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Nutrition Science in the Lab

Reference Intervals and You

Precision in lab measurements must form the bedrock of any evidence base, however, what lies beneath the surface and in the realms of the lab? We've asked our collaborator and Nutritional Biomarkers expert, Kate Guberg, to provide us with *10 Key Insights*…

1. When reviewing biomarker data in studies, the following questions are often asked: Was this expected? Do the differ from 'normal'? How do they compare with previous studies? Are they significant or the result of methodological or population differences?
2. Research does not exist in a vacuum and it is essential that results can be compared with other studies. A robust understanding of what reference intervals are, how they are determined, how they vary, and their limitations is paramount.
3. Reference intervals are derived from statistical analysis of a population. Establishment requires a sufficient number of study participants and an understanding of what that population comprises. This population is assumed to be healthy, however health is often inferred which can lead to the introduction of bias to the reference interval from subclinical or silent pathologies.
4. Reference intervals cannot be extrapolated beyond the population used to calculate them; children cannot be assessed on adult ranges, some analytes will change with age and there are sex differences with some analytes. In addition, some healthy individuals take food supplements and medicines that may interfere with assays.
5. Often the statistical approach to calculate reference intervals used is to use the mean +/- 3 standard deviations to define a reference range (parametric method). This will result in 5% of 'healthy' subjects being classified as abnormal, with the incidence of 'false abnormal' rising when several analytes are being measured together. The parametric method assumes that a population follows a normal distribution (or has been converted to a normal distribution).
6. Even given a large population, stratification factors may result in sub – populations too small to give a robust reference range. The NHANES study consisted of circa 9000 participants, but after multiple levels of stratification (such as age and sex) even this major study fails to attain sufficient numbers to produce robust reference for these subgroups.
7. The term Clinical decision point is often used interchangeably though this refers to results significant for medical reasons based on current understanding and is distinct from a reference range. **Figure 1** and **Figure 2** show how these are derived and used compared to reference ranges.
8. Reference intervals are related to the measurement method, which must be considered when comparing results utilising different analytical techniques. There have been efforts to standardise measurements such as that of Vitamin D which highlighted the variation found in methods often used in clinical laboratories versus those used in research settings. Often fast throughput methods such as ELISA assays show greater variation and less specificity than those used more often in research settings such as LC-MS. Clinical assays are often optimised around the clinically significant decision points and may show greater variation when used to analyse samples for a 'healthy' population.
9. Care should be taken when comparing results that analytical techniques are adequately identified, and where necessary cross over studies performed to characterise the differences between the methods. In the case of folate, NHANES used cut-offs derived from microbiological assays with results obtained from the immunoassay. This resulted in risks of inadequacy being exaggerated.
10. Whilst reference intervals appear simple and easily elucidated, thought must be given to ensure that decisions and conclusions from comparing biomarker data are accurate and appropriate. Check out the bibliography to find articles which cover this subject in more depth, and welcome to the reference interval rabbit hole!

Figure 1. How reference intervals and clinical decision levels are derived

Reference intervals	Clinical Decision points
"Presumably healthy" population	For what health outcome?
Population clinically confirmed to be disease free	Intervention – short term depletion or supplementation study
Consider confounders (smoking, renal function, pregnancy)	Clinical observation: % of patients with disease at cut-off
Separate reference ranges for subgroups?	Statistical modelling: risk or hazard ratio at cut-off

Taken from: Challenges and Lessons learned in Generating and Interpreting NHANES Nutritional Biomarker Data. American Society for Nutrition Adv Nutr 2017;8:290-307;doi:10.3945/an.116.014076

Figure 2. How reference intervals and clinical decision levels are used

Reference intervals	Clinical Decision points
Used when no clinical decision limit is available	Used to assess health status of patient
Used to evaluate population distribution (centre and tails)	Used to assess overall prevalence of nutritional status in population
Used to evaluate shifts in population distribution	Used to assess difference in prevalence among population subgroups
Used to assess differences among population subgroups	

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