LABORATORY QUALITY ASSURANCE

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SEROLOGICAL ASSAYS

- Determination of immune status for **epidemiological** purposes
- Methods with **highest possible sensitivity** level should be employed
  - **SENSITIVITY** – Galen (1986) - "expressed as a percentage, indicates the frequency of positive test results in patients with a particular disease" – frequency of occurrence of positive tests

TEST QUALITY

- **SENSITIVITY**
  - defined as the proportion of subjects with the disease who have a positive test for the disease
  - describes ability of an immunologic reagent to detect small amounts of antigen
- **SPECIFICITY**
  - the proportion of subjects without the disease who have a negative test
  - describes the selective reaction between antigen and its corresponding antibody

SENSITIVITY, SPECIFICITY & SEROLOGICAL ASSAYS

- The sensitivity and specificity of the assays depend greatly on the **antigen** used
- Assays that use **recombinant protein** or **synthetic peptide** antigens tend to be more specific than those using whole or disrupted virus particle

PREDICTIVE VALUE

- Predictive value is determined by sensitivity & specificity of the test and the prevalence of disease in the population being studied
- **POSITIVE PREDICTIVE VALUE**
  - the probability of disease in a patient with positive test result
- **NEGATIVE PREDICTIVE VALUE**
  - the probability of not having the disease if the test result is negative or normal

Positive predictive value (PPV) and **negative predictive value (NPV)** of a serologic test, based on an assay assumed to be 95% sensitive and 90% specific & assuming disease prevalence of 0.01%, 0.1%, 1%, 5%, 10%, and 50%

Prevalence assumption	Test results	Disease present	Disease absent	Total	PPV %	NPV %
0.01%	Positive Negative Total	95 5 100	99.990 899.910 999.900	100.085 899.915 1.000.000	0.09	99.9994
0.1%	Positive Negative Total	95 5 100	9.990 89.910 99.900	10.085 89.915 100.000	0.94	99.994
1%	Positive Negative Total	95 5 100	990 8.910 9.900	1.085 8.915 10.000	8.8	99.9
5%	Positive Negative Total	475 25 500	950 8.550 9.500	1.425 8.575 10.000	33	99.7
10%	Positive Negative Total	950 50 1000	900 8.100 9.000	1.850 8.150 10.000	51.4	99.4
50%	Positive Negative Total	950 50 1000	100 900 1.000	1.050 950 2.000	90.5	94.7

SPECIMENT HANDLING AND TRANSPORT

- Quality laboratory results begin with proper collection and handling of the specimen submitted for analysis
- Correct patients preparation, specimen collection, specimen packaging, and transportation are of vital importance

QUALITY SAMPLE

Hyperlipemic, hemolyzed, heat-inactivated samples as well as samples containing particulate matter or exhibiting obvious microbial contamination may cause erroneous results!

QUALITY CONTROL

"The aim of quality control is simply to ensure that the results generated by the test are correct.

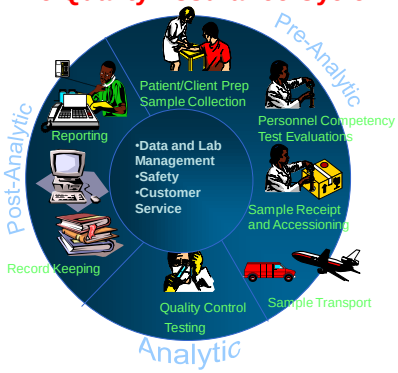
However, quality assurance is concerned with much more: that the right test is carried out on the right specimen, and that the right result and right interpretation is delivered to the right person at the right time"

ACTIVITIES WITHIN EACH PHASE OF THE TOTAL TESTING PROCESS

(MMWR 2005, Vol.54/RR13)

Before testing	During testing	After testing
Test ordering Patient identification, preparation Specimen collection, handling Preparing materials, equipment, and testing area	Control testing / checks Test performance Results interpretation Recording results	Reporting results Documenting Confirmatory testing Patient follow-up Disease reporting Biohazard waste disposal

The Quality Assurance Cycle



PERFORMANCE OF TESTING GOOD LABORATORY PRACTICE

- Precision
- Reliability
- Accuracy

LABORATORY QUALITY CONTROL

- **Quality Control** - QC refers to the measures that must be included during each assay run to verify that the test is working properly
- **Quality Assurance** - QA is defined as the overall program that ensures that the final results reported by the laboratory are correct

QUALITY ASSESSMENT

- **Quality Assessment** (also known as proficiency testing)
  - is a means to determine the quality of the results generated by the laboratory. Quality assessment is a challenge to the effectiveness of the QA and QC programs.
- Quality Assessment may be external or internal.

VARIABLES THAT AFFECT THE QUALITY OF RESULTS

- The educational background and training of the laboratory personnel
- The condition of the specimens
- The controls used in the test runs
- Reagents
- Equipment
- The interpretation of the results
- The transcription of results
- The reporting of results

The following items are essential elements of quality control that must be performed during every assay:

1. Each run must include one full set of controls
2. The controls for each test run must yield results within the limits of the manufacturer's criteria for acceptability and validity of the run.
3. All test kits must be used before the expiration date to ensure valid results
4. Physical parameters of the test such as incubation time and temperature must be followed to ensure proper performance.

SELECTING CONTROL MATERIALS
CALIBRATORS

- Has a known concentration of the substance (analyte) being measured
- Used to adjust instrument, kit, test system in order to standardize the assay
- Sometimes called a standard, although usually not a true standard

SELECTING CONTROL MATERIALS
CONTROLS

- Known concentration of the analyte
  - Use 2 or three levels of controls
  - Include with patient samples when performing a test
- Used to validate reliability of the test system

TYPES OF CONTROLS

- **INTERNAL CONTROLS**
- **EXTERNAL CONTROLS**

INTERNAL CONTROLS

INTERNAL, PROCEDURAL, OR BUILT-IN CONTROLS

- Evaluate whether certain aspects of the test system are working properly.
- They are designed to verify that the test system is working as expected, that sufficient specimen was added and, for unitized test devices, whether is migrated through the test strip properly.
- Certain test systems might have electronic internal controls to monitor electronic functions.

INTERNAL CONTROL

Reference material:

- POSITIVE CONTROL
- NEGATIVE CONTROL
- CUT-OFF CONTROL

EXTERNAL CONTROLS

- Mimic patients specimens and monitor the testing process, from specimen application to result interpretation, to assure proper test performance.
- They might be provided as liquid or other materials similar to patient specimens and might be included with the test system or purchased separately.

FREQUENCY OF CONTROL TESTING

- In the test instructions you should specify **minimum frequency** for running controls, and include recommended levels of control materials that correspond to medical decision levels.
- The appropriate control testing frequency for each test system should not be less than specified in the product insert.
- Controls should be tested concurrent with patient specimens by the personnel who routinely perform patient testing.

CORRECTIVE ACTION WHEN CONTROL TESTING FAILS

- If controls were not performed as expected, the results should not be reported until the **problem is identified and corrected**. The product insert should provide information on procedures for handling unexpected control results, identifying source of error, and manufacturers contact address for technical assistance.
- **Documenting and monitoring** control testing results provides an indication that the test was properly performed.
- Record of control results should be **periodically** reviewed to detect shifts or changes in performance over time.

TEST RESULT INTERPRETATION

- When the test is complete, interpret the results according to instructions in the product insert, as
  - QUALITATIVE
    - determines whether the substance being tested for is present or absent
  - QUANTITATIVE
    - measures the amount of a substance present

ERRORS IN MEASUREMENT

- **TRUE VALUE** - this is an ideal concept which cannot be achieved.
- **ACCEPTED TRUE VALUE** - the value approximating the true value, the difference between the two values is negligible.
- **ERROR** - the discrepancy between the result of a measurement and the true or accepted true value.

SOURCES OF ERROR

- **Input data required** - such as standards used, calibration values, and values of physical constants.
- **Inherent characteristics of the quantity being measured** - e.g. CFT and HAI titre.
- **Instruments used** - accuracy, repeatability.
- **Observer fallibility** - reading errors, blunders, equipment selection, analysis and computation errors.
- **Environment** - any external influences affecting the measurement.
- **Theory assumed** - validity of mathematical methods and approximations.

EXAMPLES OF POTENTIAL SOURCES OF ERROR TO CONSIDER THE HAZARD ANALYSIS

- **OPERATOR ERROR / HUMAN FACTORS**

- Use of incorrect specimen type
- Incorrect application of the specimen on the device
- Incorrect placement of device (e.g., non-level surface)
- Incorrect placement of reagents including strips, or other components that contain reagents
- Use of incorrect reagents, for example, reagents that are not specific for the particular device or lot, or generic reagents
- Incorrect order of reagent application
- Use of incorrect amount of reagent
- Incorrect timing analysis (e.g., specimen application, running the test, or reading results)
- Incorrect reading test results

•SPECIMEN INTEGRITY AND HANDLING

- Error in specimen collection
- Use of inappropriate anticoagulant
- Clotted specimen
- Error in specimen processing and handling
- Incorrect specimen transport and/or storage
- Presence of interfering substances
- Presence of bubbles in the specimen

•REAGENT INTEGRITY (REAGENT VIABILITY)

- Use of improperly stored reagents
- Use of outdated reagents
- Use of improperly mixed reagents
- Use of contaminated reagents

•HARDWARE, SOFTWARE AND ELECTRONICS INTEGRITY

- Power failure
- Repeated plugging and unplugging of the device
- Hardware failure
- Software failure
- Electronic failure
- Physical trauma to unit

•STABILITY OF CALIBRATION AND INTERNAL CONTROLS

- Factors that affect calibrator and calibration stability, including determination of calibration stability over time and after power failures
- Factors that may interfere with calibration

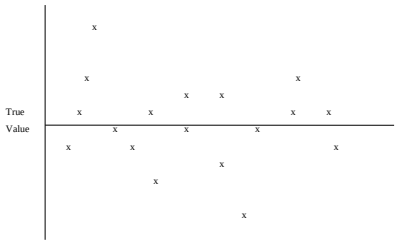
•ENVIRONMENTAL FACTORS

- Impact of key environmental factors (heat, humidity, sunlight, surface angel, device movement, etc.) on reagents, specimens, and test results
- Impact of key environmental factors (including electrical or electromagnetic interference) on instruments, if appropriate.

RANDOM ERROR

- An error which varies in an **unpredictable manner**, in magnitude and sign, when a large number of measurements of the same quantity are made under effectively identical conditions.
- Random errors create a characteristic spread of results for any test method and **cannot be accounted for** by applying corrections.
- Random errors are **difficult to eliminate** but **repetition** reduces the influences of random errors.
  - Examples of random errors include errors in pipetting and changes in incubation period.
- Random errors can be minimized by training, supervision and adherence to standard operating procedures.

RANDOM ERRORS



SYSTEMATIC ERROR

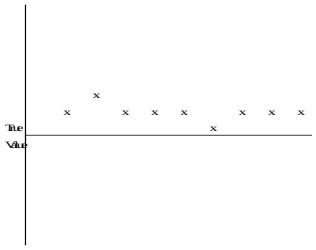
•An error which, in the course of a number of measurements of the same value of a given quantity, remains **constant** when measurements are made under the same conditions, or varies according to a definite law when conditions change.

•Systematic errors create a characteristic bias in the test results and **can be accounted for** by applying a correction.

•Systematic errors may be induced by factors such as variations in

- incubation temperature,
- blockage of plate washer,
- change in the reagent batch or
- modifications in testing method.

SYSTEMATIC ERRORS



MONITORING QC DATA

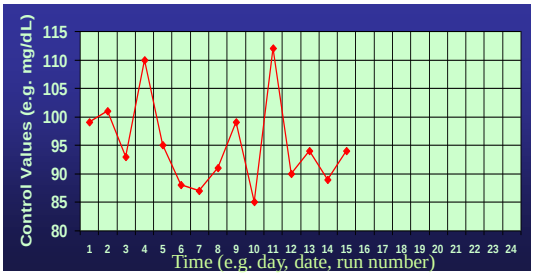
- Use Shewart chart or Levey-Jennings chart
- Plot control values each run, make decision regarding acceptability of run
- Monitor over time to evaluate the precision and accuracy of repeated measurements
- Review charts at defined intervals, take necessary action, and document

SHEWART CHART or LEVEY-JENNINGS CHART

- A graphical method for displaying control results and evaluating whether a procedure is in-control or out-of-control
- Control values are plotted versus time
- Lines are drawn from point to point to accent any trends, shifts, or random excursions

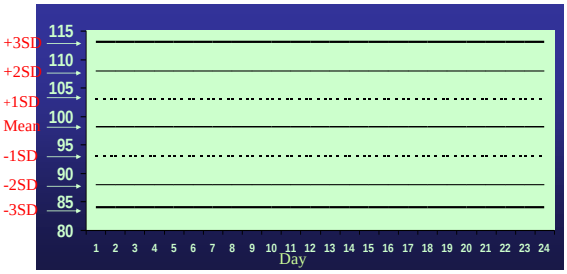
Levey-Jennings Chart -

Plot Control Values for Each Run

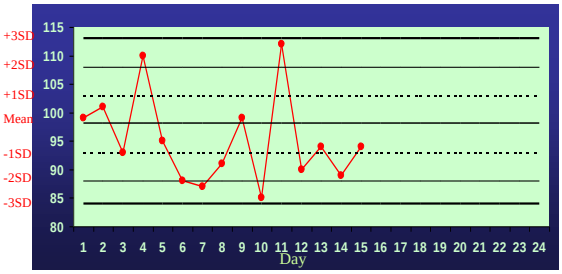


Levey-Jennings Chart

Calculate the Mean and Standard Deviation;
Record the Mean and +/- 1,2 and 3 SD Control Limits



Levey-Jennings Chart - Record and Evaluate the Control Values

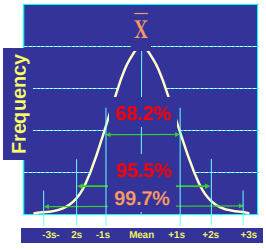


FINDINGS OVER TIME

- Ideally should have control values clustered about the mean (+/-2 SD) with little variation in the upward or downward direction
- **Imprecision** = large amount of scatter about the mean. Usually caused by errors in technique
- **Inaccuracy** = may see as a trend or a shift, usually caused by change in the testing process
- **Random error** = no pattern. Usually poor technique, malfunctioning equipment

Standard deviation and probability

- For a set of data with a normal distribution, a value will fall within a range of:
  - +/- 1 SD 68.2% of the time
  - +/- 2 SD 95.5% of the time
  - +/- 3 SD 99.7% of the time



STANDARD DEVIATION AND PROBABILITY

- In general, laboratories use the +/- 2 SD criteria for the limits of the acceptable range for a test
- When the QC measurement falls within that range, there is 95.5% confidence that the measurement is correct
- Only 4.5% of the time will a value fall outside of that range due to chance; more likely it will be due to error

INTERNAL QUALITY CONTROL PROGRAM FOR SEROLOGICAL TESTING

An internal quality control program depends on the use of:

1. internal quality control (IQC) specimens
2. Shewhart Control or Levey-Jennings Chart
3. the use of statistical methods for interpretation (Westgard rules)

1. Internal Quality Control Specimens

- IQC specimens comprises either:
  - in-house patient sera (single or pooled clinical samples),

  or

  - international serum standards with values within each clinically significant ranges.

2. SHEWHART CONTROL CHARTS

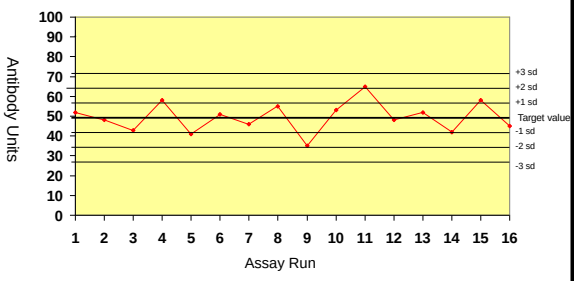
or Levey-Jennings Chart

A Shewhart Control Chart depends on the use of IQC specimens and is developed in the following manner:

- Put up the IQC specimen for at least 20 or more assay runs and record down the OD/cut-off value or antibody titre (whichever is applicable).
- Calculate the mean and standard deviations (SD)
- Make a plot with the assay run on the x-axis, and OD/cut-off or antibody titre on the y-axis.
- Draw the following lines across the y-axis: mean, -3, -2, -1, 1, 2, and 3 SD
- Plot the OD/cut-off obtained for the IQC specimen for subsequent assay runs
- Major events such as changes in the batch No. of the kit and instruments used should be recorded on the chart

SHEWHART CHART

or Levey-Jennings Chart



VZV IgG ELISA: Target Value = 49 U/ml

3. WESTGARD RULES

- The formulation of Westgard rules were based on statistical methods.
- Commonly used to analyse data in Shewhart control charts.
- Westgard rules are used to define specific performance limits for a particular assay and can be used to detect both **random** and **systematic errors**.
- There are six commonly used Westgard rules of which
  - three are **warning rules**
  - and the other three **mandatory rules**.

THE VIOLATION OF RULES

- The violation of warning rules should trigger a review of test procedures, reagent performance and equipment calibration.
- The violation of mandatory rules should results in the rejection of the results obtained with patient's serum samples in that assay.

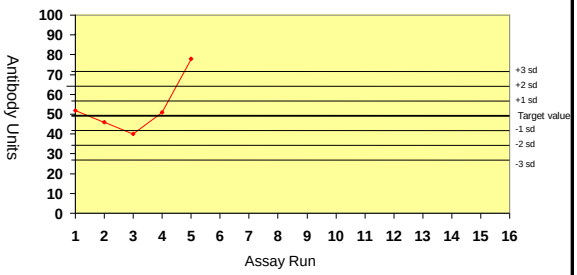
WARNING RULES

1. Warning 1_{2SD}:
   - It is violated if the IQC value exceeds the mean by ±2SD
2. Warning 2_{2SD}:
   - It detects systematic errors and is violated when two consecutive IQC values exceed the target value on the same side of the mean by ±2SD
   - Patients results cannot be reported; reject the run
3. Warning 4_{1SD}:
   - It is violated if four consecutive IQC values exceed the same limit (mean ±1SD) and this may indicate the need to perform instrument maintenance or reagent calibration.

MANDATORY RULES

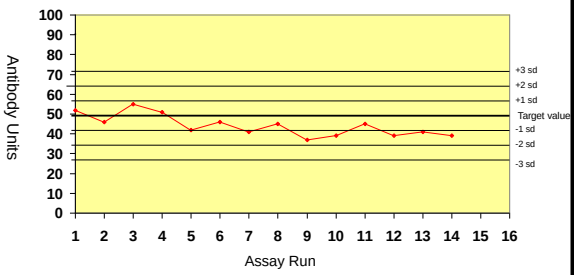
1. Mandatory 1_{3SD}:
   - It is violated when the IQC value exceeds the mean by ±3SD.
   - Run must be rejected.
2. Mandatory R_{4SD}:
   - It is only applied when the IQC is tested in duplicate (one exceeds the mean by –2SD, and the other by +2SD.
   - This rule is violated when the difference in SD between the duplicates exceeds 4SD.
   - Random error has occurred, test run must be rejected
3. Mandatory **10x**:
   - This rule is violated when the 10 consecutive IQC values are on the same side of the mean or target value. This detects systematic errors.
   - May happen when a new test batch or introduced or changes in the calibration of equipment

Westgard Rules: Mandatory 1_{3SD}



VZV IgG ELISA: Target Value = 49 U/ml

Westgard Rules: Mandatory 10x



VZV IgG ELISA: Target Value = 49 U/ml

FOLLOW-UP ACTION IN THE EVENT OF A VIOLATION

There are **three options** as to the action to be taken in the event of a violation of a Westgard rule:

1. Accept the test run in its entirety - this usually applies when only a warning rule is violated.
2. Reject the whole test run - this applies only when a mandatory rule is violated.
3. Enlarge the **greyzone** and thus re-test range for that particular assay run - this option can be considered in the event of a violation of either a warning or mandatory rule.

WHEN A RULE IS VIOLATED

- **Warning rule** = use other rules to inspect the control points
- **Rejection rule** = "out of control"
  - Stop testing
  - Identify and correct problem
  - Repeat testing on patient samples and controls
  - Do not report patient results until problem is solved and controls indicate proper performance

INTERNAL ASSESSMENT

- Objective internal assessment offers flexible, low-cost options for evaluating quality such as self-conducted inspections, supervisory review of documented problems that occur in the different phases of the testing process, review of QC documentation, and testing and reporting procedures.

Summary: How to implement a QC program?

- ✓ Establish written policies and procedures
- ✓ Assign responsibility for monitoring and reviewing
- ✓ Train staff
- ✓ Obtain control materials
- ✓ Collect data
- ✓ Set target values (mean, SD)
- ✓ Establish Levey-Jennings charts
- ✓ Routinely plot control data
- ✓ Establish and implement troubleshooting and corrective action protocols
- ✓ Establish and maintain system for documentation