CLINICAL REVIEW

Interpretating Normal Values and Reference Ranges for Laboratory Tests

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Laboratory test results drive about 70% of clinical decisions and are important in making diagnosis, prognosis, ruling out conditions, testing for propensity to disease and monitoring the course of illnesses. The requirement for releasing laboratory results to patients has altered the dynamics of doctor patient interaction. Minor variations in laboratory test results that are labeled by the laboratory as low/high/abnormal may cause unwarranted worry to the patients. The number of laboratory results that are outside the "normal range" far exceeds the clinically meaningful abnormal results due to the usually accepted methodology for ascertaining "normal values"/reference ranges, variations in methods of testing at different laboratories, variations due to age, gender, ethnicity, seasonality, and random variations. The usual process for establishing "normal values/reference ranges" entails testing at least 120 healthy individuals in a given age-group, gender, ethnicity, testing method and related health issues. The central 95% of the values is usually adopted as the normal range. This practice, by definition, labels 5% of healthy individuals as having abnormal laboratory results. This review addresses various issues that affect laboratory test results and interpretation of such results. It also addresses doctor and patient concerns about assessing and reporting laboratory results. In addition to reporting normal values along with patient results, it may be useful to include clinical significance of the findings, in simple terms, such as, no immediate concern, warrants discussion with doctor at the next visit, recommend contacting your doctor for further action. (J Am Board Fam Med 2025;38:174–179.)

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Introduction

History, physical examination, and diagnostic studies including imaging and laboratory testing are integral parts of patient evaluation. Laboratory test results drive about 70% of clinical decisions and account for 3 to 5% of the health care costs.[1,2] While reliable laboratory testing is an essential part of health care, overuse of laboratory testing is monetarily wasteful and contributes to errors. The results of laboratory tests are interpreted in comparison to the expected normal values in individuals without disorders in general and of the particular organ system in particular. However, it is not generally appreciated that the normal values reported by the testing laboratory are not a standard but derived through a process with many assumptions, differences in methods, overlay of expert opinion and other sources of variation in results. This review addresses the process of determining normal values/reference ranges, interpreting patient results in comparison to "normal" values, sources of nonclinical variation in patient results, patient access to laboratory results and potential for misdiagnosis and consternation based on apparently abnormal results that may not be clinically meaningful.[3,4] It highlights the assumptions behind the reference values to facilitate objective evaluation of

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patient results. At the same time failure to recognize the importance of an unfamiliar term may result in lack of needed action by the patient, for example, finding of lymphoma being reported in a patient being investigated for colon carcinoma.[5]

Background Information About Laboratory Testing and Normal Values

Source of "Normal/Reference" Laboratory Values

The population found suitable to donate blood is generally considered healthy and often specimens from this population are used to define "normal" values for laboratory tests results.[6] Under ideal circumstances, each laboratory is expected to determine its own "normal/reference" ranges by testing at least 120 individuals without discernible disease, from each relevant age, gender, and ethnic groups in the population served by the laboratory. This is essentially impractical. In practice, reference ranges or normal values are adopted from historic standards, textbooks and information provided by manufacturers of laboratory equipment and reagents. A source of variation is added by the different methods used for various tests. For example, there used to be up to 40-fold variation in the normal values for troponin I, depending on the instrument and method used. Even in 2024, results for troponin I vary by more than 4-fold among different methods.[7] Only 3 analytes (a chemical or molecule) have undergone international standardization, harmonization, [In the context of Laboratory Medicine, harmonization of laboratory testing refers to our ability to achieve the same result (within clinically acceptable limits) and the same interpretation irrespective of the measurement procedure used, the unit or reference interval applied, and when and/or where a measurement is made.], to mitigate the variation engendered by different methods.[8–12] The 3 harmonized analytes being Cholesterol, Creatinine and Hemoglobin A1C. The coagulation test result, INR, is not an individual analyte and reflects a calculated value that has been nearly standardized. The logistics for harmonization of any analyte are overwhelming, expensive, and subject to objections by various vendors of the test equipment and reagents.[13,14]

It bears emphasizing that the historic normal values were ascertained in metabolic units of research hospitals where patients were admitted, maintained on a standard diet and physical activity and first morning (postabsorptive, basal) specimens were used to determine the normal/reference values. For many analytes these values are lower than the values that may be seen in an ambulatory patient coming to the laboratory. As examples, (a) blood levels of creatinine kinase, a muscle enzyme, go up with normal activities, similarly (b) blood levels of alkaline phosphatase are higher after a meal.[15–17]

Process for Determining "Normal/Reference" Range

At the introduction of a new test for a new or old analyte, the regulations by the Centers for Medicare and Medicaid Services (CMS), Food and Drug Administration (FDA) and Centers for Disease Control (CDC) require following a prescribed process for validation of the test, including establishing reference range.[8] In its simplest form, 120 healthy individuals are tested. The specimen may be blood, a blood component, or another body fluid. The results from this group of people, referred to as a partition, are plotted. The lowest 2.5% and highest 2.5% of the values are excluded and the values comprising the central 95% are adopted as the reference range. This convention of using the central 95% as normal is an important cause of "abnormal" laboratory results in healthy people.[18] By definition, 5% of normal/healthy people will have an abnormal result. Thus, it is imperative that all laboratory results be interpreted in the clinical context to ensure that a person is not labeled with a diagnosis based solely on an "abnormal" laboratory value. As a corollary of the central 95% convention for normal range, if 20 tests are done on a normal/healthy person, one of the results is expected to fall outside the normal/reference range. Alternatively, the probability of no abnormal test result in a healthy subject, in a panel of 20 tests is $(0.95)^{*}20 = 0.358$. Thus, $(1\text{-}0.358 = 0.642)$, 64.2% of "normal" patients will have at least one "abnormal" test result in a panel of 20 tests.

Exceptions to Sources and Processes for Determination of "Normal/Reference" Laboratory Values

1. **Expert opinion and changing healthcare knowledge.** The central 95% of values in an apparently healthy population are sometimes modified by experts based on additional information. For example, (a) Body mass index (BMI) of the extant population is not used as the standard because it is known that patients with higher BMI are susceptible to

more diseases. The average BMI in the USA is about 30, but healthy BMI range is considered to be 18.5 to 25.[19,20] (b) normal blood pressure readings have been lowered over the years, (c) thyroid stimulating hormone level range has been narrowed over the years due to increase sensitivity and preciseness of the test methods, (d) normal range for cholesterol values has been lowered over the years to take into account additional information about outcomes. The issue of using central 95% as reference range is itself under scrutiny and variations in this theme have been proposed.[21]

2. **Age as a modifier:** In an adult woman, a hemoglobin level of 12.0 g/dL is considered normal, however, a newborn girl with that level of hemoglobin is considered to have anemia.[9] Blood levels of prostate specific antigen (PSA) increase with increasing age without the presence of prostate carcinoma.[22] Blood levels of alkaline phosphatase are higher in growing children than in adults.

   The levels of some analytes found in healthy blood donors do not reflect the usual levels in older individuals without a relevant disease. For example, the normal ranges for serum free light chains noted in blood donors generate an abnormal value in 36% of adults presenting to a tertiary care hospital, without any evidence of a monoclonal gammopathy.[23] It could be argued that normal/reference values should be derived from a "healthy" group of the same age and with similar co-morbidities as the ones affected by a relevant disease.[23–25]
3. **Gender as a modifier:** The lower limit of normal hemoglobin is 12.0 g/dL in adult women, and it is 14.0 g/dL in adult males. The lower level in women is not due to menstrual blood loss but due to the hormonal influence improving the delivery of oxygen to tissues by affecting the levels of 2-3 DPG.[26,27] Similarly, some hormone levels have gender specific normal values. Different reference ranges are adopted for HDL cholesterol, ferritin and creatinine etc. for men and women.[28–31] The reference ranges for non-binary/transgender people are still being worked out.[32]
4. **Physiological state:** As mentioned above, growing children have different values for some enzymes and hormones. Pregnant women have different normal levels for some laboratory tests than non-pregnant women.[31]
5. **Ethnicity:** Some laboratory values vary by ethnicity, just like normal height differs between the Dutch and Filipinos. Vitamin D levels are lower in persons of African origin even though they have lower incidence of bone disease. The apparently lower levels of vitamin D in African origin persons are due to genetically determined lower levels of vitamin D binding proteins, though bioavailable levels of the vitamin are normal.[33] To avoid race-based medical impressions that reflect socials influences, newer ranges are sometimes described to avoid taking race into consideration, e.g., newer formula for calculating estimated glomerular filtration rate (eGFR).[34]
6. **99th percentile rather than central 95%:** The normal upper limit of troponin is based on the 99th percentile of the value in healthy subjects. There is no range, just one value. A person with the cutoff value or lower result is normal and higher level is abnormal.[35,36]
7. **Only one normal/reference value, rather than a range:** For some analytes only values higher than a trigger point are a cause for concern and lower values have a different interpretation. For example, blood creatinine levels in men are 0.74 to 1.35 mg/dL and for women, 0.59 to 1.04 mg/dL.[14,30] Levels higher than the upper limit indicate kidney insufficiency. However, lower levels do not mean kidney hyper-efficiency but lower muscle mass or sarcopenia, as muscle is the source of creatinine.
8. **Variation by method of testing:** As mentioned earlier, testing methods for only three analytes, Cholesterol, Creatinine and Hemoglobin A1C have been standardized internationally. For all other analytes, results and normal values vary by the method of testing, the most prominent example of this is seen with troponin as noted above.[7] Some analytes are tested by different methods even within a given hospital, for example the blood hemoglobin measured by blood gas analyzers in Emergency Department is 0.5 to 1.0 g/dL higher than the result from the main laboratory. Blood glucose values determined by portable glucometers are generally lower than the plasma glucose reported by the main laboratories. Values of vitamin D vary between immunological methods and mass spectrometry. It is important to address the normal values/reference ranges particular to the laboratory where testing was done.[37] All accredited laboratories report their reference ranges with results from patient specimens.
9. **Variation among laboratories:** In addition to the variation in results due to different methods, there is additional variation in results among laboratories using the same methods and instruments, including for analytes that have undergone harmonization. One measure of such variation is the range of lower and upper acceptable limits for result from proficiency testing. Proficiency testing for regulated analytes is mandated by CMS. A deemed entity, including College of American Pathologists, sends samples to participating laboratories who test the specimens as they would test specimens from patients. The deemed entity analyzes the results from participants to ascertain if the performance of the laboratory is acceptable. The entity reports the lower and upper limits of acceptable results. The difference in lower and upper limits of acceptable results is 35% for creatinine, an analyte that has undergone harmonization. The difference in acceptable lower and upper limits for thyroid stimulating hormone is also at about 35%,

in part because data involves results from laboratories using the same method and same instrument. The 66% variation in lower and upper values for immunoglobulin G is a common level of variation in lower and upper acceptable limits.[7] This much variability in results among different laboratories may appear disconcerting, however, the variability on repeat testing in each laboratory is much narrower and argues for using the same laboratory for serial monitoring of a given analyte.

10. **Essential nutrients:** The recommended daily allowance (RDA) and by corollary, the normal values, are geared to provide a value that prevents disease in 98% of otherwise healthy individuals.[38] RDA is not designed for optimum health. Folic acid provides an excellent example of this conundrum. The Centers for Disease Control and Prevention (CDC) lists normal level of serum folate/folic acid as >4.0 ng/mL. This level prevents megaloblastic anemia in 98% of the population. A proportion of individuals with this level of serum folate have elevated serum levels of homocysteine and the values of this undesirable substance are normalized if sufficient folic acid is administered to raise serum folic acid levels to >7.0 ng/mL. FDA mandated that folic acid be added to cereals as the supplement is known to prevent neural tube defects in the fetus. However, the optimum serum folate level for maximum benefit is >13 0 ng/mL. Similarly, a serum folate level of >13.0 ng/mL has been shown to reduce ischemic strokes in hypertensive patients. Thus, there is a wide gap between the minimum essential level and the optimum level.[39,40] A similar situation has also been observed for vitamin D. A serum level of 12.0 ng/mL prevents bone disease but a proportion of people with that level have elevated levels of parathyroid hormone. If sufficient vitamin D is administered to raise the serum levels to 20.0 ng/mL, parathyroid hormone levels get normalized. In most of human history, we were exposed to sun all day, extant populations with similar exposure have vitamin D levels of 50-80 ng/mL. Thus, it could be argued that normal serum levels of vitamin D should be pegged at 50-80 ng/mL.[41,42]
11. Paradoxical better outcomes with abnormal results: Examination of modifiable risk factors and mortality revealed that all cause mortality is lower in individuals with non-HDL cholesterol of 200 mg/dL than in those with 100 mg/dL. Similarly, a person with a BMI of 30 has lower risk of death than one with a BMI of 20, despite the "desired" BMI being 18.5-25.[43]

Clinical Considerations for Reporting Laboratory Results

Laboratory test results are an integral and essential part of health care, however, the state of the art in the accuracy and precision of results warrants caution in interpreting results for diagnostic as well as monitoring of disease states. The usual medical school curriculum often does not address the details and nuances of laboratory test results and this narrative is intended to prompt a more informed review of test results. For a clinician with privileges at multiple hospitals, it is imperative that laboratory rest results be viewed in the context of that laboratory's reference ranges and similar caution needs to be exercised in patient transfers among institutions. It is generally accepted that repeat testing for tumor markers in assessing the progress of patient should be obtained from the same laboratory to avoid the imprecision from using different methods. The same dictum should be applied to other analytes that are often repeated over time and to monitor the results of treatment, for example, Thyroid Stimulating Hormone, blood lipids, Comprehensive Metabolic Panel, immunoglobulins, etc. Even for the 3 analytes, namely, creatinine, cholesterol, and hemoglobin A1c that have undergone international standardization, harmonization, the range of upper and lower acceptable values may be disconcertingly large and could be minimized by using the same laboratory for serial testing.

In addition to the reporting the reference ranges along with laboratory results, a brief statement of the clinical significance of the results should be included. For example, minor changes in Mean Corpuscular Hemoglobin (MCH) Relative Width Distribution of red cells (RDW), pCO2, Sodium, Alkaline phosphatase etc., could be annotated with a comment like, "No immediate concern." As the next level, a hemoglobin of less than 10 in males and 9 in females, fasting plasma glucose of 110 to 125, total serum proteins >9.0 and the like could have a comment like, "Discuss with your doctor at the next visit. More serious results, for example, hemoglobin <7.0, fasting plasma glucose of >130, serum creatinine >2.0 etc. could have a bolded comment like, "Contact your doctor at your earliest convenience." These statements would not replace the need for the laboratory to report critical/panic values to the health care clinician. A national organization, such as the College of American Pathologist could provide brief, uniform appendices that could be attached to the common laboratory test reports posted on the patient portals.

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