11) (12), differences in sample matrix and analytical technology, the need for population-specific data, and the unavailability of original package inserts, we conducted a complete reference interval study following CLSI guidelines to ensure accurate, clinically relevant intervals. The purpose of these studies was to establish population-based reference intervals and clinical decision limits for CH recall and follow-up testing.

METHODS

This prospective study used residual cord blood samples collected from newborns for the PMNS CH Screening Program. A total of 1,007 samples were collected over two weeks (Figure 1). For the reference interval studies, 121 samples were randomly selected after applying specific exclusionary criteria. These criteria aimed to identify samples from infants born to mothers with uncomplicated pregnancies at midwife obstetric units (MOUs) and district hospitals. By selecting samples exclusively from MOUs and district-level hospitals, mothers with comorbidities - such as elevated BMI, diabetes, or thyroid disease, which could influence a newborn's cord thyroid hormone profile - were excluded (13). Additionally, samples were excluded if they had abnormal TSH values (measured via the IRMA method), low or high birth weights, insufficient volume, improper labelling, or missing essential information. For the reference interval study, the CLSI EP28-A3c document was adhered to, whereas the CLSI EP09-A3 was utilised for the method comparison (14) (15). Due to the new assay methodology and lack of existing data, the direct method was chosen to establish accurate, population-specific reference intervals that align with our study's clinical objectives (14).

For the TSH method comparison component of the study, an additional 14 samples with TSH values outside the existing reference interval were identified to include the clinically relevant measuring range of the assay.

This study was approved by the Faculty of Health Sciences Human Research Ethics Committee (HREC study number 238/2023) at the University of Cape Town, following the Declaration of Helsinki.

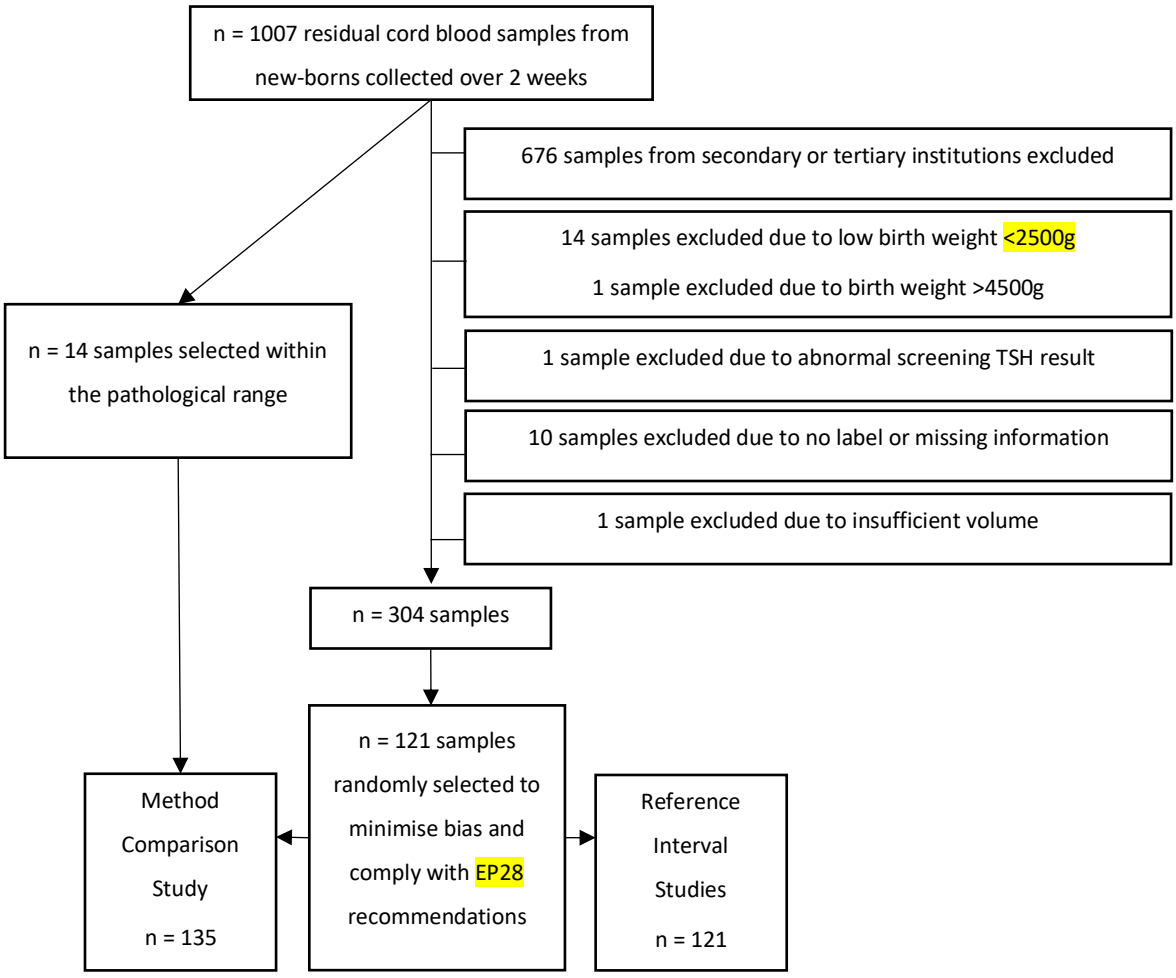

Figure 1. Flow of participants: Selection of residual newborn cord blood samples for reference interval and method comparison studies

Sample Handling

Residual cord blood samples were collected from the Red Cross Hospital Nuclear Medicine Department after TSH screening. The samples were then transferred to the NHLS at Groote Schuur Hospital on the same day, where they were screened according to exclusion criteria and analyzed on the Roche Cobas system.

Test Methods

For the reference interval studies, the samples were analysed on the Cobas® 6000 using electrochemiluminescence immunoassay (ECLIA) methodology for TSH (Elecsys TSH) and free T4 (Elecsys FT4 III). The TSH assay uses a non-competitive ('sandwich') immunoassay principle with

monoclonal antibodies, while the free T4 assay is competitive and uses polyclonal antibodies (16) (17). All samples were collected into BD Vacutainer® Blood Collection Tubes (Becton Dickinson, Franklin Lakes, New Jersey, USA), specifically serum separator tubes. In accordance with the routine procedures in a South African National Accreditation System (SANAS) accredited diagnostic laboratory, the assays underwent extensive quality control procedures throughout this study. In our laboratory, the Elecsys TSH and FT4 III immunoassay were performed with imprecision of 4.2% and 3.5%, respectively.

For the TSH method comparison, the samples were analyzed using an immunoradiometric assay (IRMA) conducted by the Department of Nuclear Medicin. Thisnon-competitive immunoassay method makes use of a ^{125}Iodine-labelled signal antibody that binds to an epitope of the TSH molecule and a biotin capture antibody that binds to a separate epitope (18). The sample is then incubated, during which time the immuno-complex is immobilised to the reactive surface of streptavidin-coated test tubes. The radioactivity is then measured using a gamma counter. The concentration of antigen is directly proportional to the radioactivity measured in the samples (18).

Statistical analysis

The study data was collected in Excel (Microsoft Corporation, Redmond, Washington, USA). Statistical analysis was performed using R Studio (version 4.3.0) (RStudio, Boston, Massachusetts, USA) and Python (Python Software Foundation, Wilmington, Delaware, USA). The data was visually assessed by histogram and tested for normality using the Shapiro-Wilk test.

For the reference interval studies, a sample number of 120 reference individuals was determined to be a good compromise between cost and confidence, as per the International Federation of Clinical Chemistry (IFCC) recommendations (16). Residual cord blood samples (n=121) were randomly selected after applying exclusionary criteria, as depicted in Figure 1. Conforming to CLSI EP28-A3c recommendations, outliers were identified and removed followed by using parametric, non-parametric, and robust methods to determine the reference intervals. Additionally, if the data was not normally distributed, it was first log-transformed before parametric reference interval determination. Outliers were detected and considered for exclusion in three steps: visual inspection of the histogram, calculation by Tukey's Fences, and, finally, when one or several outliers were excluded, we checked the remaining data for additional outliers. The reference intervals for TSH and free T4 were defined as the central 95% of laboratory test results expected in a healthy reference population (10). Confidence intervals (90%) were calculated for the lower and upper limits of the reference interval.

For the TSH method comparison study (Roche Elecsys TSH versus TSH), 14 additional samples were selected for their TSH concentrations to cover the measuring range of the assay (n=135). A sample number of 40 is usually deemed sufficient for method comparison studies, however, we decided to use all 135 samples with results available. Analyses included linear regression, with Lin's concordance correlation coefficient (CCC), and Bland-Altman difference plots.

Acceptability criteria specific to cord blood TSH and T4 are not established; therefore, we used the allowable limits for serum TSH and FT4 as the basis for the method comparison study. In the absence of specific quality specifications for cord blood samples, we used minimum specification values from The EFLM Biological Variation Database, specifically, total allowable error of 36.9% and allowable bias of 13.3% for TSH (20).

RESULTS

The birth weights of the sample population (n=121) ranged from 2500g to 4120g, demonstrating a parametric distribution. The average birth weight was 3211g, with a standard deviation (SD) of 387g.

Reference Interval Studies for TSH and FT4

The Roche Elecsys TSH values showed a non-parametric, right-skewed distribution (Shapiro-Wilk test, $p<0.001$) (Figure 2A). Although the log transformation of the data improved the shape of the curve, it was still not normally distributed ($p=0.007$). There were no outliers detected and, therefore, no points were excluded. The median (25th, 75th percentiles) was 6.13 mIU/L (4.78, 9.72).

The Roche Elecsys FT4 III values initially indicated a non-parametric distribution ($p<0.001$). However, this divergence from normality was heavily influenced by one outlier registering a free T4 value of 29.0 pmol/L (Supplementary Figure 1). Four additional outliers were determined by Tukey's fences. Removal of five outliers resulted in a normal distribution (Figure 2B; $p=0.355$). The median (p25, p75) was 16.46 pmol/L (15.24, 17.46).

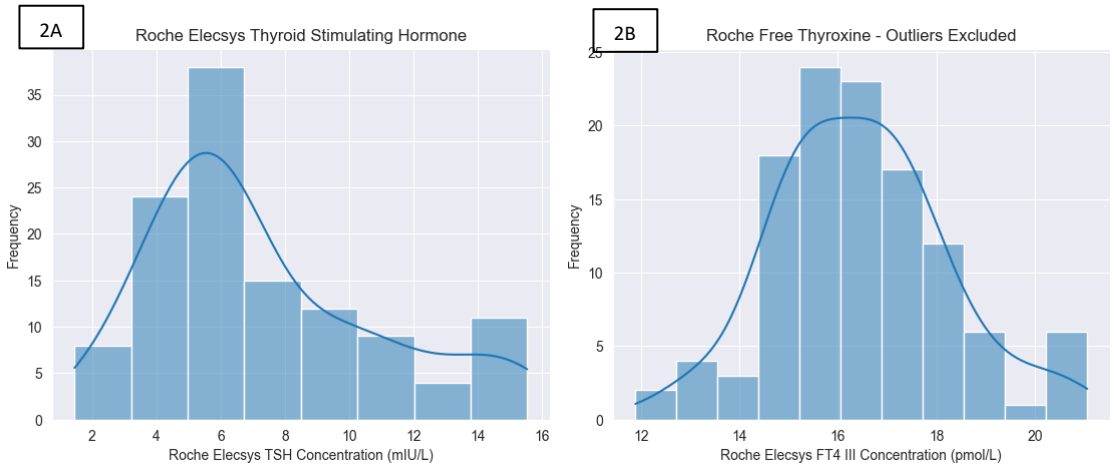

Figure 2. Histograms of Roche Elecsys TSH (2A) and Roche Elecsys FT4 III (2B) after outlier exclusion

For TSH, the non-parametric reference interval method yielded a lower limit of 1.85 mIU/L with a 90% confidence interval (90%CI) of 1.57 to 2.94 mIU/L and an upper limit of 15.35 mIU/L (90%CI 14.84 to 15.52). The non-parametric reference interval for free T4 was calculated as 13.0 pmol/L (90%CI 12.1 to 13.9) to 20.4 pmol/L (90%CI 19.41 to 21.05). The four methods for reference interval determination for FT4 provided similar results. The minor differences in lower and upper limits across the methods suggest that they provide generally similar results, especially given the overlapping confidence intervals. This indicates consistency in establishing reference intervals for FT4 among the methods. Table 1 shows the TSH and free T4 reference intervals calculated using the various methods, for comparison.

Table 1: Reference interval limits for Roche Elecsys TSH (mIU/L) and FT4 III (pmol/L)

Method	Lower limit	90% CI	Upper limit	90% CI
Thyroid Stimulating Hormone in mIU/L				
Parametric	0.28	(-0.26, 0.81)	14.37	(13.83, 14.91)
Transformed-Parametric	2.39	(2.22, 2.58)	17.58	(16.29, 18.97)
Non-Parametric	1.85	(1.56, 2.97)	15.35	(14.84, 15.52)
Robust	-1.05	(-2.14, 1.27)	13.31	(10.65, 14.61)
Free Thyroxine in pmol/L				
Parametric	13.0	(12.7, 13.2)	19.9	(19.7, 20.2)
Transformed-Parametric	13.2	(13.0, 13.5)	20.2	(19.9, 20.5)
Non-Parametric	13.0	(12.1, 13.7)	20.4	(19.4, 21.0)
Robust	13.2	(12.6, 13.8)	19.7	(19.0, 20.3)

Method Comparison Study of TSH

In the method comparison study, 135 samples were analyzed. Median (p25, p75) TSH concentrations were 6.89 mIU/L (5.07, 11.21) for Roche and 7.00 mIU/L (5.00, 12.00) for IRMA, noting that the IRMA assay was only reported to whole numbers. The range of results spanned 1.46 – 53.65 mIU/L for the Roche assay and 2.00 – 70.00 mIU/L for the IRMA assay.

When we compared the TSH values measured on the IRMA method versus the Roche Elecsys method, using linear regression (Figure 3), we found a strong level of agreement with an R-squared value of 0.98. This indicated that approximately 98% of the variance in the TSH Roche method could be explained by the TSH Isotope method. Regression equations for simple linear regression, Deming regression, and Passing-Bablok regression are shown in Table 2. The Pearson correlation coefficient was 0.99 ($p < 0.001$).

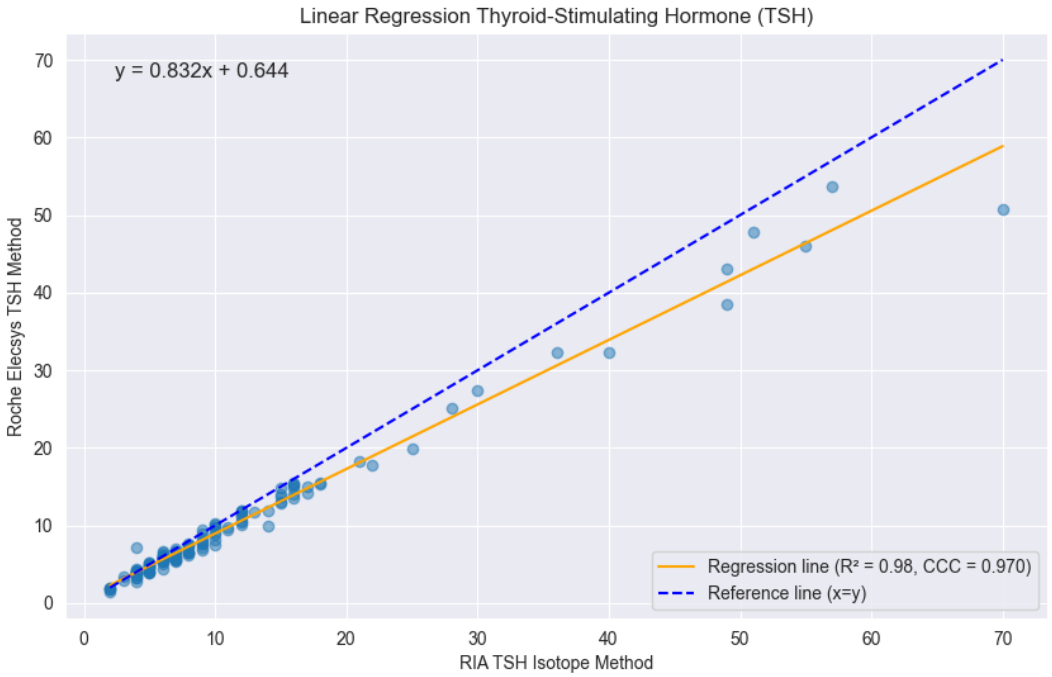

Figure 2. Linear regression of TSH measured on the IRMA method versus the Roche Elecsys method

Table 2: Regression equations for Roche Elecsys TSH (mIU/L) and IRMA TSH (mIU/L)

Method	Equation
Simple linear regression	TSH Roche = 0.832xTSH Isotope + 0.644
Deming regression	TSH Roche = 0.642×TSH Isotope + 0.443
Passing-Bablok regression	TSH Roche = 0.864×TSH Isotope + 0.355

Bland-Altman analysis was performed (Figures 4A and 4B). Most data points fell within the European Federation of Clinical Chemistry and Laboratory Medicine (EFLM) total allowable error goals for TSH (20), suggesting that the two methods were comparable. The two trendlines demonstrated that the differences at higher concentrations of TSH were larger than within the measuring range or at lower concentrations. The plots confirmed that IRMA TSH results were positively biased compared to Roche TSH results, as shown by the slope and intercept in Table 2. This bias was approximately 5.55% at low concentrations and 12.61% at high concentrations.

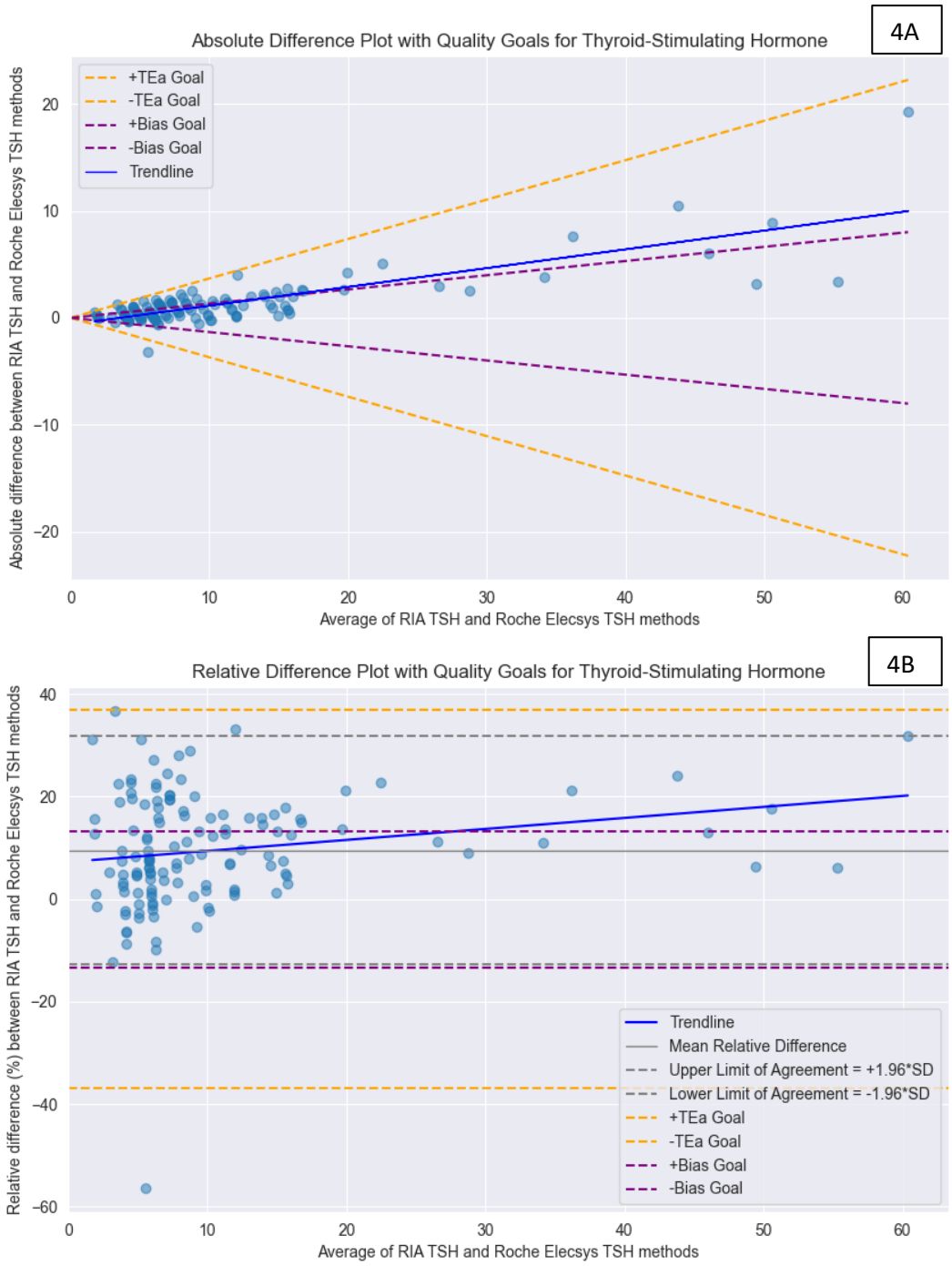

Figure 3: Bland-Altman Analysis: Absolute (4A) and Relative (4B) Difference Plots for TSH

DISCUSSION

This study aimed to define reference intervals and clinical decision thresholds for cord blood TSH and FT4 utilising automated Roche Cobas ECLIA methods. Our findings contribute significantly to the field, amidst the persistent discontinuation of RIA kits. This is especially pertinent given the limited research available in this area, with a notable gap in the context of South Africa.

The use of cord blood for CH screening in lower-middle-income countries like South Africa offers several advantages, particularly relevant to the constraints and unique challenges these regions face. Cord blood collection leverages existing childbirth practices, allowing for efficient integration into healthcare systems with limited resources. One-time collection, immediately after birth, minimises logistical challenges (such as transportation and communication barriers) and loss to follow-up. Previous research has observed a high correlation between cord blood and heel-prick blood TSH levels measured 48 hours after birth (21), supporting the practicality of cord blood as an alternative for screening purposes (22).

Our approach to establishing reference intervals closely followed the recommendations outlined in the CLSI document EP28-A3c, "Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline—Third Edition" (14). This document offers a comprehensive framework for the determination of reference intervals, ensuring that these are both clinically relevant and scientifically sound. Key features of the EP28-A3c recommendations include:

1. Selection of the reference population: We selected a reference population that accurately represented the demographic and clinical characteristics of the patient population for whom the tests would be used. This was done to ensure the clinical applicability of the determined intervals.

2. Sample size considerations: We studied ~120 reference individuals to achieve statistically valid reference intervals. This provided a good balance between statistical reliability and practical feasibility.

3. Outlier detection and treatment: We followed procedures for identifying and handling outliers to ensure accurate reference intervals. This ensured that our reference intervals were not skewed by anomalous data points.

4. Statistical methods for data analysis: Our study employed these statistical analyses, aligning with the guideline's emphasis on rigorous data analysis. We used various statistical methods for analysing the data and determining the reference intervals, including parametric, non-parametric, and robust methods. We also log-transformed the data and repeated the parametric method. These methods accounted for the distribution of the data and the presence of outliers, ensuring accurate and reliable intervals.

5. Verification of reference intervals: The EP28-A3c guideline emphasizes verifying existing reference intervals when implementing new methods or instruments, ensuring they remain valid for the test results produced by the new system. This consideration was incorporated into our method comparison study to support the transition to the Roche TSH method. We conducted a full reference interval study, eliminating the need to verify the previous reference intervals. However, we also performed a method comparison study to evaluate the medical decision limits specific to our program as part of the transition to the Roche TSH method.

These reference intervals for TSH and FT4 are consistent with those reported in other populations and are supported by the literature, including findings from studies conducted in regions with similar iodine-sufficiency statuses (Table 3). This consistency reinforces the reliability of our intervals for clinical application in CH screening. The non-normal, right-skewed distribution of TSH results mirrors previous findings, emphasising the relevance of using non-parametric methods for determining reference intervals in populations where the distribution deviates from normality. Our results also illustrate how parametric, non-parametric, and robust methods address reference intervals in data with differing distribution characteristics. This choice is further justified by comparing our results with those from similar studies, revealing congruence in the distribution and reference interval limits for TSH, underscoring the consistency of our findings across different methodologies and geographical settings (13) (23).

Mehari et al. (2013) conducted a study in Ethiopia to establish reference intervals for thyroid function tests from cord blood, acknowledging challenges similar to those in South Africa regarding early hospital discharges. Following IFCC and CLSI guidelines, they employed the ECLIA assay on the Roche Cobas 6000® for TSH, FT4, and FT3 measurements. Their study established a slightly higher TSH reference interval of 3.48 – 27.56 mIU/L and a lower limit for FT4 at 11.46 pmol/L. These differences may arise from including neonates ranging from 2000 g to >4000 g, with the largest baby weighing 4700g, while our study focused solely on "normal birth weight" neonates (2500 g – 4500 g). Additionally, we collected cord blood using serum separator tubes, whereas Mehari et al. used EDTA tubes. Furthermore, our samples were analysed within 1 day of collection without freezing, whereas

theirs were frozen at -20°C post-plasma separation and then stored at -80°C in the laboratory. There is no indication in their paper regarding the duration of sample storage before analysis.

Disparities identified between the remainder of the studies may be attributed to diverse environmental factors, such as variations in the prevalence of iodine deficiency within the population (22). Also, thyroid hormone assay characteristics may have played a significant role as the assays utilised in the studies were varied and not standardised (24). As demonstrated in Table 3, studies performed using enzyme-linked immunoabsorbent assay (ELISA) platforms, such as the studies done by Ishaku et al. and Satheesan et al., produced a lower limit as well as a wider reference range for TSH (25) (23). Furthermore, the studies were not all standardised to the CLIA EP28-A3c guidelines, with variations in several study features, including the handling of outliers and the statistical test utilised to determine the reference intervals. Our study's adherence to CLSI guidelines ensured that these intervals were scientifically robust and tailored to the specific population served by the program.

Our Free T4 data initially deviated from a normal distribution, primarily due to the presence of outliers. After the removal of outliers, the data exhibited a normal distribution, allowing for a more appropriate calculation of the parametric reference interval. This highlights the importance of outlier identification and treatment in reference interval studies. The FT4 results from our study align with a study performed in Shanghai, China by Fan et al. In this study, the cord blood of 452 singletons, term infants of mothers with normal thyroid function was assessed and the mean ± SD for all participants were 13.26±1.66 pmol/L and 13.39±1.43 pmol/L for boys and girls, respectively (26).

In our setting, we have chosen to implement the non-parametric reference interval for TSH of 1.85 - 15.35 mIU/L and for FT4 of 13.0 to 20.4 pmol/L. A TSH result of <1.00 mIU/L or >50.00 mIU/L elicits immediate recall of the neonate, whereas a TSH result of 15.36 to 50.00 reflexes further FT4 testing on the cord blood sample. The FT4 lower limit of 13.0 pmol/L serves as a cut-off for identifying patients requiring follow-up and further investigations, aligning with clinical objectives for the intended use of this assay for assessing CH risk. In the CLSI EP28-A3c guideline, the non-parametric method is recommended as the preferred choice for calculating reference intervals. This recommendation is based on its flexibility and minimal assumptions about data distribution.

Linear regression during the method comparison study showed Lin's concordance correlation coefficient (CCC) value of 0.97, indicating a high degree of concordance between the IRMA and Roche Elecsys methods for TSH measurement. This finding reinforced the strength of agreement between the two methods beyond just linear association. This finding suggests that the transition to the Cobas

methods will not only be seamless but will also maintain the integrity and reliability of TSH measurements in the CH program.

The slight underestimation by the Roche method, as indicated by the slope of less than 1 in both Deming and Passing-Bablok regressions, is clinically insignificant given the high degree of concordance and the observation that the differences were within clinically acceptable limits. Deming regression, which accounts for errors in both the independent and dependent variables, may provide a more appropriate model for comparing two methods of measurement where both have associated measurement errors. The Root Mean Squared Error (RMSE), which is a measure of the average magnitude of the errors between the observed TSH Roche values and the values predicted by the Deming regression model was 3.43, suggesting fitness for purpose. Passing-Bablok regression, which is known to be robust to outliers and does not assume a normal distribution of the errors, makes it another suitable alternative measure for method comparison studies. Overall, it offers a robust evaluation of the relationship between the two methods, uncovering differences in scale and potential systematic biases. This suggests that the transition to the Roche Cobas ECLIA method is feasible and will maintain consistency in clinical assessments.

There are several strengths to this study. By meticulously adhering to the CLSI EP28-A3c and EP09-A3 guidelines, our study not only establishes scientifically valid reference intervals and clinical decision limits but also ensures that these values are tailored to the specific needs and characteristics of the patient population served by the PMNS CH Screening Program. This adherence underscores the scientific rigour and clinical relevance of our findings, providing a solid foundation for the accurate interpretation of TSH and Free T4 measurements in newborn screening for CH in South Africa.

There are some limitations to consider, including a lack of comprehensive clinical data accompanying the residual samples and the representation of the study population. These limitations emphasise the need for caution when transferring our reference intervals to other population groups. A comparison between the previous RIA total T4 assay and the current Roche FT4 assay could not be conducted due to the sudden discontinuation of the RIA service. Additionally, a method comparison with the previous RIA method may not have added further insight, as the RIA measured total T4, while the ECLIA measures free T4. Future research may wish to explore these areas further, ensuring the broad applicability and accuracy of our findings.

CONCLUSION

Our study addresses the need to transition from RIA to ECLIA methods for CH screening in South Africa and provides essential reference intervals for accurate newborn screening interpretation. This transition is a critical step towards improving CH outcomes and reflects the broader global shift towards more advanced and safer screening methodologies. As we move forward, it is imperative to continue monitoring and validating these intervals against clinical outcomes to ensure their ongoing relevance and accuracy.

(3601 words, limit = 3000)

Table 1. Studies Performed Measuring Thyroid Hormones on Cord Blood for CH Screening

Author	Region	Analyte	Reference Interval	Analyser	Method
Abdelouahab, et al. 2013 (27)	Quebec, Canada	TSH	2.75 – 21.55 mIU/L	Advia Centaur	ECLIA
		FT4	9.46 – 16.13 pmol/L	Advia Centaur	CMIA
Al Juraibah, et al. 2019 (28)	Riyadh, Saudi Arabia	TSH	1 – 39.4 mIU/L	Abbott Architect i2000 immunoassay analyser	CMIA
		FT4	<9 pmol/L	Abbott Architect i2000 immunoassay analyser	CMIA
Ogunkeye, et al. 2007 (12)	Najran, Saudi Arabia	TSH	2.0 – 16.8 mIU/L	DELFIA	FIA
		FT4	<12 pmol/l	DELFIA	FIA
Henry, et al. 2002 (29)	Riyadh, Saudi Arabia	TSH	2.38 – 19.06 mIU/L	Boehringer-Manheim ES 700	ELISA
		FT4	11.70 – 19.64 pmol/l	Boehringer-Manheim ES 700	ELISA
Ishaku, et al. 2017 (25)	Jos, Nigeria	TSH	0.21 – 29.2 mIU/L	*Not indicated*	ELISA
		FT4	6.30 – 20.29 pmol/L*	*Not indicated*	ELISA
Jeyachandran, et al. 2017 (13)	Karakonam and Trivandrum, India	TSH	2.2 – 18 mIU/L	VITROS Eci	CLIA
Kawahara, et al. 2002 (30)	Toranomon, Japan	TSH	4.54 - 14.50 mIU/L	TSH RIA Beads kit (Dainabott)	IRMA
		FT4	13.52 – 20.72 pmol/L*	Amerlex MAB	RIA
Mehari, et al. 2016 (31)	Addis Ababa, Ethiopia	TSH	3.48 – 27.56 mIU/L	Elecsys immunoassay analyser (Cobas®)	ECLIA
		FT4	11.46 – 19.69 pmol/L*	Elecsys immunoassay analyser (Cobas®)	ECLIA
Mutlu, et al. 2011 (32)	Karadeniz, Turkey	TSH	3.48 - 27.56 mIU/L	E170 Hitachi	ECLIA
		FT4	11.46 – 19.69 pmol/L*	E170 Hitachi	ECLIA
Satheesan, et al. 2022 (23)	Kerala, India	TSH	0.63 – 17.03 mIU/L	Biorad ELISA reader and Washer	ELIS
		FT4	12.16 – 34.98 pmol/L*	Biorad ELISA reader and Washer	ELISA
Seth, et al. 2014 (33)	North India	TSH	1.4 – 29.4 mIU/L	*Not indicated*	IRMA

*Note: Values marked with an * have been converted to standard international units) Abbreviations: CLIA – chemiluminescent immunoassay; CMIA – chemiluminescent microparticle immunoassay; ECLIA electrochemiluminescent Immunoassay; ELISA - enzyme-linked immunosorbent assay; FIA – fluorescent immunoassay; FT4 – free thyroxine; IRMA – immunoradiometric assay; RIA – radioimmunoassay; TSH – thyroid-stimulating hormone*

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SUPPLEMENTARY DATA:

Congenital Hypothyroidism: Cord Blood Sample Reference Intervals for Thyroid Stimulating Hormone and Free Thyroxine on Roche Cobas® Analyser

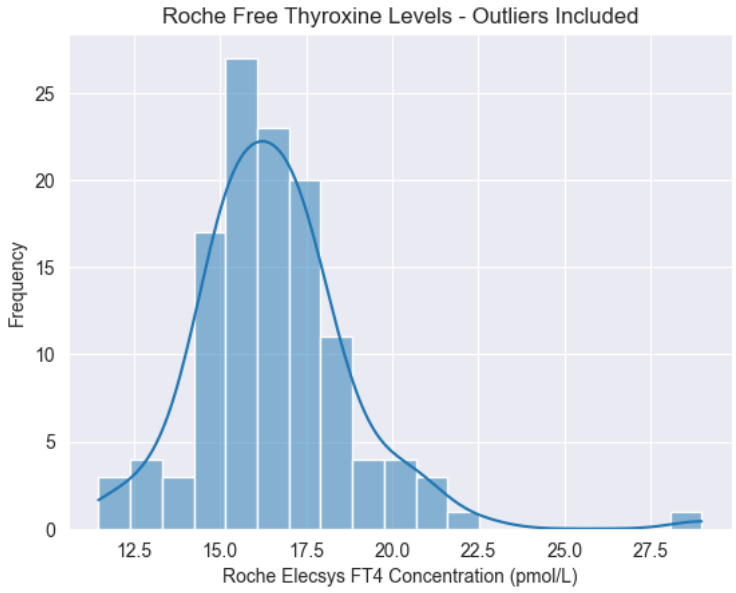
Distribution of birth weight

The birth weight distribution analysis involved 121 samples, with no identified outliers according to Tukey's Fences Test. The data exhibited a mean birth weight of 3207.51 grams, with a standard deviation of 388.36 grams and a median of 3202.5 grams. The interquartile range (IQR) was calculated as 577.5 grams, with the first quartile (Q1) at 2915 grams and the third quartile (Q3) at 3492.5 grams.

Reference interval study

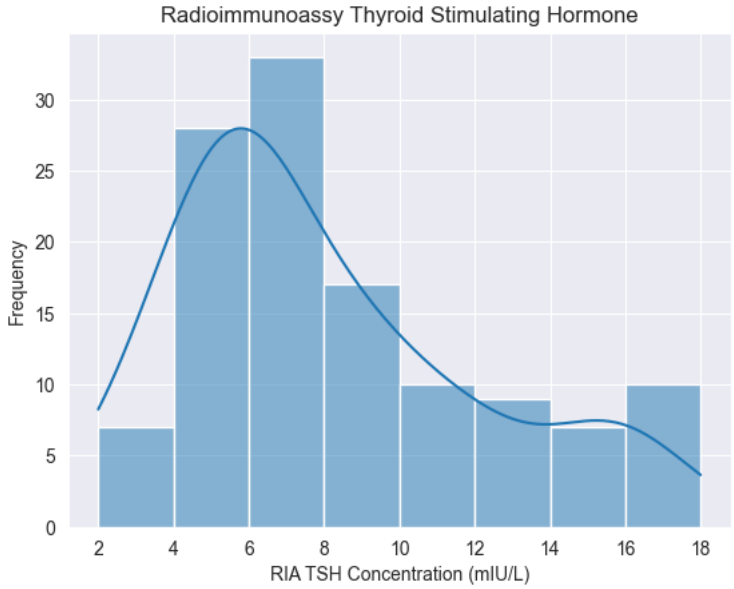
The reference interval study for TSH measurement on cord blood, conducted on the Roche Cobas® 6000, involved 121 samples, with no identified outliers. The Shapiro-Wilk test revealed non-normal, with no outliers identified using Tukey's Fences Test. The TSH measurements exhibited a mean of 7.32, a standard deviation of 3.60, and a median of 6.13.

The analysis of Free T4 on the Roche Cobas® 6000, after the removal of 5 outliers, displayed normal distribution. The mean Free T4 was 16.45, with a standard deviation of 1.77 and a median of 16.46. The Shapiro-Wilk test confirmed normality ($p > 0.05$).



Supplementary Figure 1. Histogram of FT4 as measured on the Roche Cobas® 6000, before removal of outliers

TSH measurements using the Coat-a-Count Radioimmunoassay exhibited a mean of 8.04, a standard deviation of 4.04, and a median of 7. Despite log transformation, the Shapiro-Wilk test still indicated a non-normal distribution ($p < 0.05$).



Supplementary Figure 2. Histogram of TSH measurements using the Coat-a-Count Radioimmunoassay

Supplementary Table 1. Basic statistics and tests for normality for TSH and FT4 on the Roche Cobas® 6000 and TSH on the IRMA Coat-a-Count Radioimmunoassay

Method	Sample no.	Mean	SD	Median	P25	P75	IQR	P2.5	P97.5
Roche TSH	121	7.32	3.60	6.13	4.78	9.72	4.94	1.98	15.30
Roche FT4	120	16.46	1.98	16.46	15.23	17.56	2.32	12.69	21.03
IRMA TSH	121	8.04	4.04	7.00	5.00	10.00	5.00	2.00	17.00

Method Comparison Analysis

The method comparison between TSH measured on the Coat-a-Count Radioimmunoassay (IRMA TSH) and TSH measured on the Roche Cobas® 6000 (Roche TSH) revealed a robust positive association between the two methods, as indicated by both Pearson and Spearman correlation analyses. The exceptionally low p-values obtained from both statistical tests signify a high level of concordance between the new Roche TSH method and the established IRMA TSH method, both in terms of measurement rank and value.

Table 5: Pearson & Spearman Correlation for TSH Measurement Agreement (IRMA vs. Elecsys)

Type	Correlation Coefficient	p-value
Pearson	0.991	p<0.001
Spearman	0.974	P<0.001

AUTHOR CONTRIBUTION STATEMENT

Dr Mariam Mahomed contributed to the study by writing the original draft, sample collection, sample analysis, data collection, data analysis, and reviewing and editing the manuscript.

Dr Jody Alan Rusch played a lead role in the conceptualization of the study, study design, data analysis, writing the original draft, and reviewing and editing the manuscript.

Dr Helena Vreede was involved in identifying the need for the study, conceptualization of the study, study design, data analysis, writing, and reviewing and editing the manuscript.

Ms. Bianca Southon contributed to the study by writing, reviewing and editing the manuscript.

Ms. Haylie Geffen performed the primary data analysis and guided data presentation.

Ms. Kulsoem Kasker facilitated the implementation of this study as part of a laboratory improvement project and offered continuous support and feedback throughout its duration.

Dr Michelle Carrihill provided clinical background and contributed to reviewing and editing the manuscript.

Dr Anita Brink contributed to reviewing and editing the manuscript.

AUTHOR DISCLOSURE STATEMENT

Dr Mariam Mahomed (author), registrar in the Division of Chemical Pathology, Department of Pathology, University of Cape Town, Cape Town, South Africa and C17 Chemical Pathology Laboratory, Groote Schuur Hospital, National Health Laboratory Service, Cape Town, South Africa, declares that she has no relevant or material financial interests that relate to the research described in this paper.

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Ms. Bianca Southon (author) declares that she has no relevant or material financial interests that relate to the research described in this paper.

Ms. Kulsoem Kasker (collaborator), lab manager at C17 Chemical Pathology Laboratory, Groote Schuur Hospital, National Health Laboratory Service, Cape Town, South Africa, declares that she has no relevant or material financial interests that relate to the research described in this paper.

Ms. Haylie Geffen (collaborator), data analyst at the School of Public Health, University of Cape Town, Division of Epidemiology and Biostatistics, declares that she has no relevant or material financial interests that relate to the research described in this paper.

Dr Michelle Carrihill (clinical collaborator), Paediatric Endocrinologist at Red Cross War Memorial Children's Hospital and Groote Schuur Hospital, declares that she has no relevant or material financial interests that relate to the research described in this paper.

Dr Anita Brink (clinical collaborator), Nuclear Medicine Physician at Red Cross War Memorial Children's Hospital, Department of Radiation Medicine declares that she has no relevant or material financial interests that relate to the research described in this paper.

FUNDING STATEMENT

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This project was undertaken as part of a lab improvement project at the NHLS Groote Schuur Hospital and the majority of the funding was provided for by the NHLS Groote Schuur Hospital. These costs include collection of samples, transport of samples, measurement of TSH and T4 at Groote Schuur National Health Laboratory Service, and data collection and analysis.

Funding for the publication of this project will be applied for through the UCT Open Access Funding facility.

ANNEXURE 1: PROJECT BACKGROUND

Thyroid hormones are involved in a wide array of bodily functions and the symptoms of thyroid hormone deficiency are diverse and may involve multiple systems. In adults, thyroid dysfunction is associated with neuropsychiatric complications such as restlessness, irritability, emotional lability, depression and even psychosis, encephalopathy, and coma[2]. These conditions are usually treatable with thyroid hormone supplementation. However, in neonates, the consequences of hypothyroidism are much further reaching and permanent. Congenital hypothyroidism (CH) is recognised as a preventable cause of intellectual disability and growth retardation[2]. Ideally, treatment should be initiated within two weeks of birth, as delays in treatment correlate directly with poorer outcomes[3]. CH was initially estimated to be present in 1 in 7000 new-borns worldwide but with the implementation of widespread new-born screening programmes, the prevalence was noted to be approximately 1 in 3000[4]. In populations with effective neonatal screening programmes, however, this number is estimated to be closer to 1 in 2000[5].

Due to the frequency of CH and the devastating consequences associated with this disease, most industrialised countries have implemented some variation of a newborn screening programme[6]. One of the earliest screening programmes was implemented in Italy as early as 1977, in which dried blood spots were used to estimate neonatal thyroid stimulating hormone (TSH) to screen for CH. This programme grew to cover virtually 100% of the population by the 90s and is now subject to frequent auditing and optimization to harmonize and improve the screening strategy and diagnostic approach in all affected infants[7]. Currently, in South Africa, which is considered a developing nation, there is no national state-sponsored screening programme for CH.

According to the International Society for Neonatal Screening (ISNS), neonatal screening is an accepted medical intervention aimed at detecting treatable metabolic and other disorders. It has been accepted as part of routine neonatal health care in almost all countries with well-developed medical services[6]. Newborn screening is recommended, provided that the following criteria are met, as per the ISNS guidelines: (a) there is considered to be a direct benefit to the neonate from early diagnosis; (b) the benefit is reasonably balanced against financial and other costs; (c) there is a reliable test suitable for neonatal screening; (d) there is a satisfactory system in operation to deal with diagnostic testing, counselling, treatment, and follow-up of patients identified by the test[6]. Screening for CH in South Africa fulfils most of the above criteria, with the biggest barrier currently being the follow-up of patients[4].

To understand and assess thyroid disorders occurring in the newborn, it is imperative to be familiar with the physiology of thyroid hormone production and control in the foetal and neonatal periods[3]. Fetal thyroid production remains low in the first trimester of pregnancy and the foetus is dependent on maternal thyroid hormones, which can freely cross the placenta[3]. The foetal hypothalamus begins secreting thyrotropin-releasing hormone (TRH) around the eighth week of gestation, stimulating the thyroid gland to start producing thyroxine. However, the hypothalamic, thyroid hormone axis becomes fully matured about six to eight weeks after birth. At the time of delivery, about 30% to 50% of TSH measured in cord blood is of maternal origin[3].

The transition from intrauterine to extrauterine life results in several changes to stimulate neonatal thyroid hormone production. The neonatal pituitary gland is stimulated to produce TSH in response to the temperature change from the warm intrauterine space to the cooler extrauterine environment, leading to a TSH surge[3]. TSH in neonates rises to a peak about 30 minutes after delivery and decreases to normal-term infant levels by day three to five of life. Thyroxine (T4) concentrations peak 48 hours after birth and may require several weeks to normalise[6]. Pre-term infants, (those born before 34 weeks of gestation) have lower T4 concentrations than term infants. Ideally, newborn screening should occur between the 2nd and 4th day after birth (and within the first seven days in preterm infants). Testing before this time may lead to falsely raised TSH measurements. Additionally, neonates with postpartum complications, such as sepsis or lung disease, may have a low T4 and T3, which is in keeping with sick euthyroid or non-thyroidal illness[3].

CH may be due to thyroid dysgenesis, thyroid dyshormonogenesis, central hypothyroidism, thyroid hormone resistance or maternal factors such as the use of antithyroid medication during pregnancy[3].

To prevent the neurocognitive complications associated with CH, many countries have implemented screening programmes to detect this disease. In most countries with robust newborn screening programs, this is done in the neonatal period using dried blood spot testing[8]. Tandem mass spectrometry is used in many developed countries to screen for common inherited metabolic diseases in the population, as well as screening for CH. This testing is done on heel prick or venous blood, used to make dried blood spots on day 3 – 5 of life. However, in countries where early discharge post-delivery is the current practice, cord blood TSH testing is the best screening method, with some studies showing superior sensitivity using early heel prick testing [1]. An additional barrier to the use of heel prick samples in South Africa is the large burden of postnatal loss to follow-up. A study performed from 2010 to 2013 showed that approximately 40% of neonates did not receive the three postnatal visits during the first six weeks of life as recommended by the World Health Organisation (WHO)[10].

Therefore, the current South African infrastructure and the existing evidence support the use of cord blood for screening for CH.

Currently, South Africa does not have a state-sponsored newborn screening program. In 1982, the Peninsula Maternal and Neonatal Services (PMNS) initiated a congenital hypothyroidism screening program across three maternity units[7]. Following an assessment demonstrating its cost-effectiveness, the program expanded to cover the entire PMNS region. A 2008 audit confirmed that the incidence of congenital hypothyroidism in this district aligned with global rates[7]. Additionally, a cost analysis affirmed the program's economic viability, though this did not factor in the psychosocial and ethical implications of undiagnosed cases[7]. Originally managed by the Department of Nuclear Medicine at Red Cross War Memorial Children's Hospital (RCWMCH), the program was transferred to the National Health Laboratory Service (NHLS) after the discontinuation of radioimmunoassay (RIA) kits previously used by Nuclear Medicine.

To make rational management decisions using the results from cord blood, appropriate reference intervals would need to be provided by the laboratory. Reference intervals may be affected by lifestyle, ethnicity, gender nutrition and other environmental factors[2]. However, there is currently no population-specific data available for the measurement of TSH and T4 in cord blood in South Africa. In a country as diverse as South Africa, it could even be argued that regional or subpopulation reference intervals would be more useful[2].

This study seeks to establish useful and reliable reference intervals, within the South African population, to be used as tools for clinical decision-making in the existing CH in Cape Town. Also, the results gained from this study may be used by other laboratories using the same methodology and analyser, allowing this vital program to be rolled out in other parts of the country. Having these samples run through the National Health Laboratory Service (NHLS) will allow the monitoring of the epidemiology of this disease in our country and, with ongoing auditing and review, provide more sensitive and specific reference intervals or clinical decision limits. This study is a useful step toward a more inclusive CH screening program in South Africa.

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ANNEXURE 2: HREC LETTER

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

Room 45 E-52-E-Floor- Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: hrec-submissions@uct.ac.za
Website: https://health.uct.ac.za/home/human-research-ethics

13 April 2023

HREC REF: 238/2023

Dr J Rusch
Division of Chemical Pathology
NHLS NGSH
Email: jody.rusch@uct.ac.za
Student: mariammahomed90@gmail.com

Dear Dr Rusch

PROJECT TITLE: DETERMINATION OF REFERENCE INTERVALS AND DECISION LIMITS FOR THYROID STIMULATING HORMONE AND THYROXINE ON CORD BLOOD SAMPLES-SUB-STUDY LINKED TO R017/2022-
(MASTERS' CANDIDATE-DR MARIAM MAHOMED)

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

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

Approval is granted for one year until the 30 April 2024.

- Please consider developing an HREC approved biobank for these stored samples.

Please submit a progress form, using the standardised Annual Report Form (FHS016) 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)

The HREC acknowledge that the student: Dr Mariam Mahomed will also be involved in this study.

Please quote the HREC REF 238/2023 in all your correspondence.

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

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

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FACULTY OF HEALTH SCIENCES HUMAN RESEARCH ETHICS COMMITTEE

HREC/ref 238.2023

ANNEXURE 3: JOURNAL GUIDELINES

General Manuscript Submission Guidelines and Policies for Mary Ann Liebert Journals

Last updated 2/2/2024 2:32:07 PM

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Please check your journal's requirements for file formatting. Many journals require formatting compliance only on revision; however, unless stated, the file formatting should comply with the following requirements on submission.

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The main text file, figure legends, and tables should be prepared in Microsoft Word. Some journals may accept LaTex. Please consult your individual journal instructions for guidance.

File Naming

- All file names should be in English and contain only alphanumeric characters.
- **Do not include spaces, symbols, special characters, dashes, dots, or underscores.**
- Title each file with the type of content contained in the file (e.g., manuscript.doc, tables.doc, FigureLegends.doc, Fig1.tif, SupplementalData.pdf, etc.).

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- Submission of high resolution .TIFF or .EPS figure files is preferred. Please upload as individual files.
- Cite figures consecutively in text within parentheses.
- Images should not reveal the name of a patient or a manufacturer.
- Note: Figures that will not be reproduced in color must be readable and interpretable in black and white.

Figure Legends

- A legend should be provided for each supplied figure.
- All legends should be numbered consecutively.
- Figure legends may be included at the end of the main text file or uploaded as a separate, double-spaced Word file.
- In each legend, provide explanations for any abbreviations or symbols that appear in the figure.
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- Title page* with full manuscript title, all contributing authors' names and affiliations, a short running title, a denotation of the corresponding author, and a list of 4-6 keywords/search terms,
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- Main text without embedded figures or tables and with appropriate section headings, if applicable. Most research papers should be organized as follows: Introduction, Materials and Methods, Results, Discussion, and Conclusions.
- Acknowledgments,
- Authorship confirmation/contribution statement (CRediT format is preferred)
- Author(s') disclosure (Conflict of Interest) statement(s), even when not applicable,
- Funding statement, even when not applicable,
- References,
- Tables included in the text or as a separate document,
- Figure legends at the end of the main text or in a separate Word file,
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*Double-blinded journals require a separate title page with the title, all contributing authors' names and affiliations, a denotation of the corresponding author, author acknowledgements, disclosures, and related identifying information.

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- An Institutional Review Board (IRB) approval (or waiver) statement and statement of patient consent as a separate paragraph after the methods section,
- Other relevant ethics attestations (see icmje.org for further guidance),
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One author should be designated as the corresponding author who will be responsible for communication between the authors and the journal editorial office and publisher. This individual will be responsible for ensuring all authors submit copyright forms, coordinating and responding to page proofs, and managing any other necessary contact during the peer review and production processes.

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An authorship contribution statement must be included with the manuscript. We strongly recommend that the authorship contribution statement follow the CRediT Taxonomy guidelines. (https://credit.niso.org/)

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- Data curation (Management activities to annotate (produce metadata), scrub data and maintain research data (including software code, where it is necessary for interpreting the data itself) for initial use and later re-use.)
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Example

Author 1: review and editing (equal). **Author 2**: Conceptualization (lead); writing – original draft (lead); formal analysis (lead); writing – review and editing (equal). **Author 3**: Software (lead); writing – review and editing (equal). **Author 4**: Methodology (lead); writing – review and editing (equal). **Author 5**: Conceptualization (supporting); Writing – original draft (supporting); Writing – review and editing (equal).

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If the study is judged exempt from review, a statement from the committee is required in the Materials and Methods section, including, if applicable, documentation of institutionally approved waiver of informed consent.

Ethics of Experimentation

See the following resources for studies involving human fetuses, fetal tissue, embryos, and embryonic cells:

- **NIH Grants Policy Statement**
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instead requires the author(s) to retain and archive all patient consent documentation. Upon submission of a manuscript for review, the authors must make a statement in the cover letter to the Editor/Journal which attests that

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