Analytical Validation

For Laboratory Developed Tests (LDT) laboratories must establish the test's:

- Accuracy
- Precision
- Analytical sensitivity (lower limit of target detection)
- Analytical specificity
- Reportable range of test results
- Reference intervals (normal values)

I. Analytical Validation

A. Accuracy

Per the Standards for the Reporting of Diagnostic Accuracy (STARD): The amount of agreement between the information from the test under evaluation (the index test) and the reference standard (the best available method for establishing the presence or absence of the condition of interest).

Accuracy = true result ÷ (true result + false result)

In general this can be assessed by testing blinded samples and confirming that correct result is identified.

B. Precision/Robustness

In the context of a quantitative test, could be considered a measure of precision. However, robustness expresses how well a test maintains precision when faced by specific, designed "challenge", in the form of changes in pre-analytic and analytic variables therefore reduced precision does not represent random error. Typical variables in the laboratory include sample type (e.g. EDTA blood, LiHep Blood), sample handling (e.g., transit time or conditions), sample quality, DNA concentration, instrument make and model, reagent lots, and environmental conditions (e.g., humidity, temperature). Appropriate variables should be considered and tested for each specific test. The principle of purposefully challenging tests is also applicable to both categorical and qualitative tests and should be considered in these validations as well. Robustness can be considered as a useful prediction of expected intermediate precision.

In general, this is often addressed by the same samples run within the same run (intra assay variability) and on a different run (inter assay variability).

Note laboratory, should validate all specimen types that it expects to accept.

C. Analytical Sensitivity

1. The ability of a test to detect a mutation when that mutation is present

Sensitivity = True positive ÷ (True positive + False negative)

There are good statistical (excel spreadsheets) methods available online that can be used (www.pedro.org.au/wp-content/uploads/CIcalculator.xls)

2. Also, some refer to the lower limit of detection (LoD) for the analyte of interest (i.e., the lowest concentration of analyte that the assay can detect). It is preferable to specify LoD, if important for assay.

D. Analytical Specificity

1. The ability of a test to give a normal (negative) result in specimens without the mutation being tested.

Specificity = True negative ÷ (True negative + False positive)

It is important to document confidence intervals (see above)

2. Also, some refer to the ability of a test to detect the analyte without cross-reacting with other substances. It is preferable to specify cross-reactivity, if important for assay.

E. Assay Linearity and Reportable Range

1. The range of analyte concentration for which the result is directly proportional to the concentration; or the "range of values over which the acceptability criteria for the method have been met" and errors are "within defined limits" (CLSI)
2. The reportable range includes all of the possible results that can be reported (for both qualitative and quantitative results)

   For molecular genetics assays, documentation that results can be negative, heterozygous, homozygous, hemizygous, compound heterozygous.