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LRA by ELISA/ACT™ Tests Sample Quality Control

On comparison of samples. . .Fundamentals of quality control

The quality of information provided by a test analyte is a function of the precision, accuracy, reproducibility, sensitivity, and specificity of the method used to make the measurement. This is particularly important for a functional test procedure whose result depends upon living cells induced to respond to a stimulus (*e.g.* antigen or hapten).

LRA by ELISA/ACT™ tests are *ex vivo* functional procedures for measuring lymphocyte responses to all three delayed hypersensitivity pathways. The procedure is *ex vivo* because the sample is obtained in ways that neither activate nor damage it compared to the flowing blood *in vivo*. Since lymphocyte response is affected by both preactivation and cell degradation, accurate results depend upon obtaining a specimen unmodified by the venipuncture, transport, and storage procedures.

A split sample is one taken under similar conditions to evaluate variance in the procedure when performed at different times on the same or essentially same specimen. The same specimen means that a single sample is divided and analyzed under similar conditions. An essentially same specimen refers to two or more samples taken at different times from the same person and analyzed under similar conditions. In contrast, a sequential sample refers to samples taken at the same phlebotomy session one after another.

Split samples are different from sequential samples, particularly in functional tests.

Functional procedures are meaningful when they reproduce with small variances on the same or essentially same specimen. In contrast, functional and static procedures are vulnerable to physiologic changes known to occur when a tourniquet or blood pressure cuff is in place for extended periods of time such as are required for sequential specimens to be obtained. For purposes of sample collection, more than two minutes of vein compression is enough to induce substantial physiologic changes in the specimen. Effects are more substantial in some people; less substantial in others.

It is not clinically feasible to predict for an individual how much variation is induced by sequential samples. Split sample comparisons are done under conditions that are comparable for each specimen obtained.

Reliability of procedure when repeated under constant or similar enough conditions to be reproducible depends upon the specimen conditions when the blood is drawn. This applies to when a physical chemistry measure is made, e.g. glucose, cholesterol, calcium, as well as enzyme measurements and antibody quantitations. Antibody quantitations, for example, are static tests. This means they tell the presence or absence and amount but not action or function of the antibody. For example, an antibody can be neutralizing and protective (beneficial) or can be complement activating and reactive (symptom provoking). A serum ELISA procedure for antibody quantitation is not designed to distinguish function and therefore detects both beneficial and reactive antibodies. In contrast, a functional lymphocyte response assay detects only complement activating, reactive antibodies since lymphocytes are not activated by protective, neutralizing antibodies.

Analytes that are known to be significantly (from 5-20+% depending on the subject and the conditions) affected by extended phlebotomy include:

1. Cholesterol increases due to release from cell membranes (1,2,3).
2. Triglycerides increase due to phospholipase activation (1,2,3).
3. Glucose decreases due to cell metabolism activation (1,2,3).
4. Calcium (particularly ionized) due to influx into activated cells (1,3).
5. Magnesium (particularly ionized) due to eflux from activated cells (2).
6. Potassium due to eflux from activated, acidotic cells (1,2,3).
7. Adrenaline due to release from platelet storage granules (1,2,3).
8. Cortisol due to cell hypoxic distress and activation (1,2,3).
9. Standard non-functional ELISA procedures for measuring proteins (e.g. antibodies, enzymes, and other immunoreactive elements) are susceptible to these as well as inter laboratory and intra laboratory variances (2,3,4)

LRA by ELISA/ACT tests have regular open and blind split samples performed as part of ongoing quality control (5). Reproducibility within 3% day-to-day variance on these samples demonstrates the exceptional precision of the procedure when performed according to usual sample guidelines (5). Colleagues are welcome to submit open or blind split samples to confirm the tight procedural precision of this *ex vivo* functional, comprehensive procedure.

References:

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