Definition and Guidelines to the determination of Reference Intervals in the Clinical Laboratory

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Definitions (IFCC)

- Reference individual: a person selected for testing on the basis of well defined criteria (usually imp to define person's state of health)
- Reference Population: A group consisting of all reference individuals – usually has an unknown no of members
- Reference Sample group: an adequate no of persons selected to represent the reference population
- Reference Value: Tests results obtained from a reference individual
- Reference Distribution: Distribution of reference values
- Reference Limit:Value derived from reference distribution
- Reference Interval: Interval b/w 2 reference limits

Clarifications

Reference values may be associated with

- Good health
- Certain physiological conditions eg pregnancy
- Pathological states

  In all cases, reference values allow one to relate or compare observed data to reference data from a defined population of subjects

Relationship between terms defined

Reference Individuals

Comprise a

⇩

Reference Population

from which is selected a

⇩

Reference Sample Group

on which are determined

⇩

Reference Values

on which is observed a

⇩

Reference Distribution

from which are calculated

⇩

Reference Limits

that may define

⇩

Reference Intervals

Protocol for establishing Reference Intervals

- Establish list of biological variations & interferences from literature
- Establish selection & partition criteria
- Categorize potential reference individuals based on questionnaire
- Exclude individuals based on criteria
- Decide on appropriate no in consideration of desired confidence limits
- Prepare properly &consistently selected individuals
- Collect & handle specimens properly and standardize
- Analyze samples under well defined conditions consistent with routine practice
- Inspect reference value data & prepare histogram to evaluate distribution
- Identify possible data errors and/or outliers
- Analyze reference values using selected method of estimation including partitioning into subclasses if appropriate

Examples of possible exclusion criteria

- Alcohol consumption
- Blood Donor
- B pressure (raised)
- Drug abuse
- Drug Prescription
- Environment
- Fasting/nonfasting
- Genetic factors
- hospitalization
- Illness, recent
- Lactation
- Obesity
- Occupation
- Oral Contraceptives

Possible Partitioning Factors

- Age
- Blood gp
- Circadian variation
- Diet
- Ethnic background
- Exercise
- Fasting/non fasting
- Geographic location
- Posture when sampled
- Race
- Sex
- Stage of menstrual cycle
- Time of day when sampled
- Tobacco use

Summary of Critical Factors

Biological factors

- Metabolic
- Haemodynamic
- Enzyme induction
- Cell damage

Methodological factors

- Specimen collection
- Specimen transport
- Specimen handling

Sources of variability & Standardization

Specific factors (supine vs upright

Multiple factors eg exercise ,diet, sex, pregnancy, age,tobacco use, time of sampling, ethnicity

Subject Preparation : Preanalytical factors for consideration

Subject preparation

- Prior diet
- Fasting vs non fasting
- Use of pharmalogic agents
- Drug regimen
- Sampling time
- Physical activity
- Rest period before collection
- stress

Specimen collection

- Environmental conditions during collection
- Time
- Body posture
- Specimen type
- Collection site
- Site preparation
- Blood flow
- Equipment
- Technique

Preanalytical Factors for consideration

- Specimen Handling
- Transport
- Clotting
- Separation of serum/plasma
- Storage
- Preparation for analysis

Minimum number of reference subjects

- N = (100/P) -1 where P is the difference between 2 percentiles
- Eg if you wish to compare every 2.5th percentile n = (100/2.5) – 1 = 39 – as thes small nos may not be totally representative

Reed suggests that a minimum of **120** reference subjects be used for 90% confidence limits and **153** for 95% confidence limits

Treatment of Outlying Observations

- Imp assumption here is that measures values are from a homogeneous pop
- If certain results fall outside the normal distribution , they should be retained if no analytical mistake is found esp if sample size is at least 120
- Many statistical tests available to determine if outlier is atypical. **Dixon's test** D/R commonly used where D is the diff b/w outlier & largest or smallest observation & R is the range of all observations including outliers. If value of D is greater than 1/3 of the range, R, then outlier should be rejected

Partitioning of Reference Values

- Only necessary if values are significantly different at 5% or 1% probability levels
- Suggested that a pilot study using about 60 subjects be used

Transference

- Transference of reference values to another lab with same or different geographic distributions using same or other analytical systems can occur if
- (a)comparison of analytical systems is made
- Comparability of reference population
- Additionally preparation of reference individuals and specimen collection/handling procedures need to be considered

Validation

- **3 approaches used**
- Subjectively assess and inspect pertinent factors of the original appropriate reference study eg population demographics, geographics, examination of preanalytical & analytical procedural details, method of estimating the reference interval
- Alternatively , a smaller no of reference individuals n=20 may be used provided the detailed preanalytical & analytical and statistical analysis are known – results examined for outliers using Reed's "one third " rule. Outliers should be replaced if less than 2 found and rechecked. >3 outliers, analyse another 20: if 3 results are outliers examine analytical procedures & consider possible differences b/w the 2 pops gps
- May also be assessed by using 60 reference individuals using the above principles

Conclusion

- Selection of reference individuals must be thoughtful with advance consideration to exclusion and partitioning criteria. Evaluation of health status should be documented
- All preanalytical & analytical procedures must be considered and controlled where appropriate
- Non parametric method of estimation of reference interval recommended for its simplicity and reliability
- A uniform process for detecting & discarding outlier values is recommended