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ESTABLISHING REFERENCE INTERVALS (RIS) FOR NEPALESE POPULATION: A PROTOCOL AND PROCEDURE.

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ABSTRACT
The reference intervals (RIs) given in laboratory reports have an important role for a clinicians to interpret test results in reference to values obtained for healthy populations. In Nepal reference values used in laboratories is either from scientific literature or from kit insert by manufacturers which have been established for Western or European population. It is well known that population across the globe differs physiologically, genetically; ethnically, their lifestyle, food habits and diet have great impact on the reference values. Thus, it is inappropriate to use RIs that do not represent the local population. According to International Federation for Clinical Chemistry (IFCC), each laboratory has to develop its own reference RI. This approach highlights protocol and standard operating procedure (SOPs) for conducting RI studies for a small laboratory as well as on a national scale to establish reference interval for Nepalese population using the IFCC-CRIDL recent guidelines (C28-A3).1-7

KEYWORDS: Reference value, reference range, IFCC, CLSI, Reference interval, Protocol.

INTRODUCTION
Reference interval (RIs) is the range of values provided by laboratory scientists in a convenient and practical form to support clinician in interpreting observed values for diagnosis, treatment and monitoring of a disease. Health of an individual is different in different countries, in the same country at different times and in the same individual at different ages. Thus it is relative not an absolute state. Therefore condition of individuals obtained during medical interview, clinical examination, and laboratory investigation must be compared with reference data. A standard protocol and procedure describing theory and practical aspects of reference value determination is needed to provide reliable basis for establishing reference interval.

Clinical laboratories in Nepal are depends on the RIs, provided in the kit inserts by the manufacturers or scientific literature which is established for Western/European population. It is well known that population across the globe differs physiologically, genetically; race, ethnically, lifestyle, food habits and diet have great impact on the reference values. Thus, it is inappropriate to use RIs that do not represent the local population. This article highlights procedures for establishing reference values in Nepalese population using the IFCC-CRIDL guidelines.(C28-A3).

In 20th century the term "Reference Value" was first introduced by Ralph Grasbeck,Fellman and Nils-Erik Saris in 1969[8]1 They published a paper entitled 'Normal Values and Statistics' as an initial study in the field of reference intervals (RIs)[9] In subsequent years it was realized that the terminology of 'normal values' was not adequate and even partially incorrect, so the term 'reference values' came into use. From 1987 to 1991, the International Federation of Clinical Chemistry (IFCC) published a series of 6 papers, recommending that each lab-oratory should produce its own reference range following the IFCC-CRIDL and CLSI guidelines.[10-15] This guideline entitled 'Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory' provides the necessary steps mainly for the selection of reference individuals, pre-analytical, analytical considerations and analysis of reference values for a RI establishment study.[16]

IFCC has coined some concise and well defined terms that permit unambiguous description and discussion on the subjects of reference values.[1]

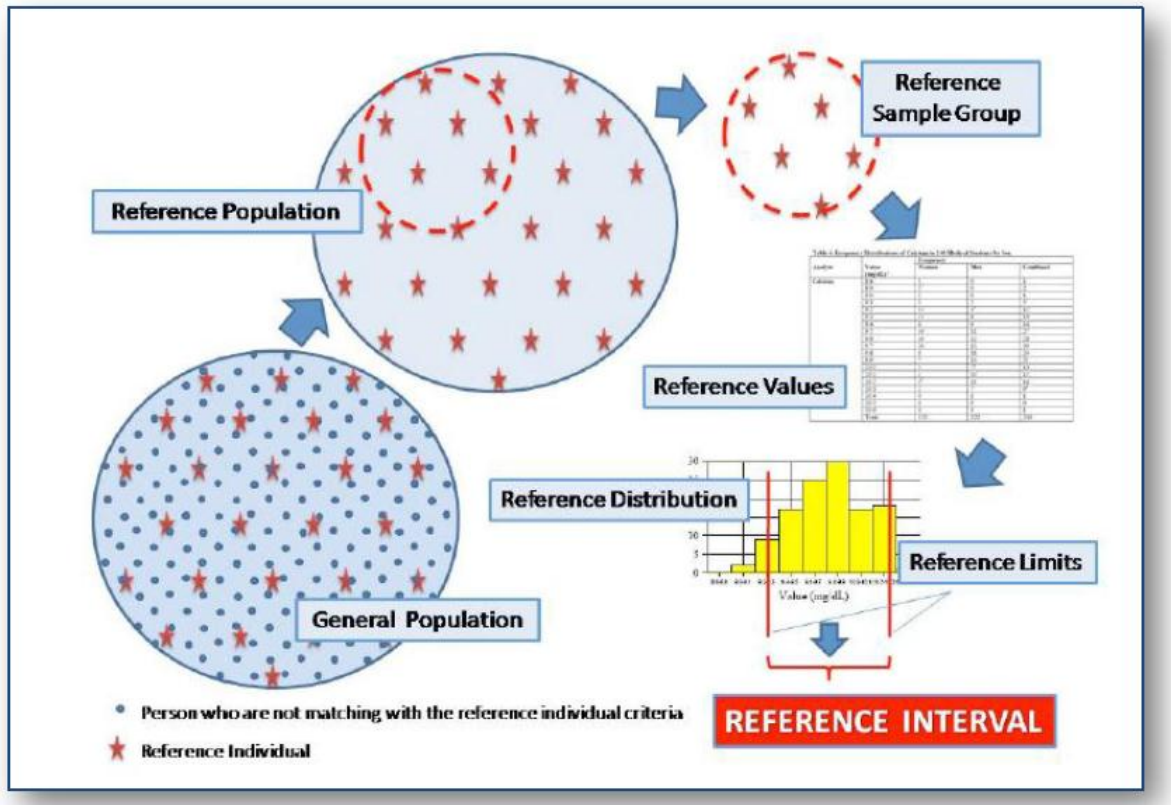

Fig. 1: Shows flow chart for selection of reference interval and determination of RI.

1. Terminology for RI establishment

i) Reference population: A group consisting of all the reference individuals. The reference population usually has an unknown number of members and therefore it is hypothetical entity.

ii) Reference sample group: An adequate number of persons selected to present the reference population. It is subset of reference population.

iii) Reference individuals: A person selected for testing on the basis of well defined criteria.

iv) Reference value: The value (test result) obtained by the observation or measurement of particular type of quantity on reference individual belonging to the reference group.

v) Reference distribution: This is statistical distribution of reference values.

vi) Reference limit: A value derived from reference distribution and used for descriptive purpose.It is common practice to define a reference limit so a stated fraction of the reference values is less than or equal or greater than or equal to respective upper or lower limit, the reference limit is descriptive of the reference values and may be distinguished from various other types of decision limits**.**

vii) Reference Interval: The interval between and including two reference limits(It is designated as the interval of values from the lower reference limits to the upper reference limit(eg.for calcium reference interval is 9.1mg/dL to 10.3mg/dL[2.27 mmol/L to 2.57 mmol/L].

3. Determination of reference limits

Practically observed value from patient sample is compared with the corresponding reference interval, which is bounded by lower and upper reference limits.[17] Parametric method is used if reference distribution of analytes shows Gaussian distribution (symmetrical). According to this, the determination of reference limits (percentile) would be calculated as values±2SD below and above the mean. If the reference distribution shows non Gaussian distribution, one may use mathematical functions that transform data to approximately Gaussian shape. In the nonparametric method the percentiles are simply determined by cutting off the required percentage of values in each tail of the reference distribution. Reference interval is computed using reference distribution.[18] There are three kinds of reference intervals: tolerance interval, prediction interval, interpercentile interval. The interpercentile interval is simple to estimate, more commonly used and recommended by the IFCC. It is defined as an interval bounded by two percentiles of the reference distribution. The reference range is usually defined as the set of values 95% of the healthy population falls within. It is an arbitrary but common convention to define the reference interval as the central 95% interval bounded by two limiting values, the lower 2.5% and upper 97.5% percentiles. The interpercentile interval can be

determined by both parametric and non-parametric statistical techniques as mentioned earlier.[18]

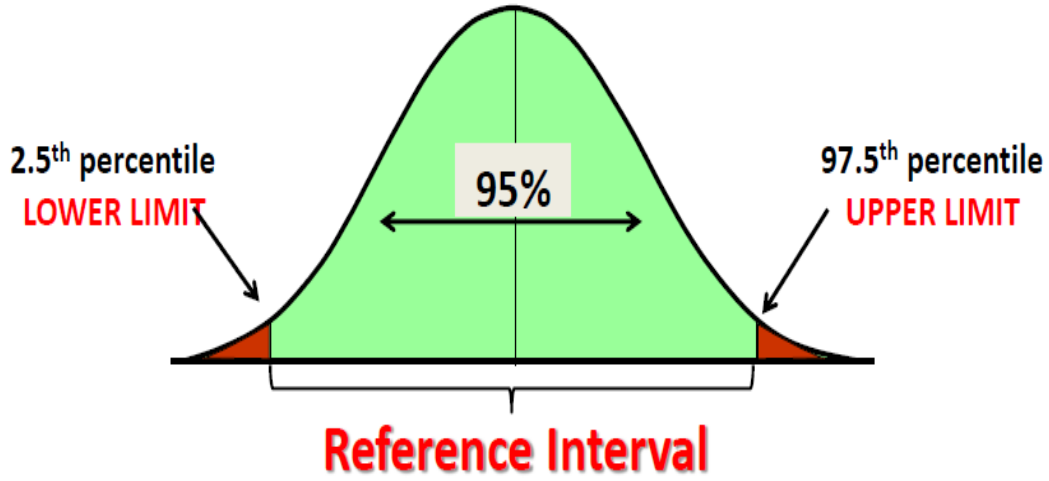

Fig. 2: 95% data for reference interval determination.

4. Organization of National RI study

1. Clinical laboratories of every country should conduct their own nationwide study to derive country-specific RIs.
2. Collaborating laboratories should recruit healthy volunteers, draw blood, and process the specimens following the common guideline published by IFCC-CIDL (C28-A3).
3. The common assay procedures should be used in each laboratories of the country to eliminate variations due to analytical methods. One or two laboratories may act as central laboratories in each country to receiving specimens from local laboratories. Central laboratory can use any assay platform for measurement.
4. RIs should be made traceable to the RMPs for standardized analytes through the measurement of standard reference materials (SRMs) or value-assigned sera.
5. For the non standardized analytes, centrally determined RIs should be converted to those of each participating laboratory through cross-checking results with those of the central laboratory using statistical approach such as linear regression.[19]
6. In order to compare test results with other countries after adjusting for various factors, a panel of sera is used (freshly prepared from reference individuals by C-RIDL for the purpose) was adopted. Using this approach, every participating laboratory would be expected to analyze this panel of sera along with the locally-acquired specimens in order to make the results comparable across the countries.[20]

4. Selection of reference individuals:

Direct method is used for the selection of reference individual as recommended by IFCC (21)In this the individuals are selected from a parent population using defined criteria. However the disadvantages are the problems and cost of obtaining a representative group of reference individuals. In contrast, in indirect method[22] the individuals are not considered. The method assumes that the values of an analyte have a distribution with a preponderant central peak, which is composed, mainly of normal values. The normal interval can be estimated by extracting the distribution of normal values from this part of the distribution. The other strategies are the priori and posteriori sampling method.[21, 22] The priori (prospective) strategy is best suited for smaller studies and individuals fulfilling defined inclusion criteria are selected for sample collection. On the other hand, a posteriori (retrospective) methods consist of database containing both analysis results and information on a large number of individuals. Values of individuals fulfilling defined inclusion/exclusion criteria are selected.

Exclusion and Inclusion criteria

a) Exclusion criteria

i) Known diabetes on oral therapy or insulin (diet alone is acceptable).
ii).History of chronic liver or kidney disease.
iii) History of being a hospitalization during the previous 4 weeks.
iv) Blood donation in the previous 3 months.
v) Known carrier state for HBV, HCV, or HIV.
vi) Female participants who are pregnant, breastfeeding, or within 1 year after childbirth.
vii) Any other disease or disorder that influences the results of the study.

b) Inclusion criteria

i) The participants should be apparently healthy.
ii) The participants should age and sex matched (18–65) years.
ii) The participants ideally should not be taking any medication. Any subject taking medications or vitamin supplements should have them recorded (name, dose, and frequency) so that secondary exclusion after measurement can be done as required.

The following medications are permitted but should be recorded: contraceptive pills or estrogens and thyroxine, if the subject is well replaced (i.e., TSH is lower than the upper reference limit), are permitted, but they should be recorded.

c) Ethnicity

Information on ethnicity can be collected from the volunteers.

d) Sample size

To establish country specific RIs the minimum sample size is 500 (male and female: 250×2) or more, which is greater than twice the minimum number recommended by C28-A3 [120 ×2 (men and women)], so that country-specific RIs can be obtained in a more reproducible manner. This number is adequate for making between-country comparisons of test results with a power of detecting a difference of two means equivalent to 0.25 times SD comprising the RI (SD_{RI}), which corresponds to a bias of 0.25 times between individual variation, allowing errors of $\alpha<0.05$ and $\beta<0.2$ in the statistical hypothesis testing done separately for each gender. If there is an interest in exploring regional within country variations, it is recommended to obtain at least 120 (men and women, 60×2) samples from each local area to acquire adequate power to test for a difference of two means equivalent to $0.5\times SD_{RI}$ by the above specification.[23]

5. Target Analytes

i)Clinical Chemistry

Enzymes: AST, ALT, ALP, LD, GGT, CK, and amylase,Electrolytes: sodium, potassium, chloride, calcium, inorganic phosphate, iron and magnesium,Miscellaneous: total protein, albumin, creatinine, urea, uric acid, total bilirubin, and glucose,Lipids: Triglycerides, Total Cholesterol, HDL-C, and LDL-C.

ii)Immunoturbidimetry

This includes CRP, IgG, IgA, IgM, C3, C4, transferrin, TTR (prealbumin), and cystatin C.

iii)Immunoassays

It is preferred that the following nonstandardized but commonly measured analytes also be measured in common to allow international comparison of results: Tumor markers: Ferritin, AFP, CEA, CA125, and PSA Endocrinology: TSH, prolactin, cortisol, and PTH, Miscellaneous: vitamin B12 and folate, CA19-9, CA15-3, fT4, and fT3 have been excluded due to known method related variation and failure of their test results to be made comparable based on previous cross-check testing. Those analytes that are very unstable or require a special sampling tube should also be excluded.

6. How to prepare individual for sample collection.[22, 25]

Specimens for the production of reference value should be collected under condition as similar as clinical practice. As several factors cause increased variability of analytes, it is necessary to standardize the pre-analytical procedures. Biological factors, meals, pharmalogically active substances, hormone supplementation therapy, stress and exercise, change in posture, tourniquet pressur can bring about an increase in the concentration of proteins, calcium, fatty acids and bilirubin. Intake of ethanol, anti-convasant drugs may induce synthesis of liver enzymes Y-GT which may increase the clearance of many substances, thus affecting their concentration in serum.

7. Invitation to participants for enrollment in the study

It is advised to advertise the study by posters in the indoor and outdoor areas of hospital and by electronic invitations sent to staff members within the participating health care institution. It is also advisable to hold meetings to explain the clinical and scientific importance of the study and benefits for the laboratory and volunteers to obtain cooperation.Give each volunteer the following.

1. An invitationfor the study.
2. An explanation of the study (inclusion/exclusion criteria).
3. A consent form (written in the local language, in accordance with the guidelines of the institutional IRB committee).
4. Procedures for participation.

8. Informed consent

Detail written and verbal information must be presented to the participants about the exact nature, benefit and risk of the study. It should be clearly stated that the participant is free to withdraw from the study at any time for any reason. The participant must be allowed as much time as desired to consider the information and allowed an opportunity to question the investigator, their physicians or other independent parties to help decide whether they will participate in the study. Written informed consent should then be obtained, including the personal signature and date for both the participant and the person who presented and obtained the informed consent.

9. Tabulation of volunteers by age and gender

One who agrees to participate in the study by reading the invitation is expected to contact the local representative. A balanced distribution of gender and age should be ensured by tabulating volunteers as shown below (Table 1). All participants must be older than 18 years. The main target range of ages is 18 to 65 years, for which an even distribution of age and gender is of the utmost importance to have comparability of test results across regions. Individuals older than 65 years are also sought, as long as they match the inclusion criteria.

Approximately 20% of the total number should be in this age group. The primary objective of including this age range is to evaluate age related changes in test results, and thus, there is no need to balance the gender distribution of this group.

10. Appointment and preparation for blood collection

It is advisable to make the appointment for the drawing of blood for most of the subgroups in the above table must be recruited. Make appointments between 7:00 and 10:00 AM with 30 min intervals (i.e., 7:00, 7:30 8:30, 9:00, 9:30, 10: 00).

Sampling schedule and labeling

Table1	18–29 years	30–39 years	40–49 years	50–64 years	60-65 years
Male	01 001–01 010	01 011–01 020	01 021–01 030	01 031–01 040	01 041–01 050
Female	01 051–01 060	01 061–01 070	01 071–01 080	01 081–01090	01090–01 100

Prepare a sampling schedule as shown below, setting the date and time of sampling together with the volunteer's name and ID number. The ID number will be generated as AA-LL-###, with AA representing the area code within the country; LL, the laboratory code; and ###, the proper number of the sample.

11.Reminders for volunteers in preparation for sampling

1. Make an appointment for blood collection when the volunteer agrees to participate.
2. Remind each volunteer of the following requirements before sampling:
3. Avoid unusual strenuous exercise for 3 days before the sampling.
4. Avoid sampling on the day after working a night shift.
5. Fast overnight (at least 10 h before the sampling).
6. Avoid excessive eating and/or alcohol intake the night before the sampling.
7. Avoid smoking just before the blood collection.

12.Procedures on the day of blood collection

i) Collection of questionnaire

All the participants will be given questionnaire at the time of phlebotomy. Participants will have their height, weight, and abdominal circumference measured by the local coordinators who also may help them complete the questionnaire. The ID labels should be pasted on both the questionnaire and consent forms. The participants' consent form and the questionnaire will be kept confidential by the local representative of the study.

ii) Preparation of equipment for sampling and storage

The type of blood collection tubes, either plain or gel-separator tubes, must be determined in each laboratory; it is preferable to use the same sampling equipment as is used for routine testing. Assays for the most commonly measured analytes are not influenced by the tube type, except for some drugs or hydrophobic analytes[26-28] however, it is recommended that each central laboratory investigates the possible differences in test results between the plain and gel-separator tubes by comparative measurements of several specimens. The type of tube being used must be recorded.ID labels should be pasted onto vacuum blood collection tubes and storage containers. Make sure that the IDs match with those on the corresponding questionnaire and consent form for each participant.

iii) Preparation of volunteers immediately before sampling

Volunteers should rest in a sitting position at least for 30 min before drawing blood. Hasty sampling after a volunteer rushes in causes stress-induced (inorganic phosphate, glucose, etc.) and postural changes (almost all proteins) in test results. Smoking cigarettes just before blood collection is not allowed because smoking is known to affect the values for some enzymes (LDH and amylase) and glucose.[28]

iv) Procedures for drawing blood

Apply the tourniquet 7–10 cm above the venipuncture site. The pressure should be set below the diastolic blood pressure for the smooth pooling of blood in the periphery. Never leave the tourniquet for longer than 1 min. Do not clench the fist while drawing blood; this causes false elevation of serum potassium. Draw the volume of blood required (The amount of blood to be drawn can be determined in each country according to the analytes being tested locally). Invert each tube 180 (upside down) at least five times (in the presence of a clot activator in the sampling tubes).If the blood draw is interrupted before a tube is completely filled, the remaining vacuum should be filled with air to avoid vacuum induced hemolysis.[29]

v) Preparation of the serum and its aliquots

Blood filled tubes should not be exposed in direct sunlight or in low temperatures. Leave it at room temperature for 30–60 min after sampling then centrifuge the specimen at 1200 *g* for 10 min.ID label on the blood collection tubes and on the serum containers must be matched (2 mL each).Transfer the serum into each of the containers.

vi) Storage and shipment of the specimens

The serum aliquots (1–2 mL) should be well sealed and stored at −80°C within 2 h. All aliquots that are not locally used will be sent to central laboratory in dry ice for collective measurement.

vii) Thawing and preparation of samples for analysis

On the day of analysis, the samples must be thawed by letting them stand at room temperature for at least 1 h,

avoiding direct sunlight because of its effect on bilirubin concentrations. Homogenization is then achieved by inverting the samples 10 times. Analysis must be performed within 4 h from the start of thawing.

viii)Procedure for cross-check testing

To share the RIs derived for non-standardzed analytes such as protein hormones and tumor markers, cross-check testing should be conducted by asking each participating laboratory to retain one or two aliquots of serum each from a part or all of the volunteers and to measure them locally near the time of the centralized assay for cross-comparison of the results.[30]

ix)The number of samples for cross-check testing

The number of samples required for the cross-check depends on the accuracy specification set by each laboratory. The recommended number is ≥ 10 for the standardized analytes and ≥ 20 for the non-standardized analytes.[31] Procedures for the cross-check sample collection and storage will be the same as previously stated. Cross-check testing samples should be selected randomly. In addition, the samples for the cross-check should be divided into multiple parts (at least four) and measured on different days in order to reduce the effect of between day errors on the conversion.

x) Requirements for the central laboratories

One or two laboratories in each country should be chosen as a central laboratory that will provide collective measurements. The requirements for the central laboratory should be specified so that it (1) implements reliable measures for both short- and long-term quality control, (2) ensures traceability of test results for the standardized analytes based on CRMs and value assigned sera for enzymes, and (3) participates in the alignment of test results across central laboratories, using the reference panel of sera.

xi)Standardization of assays

To ensure essential traceability of test results, internationally qualified standards or CRMs should be measured for the standardized analytes.

xii)Cross-comparison of values

A panel of sera composed of 40 sera prepared from healthy individuals should be divided into at least four parts and tested on different days to avoid bias attributable to between-day variation of the test results.[33] The between-day variation of test results should also be monitored by the QC specimens and recorded.

13.Data analysis

i) Analyses of source of variation of test results

Multiple Regression Analysis (MRA) should be performed for every analyte to identify factors which affect the test results.[34] The possible factor are: gender, age, BMI, smoking status, level of alcohol consumption, frequency and strength of physical exercise, dietary status, ethnic group and, if available, the ABO blood group.

ii)Partitioning criteria

The magnitude of between country SD (SDcntr), between-sex SD (SDsex), between-age SD (SDage), and net between-individual SD (SDindiv) should be computed by 3-level nested ANOVA. Ratios of SDcntr over SDindiv 0.3 can be used as a guide to judge the presence of significant regionality in test results.[34] However, implication of regional differences should be evaluated by multiple regression analysis as described.

ii) Report of test results

iii) Only after all the specimens have been analyzed and the RIs have been derived should the test results be returned to the volunteers. A sheet with a given participant's printed test results should be sealed in an envelope with the ID label on the outside. The local representative should be asked to give this to the corresponding individual by referring to the name from the informed consent form with the same ID number. Each individual's test results and the newly-derived, country-specific RIs will be reported to the volunteers after all the measurements have been completed, together with an explanatory sheet for the interpretation of the test results.

iv)Derivation of RI

According to the IFCC it is necessary for each laboratory to determine their own set of reference limits. In Nepal most of the laboratories follow reference intervals established in the western population. The reference intervals can be different due to variation in diet, lifestyle and ethnicity in western and Nepalese population. So far we have not come across any literature on Nepali reference intervals even on internet search did not yield any finding. Therefore we need to conduct a nationwide study to establish reference intervals for our population.For the derivation of RIs, the parametric method will be used after excluding samples with abnormal test results (secondary exclusion, as discussed above) and then normalizing the data by power transformation using the modified Box-Cox formula.[34] For tertiary exclusion, a multivariate iterative method called LAVE may be applied at the time of computing RIs.[35,36,37, 38, 34]

V)Analytical Procedure and Quality control

It is mandatory to mention the essential components while establishing reference interval. i) Method, information on equipment, reagents, calibration standard, calculation methods ii) Reliability criteria ii) Quality control. The analyser has to be calibrated with the reference materials provided by the manufacturers. Change in calibration curve and specificity of the analytical method can be detected by using a number of accuracy control specimens, at both normal and pathological levels of concentrations for the various

analytes. During the course of study the study there should be no change in the equipment, reagents, calibration standards and controls.In order to maintain the required precision an adequate number of control specimens should be included at fixed or random position in each analytical run[32] Ideally precision controls should be employed at different level of concentration. In addition to standard quality control materials supplied by manufacturers, each laboratory will prepare multiple commutable specimens (mini-panel) for QC monitoring. The desirable limits of bias should be specified before-hand. The desirable limits for between- and within-day CV are set as 1/2 of CV_I (within-individual CV listed in the Westgard website: www.westgard.com/biodatabase1.htm).

Vi) Presentation of an observed value in relation to reference values[39]

An observed value may be compared with reference values. Reference values must be made available to the clinicians in the form of table graphs or figures either of result reports laboratory format or in the form of publication. A printed set of reference data on report form is recommended if researcher can insure that it is relevant to the observed value to be reported. Partitioned reference value must be reported in different sets according to age, sex, activity, physiology etc.

On the basis of reference two limits of reference intervals, observed values can be classified into three groups. It is convenient to bold the abnormal reports by L for Low and H for high observed value.

a) Abnormally low when value is below reference limits(<LL)
b) Normal when observed value is equal or between two limits(LL-UL)
c) Abnormally high when the observed value is above the upper Limit(>UP)

14. DISCUSSIONS

Although RI is crucial in the clinical decision-making process, its use is still incomplete in most clinical laboratories.[40] Derivation of RIs for a country by conducting a multicenter study following a common protocol is probably the most effective way to seek globally applicable common RIs.[41] The success of globalization depends on the absence of regional and/or ethnic differences in test results, whereas their presence provides invaluable information regarding those differences. In order to derive scientifically valid results for comparisons across the world, nationwide multicenter studies must be conducted in each country following a common protocol.

Objective of this study is to produce common guidelines which can be followed by any laboratory anywhere in nation and abroad, thus ensuring repeatability and transferability of reference values. To achieve this, CSLI/IFCC developed C28-A3 (Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory) was used as the basic guidelines so that every participating laboratory follows exactly the same protocol. This study is planned for a multicenter, multicounty study.

Protocol has been modified regarding the inclusion/exclusion criteria in order to show how healthy reference individual are defined during selection by different cultures and investigators. Ethnic origins, alcohol consumption, physical activities, menstrual cycle, and medications were included in the questionnaire to see how these factors influence test values.

In the previous guidelines minimum sample size for each country was 240 volunteers (120 of each gender) but in this protocol (at least 500 reference individuals) required to calculate RIs, and partitioning.[42] Pannel of sera used for harmonizing results across the laboratories. In order to enable comparison of test results between the central laboratory and each of the local laboratories, the cross-check testing procedure is also described in detail at each stage.[43, 44] thus allowing comparison and transfer of the centrally derived RI for the local laboratories.

The protocol and procedure described in this study give detailed coverage of the scientific criteria and requirements to get valid results. They can be used, modified, and/ or adopted for similar multicenter studies to establish common RIs. The appropriateness of the protocol described here will be evaluated through their implementation in the nationwide multicenter study on reference intervals.

15.Future aspect of this study

Apart from establishing the reference values in healthy Nepalese population, we should establish reference intervals for pediatric, geriatrics, pregnant, smokers, alcoholics which effects several biochemistry parameters. Reference intervals in these populations give an idea of influence of physiological changes on the various analytes in comparison to reference values of our healthy population.

CONCLUSION

Nationwide multicenter study must be conducted to establish common reference interval for the country following this protocol and procedures. With cooperation of all the tertiary level laboratories in the country, it is possible to obtain large sample sizes, in order to gain confidence about the estimated limits and investigate variations among age, gender, race, environmental/physiological conditions. It would be better to refer IFCC, C-RIDL,CLSI and C28-A3 guidelines to conduct study for establishing reference intervals even for a local small capacity laboratory.

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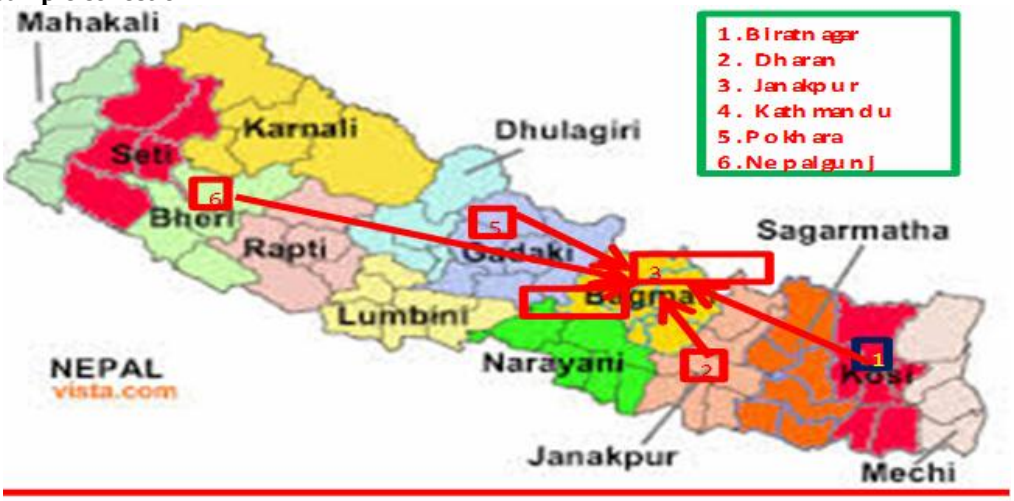
Centers of Sample collection



A sample health status questionnaire is shown above.

General Health Screening Questionnaire

1. Do you consider yourself **healthy**? ( ) yes, ( ) No
2. Are you in between **18 -65 years** of age? ( ) yes, ( ) years.

Exclusion Criteria

Do you have **diabetes** and are treated with oral therapy or insulin? **( ) yes ( ) No**
Do you have or have you had **chronic liver or kidney disease? ( ) yes ( ) No**
Do you have results from a **blood test that point to a severe disease**? **( ) yes ( ) No**
Have you been **hospitalized or seriously ill in the past 4 weeks**? **( ) yes ( ) No**
Have you given **blood as a donor in the previous 3 months**? **( ) yes ( ) No**
Are you a known carrier of **HBV, HCV, or HIV**? **( ) yes ( ) No**
Are you **pregnant or within one year after childbirth**? **( ) yes ( ) No**
Have you **participated in research study** involving an investigational drug in the past 12 weeks? **( ) yes ( ) No**

DEMOGRAPHICS

Are you f**asting**? ( ) yes ( ) No. **Last food intake**: ( ) AM/PM
Age: ( ) years Gender: ( ) Male, ( ) Female ( ) Today's date: Time of collection Place of Birth: Place of Residence:
Body **Hight**: ( ) cm Body **weight:** ( ) Kg **Abdominal circumference**: (….) cm
ABP blood type(blood group): ( ) A, ( ) B, ( ) AB, ( ) O, ( ) Not Known **Do if not known…….**

HEALTH STATUS AND MEDICAL HISTORY

1.Are you **vegetarian** (no meat or fish): ( )No, ( ) Yes
2. Do you eat a **special diet**? ( ) No, ( ) Yes **If yes, what?**
3. Have you been **sick** within the past 4 weeks? ( )No, ( ) Yes yes, describe the illness and when.
4: Have you been **hospitalized in the last 6 months?** ( )No, ( ) Yes

−−If yes, what for and when?
5: Do you have **any allergic condition** (pollinosis, atopic dermatitis, asthma,etc) ? ( )No ( ) Yes −−If yes, what? (describe the condition, is it currently active?)
6: Do you have **high blood pressure**? ( )No, ( ) Yes
7: Are you **currently under a doctor's care**? ( )No, ( ) Yes−−If yes, what for?
8: Are you taking any **prescribed medication on a regular basis**? ( )No, ( ) Yes (including **diet pills, anti-hypertensive**, **antacid**s, or **allergy medicine**, etc.) −−If yes, what? (describe the name, dose, and frequency for each medicine.)
9: Do you take vitamin supplements or herbal remedies? ( )No, ( ) Yes −−If yes, what? (describe the name, dose, and frequency for each medicine.)
10: Have you taken any pain relievers in the past 4 weeks? ( )No, ( ) Yes
11: Are you exposed **to any hazardous chemicals in your job**? ( )No, ( ) Yes −−If yes, what?

WOMEN

1: When was your **last period**? (……) date
2: How is **your menstrual cycle? (**)regular, ( ) irregular, ( ) menopause, ( ) under hormone therapy or taking contraceptive −−If "**regular**", what is the averge lengh of the cycle? (……..) days.

ALCOHOL

1: Have you had a drink in the last 48 hours? −−If yes, what?
2: How much do you drink in a typical week? Beer ( ) liter Wines ( ) glasses (assume 6 glasses per standard sized bottle) Spirit/Hard drink (Vodaka,Riyal stage, local ( ) ml
3: For how many months/years has this been typical? (…..) Years

SMOKING

1: How many cigarettes do you smoke? ( ) per day, ( ) per week
4: How many years have you smoked? ( ) years
5. If you quit smoking within a year, please describe when you quit, and how much you smoked (amount and years). (……..)

PHYSICAL ACTIVITIES AND EXERCISE

1.In a usual day, how many hours do you stand: (1)(…) hours/day (include exercise, walking) In a usual day, how many hours do you sit: (2) (…) hours/day **Note: 24 hours = (1) + (2) + (hours of sleep or other lying posture/day)**
2. Do you exercise regularly? ( )No, ( ) Yes If yes, how many days per week? (…… .) days/week Please describe your regular exercise (name or type of exercise, duration/day).
3.How many days in the past week have you performed physical activities during your work or exercise when your heart has beat faster and your breathing was harder than normal for a total of 30 minutes or more per day? (……..) days /week
How many days in a typical week have you performed such strenuous activity? (……. .) days /week

I authorized access to my personal health information (medical record) as explained in this form and I have agreed to participate in this research study.

_____ _____ _____

Name of Participant Signature Date

It can be customized to local needs by adding and removing query items. The essential items required for the worldwide comparison are BMI, special diet, records of medicines and/or supplements regularly taken, status of menstruation, habits of smoking, alcohol consumption per week (roughly expressed grams of ethanol), and frequency and strength of physical exercise. The information will be used for analyzing sources of variation in test results and for judging the necessity of a secondary exclusion.

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