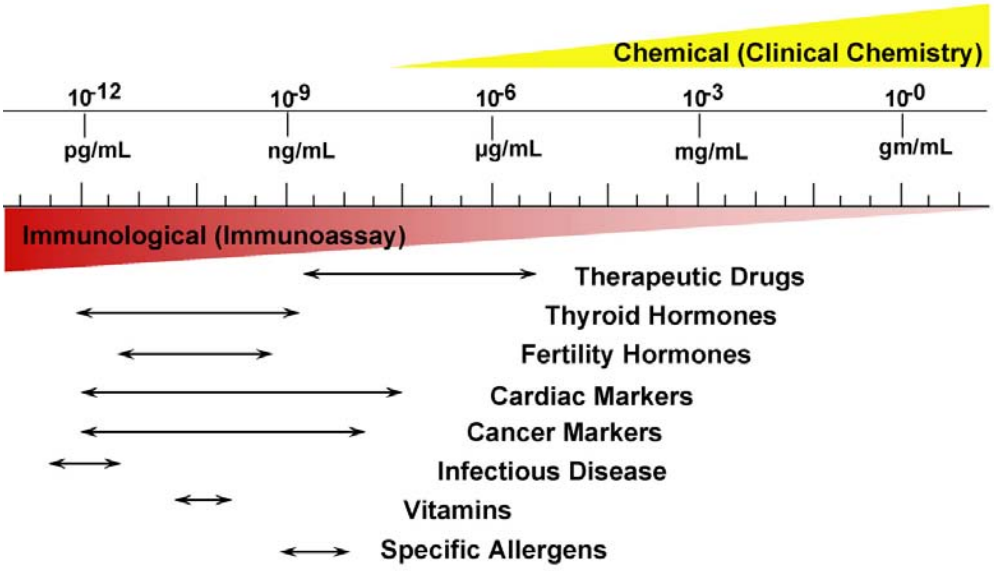
TECHNICAL INFORMATION

ESTABLISHING APPROPRIATE QUALITY CONTROL RANGES FOR IMMUNOASSAY TESTS

INTRODUCTION

An immunoassay is a biochemical test which uses an antibody / antigen reaction to measure the concentration of a substance in a biological liquid (typically serum, plasma, or urine). Because antibody / antigen binding reactions are specific, immunoassays can detect very low concentrations of analyte.

Immunoassay testing is more sensitive than standard chemistry assays. Assays performed on general chemistry systems typically measure analytes in the range of µg/mL to gm/mL, while immunoassay testing typically measures analytes in the range of pg/mL to µg/mL. This difference in sensitivity is an effect of the specific reaction each method employs. Most general chemistry assays utilize chemical reactions, which produce a change in color that is measured by a spectrophotometer. Automated immunoassays, such as those performed on the Access Family of Immunoassay Systems, use an enzyme-mediated chemiluminescent reaction, which produces light that is measured by a luminometer. This allows for detection of lower concentrations and a broader range of analyte in a sample. The diagram below compares the measuring ranges of both technologies.



LAB FORWARD

IMPRECISION

The distinct nature of the biological materials used to produce immunoassay reagents, calibrators, and quality controls (QC) contribute to normal variability between lots. This variability, combined with the very low concentrations of analyte immunoassays are designed to measure, typically yields overall imprecision parameters which are higher than chemistry assays. It is important to account for this expected variability when establishing QC ranges for an immunoassay system.

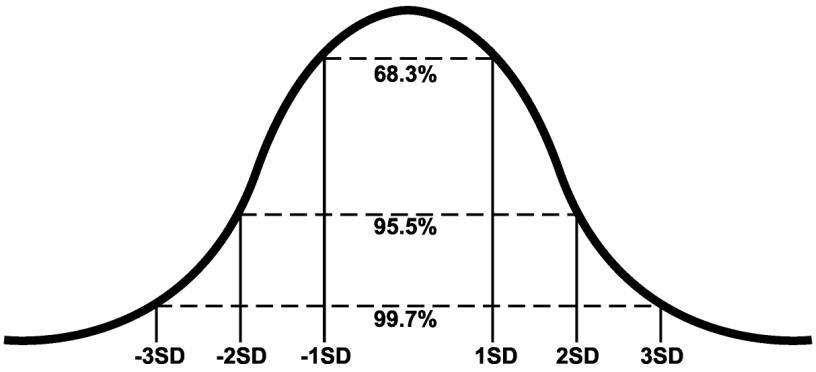
The standard deviation (SD) and the coefficient of variation (CV) are often used to establish QC ranges from the control data set. The standard deviation is a measure of the spread around the mean of that data set. The coefficient of variation is the standard deviation expressed as a percentage of the mean.

$$SD = \sqrt{\frac{\Sigma(\bar{x} - x)^2}{n - 1}} \qquad \%CV = (SD / Mean) \times 100$$

CVs are useful when comparing the precision of QC samples at different concentrations of an analyte. CVs for chemistry assays are typically 3-6%, while typical CVs for immunoassays are 8-12%. In some cases immunoassay CVs can be as high as 20%, especially at the lower limit of the assay's analytical range. Each assay designed for the Access Family of Immunoassay Systems has its own reagent Instructions for Use (IFU), which provides representative precision data which can be used to approximate assay performance. You can also consult other sources, such as peer surveys or quality control peer group reports, to review expected assay performance results.

Assuming that the set of data used to determine QC ranges represents a normal population distribution, laboratories often use a QC range of ± 2 SDs from the mean. Any results outside this 2 SD range are an indication that the method may be out of control. It is important to understand that a normal distribution of data will include a number of points which are outside the expected distribution limits (2SD), as demonstrated in the graph below. Approximately 5% of the time there will be values which fall outside of the expected 2SD limit. To prevent unnecessary troubleshooting, laboratories can implement additional quality control measures, such as Westgard Rules, to further define when systems are out of control. These rules help determine whether QC data demonstrates true shifts and trends, or is part of the normal distribution.

Normal Distribution



GETTING STARTED: ESTABLISHING QC RANGES FOR A NEW ASSAY OR QC MATERIAL

It is recommended that QC ranges are established over a period of time which reflects the expected overall imprecision for a particular assay. When collecting data, try to include as many of the following variables as possible:

- Multiple reagent lots
- Multiple calibrator lots
- Multiple calibrations
- Multiple vials of QC material (especially lyophilized material, which introduces operator variability when the material is reconstituted)
- Different shipments of the same lot number of QC material
- Data collected on separate shifts, by different operators over multiple days
- Data collected from each instrument used for establishing the new QC ranges

The following procedure is a recommendation which can be used to supplement standard laboratory procedures. It is not intended to replace existing laboratory protocols.

STEPS:

1. Prepare a minimum of two quality control levels according to the control manufacturer's instructions.
2. Assign temporary ranges from the published means and SDs listed in the manufacturer's quality control IFU. These ranges should only be used as a guideline until a sufficient number of measurements has been gathered.
3. Collect data for a minimum of 10 days on each instrument, until at least 20 measurements have been obtained for each QC level.
4. Calculate the means, SDs, and CVs for each control level. If multiple instruments are used for reporting patient results, it is best to pool the QC data from each instrument when calculating an overall observed mean and SD. This allows for QC acceptability criteria that also incorporates instrument-to-instrument variability.
5. Compare these results against the ranges listed in the QC IFU, peer group data (if available), and the expected imprecision parameters listed in the IFU for each assay. If your observed results fall outside of these expected guidelines, contact Customer Technical Support for further assistance before proceeding.
6. Enter the calculated means and SDs into the instrument or other QC management program.
7. Continue to update the mean and SD results as additional data is collected. This should be done frequently during the first few months of running a new test method or instrument to successfully incorporate each expected variable.

NOTE

If you collect data over too short of a time period, the QC ranges you establish may not reflect each expected variable. Consequently, this increases the likelihood of incorrectly rejecting tests, and may cause laboratory personnel to frequently repeat QC, recalibrate the assay, needlessly discard reagents, or spend unnecessary time troubleshooting instrumentation.

MONITORING, ADJUSTMENTS, AND CHANGING QUALITY CONTROL LOTS

Over time more variables are introduced to the testing system, resulting in changes to the means and SDs of the established QC ranges. Due to the inherent biological variability associated with immunoassay products, it is not unexpected that changes in reagent or calibrator lots may result in slight shifts of QC recovery. The Clinical and Laboratory Standards Institute (CLSI) recognizes the need for monitoring QC results, and recommends periodically recalculating and adjusting QC statistics. When doing so, it is important to include all valid data collected since the material was put into use. When re-evaluating QC ranges, only omit data points caused by known operator or instrument error. Do not delete data points simply because they lie outside a 2 SD range. These outlying points may be part of the normal distribution, and eliminating them from the data set will not account for true variability.

When evaluating a new lot of the same QC material, it is recommended that data is collected alongside the current QC lot. Follow the same procedure described previously to determine the mean, SD, and CV for each QC level. The existing QC lot SDs, which have been developed over a long period of time, are a good indication of the variation to expect from the new QC lot, assuming that the means do not differ significantly.

ADDITIONAL QUALITY CONTROL INFORMATION AND SUPPORT

For Bio-Rad controls:

Bio-Rad Laboratories
http://www.bio-rad.com
1-800-2-BIORAD (800-224-6723)

For MAS brand controls:

Thermo Scientific
http://www.thermoscientific.com
1-800-232-3342

REFERENCES

> Clinical and Laboratory Standards Institute (CLSI). 2006. C24-A3, Approved guideline - Statistical quality control for quantitative measurement procedures: Principles and definitions.

> Westgard JO. 2010. Basic QC practices. 3rd ed. Madison, WI: Westgard Quality Corporation.

> *Information about Multirules and "Westgard Rules"*: http://www.westgard.com.