Quality control in Immunoassay

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Quality Control(definition)

- **quality control (QC) – part of quality management focused on fulfilling quality requirements**
- **Definition in Medical Laboratory:**

1. **the set of procedures based on measurement of a stable material that is similar to** the intended patient specimen, to monitor the ongoing performance of a measurement procedure and detect change in that performance relative to stable baseline analytical performance;
2. **QC** includes testing QC materials, charting the results and analyzing them to identify sources of error, and evaluating and documenting any remedial action taken as a result of this analysis.

QC- Statistical Quality control(SQC or SPC)

- **periodic measurement of stable QC materials, designed to mimic as much as possible the analytical behavior of patient samples**
  - Determine Target Values and Standard Deviations for Quality Control Materials that Represent Stable Analytical Performance.

QC- Statistical Quality control(Standard Deviation)

- **Stable Total Imprecision (Standard Deviation) for Each Control Material**
  - **Repeatability**
  - **within-laboratory precision**
  - **SD based on QC data obtained from a long enough time(electronic noise, pipette performance, detector performance, temperature control, daily recalibration)**
  - **Minimum data: 30**
  - **Pooled Standard Deviation**

- $$SD_{pooled} = \sqrt{\frac{(n_1-1)SD_1^2+(n_2-1)SD_2^2+\cdots+(n_k-1)SD_k^2}{n_1+n_2+\cdots+n_k-K}}$$

Pooled Standard Deviation (worked example)

در آزمایشگاهی انحراف معیار نتایج سنجش TSH از روز اول تا بیستم ماه معادل 0.2 mIU/L برگرفته از نتایج 20 بار اندازه گیری برروی نمونه کنترلی با میانگین 2.5 mIU/Lبدست آمده است. در روز بیست و یکم ماه برروی دستگاه پردازشگر الایزا این آزمایشگاه از سوی شرکت پشتیبان تعمیر و نگهداری انجام پذیرفت. پس از تعمیر و نگهداری تا روز بیست و پنجم ماه بعد انحراف معیار 0.23 mIU/Lاز 35 بار اندازه گیری برروی نمونه کنترلی با میانگین 2.67 mIU/L بدست آمد. در روز بیست و پنجم شناسه ساخت (LOT No) معرف ها تعویض و تا روز دهم ماه بعد انحراف معیار 0.25 mIU/L ماه از 15 بار آزمایش برروی نمونه کنترلی با میانگین 2.37 mIUl/L بدست آمد. از آنجایی که در سه بازه زمانی یادشده میانگین نتایج تغییر عمده ای نداشته است. (کمتر از RCV معادل سه انحراف معیار) انحراف معیار تجمعی سنجش TSH این آزمایشگاه طی بازه زمانی ۷۰ روزه یادشده برابر است با:

$$SD_{pooled} = \sqrt{\frac{(20-1)\times 0.2^2 + (35-1)\times 0.23^2 + (15-1)\times 0.25^2}{20+35+15-3}}$$

$$SD_{pooled} = 0.23\ mIU/mL$$

QC- Statistical Quality control(SQC or SPC)

- **Target Value for Each Control Material**
- **Minimum of 10 measurement in 10 days in parallel with previous Lot for preliminary target assignment**
- **Updated as soon as more data become available**
- **Adjusting the Target Value During the Life of the Lot of Quality Control Material**
- **Reagent lot changes some maintenance procedures, or deterioration of a Analyte during the expected shelf life of a QC material**

Quality Control(Control Material)

- **Appropriate concentrations,**
- **product stability and shelf life.**
- **Appropriate level (3 level for TSH and 2 level for most tumor markers)**
- **Commutability (Additives to stabilize agents)**
- **obtain enough homogeneous and stable control material(e.g one year or more to evaluate stability of a Measuring System)**
- **In-kit controls(Can not be used with other Methods)**
- **Third party vendor control martials(Limitation of assigned value)**
- **Change CV% to SD**

$$SD = \frac{CV \times 100}{\overline{X}}$$

Set Quality Specification Goals

- Six different approaches for setting quality goals
  - i) reference intervals,(Tonks Method)
  - (ii) opinions of clinicians,
  - (iii) the state of the art,
  - (iv) the views of expert individuals and groups,
  - (v) data on biological variation and Desirable Analytical Quality Specifications for Imprecision, Bias and Total Error Upon Biological Variation.pdf
  - (vi) analysis of the effect of performance on interpretation in specific clinical situation

$$2CV = \left(\frac{{}^{1}/_{4}\, refrence\ interval}{mean\ of\ reference\ interval} \right) \times 100$$

Quality goals Based on Biological variation

- $Excellent\ \ CV_A =< 0.1\ CV_I$
- $Optimum\ CV_A =< 0.25\ CV_I$
- $Desirable\ CV_A =< 0.5\ CV_I$
- $Minimum\ CV_A =< 0.75\ CV_I$
- $Optimum\ B_A = 0.125\sqrt{CV_I^2 + CV_G^2}$
- $Desirable\ B_A = 0.25\sqrt{CV_I^2 + CV_G^2}$
- $Minimum\ B_A = 0.375\sqrt{CV_I^2 + CV_G^2}$
- $ATE = 1.65CV_A + B_A$

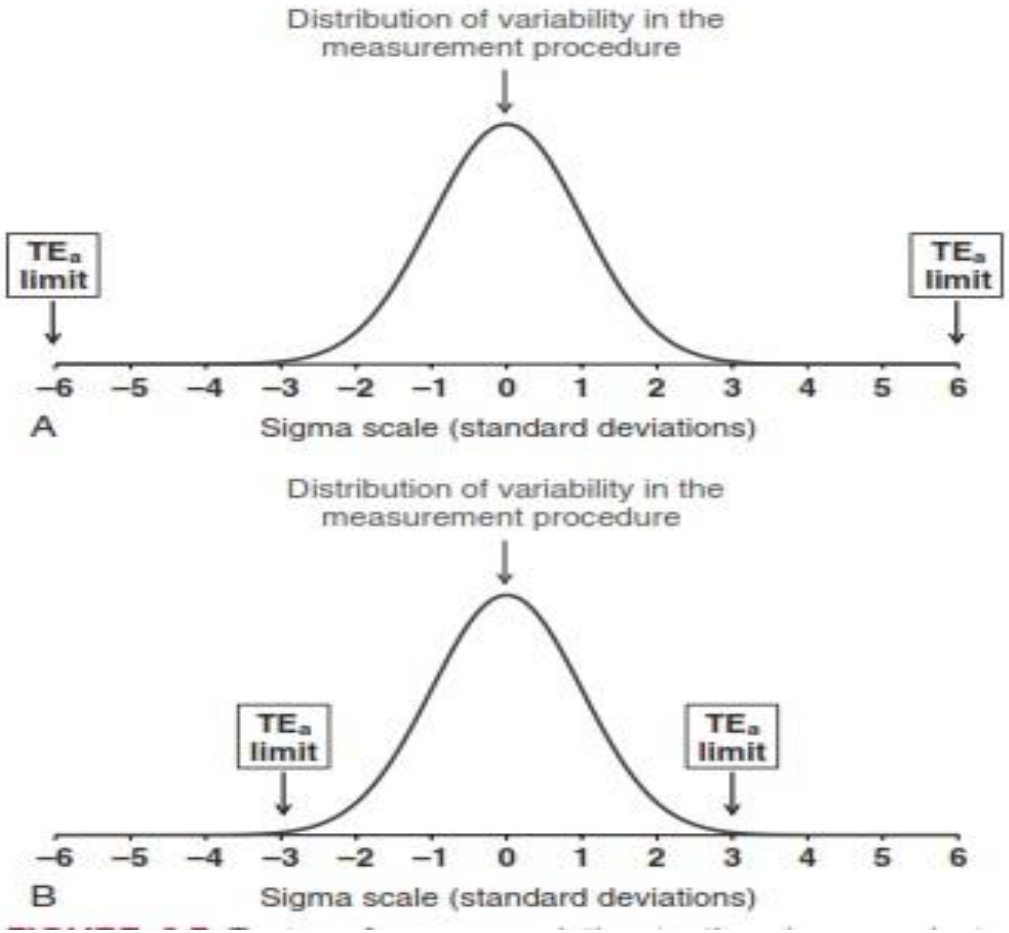
Quality control Rules

Rule	Meaning	Detects
1_{2s}	One observation exceeds 2 SDs from the target value	Bias or imprecision
1_{3s}	One observation exceeds 3 SDs from the target value	Bias or large imprecision
$1_{2s}(1_{2.5s})$	Two sequential observations, or observations for two QC samples measured at approximately the same time, exceed 2 SDs (or 2.5 SDs) from the target value in the same direction	Bias
$2\ of\ 3_{2s}$	Two observations for three QC samples measured at approximately the same time exceed 2 SDs from the target value in the same direction. Note that this type of rule is used when three QC materials are used for a measurement procedure	Bias
R_{4s}	Range between observations for two QC samples measured at approximately the same time, or for two sequential observations of the same QC sample, exceeds 4 SDs.	Imprecision

Quality control Rules(contd.)

Rule	Meaning	Detects
$10_X or\ 10_m$	Ten sequential observations for the same QC sample are on the same side of the target value (x or mean). excessive false−alert rate.	Not recommended
$8_{1s}(8_{1.5s})$	Eight sequential observations for the same QC sample exceed 1 SD (or 1.5 SD) in the same direction from the target value.	Bias trend
CUSUM	CUSUM of SDI for the current and previous results.	Bias trend
EWMA	EWMA for the current and previous results with newer results having more influence (weight).	Bias trend

QC-sigma metric



QC-sigma metric

Probability of Acceptable or Erroneous Results Based on the Sigma Scale

Sigma Value	Percent of Results Within Specification	Percent of Results With an Error (Defect)	Errors (Defects) per Million Opportunities
1	68	32	317,311
2	95.5	4.5	45,500
3	99.7	0.3	2,700
4	99.994	0.006	63
5	99.99994	0.00006	0.6
6	99.9999998	0.0000002	0.002

QC-sigma metric(Cont'd)

- Calculated for each measurement procedure (designing an appropriate QC plan)
  - Selecting QC rule
  - QC sample testing frequency,

$$Sigma = \frac{TAE - bias}{SD}$$

QC- trueness control

- Trueness and accuracy definition
- Trueness control using certified Reference Material(Too expensive for routine use)
- In most cases of Immunoassay, analytes are not sufficiently well-defined(are not metrologically traceable to SI units)
- **Interlaboratory comparisons**
  - **Interlaboratory QC**
  - **Proficiency testing -External quality assessment (program)**

PT-EQA(EQAP)

- Samples with unknown commutability
- Results grouped Based on priority Instrument/analytical principle/Method
- Peer group
- Outliers trimmed and target value assigned to each peer group
- Z-score or SDI calculated as follow:

$$Z_i(SDI) = \frac{X_i - X_{pt}}{\sigma_{pt}}$$

- σ_{pt} calculated from participants results or consensus value(CCV)

PT-EQA(EQAP) – A worked Example

- In an EQAP for human chorionic Gonadotropin:
  - Target Value for XXX group(X_{EQA}): 160 U/L
  - Lab YYY result: 165 U/L
  - $CCV_{hCG} = 10$
  - $\sigma_{EQA} = \frac{CCV \times X_{EQA}}{100} = 16$
  - $Z_i(SDI) = \frac{165-160}{16} = 0.31$

Excellent: SDI<=0.99
Acceptable: 1<=SDI<=2.0
Questionable: 2.0<=SDI<=2.99
Unacceptable: SDI>=3.0

QC-Pre-analytical Factors

- **32% to 75% of all testing errors occur in the preanalytic phase**
  - **Diurnal variation GH- cortisol**
  - **Exercise and stress(prolactin-cortisol-T4)**
  - **Inadequate patient preparation(fasting, improper timing- Insulin, GH,Cortisol)**
  - **Hemoconcentration from prolonged tourniquet time(FT4)**
  - **Improper transport to lab (ACTH)**
  - **Processing errors: Incomplete centrifugation(clot particle-physical barrier in microwell)**

QC-Analytical specificity-Interferences

- Ability of an assay procedure to determine the concentration of target analyte without influence from interfering substances
- $X_i = True\ Value + cal\ bias + Random\ Bias + \epsilon_i$
- Random-Bias=Sample-Related Bias
- General Interferences: Lipemia, Hemolysis, Icteric sample-Immunoassay are less affected by these type of interferences due to B/F seperation

QC-Analytical specificity- Immunoassay Interferences

- Heterophilic Antibodies (HAMA)
- Rheumatoid Factor
- Cornstarch in powdered gloves(ELFA)
- Macromolecules with serum proteins(Macro-Prolactine, Macro-TSH)
- Cross-reaction
- Nonsense Assay for detecting Immunoassay interferences
- Co-operation with IVD Producer

Testing for interference in suspected samples.

- **Use of an alternate immunoassay that preferably uses antibody raised to a different species**
- **Measurement before and after addition of a blocking reagent (Non Immune mouse Immunoglobulin)**
- **Unexpected result in dilution study**
- **Choosing appropriate diluent**
- **Sample pre-treatment (Precipitation with 25% PEG-6000 and reassay of supernatant- unexpected recovery)**
- **inconsistency with clinical findings**

QC- Validation and Verification

- **Validation – confirmation, through the provision of objective evidence, that requirements for a specific intended use or application have been fulfilled.**
  - non-standard methods;
  - laboratory designed or developed methods;
  - standard methods used outside their intended scope
  - validated methods subsequently modified.

QC- Validation and Verification

- **verification – confirmation, through the provision of objective evidence, that specified requirements have been fulfilled**
  - **The laboratory shall confirm that the performance claims such as precission, trueness, measuring interval, linearity, Detection limit and …for the examination procedure have been met.**
  - **The performance claims for the examination procedure confirmed during the verification process shall be those relevant to the intended use of the examination results**

Performance characteristics of Measurement procedure

These characteristics are more applicable for qualitative tests or those which have qualitative interpretation

$$Clinical\ sensitivity(Diagnostic\ sensitivity) = \frac{TP}{TP + FN} \times 100$$

$$Clinical\ Specificity(Diagnostic\ Specificity) = \frac{TN}{TN + FP} \times 100$$

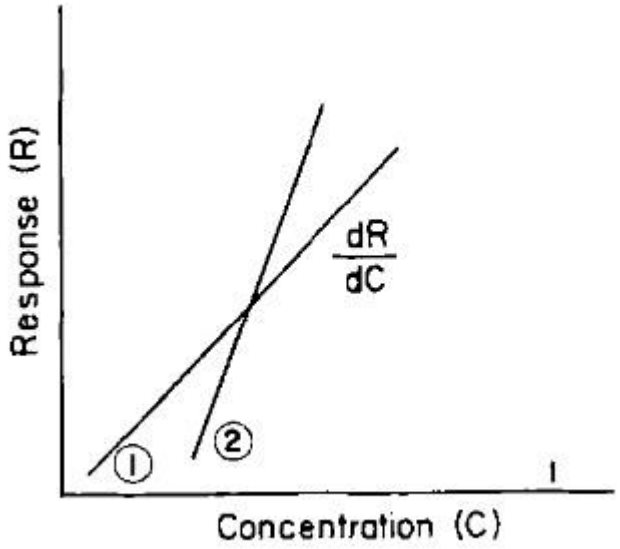
$$Diagnostic\ Accuracy = \frac{TN + TP}{TN + FN + TP + FP}$$

$$Positive\ predictive\ value = \frac{TP}{TP + FP}$$

$$Negative\ Predictive\ value = \frac{TN}{TN + FN}$$

Two latter ones depends on prevalence of diseases

Performance characteristics- Analytical sensitivity & detectability



The smallest difference that will be statistically significant equals $2\sqrt{2}SD_A$

Performance characteristics- Linearity evaluation

- relationship between measured and expected values over the analytical measurement range.
- Make several dilution with appropriate matrix and evaluate relation between expected and measured value (Bias and recovery)
- Regression analysis between expected(True) value on x axis and measured value on y axis

$$\text{Recovery}\% = \frac{\text{Observed}}{\text{Expected}} \times 100$$

$$\text{Bias}\% = \frac{\text{Observed} - \text{Expected}}{\text{Expected}} \times 100$$

Bias evaluation-proportional error

- Recovery evaluation
  - Recovery Studies are used to establish what portion (percent) of the analyte in the specimen is being measured. Ideally 100%

$$Concentration\ analyte\ added = high\ analyte\ Conc \times \frac{vol.analyte}{total\ volume}$$

$$hCG\ added = 1000\frac{IU}{L} \times \frac{0.1}{1} = 100$$

$$\%Recovery = \frac{Concentration\ Recovered}{Concentration\ Added} \times 100$$

$$\%hCG\ Recovery = \frac{179}{185} \times 100 = \%96.75$$

$$Proportional\ error = 1 - Recovery = 1 - 0.9675 = 0.0325$$

$$Proportional\ error = 3.25\%$$