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ORIGINAL RESEARCH

Establishment of reference intervals of biochemical analytes for South African adults: A study conducted as part of the IFCC global multicentre study on reference values. Assessment of methods for derivation and comparison of reference intervals

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Objective: This study was part of International Federation of Clinical Chemistry (IFCC) global study establishing reference intervals (RIs) of analytes for a South African (SA) population in consideration of ethnicity, gender and age.

Design: 1 143 healthy participants aged ≥ 18–65 years were recruited (551 African, 383 Caucasian and 209 Mixed Ancestry). Samples were analysed for 40 chemistry and immunochemistry analytes. Sources of variation (SV) of RIs were derived using multiple regression analysis (MRA). Partitioning RIs by ethnicity, gender and age was judged by the standard deviation ratio (SDR). Latent abnormal value exclusion (LAVE) was applied to exclude subjects with underlying latent disease, before deriving RIs by both parametric (P) and non-parametric (NP) methods.

Results: Partitioning of RIs by gender was required in 23 analytes: albumin, urea, uric acid, creatinine, total bilirubin, calcium, inorganic phosphate, magnesium, total cholesterol, triglycerides, HDL-C, LDL-C, Lipase, ALP, CK, γGT, cholinesterase, ferritin, transferrin, C-RP, IgA, IgM and free T3. Partitioning of RIs for ethnicity was required for: urea, total cholesterol, LDL-C, AST, amylase, C-RP, IgG and IgA. Partitioning of RIs by age: 1) all genders: total cholesterol, LDL-C, 2) urea in females and 3) PSA in males. The LAVE method saw prominent changes in RIs in seven analytes: TG, AST, ALT, GGT, CK, C-RP and ferritin, while the superiority of the P method over the NP method was shown.

Conclusion: Our findings emphasised the need to partition some analytes by ethnicity, age and gender in SA.

Keywords: reference intervals, standard deviation ration, latent abnormal vales exclusion, parametric method, non-parametric method, International Federation of Clinical Chemistry and Laboratory Medicine

Introduction

Reference intervals (RIs) play an important role in medical decision making, especially for diagnosis and monitoring disease.[1] Therefore, determination of reliable RIs is one of the most important missions of clinical laboratory. Laboratories should derive population-specific RIs from healthy individuals of same population as patients where these patient samples are analysed by that particular laboratory. Besides, each laboratory is required to verify the RIs in use regularly.[1] However, the selection and recruitment of a sufficient number of participants are very challenging, time-consuming and expensive. In some instances, laboratories utilise RIs supplied by in vitro diagnostic manufacturers for their patient RIs; however, these RIs may not be suitable for the laboratory's patient population.[2] Differences may be seen in the RIs depending on populations living under different environmental and dietary conditions.[3] To date there has been no study for establishing RIs for the South African population in consideration of ethnicity, age and gender.

Therefore, we joined the international multi-centre project led by the Committee on Reference Intervals and Decision Limits (C-RIDL) of the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) to establish RIs in South Africa. We compared these values with the rest of the global study. Other collaborating countries include the United States of America, Turkey, Japan, the United Kingdom, China, India, Saudi Arabia, Argentina, Russia, Philippines, Nepal, Pakistan, Kenya, Nigeria, and Bangladesh. We employed up-to-date analytical methods and the latest automated instruments for measurements of

commonly tested analytes. We expected that the project will provide us with a unique opportunity to compare South Africa's RIs with those from countries of widely different demographic profiles. The study also provided an opportunity to investigate the controversies over the need for secondary exclusion of individuals with underlying undiagnosed disease conditions by use of the latent abnormal values exclusion (LAVE) method and the use of parametric (P) and nonparametric (NP) methods for derivation of RIs.[4,5] The main goal of this study was to derive country-specific RIs for the adult South African population utilising the common protocol proposed by the C-RIDL.[1]

Materials and methods

Ethics approval was obtained from the Faculty of Health and Wellness Sciences' Research and Ethics Committee, Cape Peninsula University of Technology, Reference Number: CPUT/NHREC: REC-230408-014 as well as the University of Stellenbosch's Health Research Ethics Committee protocol number: S12/05/147. Each participant was handed a study information leaflet, informing them of the purpose of the study as well as any risks involved when participating in such a study. Written informed consent was obtained from each participant.

The study design was adopted from a previously described protocol as used in other countries and is briefly described below.[1] All participants had to be South African-born citizens regardless of their ethnic origin. Those originally born in the former South West Africa (a province of South Africa in the early 1980s), now Namibia, and who continued to live in South Africa were also allowed to participate. The participants had to be subjectively feeling well therefore regarding themselves as healthy. The target range of age was between 18 and 65 years. The participants over the age of 65 were also recruited, although their test results were not used for the derivation of RIs but for analyses on sources of variation (SVs) of RIs. Ideally, each participant should not have been taking any medication for chronic conditions. However, for this study we allowed participants taking up to three medications for any chronic conditions, provided they did not suffer from major illness described below. Since South Africa has high obesity rates,[6] participants with body mass index (BMI) of ≥ 30 kg/m^2 were not included. Participants were excluded if they suffered from or had any of the following conditions: 1) diagnosed with diabetes and/or diabetes treatment; 2) reported history of chronic liver or kidney disease; 3) the results obtained in this study clearly indicated the presence of a severe disease; 4) had been hospitalised or been seriously ill during the preceding four weeks of participation; 5) donated blood in the last three months; 6) known carrier state of hepatitis B virus (HBV), hepatitis C virus (HCV) or HIV-positive; 7) pregnant or within one year after childbirth and 8) participated in another research study involving an investigational product in the past 12 weeks. The study was conducted between October 2012 and February 2015 as a multi-centre study with a centralised scheme of analysis.[1] Recruitment took place in three core areas located in different provinces in South Africa namely: Cape Town, Western Cape Province, Walter Sisulu University in Mthatha, Eastern Cape Province, and University of Witwatersrand in Johannesburg, Gauteng Province. Each core area was responsible for sample separation, aliquoting, and freezing at -80 °C, as described below.

Anthropometric measurements: weight (kg), height (m), waist circumference (Waist-C; cm) and blood pressure measurements were performed prior to sample collection. Each participant had to be seated calmly for at least 20 minutes prior to sample collection for the equal distribution of circulation proteins. Body mass index (BMI) was calculated as weight/height^2 (kg/m^2). A comprehensive health status survey was conducted via a detailed questionnaire adapted from the one used in the previous Asian project to obtain information on BMI, ABO blood type, alcohol consumption, smoking habits, exercise, recent episodes of infection or allergy, menstrual status.[7] This was completed by each participant while seated as described above. For this study, we added additional items to the questionnaire on the intake and frequency of oily fish per week, duration of sun exposure per day and which parts of their bodies had the sun exposure as some Vitamin D (VitD) tests and skin pigment reading measurements were done on a small group of participants to determine their VitD status. For male participants, an additional question was asked on dysuria during the past three months whilst for female participants a separate question was asked on the regularity and duration of their menstrual cycle and on hormone replacement therapy.

Participants were requested to avoid excessive physical exertion or exercise three days prior to sample collection. Furthermore, they were requested to avoid excessive eating and drinking the night before sampling, and to fast at least for 10–12 hours. Approximately 40.0 mL of blood was drawn into three 9.0 mL SST III Vacutainers with clot activator and gel, one 4.0 mL lithium heparin tube, one 5.0 mL fluoride tube for glucose analysis and one 4 mL EDTA sample for full blood count from each participant. Samples requiring centrifugation were centrifuged at 4 000 rpms for 15 minutes. They were then aliquoted and labelled for freezing at -80 °C until the day of analysis. The remote sites at Mthatha and Johannesburg sent their aliquoted samples on dry ice to PathCare Laboratories (ISO 15189 accredited laboratory) in Cape Town for storage at -80 °C until the day of analysis. This was analysed together with the samples prepared at the Cape Town site. The reason for the choice for a centralised scheme of analysis was because all specimens were analysed collectively in batches of 100–300 samples. Measurements comprised of standardised analytes (enzymes, major serum proteins, lipids and electrolytes) as well as non-standardised analytes (ferritin, thyroid function tests, VitD, prostate spesific antigen [PSA]). The analytes depending on the assay principle of chemical reaction or immune-turbidimetry were measured using an automated Beckman Coulter DXC analyser for the specimens obtained between 2012 and 2013 or using an AU analyser for the specimens obtained in 2014, while those analytes depending on labelled immunoassays were measured by Beckman Coulter DxI analyser from the start of analysis in 2012.

For the purpose of this study, dedicated QC monitoring was undertaken for standardised and non-standardised reagents. This was achieved by using multiple specimens that were

prepared as suggested by the C-RIDL common protocol and standard operating procedure (SOP).[1] Accordingly, a mini-panel of sera from five healthy individuals (two males and three females of different ethnic groups and ages) was prepared and measured with every batch of 100 participant samples over the period of collective measurements. This was done to closely monitor between-day variations of test results.[5] Furthermore, two panels of sera manufactured by the C-RIDL in 2011 and 2014 respectively, were obtained and analysed 1) to ensure standardisation of RIs through recalibration based on assigned values set to the serum panels, 2) to align RIs of samples obtained between the two periods, and 3) to allow for comparison of RIs with those of the results from other countries. The analytes with assigned values in the panels are reported in the previous reports.[5]

All questionnaire data were captured on Microsoft Access and exported to a Microsoft Excel spreadsheet. Patient test results were exported to a Microsoft Excel spreadsheet and the two spreadsheets were combined and analysed using the same statistical methods described in the previous studies.[4] The graphical figures in presenting the results were all made by use of StatFlex Version 6 with flexible graphical capability (Artech Co., Ltd, Osaka, Japan). By use of the 3-level nested analysis of variance (ANOVA), the magnitude of variations of test results that was attributed to ethnicity, gender, and age. These variations were expressed as standard deviation ratio (SDR) namely: between-ethnicity SDR (SDR_{rc}), between-gender SDR (SDR_{sex}) and between-age group SDR (SDR_{age}) after partitioning age at 30, 40, and 50 years.[4] The 2-level nested ANOVA was also perfomed to predict SDR_{rc} and SDR_{age} separately for each gender. SDR values ≥ 0.40 were regarded as a guide for judging the need for partitioning RIs by a given factor.[4,5] Regarding the analyses of differences and the computation of SDR_{rc}, these were performed in two ways: in three ethnic groups of African (Afr), Mixed Ancestry (MA), and Caucasian (Cau) and in two race groups of Afr and Non-African (Non-Afr) by combining MA and Cau groups. In this study, it was inevitable that participants with latent diseases of common occurrence such as metabolic syndrome, inflammation, and anaemia were included in the sample population. Furthermore, there were participants who failed to adhere to the basal conditions required for this study or who were not truthful in answering the health screening questionnaire. Therefore, to overcome these inevitable problems, the latent abnormal value exclusion (LAVE) method was required for some analytes.[4,8] LAVE is an iterative optimisation method for refining RIs by excluding those subjects who have abnormal results in a set of reference anlaytes (other than the ones under derivation of the RIs). As the reference test items, we set 11 analytes namely: albumin (Alb), uric acid (UA), triglycerides (TG), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutemyl transferase (GGT), lactate dehydrogenase (LDH), creatinine kinase (CK), and c-reactive protein (CRP). We allowed up to one abnormal value in them.

The scheme for derivation of RIs and the 90% confidence interval (CI) of the RI upper and lower limits for this study was essentially the same as reported by Ichihara et al. in 2017.[8] RIs were determined using both the parametric (P) method and non-parametric (NP) method. For the P method, the RIs were transformed to a Gaussian distribution by making use of the modified Box-Cox power transformation equation. For the NP method, the RIs were first sorted and the 2.5 and 97.5 percentile points were determined. 90% CIs for P and NP methods were predicted by use of the bootstrap method with repeated resampling of 100 times. Therefore, the final RIs' low limit (LL), upper limit (UL) and their midpoint were smoothed by taking the averages of their resampled values.

Results

The overall number of participants recruited was 1 433. However, there was an imbalance in age distribution with a higher proportion in 18 to 29-year-olds of both genders. Therefore, for data analyses, we first applied a random filter to delete 188 participants to even out the sample size for each decade of age and gender. As another preliminary step for data validation, we scanned obvious extreme values analyte by analyte and found one participant with TG > 5.0 mmol/L, one participant with ALT and AST > 130 U/L, two participants with free thyroxine (FT4) > 18 pmol/L, one participant with Alb < 25 g/L, 28 participants with

Table I: General characteristics of participants

Race	Gender	*N*	Age					*P*	*N*	BMI	SDR	*N*	Waist - C	SDR	*N*	SBP	SDR	*N*	DBP	SDR	Smoke
			18 - 29	30 - 39	40 - 49	50 - 59	60 +														
Afr	**M**	291	77	79	60	57	18	0.281	285	26.1 ± 5.8	0.177	276	90 ± 14.6	0.000	171	130 ± 17.5	0.112	171	83 ± 10.5	0.000	6.6 %
	F	260	80	72	45	46	17		250	27.7 ± 5.9		246	89 ± 14.0		229	126 ± 19.5		228	82 ± 11.3		0.9 %
Mix	**M**	74	24	17	15	14	4	0.566	72	25.7 ± 3.9	0.000	71	89 ± 10.7	0.334	71	124 ± 14.7	0.124	71	84 ± 10.8	0.288	16.9%
	F	135	47	30	34	17	7		132	25.3 ± 4.6		130	83 ± 12.2		133	120 ± 18.3		133	80 ± 11.2		19.7%
Cau	**M**	125	44	28	28	19	6	0.317	124	26.0 ± 3.6	0.292	123	89 ± 11.3	**0.439**	124	129 ± 16.7	**0.643**	124	81 ± 11.6	0.245	10.8%
	F	258	90	49	46	46	27		256	24.3 ± 4.1		254	82 ± 11.5		254	115 ± 14.0		253	77 ± 10.1		9.0 %
Total		1143	362	275	228	199	79		1119	25.9 ± 5.2		1100	87 ± 13.3		982	123 ± 17.9		980	81 ± 11.1		10.3%

immunoglobulin G (IgG) > 30 g/L, and one participant with CRP > 60 mg/L. Therefore, we also deleted a total of 33 individuals with multiple extreme values in one or more analytes. Since we assumed the high IgG and low Alb is highly suggestive of latent infection, we applied another step to exclude individuals with IgG > 20 g/L and Alb < 30 g/L or CRP > 5.0 mg/L, which led to further reduction in data size of 69. Therefore, the final data size for use in the subsequent data analyses was 1 143 (1 43-188-33-69).

Table I summarises the population characteristics of the participants stratified according to ethnicity, gender, age, BMI, Waite-C, systolic blood pressure (SBP; mmHg), diastolic blood pressure (DBP; mmHg), and smoking habit (%). Out of 1 143 participants, 43% were males. The ethnic breakdown of

Table II: The magnitude of three major sources of variation: gender (SDR_{sex}), race (SDR_{rc}), and age (SDR_{age}) by use of 3 - level and 2 - level nested ANOVA respectively.

Analyte	3 level nested ANOVA			2 level nested ANOVA		
	SDR_{sex}	SDR_{rc} (M, F)	SDR_{age} (M, F)	SDR_{sex}	SDR_{rc} (M, F)	SDR_{age} (M, F)
TP	0.00	**0.52** (**0.49; 0.54**)	0.06 (0.04; 0.08)	0.00	**0.49** (**0.46**; **0.51**)	0.04 (0.00; 0.06)
Alb	**0.42**	0.16 (0.19; 0.11)	0.00 (0.32; 0.11)	**0.42**	0.20 (0.29; 0.14)	0.00 (0.33; 0.13)
Urea	0.00	0.47 (0.62; 0.27)	0.25 (0.21; **0.45**)	0.00	**0.49** (**0.65**; 0.30)	0.24 (0.22; **0.45**)
UA	**0.87**	0.12 (0.21; 0.00)	0.24 (0.28; **0.40**)	**0.88**	0.09 (0.20; 0.00)	0.24 (0.26**; 0.40**)
Cre	**1.00**	0.26 (0.33; 0.17)	0.00 (0.10; 0.06)	**1.01**	0.34 (0.33; 0.40)	0.00 (0.14; 0.05)
TBil	0.00	**0.51** (**0.67**; 0.37)	0.00 (0.24; 0.15)	0.12	**0.50** (**0.65**; 0.39)	0.00 (0.22; 0.15)
Na	0.03	0.04 (0.00; 0.00)	0.00 (0.34; 0.25)	0.03	0.04 (0.00; 0.00)	0.00 (0.33; 0.26)
K	0.00	0.21 (0.24; 0.18)	0.00 (0.03; 0.08)	0.00	0.18 (0.22; 0.16)	0.00 (0.00; 0.02)
Cl	0.00	0.15 (0.18; 0.06)	0.00 (0.34; 0.12)	0.00	0.12 (0.15; 0.06)	0.00 (0.33; 0.10)
Ca	0.32	0.00 (0.00; 0.00)	0.24 (0.22; 0.26)	0.32	0.00 (0.00; 0.00)	0.24 (0.20; 0.27)
IP	0.09	0.00 (0.00; 0.00)	0.30 (**0.40**; 0.38)	0.10	0.00 (0.00; 0.00)	0.30 (**0.42**; 0.37)
Mg	0.00	0.05 (0.04; 0.00)	0.00 (0.16; 0.23)	0.00	0.10 (0.13; 0.00)	0.00 (0.15; 0.23)
TCho	0.00	**0.50** (**0.53**; **0.50**)	**0.47** (**0.51**; **0.52**)	0.00	**0.43** (**0.47**; **0.41**)	**0.48** (**0.53**; **0.52**)
TG	0.24	0.00 (0.00; 0.00)	**0.49** (**0.48; 0.50**)	0.25	0.00 (0.00; 0.00)	**0.51** (**0.48**; **0.53**)
HDL-C	**0.37**	0.27 (0.00; 0.35)	0.00 (0.14; 0.15)	**0.38**	0.31 (0.00; 0.38)	0.00 (0.14; 0.11)
LDL-C	0.00	**0.63** (**0.72**; **0.56**)	0.25 (**0.41**; **0.47**)	0.00	**0.55** (**0.67**; **0.47**)	0.26 (**0.42**; **0.47**)
Lip	0.00	0.00 (0.00; 0.00)	0.00 (0.23; 0.22)	0.00	0.10 (0.00; 0.09)	0.00 (0.22; 0.22)
ALT	0.09	**0.50** (**0.62**; 0.37)	0.10 (0.18; 0.26)	0.23	**0.47** (**0.58**; 0.36)	0.07 (0.18; 0.24)
AST	0.33	0.23 (0.21; 0.35)	0.00 (0.25; 0.26)	0.34	0.23 (0.21; 0.34)	0.00 (0.25; 0.24)
ALP	0.24	0.24 (0.18; 0.22)	0.00 (0.16; **0.40**)	0.26	0.22 (0.15; 0.17)	0.00 (0.17; 0.41)
AMY	0.00	**0.60** (**0.84**; **0.59**)	0.00 (0.00; 0.12)	0.00	**0.59** (**0.85**; **0.59**)	0.00 (0.00; 0.11)
LDH	0.00	0.13 (0.20; 0.00)	0.00 (0.14; 0.17)	0.00	0.14 (0.18; 0.00)	0.00 (0.12; 0.18)
CK	**0.55**	0.23 (0.00; 0.33)	0.00 (0.24; 0.00)	**0.56**	0.21 (0.00; 0.31)	0.00 (0.24; 0.00)
GGT	0.08	0.07 (0.27; 0.18)	0.00 (0.31; 0.37)	0.09	0.05 (0.24; 0.14)	0.00 (0.31; 0.38)
CRP	0.00	0.22 (0.23; 0.21)	0.21 (0.20; 0.27)	0.00	0.22 (0.20; 0.24)	0.21 (0.18; 0.28)
ChE	0.00	**0.40** (**0.57**; 0.00)	0.21 (0.20; **0.42**)	0.15	0.35 (**0.53**; 0.00)	0.21 (0.18; **0.43**)
Fe	0.27	0.19 (0.28; 0.34)	0.00 (0.04; 0.00)	0.30	0.24 (0.27; 0.42)	0.00 (0.00; 0.10)
Ferritin	**0.71**	0.00 (0.00; 0.00)	**0.46** (0.22; **0.57**)	**0.73**	0.00 (0.11; 0.00)	**0.47** (0.23; **0.58**)
Tf	0.32	0.00 (0.00; 0.00)	0.11 (0.00; 0.28)	0.32	0.00 (0.00; 0.00)	0.13 (0.00; 0.30)
IgA	0.00	**0.49** (0.37; **0.56**)	0.00 (0.25; 0.31)	0.00	**0.48** (**0.43**; **0.52**)	0.00 (0.23; 0.31)
IgG	0.00	**0.46** (**1.05**; **0.94**)	0.00 (0.00; 0.06)	0.00	**0.45** (**1.06**; **0.96**)	0.00 (0.07; 0.00)
IgM	0.37	0.00 (0.17; 0.00)	0.26 (0.16; 0.33)	0.37	0.00 (0.18; 0.00)	0.26 (0.17; 0.33)
FT3	0.30	0.28 (0.18; 0.32)	0.00 (0.14; 0.16)	0.32	0.28 (0.16; 0.33)	0.00 (0.15; 0.18)
FT4	0.02	0.18 (0.00; 0.23)	0.15 (0.20; 0.13)	0.06	0.18 (0.00; 0.25)	0.17 (0.21; 0.15)
TSH	0.00	0.00 (0.00; 0.00)	0.00 (0.13; 0.11)	0.00	0.10 (0.00; 0.14)	0.00 (0.17; 0.08)
Thyro	0.00	0.13 (0.16; 0.14)	0.00 (0.00; 0.00)	0.00	0.12 (0.14; 0.12)	0.00 (0.00; 0.09)
TgAb	0.00	0.05 (0.10; 0.00)	0.04 (0.00; 0.09)	0.00	0.09 (0.16; 0.00)	0.00 (0.00; 0.07)
PSA	------	----- (0.00; N/A)	----- (**0.47;** N/A)	-----	----- (0.00; N/A)	----- (**0.46;** N/A)

the participants was: 551 (48%) Afr (294 males), 209 (18%) MA (74 males) and 383 (34%) Cau (125 males). Participants with a smoking habit were 10.3% overall with MA showing the highest percentage (males: 16.9% and females 19.7%).

As explained earlier, we evaluated SDR_{rc} in two ways: one by dividing RIs by three racial groups (Afr, Mix, and Cau) and the other by two racial groups (Afr and Nafr [Cau+Mix]), with derivation of SDR_{rc3} and SDR_{rc2}, respectively. For each method, we first applied 3-level nested ANOVA for gender, ethnic, and age groups (partitioned at 30, 40, and 50-years-old), then, applied 2-level nested ANOVA separately for each gender to derive gender-specific SDR_{rc} and SDR_{age}. The magnitude of three major sources of variation: gender, race, and age were calculated for each analyte as SDR_{sex}, SDR_{rc}, and SDR_{age} as shown in Table II. In

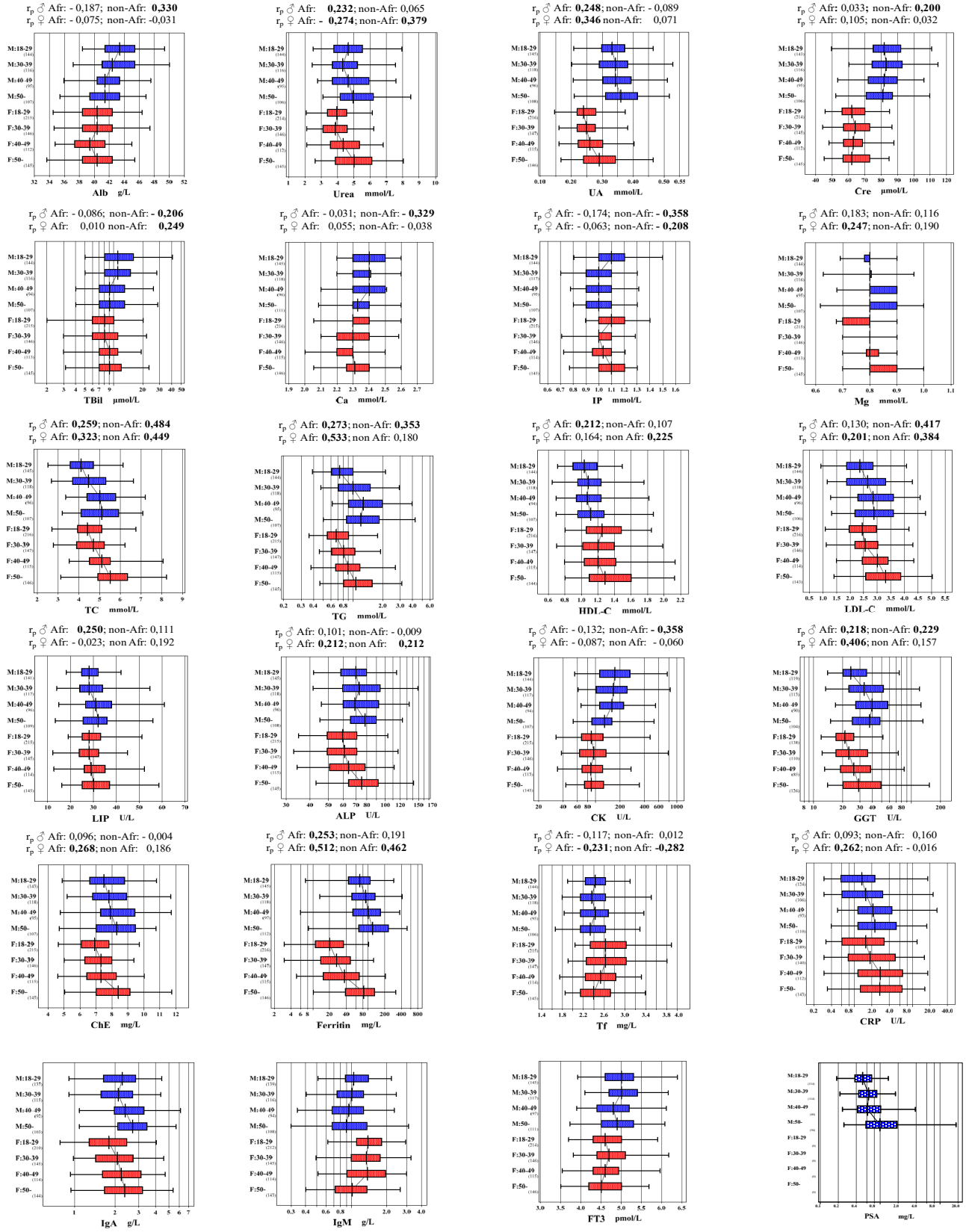

Figure 1: Gender related changes (24 analytes) and age related changes (8 analytes) of RIs.

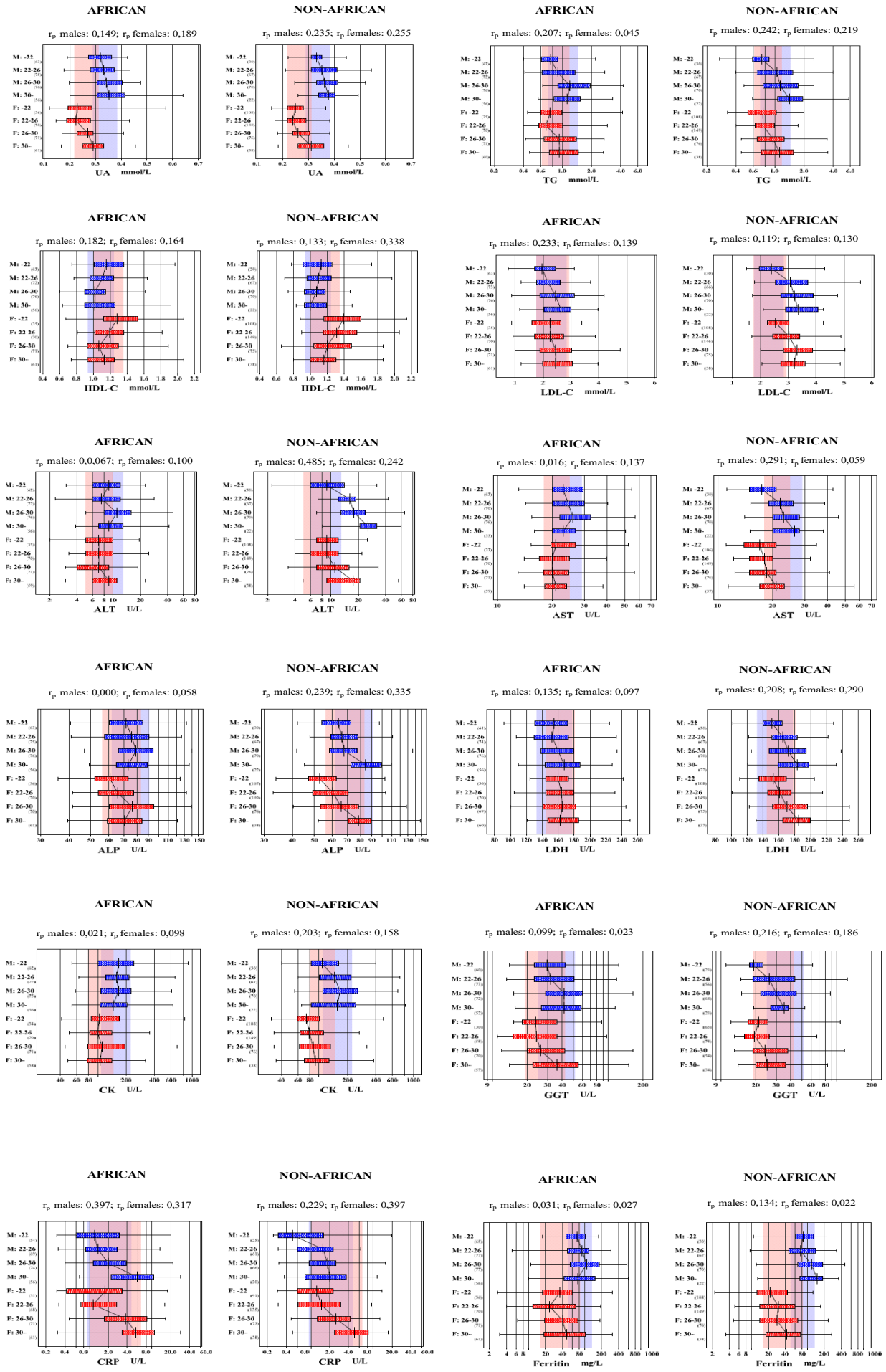

Figure 2: BMI related changeds of 12 analytes stratified according to gender and ethnicity

general, there were not many differences between SDR_{rc2} and SDR_{rc3} except for total bilirubin (TBil), LDL-C, amylase (AMY), and TC. However, because of smaller data sizes of RIs in the MA group, the final derivation of RIs, we adopted to two race group options for determining ethnic-specific RIs.

Although SDR_{age} was computed by stratifying RIs by the following four age groups: 18–29; 30–39; 40–49, and 50-years-old, because of too small a number in each subgroup, we chose to partition RIs at age 45. Gender-related changes were seen in 24 analytes namely: Alb, urea, UA, creatinine (Cre), TBil, calcium (Ca), inorganic phosphate (IP), magnesium (Mg), TC, TG, HDL-C, LDL-C, lipase (Lip), alkaline phosphatase (ALP), CK, GGT, cholinesterase (ChE), ferritin, transferrin (Tf), C-RP, immunoglobulin A (IgA), immunoglobulin M (IgM), free triiodothyronine (FT3) and PSA in males as seen in Figure 1. However, age-related RIs were derived for eight analytes namely: 1) in males: PSA; 2) in females: urea, UA, ALP, ChE and ferritin and 3) both genders: TC and LDL-C. Furthermore, partitioning by ethnicity of RIs was required for 10 analytes namely: total protein (TP), urea, TBili, TC, LDL-C, ALT, ChE, Amy, IgG and IgA. BMI-related changes were observed in 12 analytes when comparing Afr with non-Afr ethnic groups namely: UA, TG, HDL-C, LDL-C, ALT, AST, ALP, LDH, CK, GGT, C-RP and ferritin as seen in Figure 2. Figure 2 also shows the association of age and ethnicity separated by gender on test results by means of multiple regression analysis (MRA), whereby $r_p \geq 0.20$ was considered as significant. The RIs derived for South Africa can be seen in Table III for all analytes. To simply these findings, Table IV summarises the major analytes that require RIs separation for age, ethnicity, and gender.

The between-day and within-day CV SD of each analyte between both the DXC and AU analysers are shown in Table V. As described earlier, this project measured both panel I and panel II manufactured by C-RIDL on two separate occasions during the course of the study. In order to perform proper recalibration or alignment of results, the precision of RI limits, LL and UL were recalculated after calculating the RIs with or without LAVE. The LAVE+ methods were applied in seven analytes and these are shown in Table III.

Since the panel of sera has values assigned for confirming the status of standardisation of our assays, we compared our test results with them as shown in Figure 3 and Figure 4 respectively. The need for recalibration was judged by a ratio of bias for the lower limit (LL) or upper limit (UL), ΔLL or ΔUL, to the SD comprising the RI, which corresponds to between-individual SD, as follows:

$$\Delta LL\ ratio = | LL_{-} - LL_{+} | / (UL_{+} - LL_{+})/3.92$$

$$\Delta UL\ ratio = | UL — UL+ | / (UL+ - LL+)/3.92$$

where LL_{+} and LL_{-} (or UL_{+} and UL_{-}) represent LL (or UL) with and without recalibration. We set the critical value for ΔLL (or ΔUL) ratio as 0.375 in analogy to the theory of allowable analytical bias in laboratory tests because the numerator of ΔLL (or ΔUL) is a bias or change caused by the recalibration. By this reasoning, five

Table III: South African derived RI's all analytes all ethnic groups partitioned by age and gender. Values in gray background correspond to 90% confidence interval (CI) of the limits (Lower Limit = LL and Upper Limit = UL) of the RIs.

					LL 90% CI		RI			UL 90% CI	
Item	**Sex**	**Method**	**Age**	**N**	**LL-L**	**LL-H**	**LL**	**Mean**	**UL**	**UL-L**	**UL-H**
TP	**M + F**	**LAVE(–)**	All	1034	62	64	**63**	**72**	**82**	81	82
Alb	**M**	**LAVE(–)**	All	449	36	37	**37**	**42**	**49**	48	49
	F		All	587	34	35	**34**	**40**	**47**	46	47
Urea	**M**	**LAVE(–)**	All	449	2.56	2.79	**2.66**	**4.66**	**7.93**	7.63	8.32
	F		<45	402	2.01	2.29	**2.16**	**3.92**	**6.29**	6.01	6.57
	F		≥45	196	2.39	2.78	**2.55**	**4.72**	**8.15**	7.70	8.74
UA	**M**	**LAVE(–)**	All	448	196	226	**208**	**344**	**489**	474	508
	F		<45	405	145	167	**156**	**249**	**373**	357	391
	F		≥45	195	169	195	**176**	**279**	**466**	441	497
Cre	**M**	**LAVE(–)**	All	448	53	58	**55**	**82**	**110**	107	113
	F		All	585	44	47	**45**	**63**	**86**	84	88
TBil	**M + F**	**LAVE(–)**	All	1017	3.1	4.1	**3.8**	**9.0**	**22.2**	20.7	24.5
Na	**M + F**	**LAVE(–)**	All	1017	130	131	**131**	**139**	**144**	143	144
K	**M + F**	**LAVE(–)**	All	1036	3.6	3.6	**3.6**	**4.3**	**5.1**	5.1	5.2
Cl	**M + F**	**LAVE(–)**	All	1029	96	98	**97**	**105**	**112**	111	112
Ca	**M + F**	**LAVE(–)**	All	1035	2.07	2.12	**2.10**	**2.34**	**2.58**	2.57	2.60
IP	**M + F**	**LAVE(–)**	All	1037	0.75	0.79	**0.76**	**1.07**	**1.36**	1.34	1.39
Mg	**M + F**	**LAVE(–)**	All	1037	0.52	0.67	**0.65**	**0.81**	**0.94**	0.92	0.96
TC	**M + F**	**LAVE(–)**	<45	716	2.70	2.92	**2.81**	**4.51**	**6.53**	6.34	6.84
	M + F		≥45	337	3.02	3.34	**3.17**	**5.30**	**7.66**	7.42	7.95
TG	**M + F**	**LAVE(+)**	<45	621	0.38	0.43	**0.41**	**0.80**	**2.42**	2.13	2.67
	M + F		≥45	265	0.48	0.57	**0.50**	**1.06**	**2.90**	2.53	3.40
HDL-C	**M**	**LAVE(–)**	All	446	0.67	0.76	0.71	1.08	1.64	1.56	1.73
	F		All	583	0.74	0.82	0.78	1.24	1.99	1.93	2.07
LDL-C	**M + F**	**LAVE(–)**	<45	719	1.02	1.18	**1.11**	**2.54**	**4.20**	4.04	4.36
	M + F		≥45	337	1.24	1.50	**1.40**	**3.04**	**5.12**	4.88	5.34

Table III: Continued

					LL 90% CI			RI		UL 90% CI	
Item	**Sex**	**Method**	**Age**	**N**	**LL-L**	**LL-H**	**LL**	**Mean**	**UL**	**UL-L**	**UL-H**
AST	**M**	**LAVE(+)**	All	373	14	15	**14**	**23**	**42**	40	45
	F		All	509	12	13	**12**	**19**	**31**	29	33
ALT	**M**	**LAVE(+)**	All	367	2.9	4.7	**3.8**	**11.8**	**40.8**	33.6	47.4
	F		All	500	2.7	3.4	**2.8**	**8.2**	**22.3**	20.7	24.5
LDH	**M + F**	**LAVE(–)**	All	1031	103	113	**109**	**162**	**229**	223	235
ALP	**M**	**LAVE(–)**	All	443	44	49	**47**	**73**	**126**	119	135
	F		<45	392	35	40	**37**	**60**	**110**	101	121
	F		≥45	191	37	49	**45**	**74**	**135**	123	155
GGT	**M**	**LAVE(+)**	All	341	14	15	**14**	**32**	**99**	87	111
	F		<45	253	11	14	**13**	**22**	**66**	56	79
	F		≥45	125	13	14	**14**	**27**	**90**	73	110
CK	**M**	**LAVE(+)**	All	366	52	65	**58**	**156**	**569**	481	668
	F		All	506	35	44	**39**	**89**	**271**	240	341
LIP	**M + F**	**LAVE(–)**	All	1017	15	19	**17**	**29**	**50**	47	52
ChE	**M**	**LAVE(–)**	All	450	4.65	5.23	**4.88**	**7.91**	**11.4**	11.0	11.8
	F		<45	404	4.39	4.85	**4.62**	**7.09**	**9.6**	9.3	9.9
	F		≥45	196	4.52	5.41	**4.86**	**7.98**	**11.3**	10.9	11.9
CRP	**M + F**	**LAVE(+)**	All	822	0.29	0.34	**0.32**	**1.79**	**17.76**	15.02	21.14
Fe	**M + F**	**LAVE(–)**	All	1032	4.7	6.3	**5.6**	**15.2**	**30.2**	29.4	31.6
Ferritin	**M**	**LAVE(+)**	All	447	9.2	19.0	**13.1**	**90**	**374**	331	443
	F		<45	345	2.8	4.5	**3.1**	**23**	**112**	90	134
	F		≥45	149	5.3	9.9	**7.0**	**65**	**247**	209	286
Tf	**M + F**	**LAVE(–)**	All	1036	1.82	1.90	**1.85**	**2.48**	**3.53**	3.45	3.63
IgA	**M + F**	**LAVE(–)**	All	1013	0.9	0.9	**0.9**	**2.2**	**4.8**	4.6	5.1
IgG	**M + F**	**LAVE(–)**	All	1016	7.3	7.8	**7.6**	**12.5**	**19.3**	19.0	19.8
IgM	**M**	**LAVE(–)**	All	433	0.35	0.42	**0.39**	**1.00**	**2.21**	2.06	2.41
	F		All	576	0.45	0.54	**0.50**	**1.28**	**2.89**	2.77	3.08
FT3	**M + F**	**LAVE(–)**	All	1032	3.68	3.85	**3.76**	**4.77**	**6.03**	5.95	6.17
FT4	**M + F**	**LAVE(–)**	All	1035	7.7	8.0	**7.8**	**10.5**	**13.9**	13.6	14.2
TSH	**M + F**	**LAVE(–)**	All	847	0.41	0.50	**0.46**	**1.36**	**3.63**	3.40	3.94
Thyro	**M + F**	**LAVE(–)**	All	858	0.00	0.00	**0.00**	**0.13**	**1.11**	0.81	2.23
TgAb	**M + F**	**LAVE(–)**	All	960	0.00	0.04	**0.01**	**0.62**	**4.70**	3.29	336
PSA	**M**	**LAVE(–)**	<45	287	0.20	0.24	**0.22**	**0.57**	**1.71**	1.51	1.96
	M		≥45	119	0.14	0.29	**0.21**	**0.93**	**9.02**	5.14	15.06

Table IV: Selected RIs partitioned by ethnicity, gender and age; Afr versus non – Afr. Values in gray background correspond to 90% confidence interval (CI) of the limits (Lower Limit = LL and Upper Limit=UL) of the RIs.

						AFRICAN							NON - AFRICAN							
						LL 90% CI		RI			UL 90% CI			LL 90% CI		RI			UL 90% CI	
Item	**Units**	**Method**	**Gender**	**Age**	**n**	**LL-L**	**LL-H**	**LL**	**Mean**	**UL**	**UL-L**	**UL-H**	**n**	**LL-L**	**LL-H**	**LL**	**Mean**	**UL**	**LL-L**	**LL-H**
TP	g/L	LAVE (-)	**M + F**	All	502	**63**	**66**	64	74	83	**83**	**85**	532	**62**	**63**	62	70	78	**77**	**79**
			M	All	626	**2.38**	**2.69**	2.51	4.22	6.93	**6.59**	**7.58**	185	**3.06**	**3.45**	3.28	5.36	8.31	**7.90**	**8.92**
Urea	mmol/L	LAVE (-)	**F**	< 45	185	**1.75**	**2.15**	3.28	5.36	8.31	**5.71**	**6.61**	165	**2.32**	**2.64**	1.95	3.6	6.14	**5.96**	**6.80**
				≥ 45	165	**2.29**	**2.97**	1.95	3.6	6.14	**7.30**	**9.04**	238	**2.33**	**3.06**	2.48	4.14	6.37	**7.55**	**8.68**
Cre	µmol/L	LAVE (-)	**M**	All	263	**55**	**62**	58	83	114	**110**	**120**	184	**62**	**74**	69	91	117	**113**	**122**
			F		184	**46**	**50**	69	91	117	**87**	**93**	238	**50**	**54**	48	65	89	**89**	**95**
TBil	µmol/L	LAVE (-)	**M + F**	All	499	**2.9**	**4.2**	3.5	7.8	19.1	**17.3**	**21.3**	524	**3.1**	**4.9**	4.3	10.5	24.1	**22.1**	**28.4**
TC	mmol/L	LAVE (-)	**M + F**	< 45	347	**2.42**	**2.72**	2.59	4.12	6.05	**5.87**	**6.31**	369	**3.27**	**3.56**	3.42	4.86	6.82	**6.58**	**7.20**
			M + F	≥ 45	164	**2.86**	**3.24**	3.04	4.76	7.39	**7.06**	**7.80**	173	**3.67**	**4.17**	3.90	5.73	7.81	**7.49**	**8.18**
LDL-C	mmol/L	LAVE (-)	**M + F**	< 45	348	**0.80**	**1.00**	0.9	2.2	3.67	**3.55**	**3.79**	371	**1.59**	**1.75**	1.65	2.83	4.47	**4.32**	**4.72**
			M + F	≥ 45	371	**1.15**	**1.41**	1.65	2.83	4.47	**4.41**	**5.05**	164	**1.80**	**2.17**	1.27	2.51	4.68	**5.02**	**5.65**
ALT	IU/L	LAVE (+)	**M**	All	211	**2.8**	**4.8**	3.8	10.3	31	**25.9**	**38.8**	155	**4.1**	**8.3**	6.7	19.9	53	**45.6**	**65.0**
			F		155	**2.4**	**3.1**	6.7	19.9	53	**18.4**	**23.7**	207	**3.1**	**4.4**	2.6	7.8	21.1	**28.1**	**34.3**
AMY	IU/L	LAVE (-)	**M + F**	All	502	**39**	**46**	41	84	161	**152**	**173**	526	**25**	**31**	28	59	111	**104**	**117**
ChE	kU/L	LAVE (-)	**M**	All	264	**4.5**	**5.1**	4.7	7.4	10.6	**10.2**	**11.0**	185	**5.5**	**6.3**	5.8	8.7	11.9	**11.4**	**12.4**
			F		185	**4.2**	**4.8**	4.5	7.1	10.1	**9.8**	**10.4**	240	**4.6**	**5.2**	4.9	7.4	10.6	**10.3**	**11.0**
IgG	g/L	LAVE (-)	**M + F**	All	483	**9.8**	**10.3**	10.1	14.5	19.6	**19.3**	**20.1**	531	**7.0**	**7.5**	7.3	10.9	16.9	**16.4**	**17.6**
IgA	g/L	LAVE (-)	**M + F**	All	483	**1.0**	**1.2**	1.1	2.5	5.3	**4.9**	**5.7**	528	**0.8**	**0.9**	0.8	1.8	4.2	**4.0**	**4.5**

Table V: List of analytes with methods and between run SD and CV; comparison between the Beckman Coulter DXC,AU and DXI analysers shown respectively.

			Beckman Coulter DXC		Beckman Coulter AU	
Analyte	**Abbreviation**	**Method**	**Between Run SD**	**Between Run CV**	**Between Run SD**	**Between Run CV**
Sodium	Na	Ion selective electrode / diluted (indirect)	1.7	0.0	1,2	0,0
Potassium	K	Ion selective electrode / diluted (indirect)	0.3	0.1	0,2	0,1
Chloride	Cl	Ion selective electrode / diluted (indirect)	2.4	0.0	1,6	0,0
Urea	Urea	Urease	1.3	0.3	0,4	0,1
Creatinine	Cre	Modified kinetic Jaffè	11.7	0.2	13,0	0,2
Total Protein	TP	Buiret	4.7	0.1	2,8	0,0
Albumin	Alb	Bromocresol Green dye binding	1.8	0.0	1,6	0,0
Total Bilirubin	Tbil	Diazonium salt	2.4	0.3	4,4	0,5
GGT	GGT	Gamma-glutamyl-3-carboxy-4-nitroanilide	16.3	0.7	9,0	0,4
ALP	ALP	P-nitro-phenylphosphate hydrolysis	19.5	0.3	8,2	0,1
LD	LD	Lactate to Pyruvate	35.5	0.2	14,4	0,1
Calcium	Ca	Arsenazo III dye	0.1	0.0	0,1	0,0
Magnesium	Mg	Xylidyl blue	0.1	0.1	0,0	0,1
Phosphate	IP	Molybdate hydolysis	0.1	0.1	0,0	0,0
Glucose	GLU	Hexokinase	0.9	0.2	0.8	0.1
Lipase	Lip	1, 2-Diglyceride hydrolysis	18.5	0.4	13,2	0,5
Cholesterol	TC	Cholesterol oxidase	1.0	0.2	1,6	0,3
Triglycerides	Trig	Glycerol phosphate oxidase	0.6	0.4	0,5	0,4
HDL-C	HDL-C	Two phase selective accelerator detergent	0.5	0.4	0,5	0,3
LDL-C	LDL-C	Two phase selective accelerator detergent	0.8	0.3	1,1	0,4
Uric acid	UA	Modified Trinder reaction with Uricase	0.1	0.2	0,0	0,1
hsCRP	C-RP	Turbidimetry	3.4	0.8	2,0	0,8
Amylase	AMY	2-chloro-4-nitrophenyl-α-D-maltotrioside	20.2	0.3	21,4	0,4
IgA	IgA	Turbidimetry	0.5	0.3	1,3	0,5
IgG	IgG	Turbidimetry	4.5	0.4	1,5	0,2
IgM	IgM	Turbidimetry	0.3	0.3	0,5	0,5
ALT	ALT	NADH (without P-5—P)	10.7	0.8	17,4	0,8
AST	AST	NADH (without P-5—P)	4.5	0.2	8,9	0,4
Cholinesterase	Che	Butyrylthiocholine hydrolysis	1.2	0.2	1.2	0.2
CK	CK	Creatine phosphate dephosphorilysation	65.0	0.5	119,0	0,6
Iron	Fe	2, 4, 6-Tri-(2-pyridyl)-5-triazine chromogen	8.6	0.6	4,1	0,2
Transferrin	Tf	Turbidimetry	4.0	1.3	0,4	0,2
			Beckman Coulter DXI			
			Between Run SD	**Between run CV**		
TG-Ab	TG-Ab	Two-site immune – enzymatic immunoassay	7.4	4.8		
TPO-Ab	TPO-Ab	Two-site immune – enzymatic immunoassay	1.1	1.1		
TSH	TSH	Two-site immune – enzymatic immunoassay	0.7	0.5		
FT4	FT4	Two-site immune – enzymatic immunoassay	1.5	0.1		
FT3	FT3	Two-site immune – enzymatic immunoassay	0.6	0.1		
Ferritin	Fer	Turbidimetry	40.3	0.6		
PSA	PSA	Two-site immune – enzymatic immunoassay	0.6	1.6		

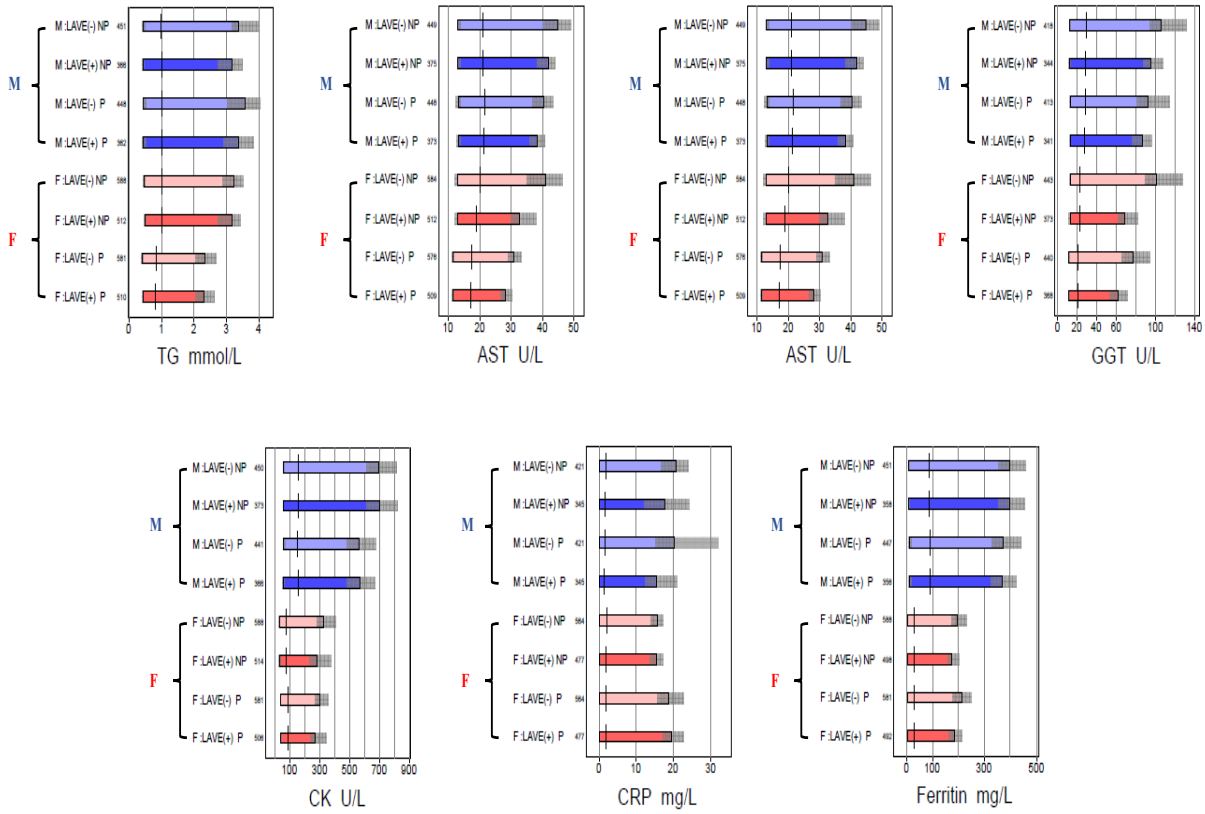

Figure 3: Analytes whereby LAVE (+) was applied in the derivation of RIs stratified by gender; P methods versus NP method.

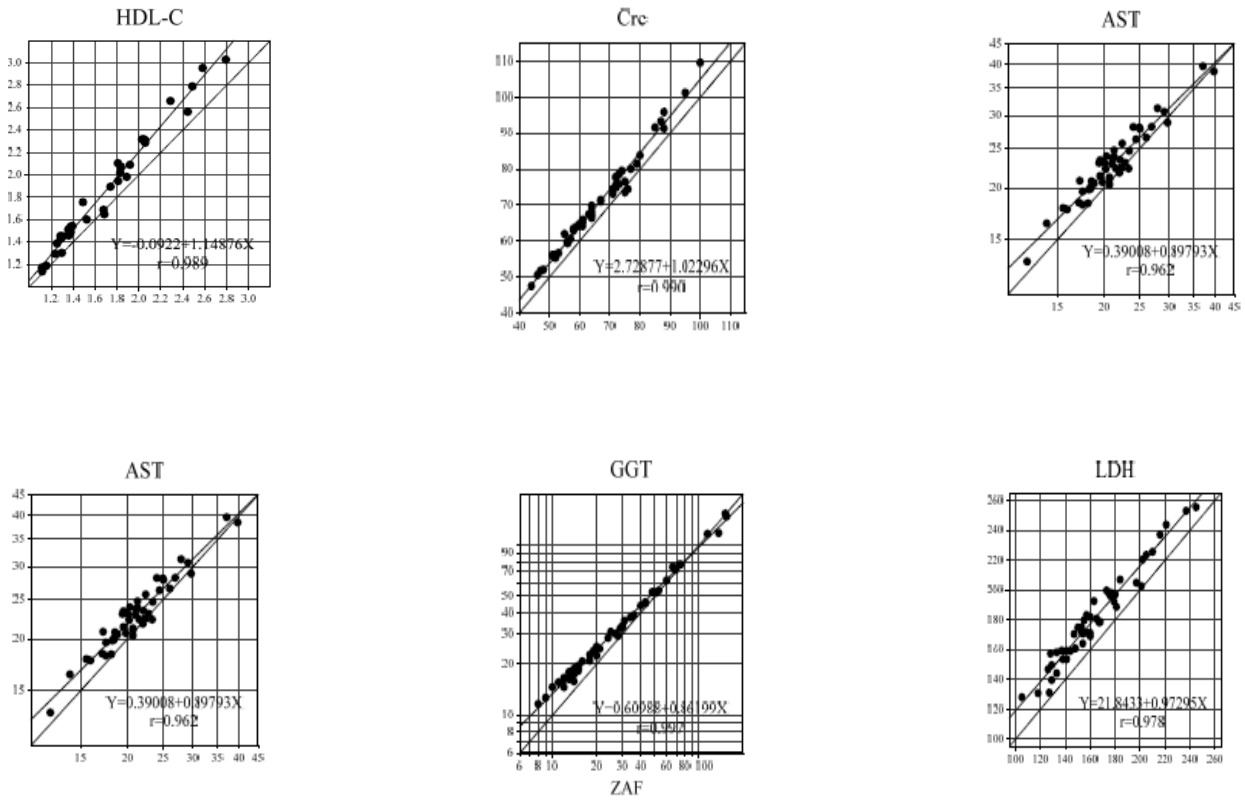

Figure 4: RIs requiring recalibration after comparison of test results with assigned values for panel of sera: HDL in panel of sera I and Cre, AST, ALT, GGT and LDH in panel of sera II.

analytes, Cre, AST, ALT, LDH, and GGT, were judged as requiring recalibration of RIs based on the linear regression coefficients.

Discussion

As a part of the global multi-centre collaborative project for derivation of RIs, this study was the largest and first study of this nature for the South African population to establish RIs for the majority of general biochemistry analytes. Moreover, this study was conducted using a standardised and harmonised protocol.[1] In this study, we successfully derived RIs for the South African population by comparing the values obtained from three ethnic groups. We proved that the P method is superior in the determination of RIs to the NP. Furthermore, we also showed the need for LAVE in derivation of RIs. Previous reports have shown that the P method based on the modified Box-Cox formula remains the method of choice over and above the NP method for the derivation of RIs in almost all settings.[8]

The use of LAVE has several advantages namely: 1) truncation of reference distributions does not occur, because this method excludes those participants whose results are outside of the RI of any given analyte, other than the analyte for which the RI is being derived, and 2) there is no effect at all on analytes whose values are on rare occasions just outside the normal RI of the analyte.[8] There is, however, a disadvantage of using LAVE because when applied it causes a reduction in the number of participants whose results are included in the final computation of the RIs.

During the process of defining RIs for this project, the investigators had to adopt a strict policy whereby the abnormal results of participants had to be excluded due to underlying disease. Many pathological conditions exist that can influence each analyte value.[2] However, we presumed that if this prevalence is low amongst apparently healthy individuals, the abnormal results from such participants will not have a major influence on the determination of RIs. Careful considerarion was required for those participants with latent but highly prevalent disorders such as metabolic syndrome and undiagnosed diabetes mellitus in the MA population group in South Africa.[9] Therefore, the LAVE method was applied in our study to exclude those participants with abnormal results attributed to those conditions as the performance of LAVE done in previous studies of this nature were well characterised.[2,10,11]

Ethnic differences in RIs were noted in many analytes. The most conspicuous were ALT, LDL-C, and IgG. For ALT, there have been many reports from epidemiological data based on population sampling schemes whereby very low ALT RIs were observed in African Americans which, in turn, makes them less susceptable to non-alcoholic fatty liver disease.[12-15] In this study, we confirmed the tendency of low ALT among Afr participants from apparently heatlhy individuals. The investigators thereby deduct from this finding that a possible genetic factor might contribute to lower ALT in the Afr population, regardless of their location globally. For LDL-C, there were significant ethnic differences seen in RIs between Afr and non-Afr. In the non-Afr group the RIs were higher in both males and females in comparison to the Afr population. This tendency has also been shown in other reports.[16,17]

The differences in RIs between Cau and MA populations were generally smaller when compared to Afr. In this study we were able to combine the values for Cau and MA to determine generic RIs for these combined population groups which were named non-Afr. Previous research has also demonstrated that the MA population of the Western Cape shares similar RIs to those of Cau descent.[18] The MA people of Cape Town are a unique population group comprised of the original indigenous Khoisan people (32–43%), Bantu-speaking Afr (20–36%), Europeans (21–28%) and Asians (9–11%).[19] According to the last census of 2011, this group makes up 48% of the Western Cape population.[20] Although previous studies have reported similar findings in Hispanics who are also of MA,[21] studies in southern Africa have usually combined those of MA with those of Cau.[22] This study therefore adds valuable information for this region and could be significant for other MA populations in Africa.

Significantly higher AMY RIs were seen in both males and females in Afr when compared to the non-Afr group. Perry et al. showed that the copy number of the salivary amylase gene (*AMY1*) is correlated positively with salivary amylase protein level and that individuals from populations with high-starch diets have, on average, more *AMY1* copies than those with traditionally low-starch diets.[23] Rural and urban Afr have been reported to consume large quantities of starchy foods prepared from maize, potatoes, rice and bread.[24] One may therefore surmise from these studies that the high AMY RIs in Afr may likely be due to the differences in diet.

This study has also shown that partitioning of RIs by gender was indicated in six analytes namely: Alb, UA, Cre, HDL-C, CK and ferritin (Ferr). The same findings were seen when compared to the global study either in a prominent, or moderate manner.[15] Furthermore, it has been reported that females have more age-related changes in RIs, especially after menopause.[15] Thus menopause-specific RIs have been recommended for many diagnostic biochemical tests when interpreting results for optimal patient management.[25] Unique observations seen in this study were a high BMI r_p for T3 in non-Afr, a strong negative r_p of age for CK in non-Afr and a low r_p of age for LDH in both ethic groups.

Three Asian studies revealed large regional differences in IgG among healthy individuals.[7,26,27] The same finding was seen in the harmonised global study[15] as well as in this study. This is probably due to latent infection that could not be elucidated in this study. One of the challenges in this study was to determine which participants to exclude due to the presence of underlying latent disease. By utilising cut-off values for C-RP, Alb and IgG as described earlier, this limitation was overcome with great success.

The major strength of this study, which has been different from previous ones in this region, is that the MA participants were separated from those of Cau descent rather than grouping these two important population groups together. In addition, the strict guidelines of the C-RIDL were followed ensuring that only healthy individuals were included. This ensures that the

results can be compared to other countries participating in the international multi-centre project (Omuse, et al., 2018). Future research should aim to include higher numbers of MA and Cau population groups and strive to generate RIs for adolescents and those younger than 18 years.

Africa has many diverse population groups, and it has been recommended that RIs be generated for each region on the continent. The demographics of South Africa, Africa and the world are constantly changing and therefore laboratory professionals should be constantly aware of the influence gender, ethnicity, age and BMI have on the interpretation of laboratory results which has further been substantiated in other research.[18]

Conclusion

In conclusion, our study showed that partitioning of RIs by gender, ethnicity and age was necessary for many analytes. Furthermore, we also showed that BMI has an effect in some analytes in this South African population. Regarding the controversies over the use of optimal statistical methods for derivation of the RIs, this study supported the use of the P method over NP method as being more optimal and the need for application of LAVE method for analytes which are easily influenced by the presence of latent infection among apparently healthy individuals. Further investigation into ethnic differences in RIs would be of great value when comparing South Africa's results with those of the other ongoing projects in Africa, particularly considering that South Africa is the destination of choice in Africa for medical treatment.

Data availability

Data from this study is available through a secure online platform upon reasonable requests directed to the corresponding author (FCS) and after approval of a proposal.

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Conflict of interest

The authors declare no conflict of interest.

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Ethics

Ethics approval was obtained from the Faculty of Health and Wellness Sciences' Research and Ethics Committee, Cape Peninsula University of Technology, Reference Number: CPUT/NHREC: REC-230408-014 as well as the University of Stellenbosch's Health Research Ethics Committee protocol number: S12/05/147.

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References

1. Ozarda Y, Ichihara K, Barth J, Klee G. Committee on reference intervals and decision limits (C-RIDL), IFCC. Protocol and standard operating procedures for common use in the worldwide multicenter study on reference values. Clin Chem Lab Med. 2013;51:1027-1040. https://doi.org/10.1515/cclm-2013-0249.
2. Borai A, Ichihara K, Masaud AA, et al. Establishment of reference intervals of clinical chemistry analytes for the adult population in Saudi Arabia: a study conducted as part of the IFCC global study on reference values. Clin Chem Lab Med. 2016;54:843-855. https://doi.org/10.1515/cclm-2015-0490.
3. Johnson AM, Petersen PH, Whicher JT, Carlstrom A, MacLennan S. Reference intervals for serum proteins: similarities and differences between adult Caucasian and Asian Indian males in Yorkshire, UK. Clin Chem Lab Med. 2004;42:792-799. https://doi.org/10.1515/CCLM.2004.132.
4. Ichihara K, Boyd J, on behalf of IFCC Committee on reference intervals and decision limits (C-RIDL). An appraisal of statistical procedures used in derivation of reference intervals. Clin Chem Lab Med. 2010;48:1537-1551. https://doi.org/10.1515/CCLM.2010.319.
5. Ichihara K. Statistical considerations for harmonization of the global multicenter study on reference intervals. Clin Chim Acta. 2014;432:108-118. https://doi.org/10.1016/j.cca.2014.01.025.
6. Cois A, Day C. Obesity trends and risk factors in the South African adult population. BMC Obes. 2015;2:42. https://doi.org/10.1186/s40608-015-0072-2.
7. Ichihara K, Ceriotti F, Kazuo M, et al. The Asian project for collaborative derivation of reference intervals: (2) results of non-standardized analytes and transference of reference intervals to participating laboratories on basis of cross-comparison of test results. Clin Chem Lab Med. 2013;51:1443-1457. https://doi.org/10.1515/cclm-2012-0422.
8. Ichihara K, Orzada Y, Barth JH, et al. A global multicenter study on reference values: 1. Assessment of methods for derivation and comparison of reference intervals. Clin Chim Acta. 2017;467:70-82. https://doi.org/10.1016/j.cca.2016.09.016.
9. Erasmus RT, Soita DJ, Hassan MS, et al. High prevalence of diabetes mellitus and metabolic syndrome in a South African coloured population: Baseline data of a study in Bellville, Cape Town. S Afr Med J. 2012;102:841-844. https://doi.org/10.7196/SAMJ.5670.

10. Ozarda Y, Ichihara K, Aslan D, et al. A multicenter nationwide reference intervals study for common biochemical analytes in Turkey using Abbott analyzers. Clin Chem Lab Med. 2014;52:1823-1833. https://doi.org/10.1515/cclm-2014-0228.
11. Yamakadoa M, Ichihara K, Matsumoto Y, et al. Derivation of gender and age-specific reference intervals from fully normal Japanese individuals and the implications for health screening. Clin Chim Acta. 2015:447:105-114. https://doi.org/10.1016/j.cca.2015.04.037.
12. Browning JD, Szczepaniak LS, Dobbins R, et al. Prevalence of hepatic stenosis in an urban population in the United States: impact of ethnicity. Hepatology. 2004;40:1387-1395. https://doi.org/10.1002/hep.20466.
13. Pan JJ, Fallon MB. Gender and racial differences in non-alcoholic fatty liver disease. World J Hepatol. 2014;6:274-283. https://doi.org/10.4254/wjh.v6.i5.274.
14. Guerrero R, Vega GL, Grundy SM, Browning JD. Ethnic differences in hepatic stenosis: an insulin resistance paradox? Hepatology. 2009;49:791-801. https://doi.org/10.1002/hep.22726.
15. Ichihara K, Ozarda Y, Barth JH, et al. A global multicenter study on reference values: 2. Eploration of sources of variation across countries. Clin Chim Acta. 2017;467:83-97. https://doi.org/10.1016/j.cca.2016.09.015.
16. Ellman N, Keswell D, Collins M, Tootla M, Goedecke JH. Ethnic differences in the association between lipid metabolism genes and lipid levels in black and white South African women. Atherosclerosis. 2015:240:311-317. https://doi.org/10.1016/j.atherosclerosis.2015.03.027.
17. Sliwa K, Lyons JG, Carrington MJ, et al. Different lipid profiles according to ethnicity in the heart of SOWETO study cohort of de novo presentations of heart disease. Cardiovasc J Afr. 2012;23:389-395. https://doi.org/10.5830/CVJA-2012-036.
18. Smit FC, Davison GM, Hoffmann M, et al. Full blood count and white cell differential count reference ranges obtained from a healthy urban South African population residing in the Western Cape of South Africa. International Journal of Laboratory Haematology 2019;00:1-7. https://doi.org/10.1111/ijlh.13076.
19. De Wit E, Delport W, Rugamika C, et al. Genome-wide analysis of the structure of the South African Coloured Population in the Western Cape. Human Genetics. 2010;128(2):145-153. https://doi.org/10.1007/s00439-010-0836-1.
20. Statistics South Africa. 2011. Provincial Profile: Western Cape.; Report No: 03-01-70 (2011).
21. Lim E, Miyamura J, Chen J. Racial/ethnic-specific reference intervals for common laboratory tests: a comparison among Asians, Blacks, Hispanics, and White. Hawaii J Med Public Health. 2015;74(9):302-310.
22. Lawrie D, Coetzee L, Becker P, et al. Local Reference Ranges for Full Blood Count and CD4 Lymphocyte Count Testing. S Afr Med J. 2009;99(4):243-248.
23. Perry GH, Dominy NJ, Claw KG, et al. Diet and the evolution of human amylase gene copy number variation. Nat Genet. 2007;39:1256-1260. https://doi.org/10.1038/ng2123.
24. Love P, Maunder E, Green M, et al. South African food-based dietary guidelines: Testing of the preliminary guidelines among women in KwaZulu-Natal and the Western Cape. S Afr Clin Nutr. 2001;14:9-19.
25. Honour JW. Biochemistry of menopause. Ann Clin Biochem. 2018;55:18-33. https://doi.org/10.1177/0004563217739930.
26. Ichihara K, Itoh Y, Lam CWK, et al. Sources of variation of commonly measured serum analytes in 6 Asian cities and consideration of common reference intervals. Clin Chem. 2008;54:356-365. https://doi.org/10.1373/clinchem.2007.091843.
27. Ichihara K, Itoh Y, Min K, et al. Diagnostic and epidemiological implications of regional differences in serum concentrations of proteins observed in six Asian cities. Clin Chem Lab Med. 2004;42:800-809. https://doi.org/10.1515/CCLM.2004.133.