

# **On-Going Quality Control of Molecular Assays**

**October 18, 2011**  
**Astrid Petrich**

**SickKids**

# Why do it?

## Purpose of QC

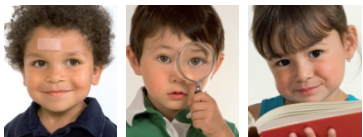
Allow people to do good work by giving them a way to check that everything is working properly

## Statistical QC (one component of overall QC)

Provides a way of looking at results of a work process and being able to identify when the results exceed the variation expected under stable routine operation.

Allows us to identify when something has gone wrong and that the process needs to be fixed.

Requirement for Laboratory Accreditation

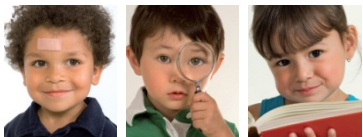


# OLA Requirement-Our Minor

“In molecular microbiology, the QC charts are incomplete and not plotted on Levey-Jennings charts or similar quality control tracking mechanism.”

Need to have something in place for all Quantitative Real-time PCR assays

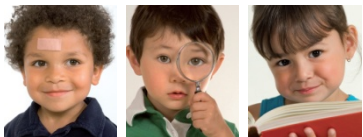
Also need for quantitative assays non-molecular (ie. Serology)



# OLA Guidelines

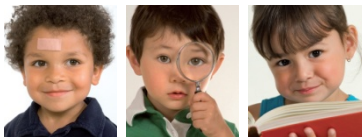
Require Levey-Jennings charts as a quality control tracking mechanism which should include:

- An appropriate time scale based on # of runs
- A full range of control limits indicated as grid lines for +/- 1, 2 and 3 standard deviations (SD)
- Mean and SD lines identified with their concentrations
- Analyte name
- Lot numbers and expiry date of test
- Quality control material
- Lot numbers and expiry date of controls
- Date of each run
- Technologist reviewing
- Interventions



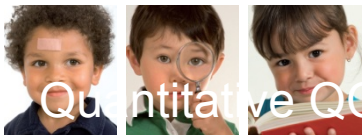
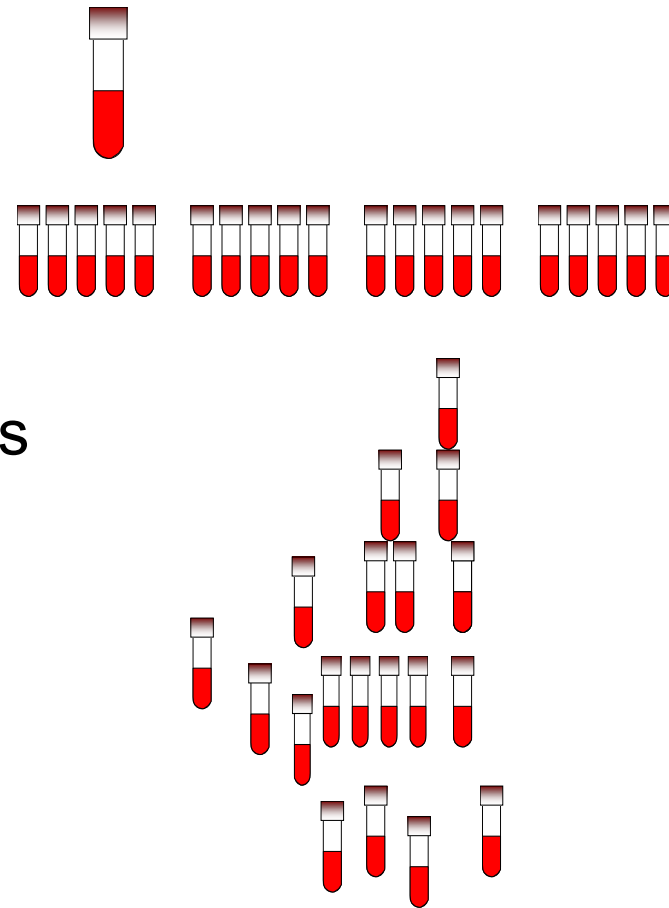
# QC Implementation

- Define the quality required for the test:
  - Negative controls need to work
  - Positive controls need to work
  - When we quantitate-controls fall within standard deviations
  - Proficiency testing
- Assess method performance during initial evaluation of assay or from on-going QC
  - Need to have appropriate control materials
  - Calculate mean and standard deviation over at least 10 days (runs), 20 data points
- Controls are different than standards (though it may be useful to follow similar performance evaluations)



# Steps in Implementing Quantitative QC

- obtain control material
- run each control 20 times over 30 days
- We are going back to previous runs and plotting
- calculate mean and  $\pm 1, 2, 3$  Standard Deviations

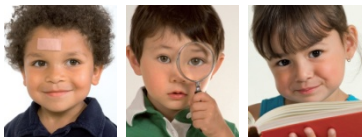


## QC Implementation cont'd

Prepare control charts (Levey-Jennings) or set up a table that allows computerized monitoring

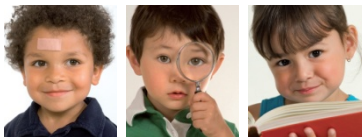
Have guidelines that indicate:

- When control samples are to be analyzed
- How many samples are to be analyzed
- How the control results are to be interpreted and by whom
- What to do when they are “out of control”



# Routine QC Operation

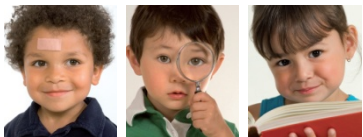
- Include control material with each analytical run
- Record the control results and plot on control charts
- Review and interpret the control results to determine control status
- Need decision criteria or control rules for standardized interpretation (Westgard Rules)
- If the controls meet the criteria for acceptance, then you can report out results





# Kinds of Error

- Random error
  - As seen by Gaussian curve
  - Statistically expected to occur at calculated frequency.
- Systemic error
  - Levey-Jenning plots are a method to determine when the error that is occurring is happening more often or in a manner that is not consistent with calculated rates of error.



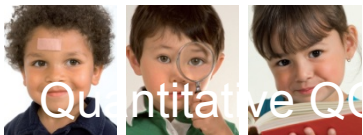
# Measurement of Variability

**Variability is a normal occurrence when a control is tested repeatedly**

Affected by:

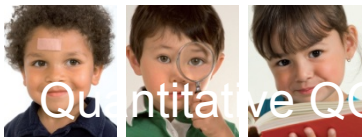
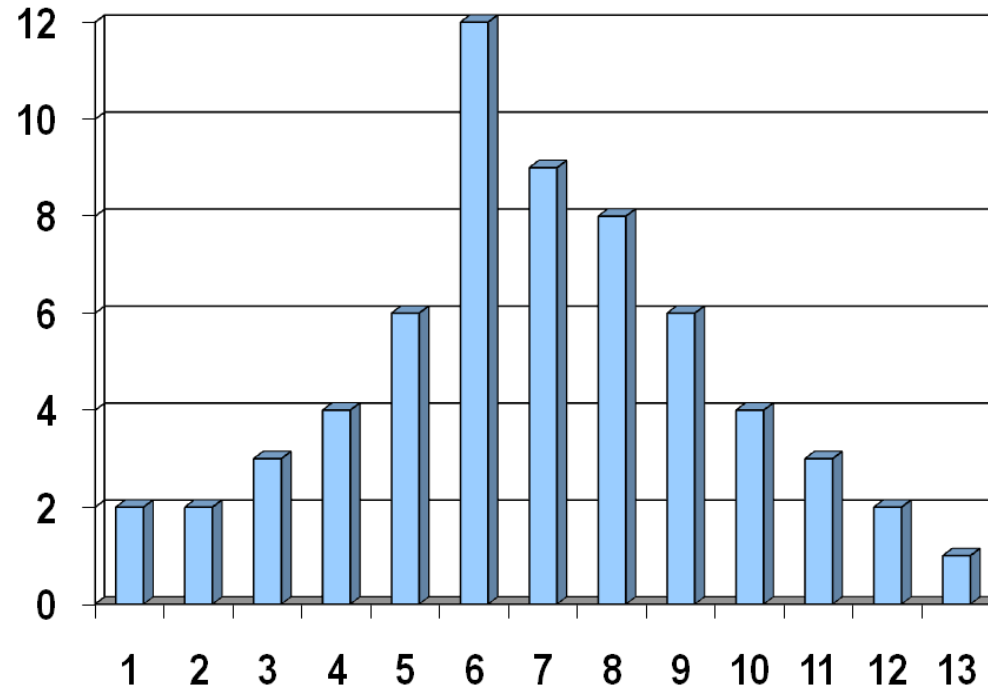


The goal is to differentiate between variability due to chance from that due to error



# Measures of Central Tendency

Although variable, sets of data are distributed around a central value



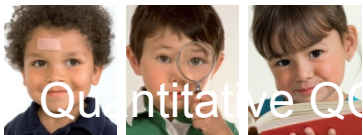
Quantitative Q

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# Measures of Central Tendency

<b>Mode</b>	the value which occurs with the greatest frequency
<b>Median</b>	the value at the center or midpoint of the observations
<b>Mean</b>	the calculated average of the values

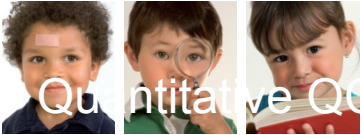
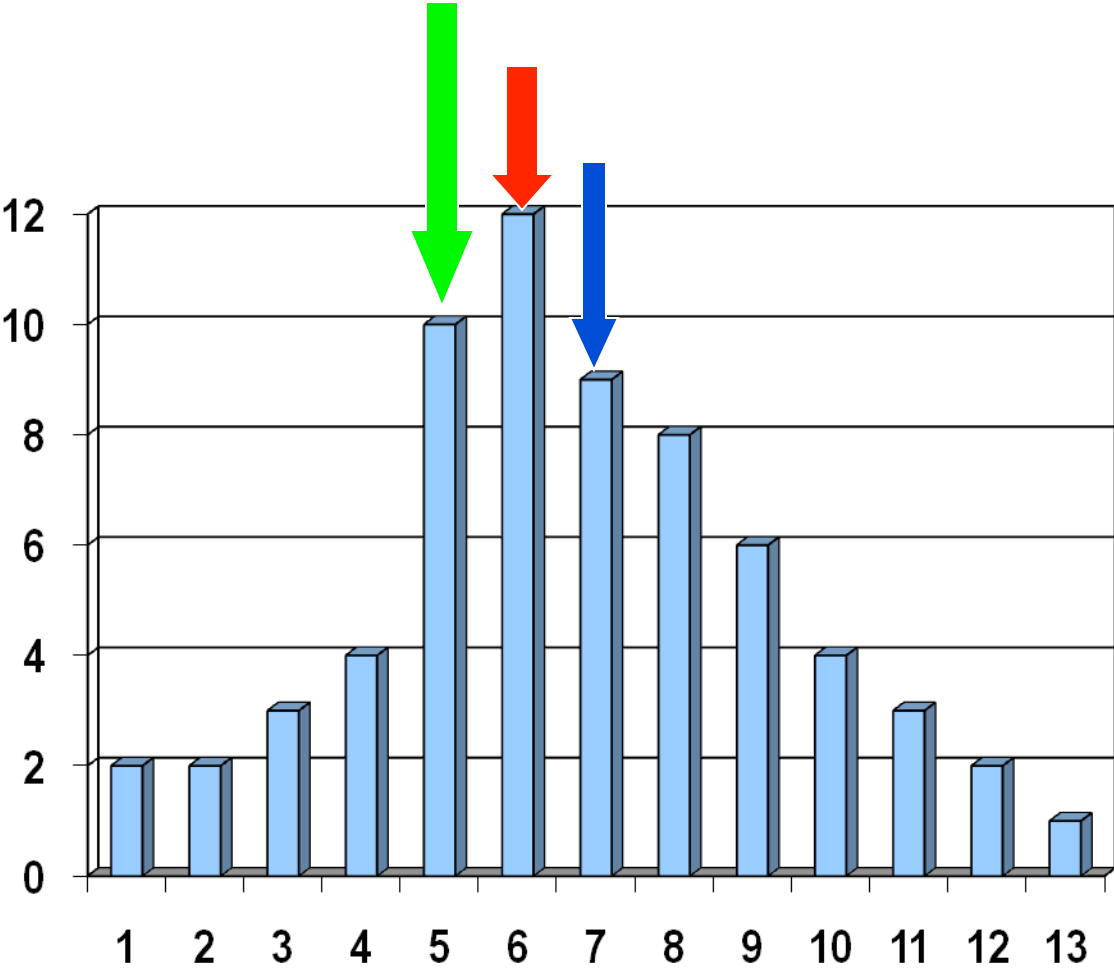


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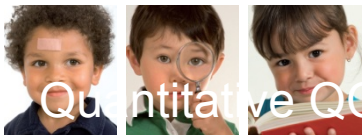
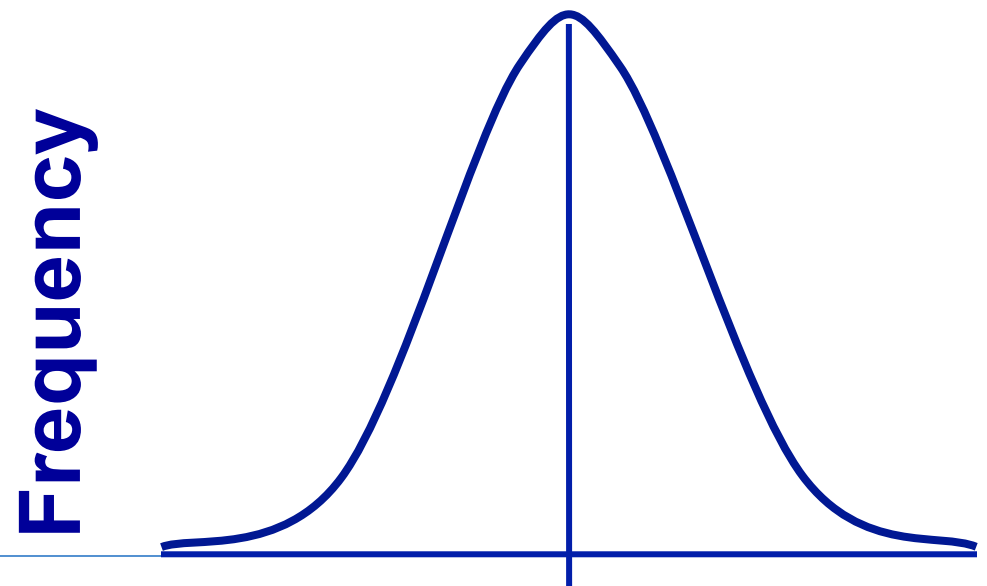
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# Not all central values are the same



# Normal distribution

- all values symmetrically distributed around the mean
- characteristic “bell-shaped” curve
- assumed for all quality control statistics



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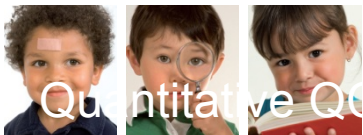
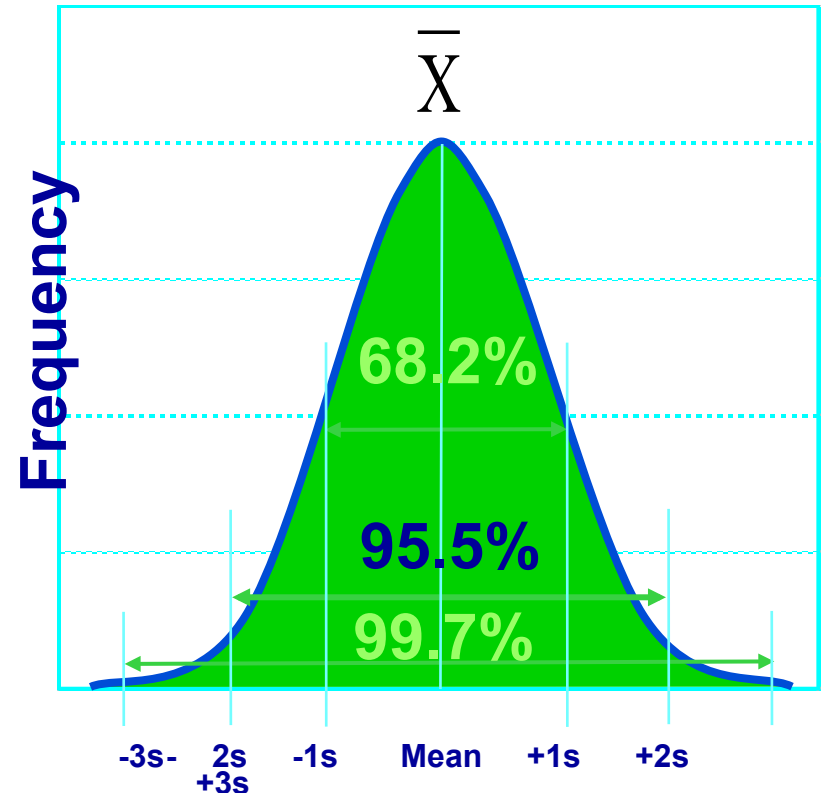
# Standard Deviation and Probability

For a set of data with a **normal distribution**, a random measurement will fall within:

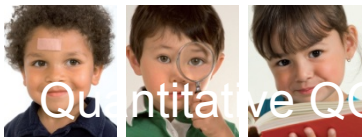
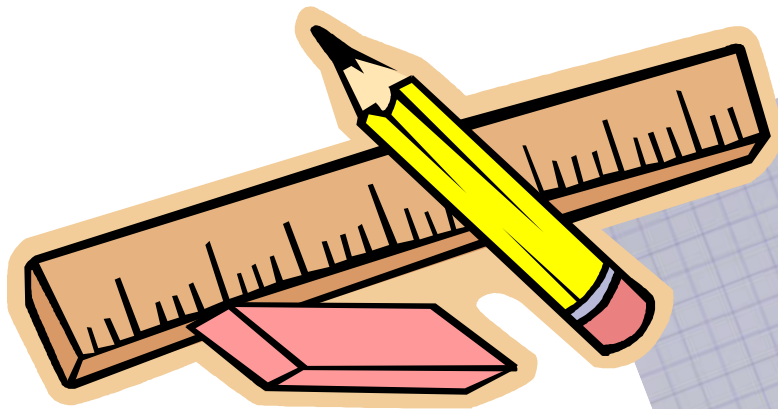
+ 1 SD 68.3% of the time

+ 2 SD 95.5% of the time

+ 3 SD 99.7% of the time



# Levey-Jennings Plot



Quantitative QC

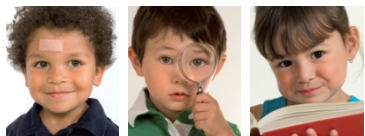
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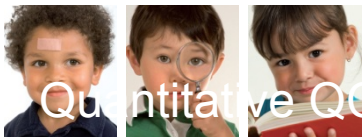


# Run Log Forms

Run Log: Real Time PCR Standards																					
				BK quant				Adenovirus				EBV		CMV		Parvovirus B19					
Date (yymmdd):		Instrument #:				QC Lot#/ Setting:				SI U		Date (yy dd):									
		Run 1	Run 2	Run 3	Run 4	Run 5	Run 6	Run 7	Run 8	Run 9	Run 10	Min	Max	Mean	Std Dev			Run 1	Run 2	Run 3	Run 4
		Ct														Ct					
S4																S4					
S3																S3					
S2																S2					
S1																S1					
Calc Conc (IU/ml)																Calc Conc (IU/ml)					
S4																S4					
S3																S3					
S2																S2					
S1																S1					
% Var																% Var					



# Draw lines for Mean and SDs (calculated from 20 controls)

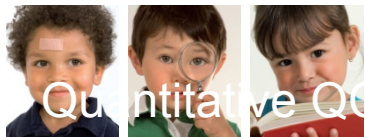
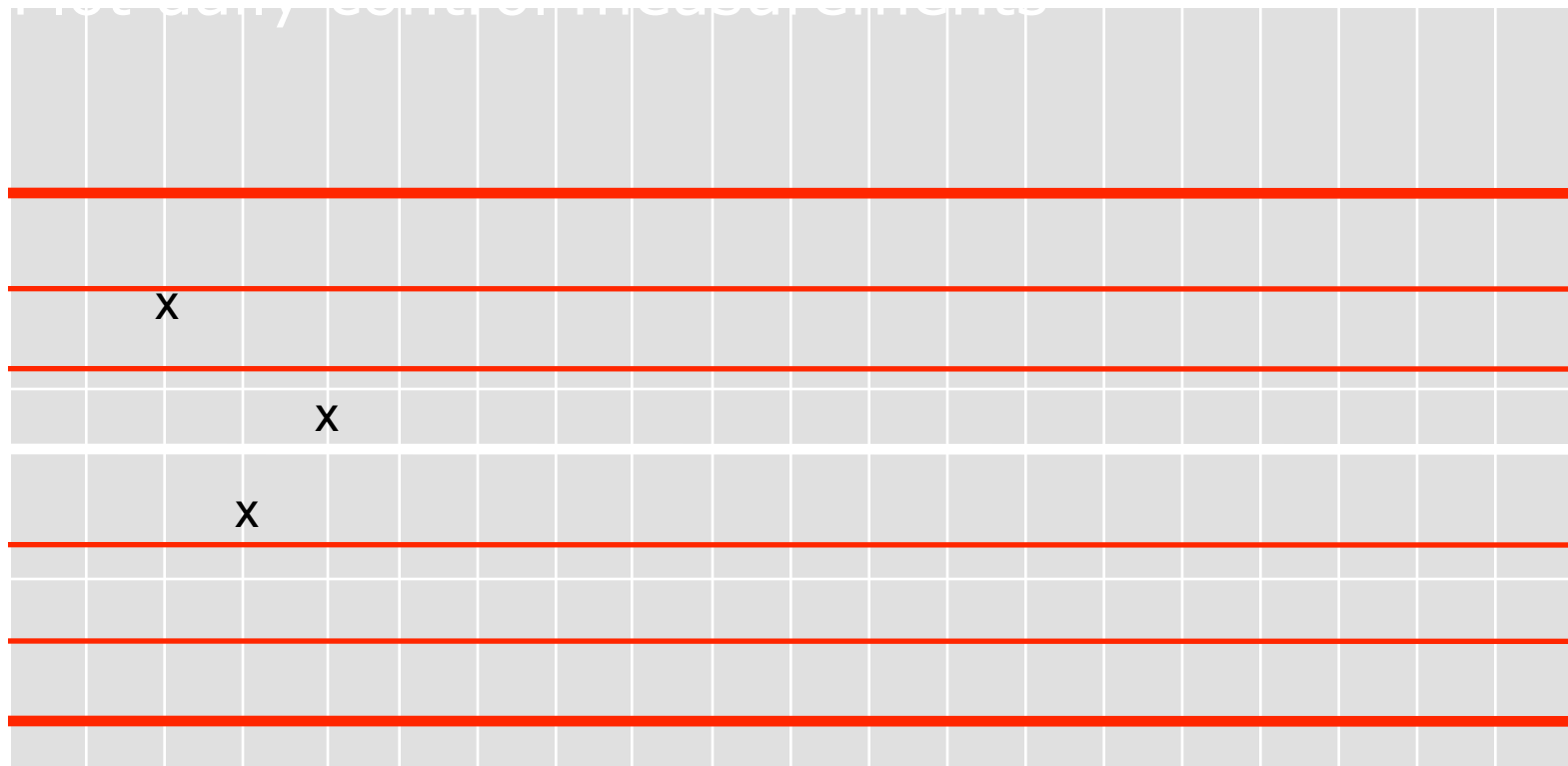


Quantitative Qc

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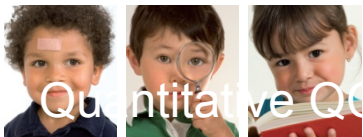
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# Levey-Jennings Chart



# Number of Controls

- Interpretation depends on number of controls run with patients' samples.
  - **Good:** If one control:
    - accept results if control is within  $\pm 2SD$  unless shift or trend (many random errors)
  - **Better:** If 2 or more controls
    - apply Westgard multirule system



# An example of Westgard rules

## Warning Rule

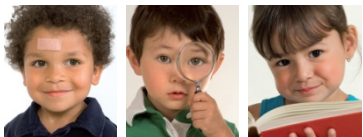
- 1 point is outside 2 SD ( $1_{2s}$ )

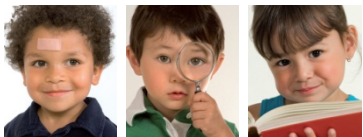
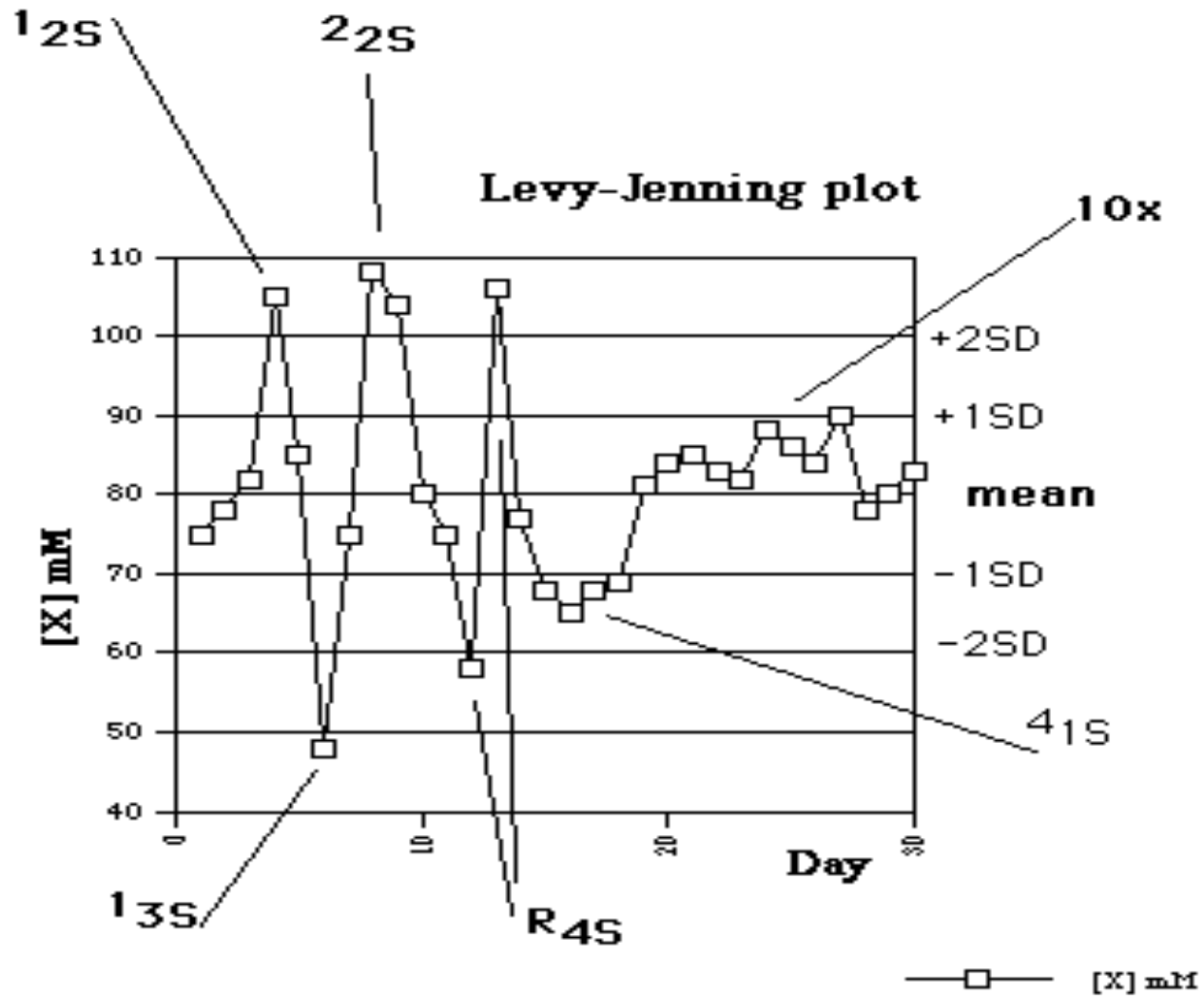
## Rejection Rule

- 1 point is outside 3 SD ( $1_{3s}$ )
- 2 consecutive points are outside 2 SD on the same side of the center line ( $2_{2s}$ )
- Range of 2 points is greater than 4 SD ( $R_{4s}$ )
- 4 consecutive points exceed 1 SD on the same side of the center line ( $4_{1s}$ )
- 10 consecutive points are above or below the mean (10x)

We are in the process of trying to define ours (one control)

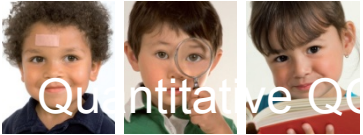
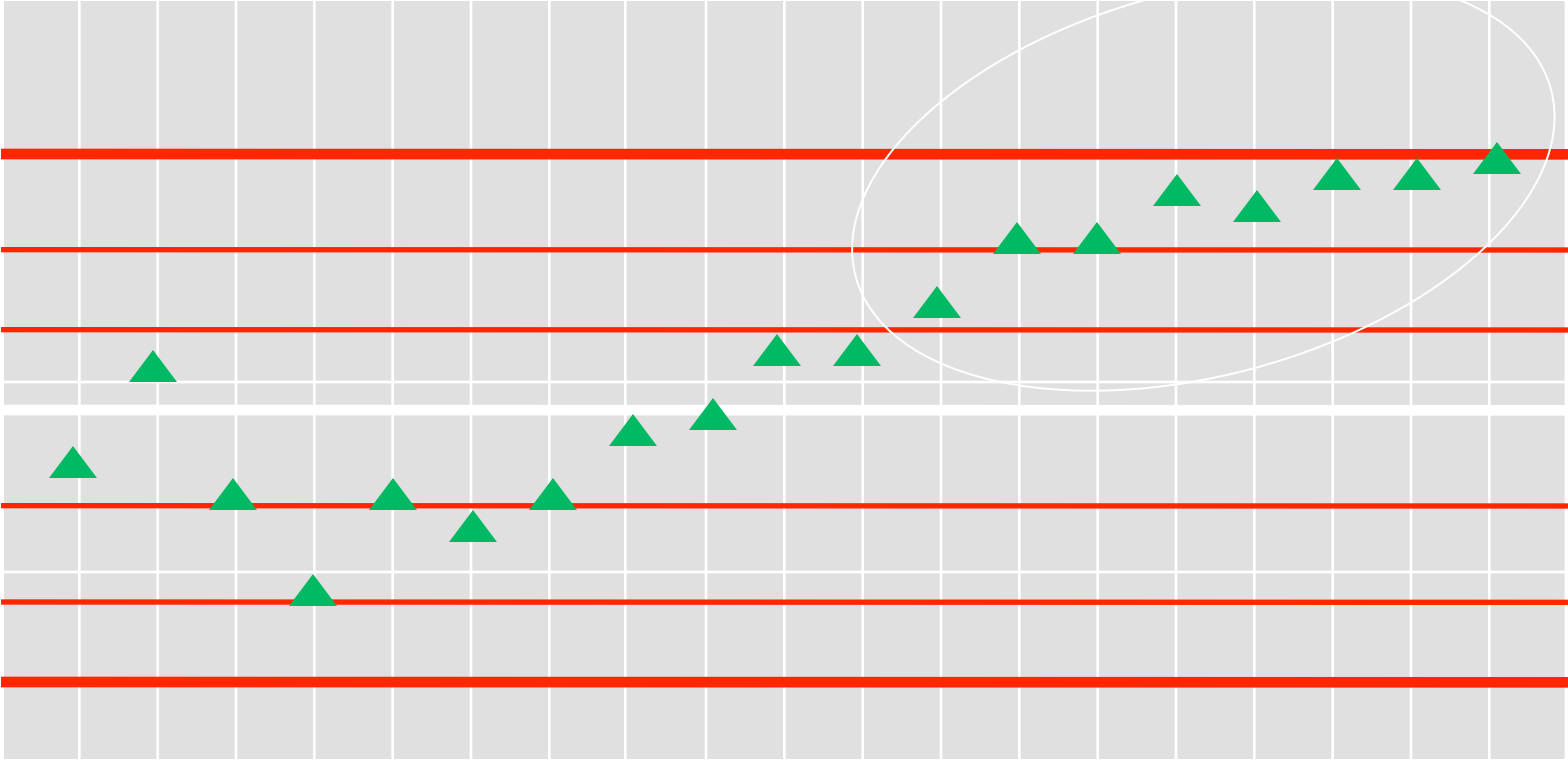
Do we define something similar for our standards or do we use a different method (variance)?





# Levey-Jennings Chart

## Trend

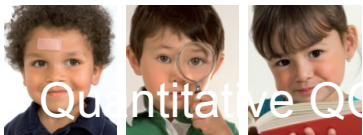


## If QC is out of control

### **STOP** testing

- identify and correct problem
- repeat testing on patient samples and controls after correction

**Do not report patient results** until problem is solved and controls indicate proper performance





# What do you do when your controls are “out of control?”

CLIA requires that laboratories develop procedures to manage an out-of-control situation.

Review procedures used

Search for recent events that could cause change such as:

- New reagent kit or lot

- New control bottle

- Instrumentation component replacement

- Instrument maintenance

- Instrument move

Examine the environmental conditions (temperature, humidity, etc.)

Prepare new control materials

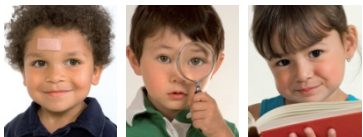
Follow manufacturers troubleshooting guide

Contact manufacturers of:

- Instrumentation

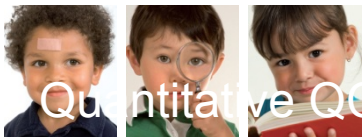
- Reagent materials

- Control materials



# Other Possible Problems

- degradation of reagents or kits
- control material degradation
- operator error
- equipment failure
- calibration error



# OMM5010a/01 Guidelines for internal Quality Control in Molecular Microbiology

## Molecular Microbiology – Quality Control

### Monthly Review

Assay:  
(yymmdd):Initials

Reviewed By: Date

From Date (yymmdd):  
To Date (yymmdd):  
Review Date (yymmdd):

Nursrin Dewsi  
Dr. A. Petrich  
Dr. Y. Yau

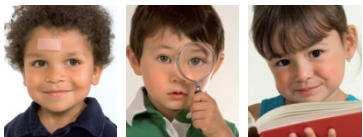
**Flagged Results / Observations:**

**Investigation - Note any previous trends/unacceptable results for this test:**

**Classification of Problem:**

Problem with QC Testing  
Problem with QC Material  
Methodologic  
Technical

**Corrective Action to Prevent Recurrence:**

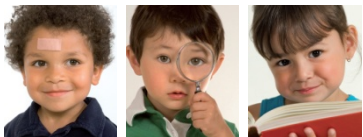


# Summary

A quality control program for quantitative tests is essential. It should:

- monitor all quantitative and qualitative tests that can have numerical values (real-time assays, Ct)
- have written policies and procedures, followed by laboratory staff
- assign someone to monitor and review QC data
- use statistical analysis
- provide for troubleshooting and corrective actions

Documentation of all of this

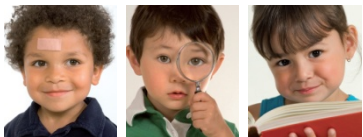


# Key Messages

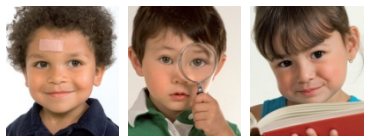
A QC program allows the laboratory to differentiate between normal variation and error.

The QC program monitors the accuracy and precision of laboratory assays.

The results of patient testing should never be released if the QC results for the test run do not meet the laboratory target values.



Thank you!



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