The Role of QC in Sample Analysis

ECMC QATS Group - GCP Training Day

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Good Clinical Practice Guide

TSO   2012

Chapter 13: Clinical trials samples – analysis and evaluation

Throughout this chapter, specific terminology - 'must', 'required' or 'requirement' – has been used to interpret activities that are legislative requirements. These terms have been number-coded in the text where used, and the corresponding reference in the legislation can be found below.

Legislative References
1. Regulation 28 (1) of SI 2004/1031
2. Regulation 29 of SI 2004/1031
3. Schedule 1, Part 2 (4) of SI 2004/1031
4. Schedule 1, Part 2 (2) of SI 2004/1031
5. Schedule 1, Part 2 (9) of SI 2004/1031
6. Regulation 31A (4) of SI 2004/1031
7. Regulation 31A (7) of SI 2004/1031
8. Schedule 1, Part 2 (13) of SI 2004/1031

13.6 Maintaining quality within the laboratory

The quality of laboratory work is of utmost importance………Quality requirements…..can be split into two functions: quality control (QC) and QA. Both are equally important but have a different focus and purpose.

13.6.1 Quality control

The accuracy of all laboratory processes should be subject to **<u>some</u>** level of QC checks

14.3 Quality control

QC is a fundamental approach to verify compliance with the Clinical Trials Regulations (Schedule 1, Part 2 (4) and (9) of SI 2004/1031) and should be implemented.

QC is an activity that involves the review of factors in a process as the process is occurring.

Expectations relating to acceptable standards of QC should be documented; this would usually define **acceptable error rates** and is recommended that the process defines the **actions to be take**n where the QC checks show a failure to meet the acceptable predefined standard

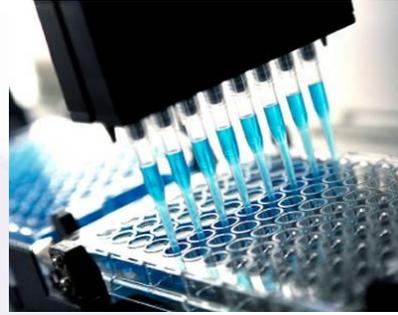# Quality Control Applies to Each Stage in Patient Sample Analysis

Collection Storage **Analysis** Data Capture Interpretation

National Academy of Clinical Biochemistry Laboratory Medicine

Quality Requirements

Errors in Laboratory Tests
Pre-Analytical Phase: 30-75%
Analytical Phase: 13-31%

GCP Guide

13.3.3 Data Recording

(Data) QC checks must[6] be documented and retained

The confirmation by the provision of objective evidence that the particular requirements for a specific intended use are fulfilled*

*ISO 17025 and 9000

- **QCs** are samples of known concentration/number/staining pattern/Δct/etc run together with the patient samples to objectively evaluate assay performance

- They should resemble as closely as possible the test samples and are therefore meant to replicate the behaviour of patient samples in the assay

- Effectively, QCs function to measure the level of error/uncertainty present in an assay

- **Acceptance Criteria** are the **control limits** that are set for the expected performance of QCs - based on intended purpose -   and the operate in conjunction with a **Decision Rule**

QC Monitoring Over Time: Focus on Control Charts and Decision Rules

Shorter Term; Focus on Acceptance Criteria

Diagnostic
Laboratories

CROs - GLP
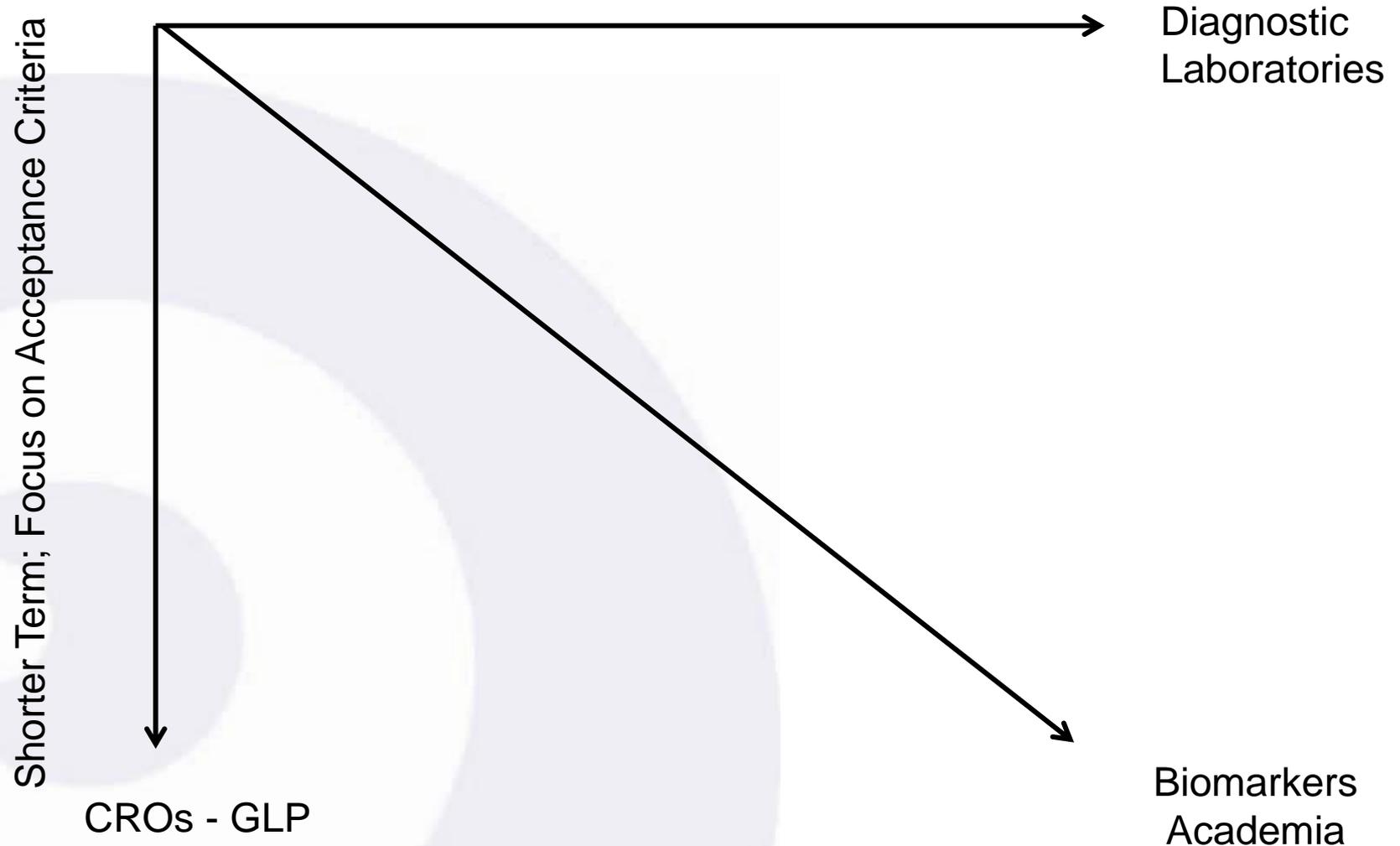
Biomarkers
Academia

1. Qualification of Multiplex ELISA Determination of Angiogenesis Analytes as Predictive Biomarkers of Response to Anti-Vascular Drugs

2. Enumeration of Circulating Tumour Cells (CTC) in Patient Stratification in a Proposed new Trial in Colorectal Cancer

3. Immunohistochemistry to Determine Pharmacodynamic Changes in the Expression of the Androgen Receptor in CTC after Treatment of Prostate Cancer Patient with a Phase I Drug

To be Followed Up in More Detail at the Data Quality Workshop*

Fit-for-Purpose Quality Control Decision Matrix
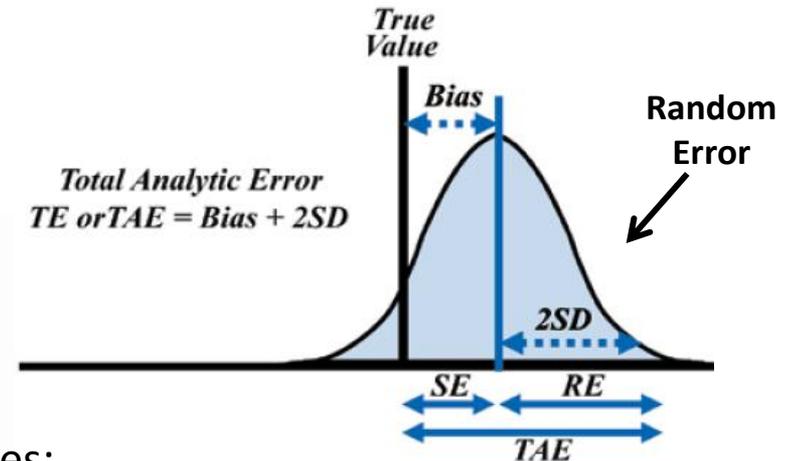## Example 1 – Multiplex ELISA
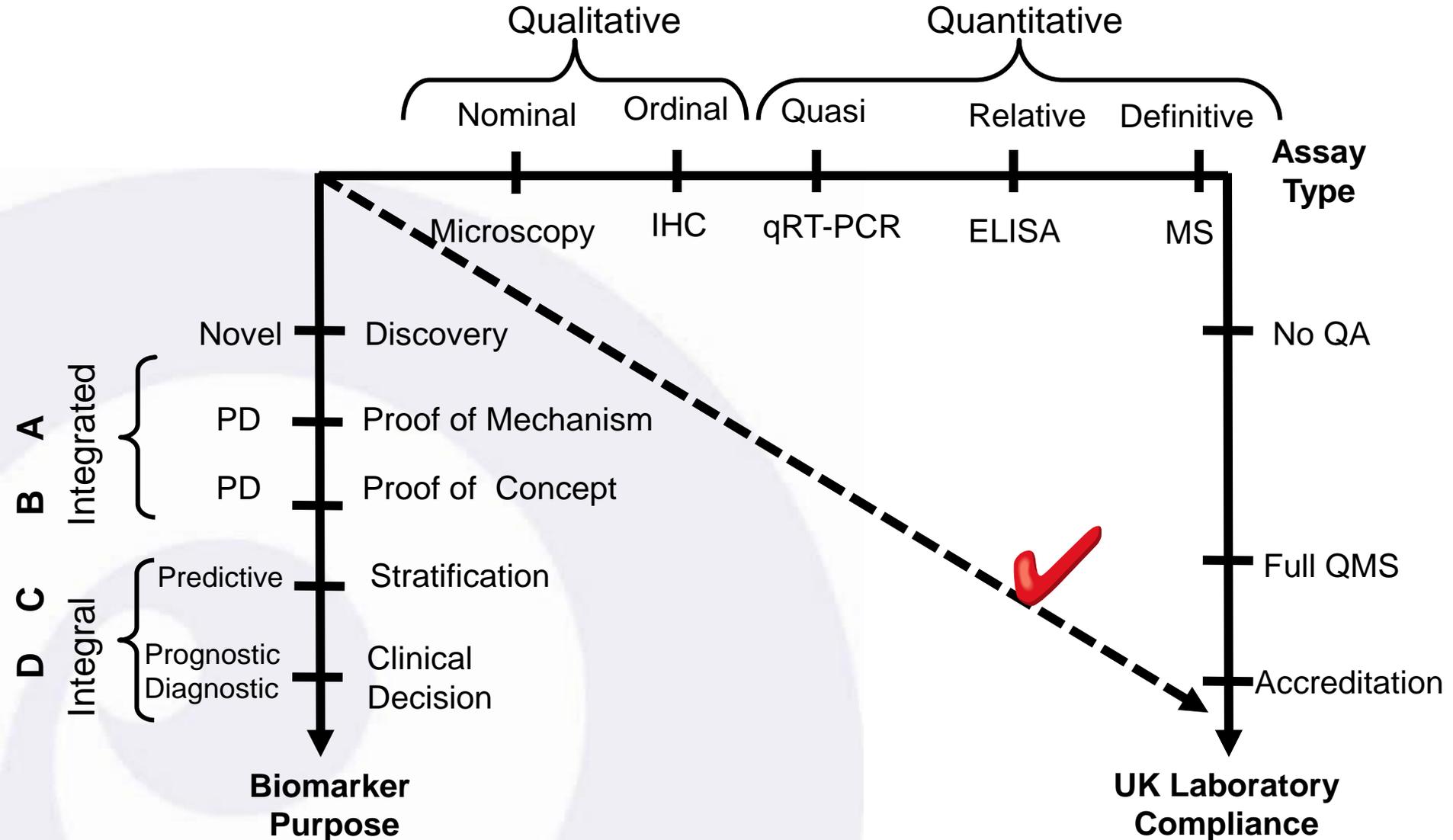
QC Approach to a Relative Quantitative Biomarker Assay

Control Limits: Levi-Jennings Plots
Decision: Westgard Rules
Assessment: Sigma Metric

True Value

Bias

Random Error

Total Analytic Error
TE or TAE = Bias + 2SD
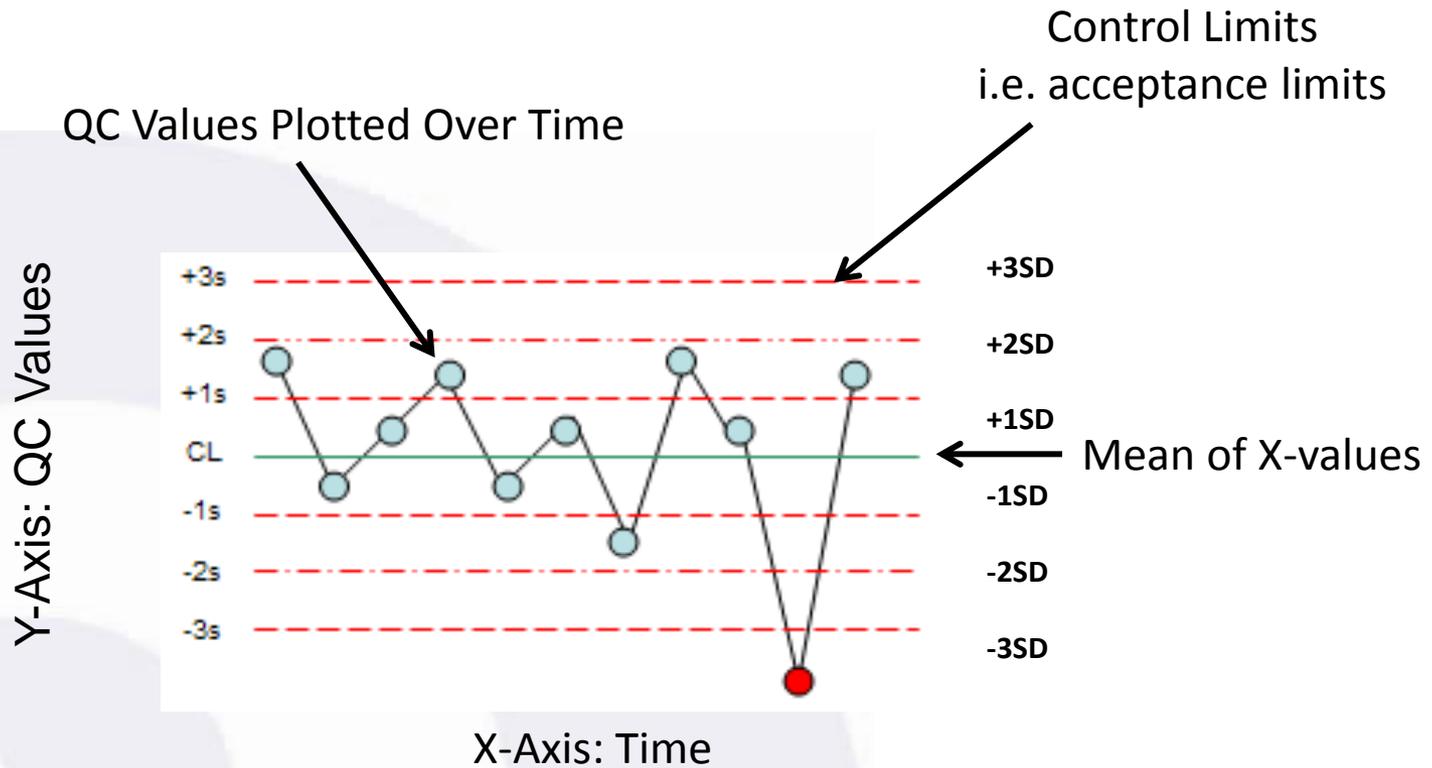
2SD

SE   RE

TAE

Westgard Rules:

- ✓ Based on the concept of Total Error (TAE): the combination of Systematic Error (SE, i.e. Bias) and Random Error (Imprecision) – Therefore ideal for a Relative Quantitative Assay
- ✓ Consists of a series of Decision rules applied in a sequence of increasing analytical rigor
- ✓ The rules have been developed based on power calculations, computer simulations and actual practice and optimised to maximise detection of true assay failures and minimise rejection of valid assays
- ✓ The rules work in conjunction with the Sigma Metric to optimise QC
- ✓ Can be customised to meet the requirements of fit-for-purpose approach to QC
- ✓ Live monitoring of assay performance
- ✓ Identifies the nature of analytical error and thus potentially facilitates correction

Levi-Jennings Control Plots for Monitoring the Progress of Biomarker Assays Over Time

QC Values Plotted Over Time

Control Limits
i.e. acceptance limits

Y-Axis: QC Values

		+3SD
+3s		
+2s		+2SD
+1s		+1SD
CL		Mean of X-values
-1s		-1SD
-2s		-2SD
-3s		-3SD

X-Axis: Time

1_{3s} refers to a control rule that is commonly used with a Levey-Jennings chart when the control limits are set as the mean plus 3s and the mean minus 3s. A run is rejected when a single control measurement exceeds the mean plus 3s or the mean minus 3s control limit.

2_{2s} - reject when 2 consecutive control measurements exceed the same mean plus 2s or the same mean minus 2s control limit.
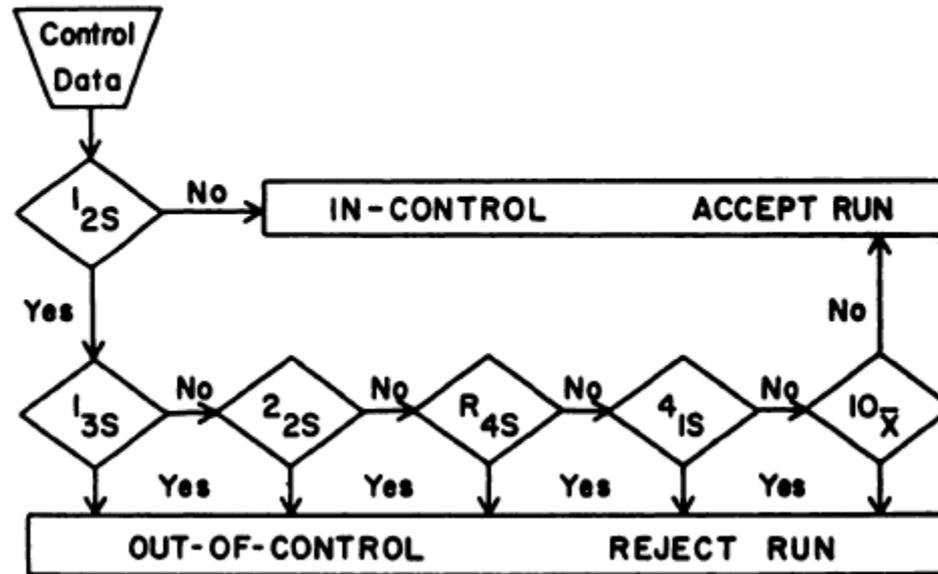
4_{1s} - reject when 4 consecutive control measurements exceed the same mean plus 1s or the same mean minus 1s control limit.

8_x - reject when 8 consecutive control measurements fall on one side of the mean.

7_T - reject when seven control measurements trend in the same direction, i.e., get progressively higher or progressively lower.
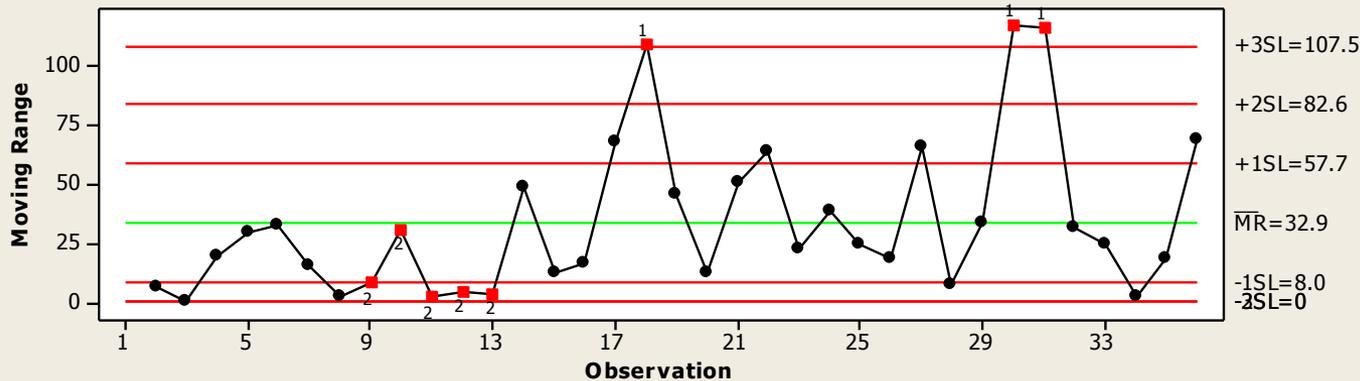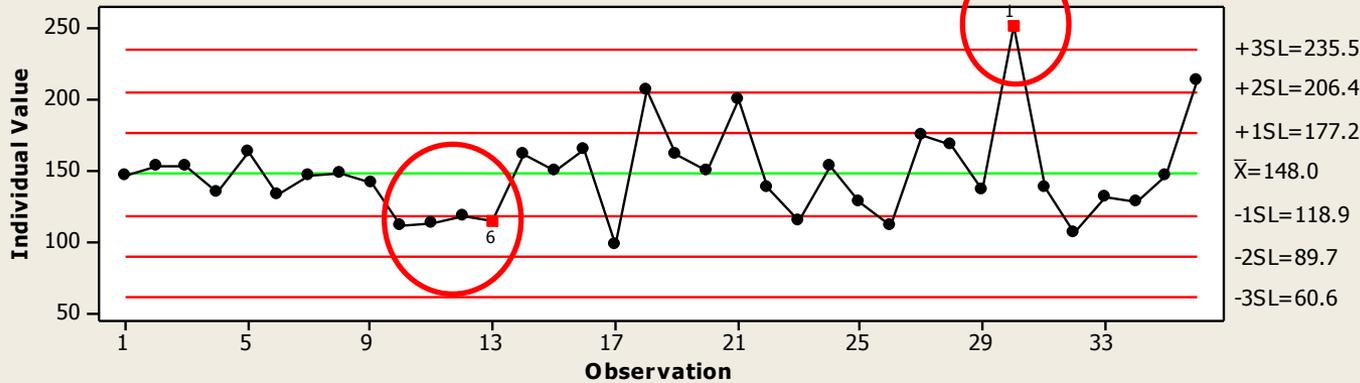
Least Rigorous   Most Rigorous

Matrix and Rules Based on An Assay with 2 Different QC Concentrations Run in Duplicate i.e. 4 QC values

A greater number of QCs will affect the Power Calculation and change the rules

Analysis of Levi-Jennings Plots and Westgard Rules of Multiplex QC Data for FGF beta Evaluated Utilising Minitab

Test 1 = 1_{3S}

Test 2 = 8_X

Test 3 = 7_T

Test 5 = 2_{S2}

Test 6 = 4_{1S}

Error Detection
Test 1 = Random
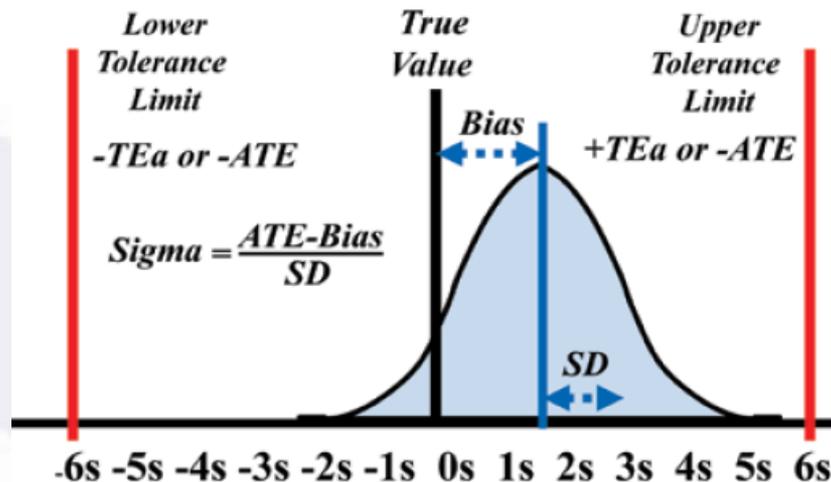Test 2 = Systematic
Test 3 = Systematic
Test 5 = Systematic
Test 6 = Systematic

Sigma-metric Calculation

Lower Tolerance Limit

-TEa or -ATE

True Value

Bias

Upper Tolerance Limit

+TEa or -ATE

$$Sigma = \frac{ATE-Bias}{SD}$$

SD

-6s -5s -4s -3s -2s -1s 0s 1s 2s 3s 4s 5s 6s

Example Calculation – Taken from Westgard QC Web-Site
The CLIA criterion for acceptable performance for cholesterol is 10%. If a laboratory method shows a bias of 2.0% on proficiency testing surveys and a CV of 2.0% on internal QC results, the Sigma-metric is (10-2/2) = 4
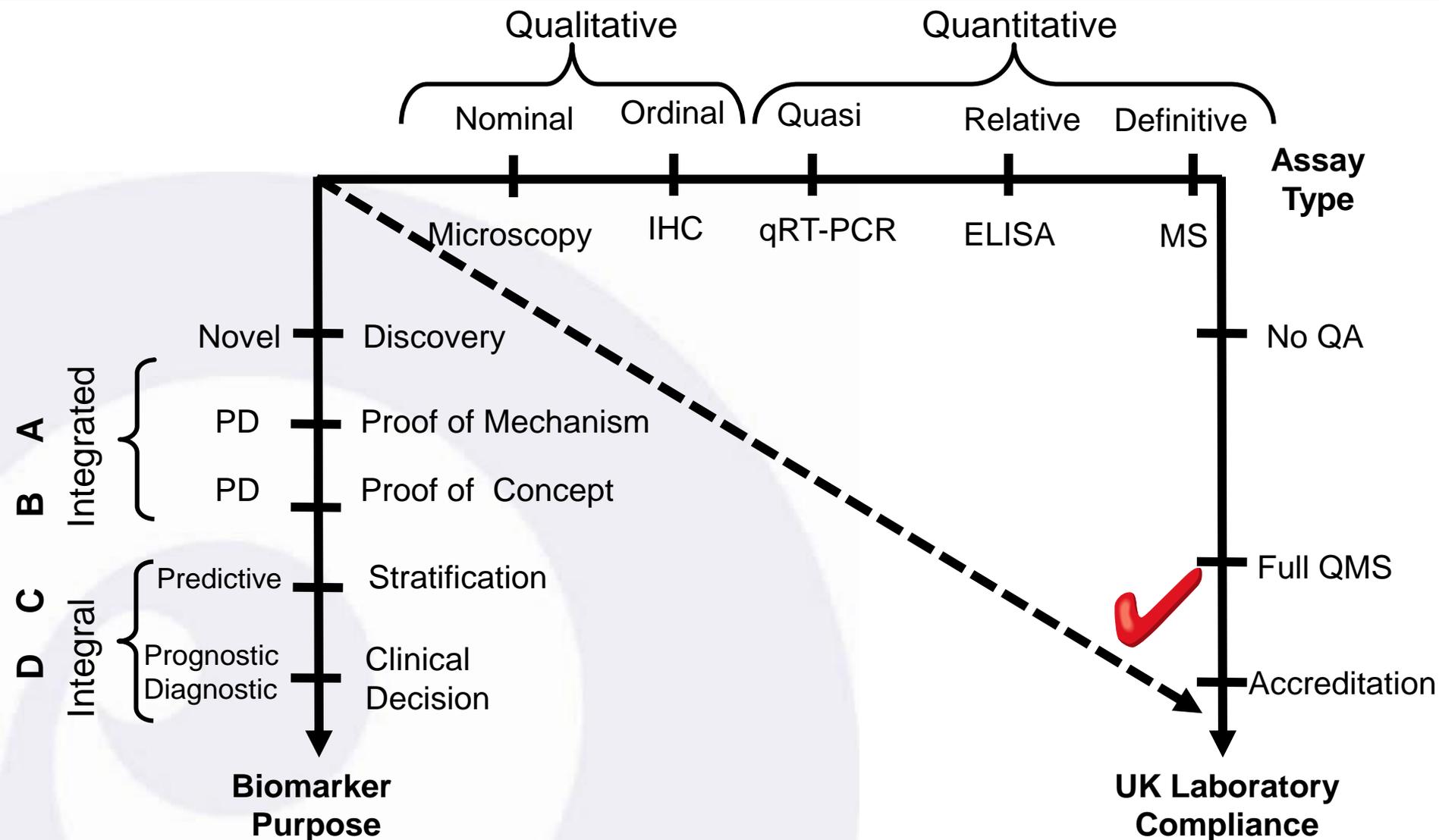
Sigma = 1-2, Poor/Very Poor, high level QC required
Sigma = 3-4, Moderate/Good, regular level of QC required
Sigma = 5-6, Very Good, less QC required

Fit-for-Purpose Quality Control Decision Matrix
## Example 2– CTC Enumeration for Patient Stratification

Qualitative   Quantitative

Nominal   Ordinal   Quasi   Relative   Definitive

Assay Type

Microscopy   IHC   qRT-PCR   ELISA   MS

Novel — Discovery

PD — Proof of Mechanism

PD — Proof of Concept

Predictive — Stratification

Prognostic / Diagnostic — Clinical Decision

Integrated   A   B

Integral   C   D

No QA

Full QMS

Accreditation

Biomarker Purpose

UK Laboratory Compliance

The Most Important Parameter of a Definitive Quantitative Assay is:
Analytical Accuracy

The Ability to Measure the True Concentration/Amount Present in the Patient Sample
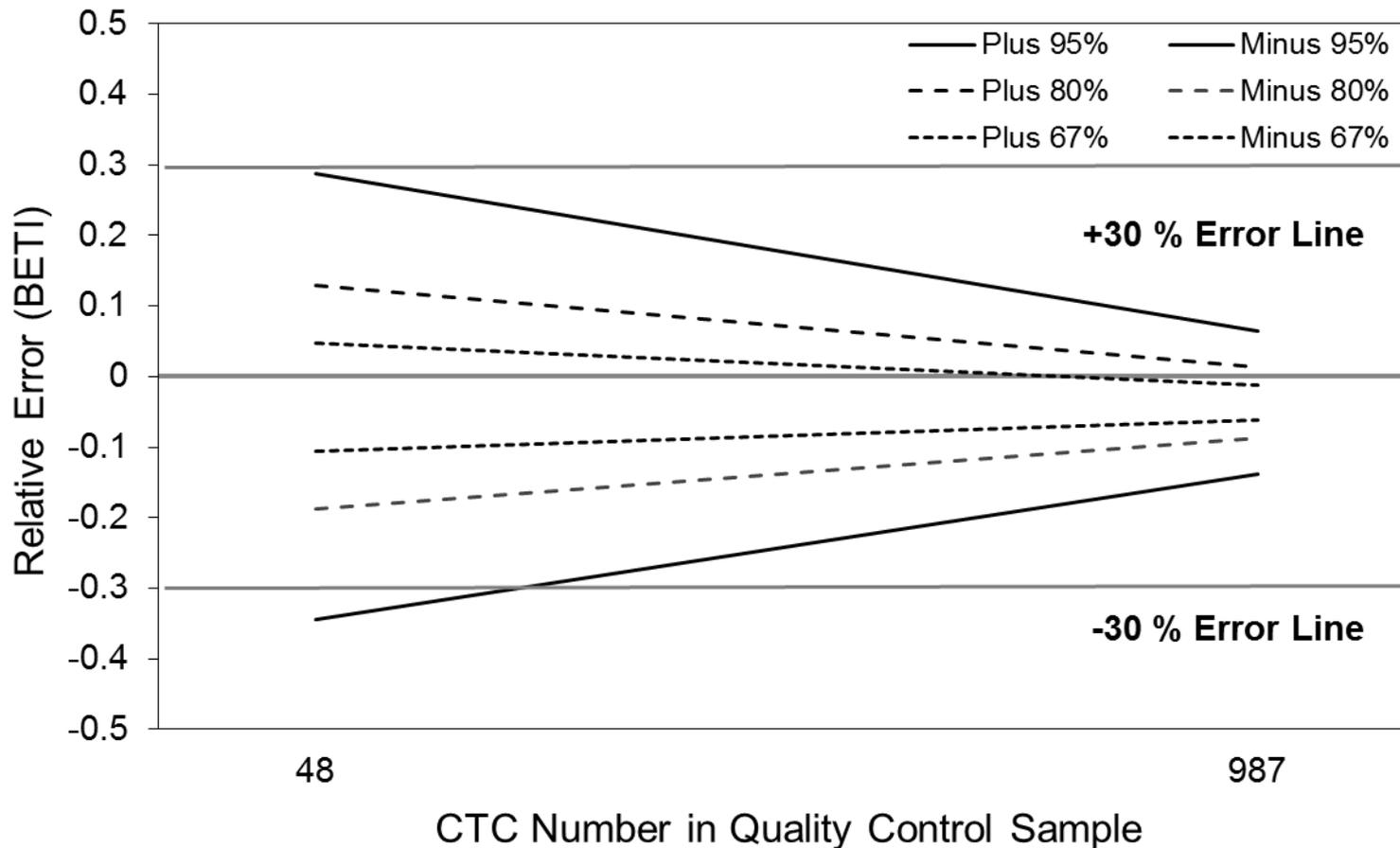
True Value ●
Assays Values ▪

Absolute Quantitation

Relative Quantitation
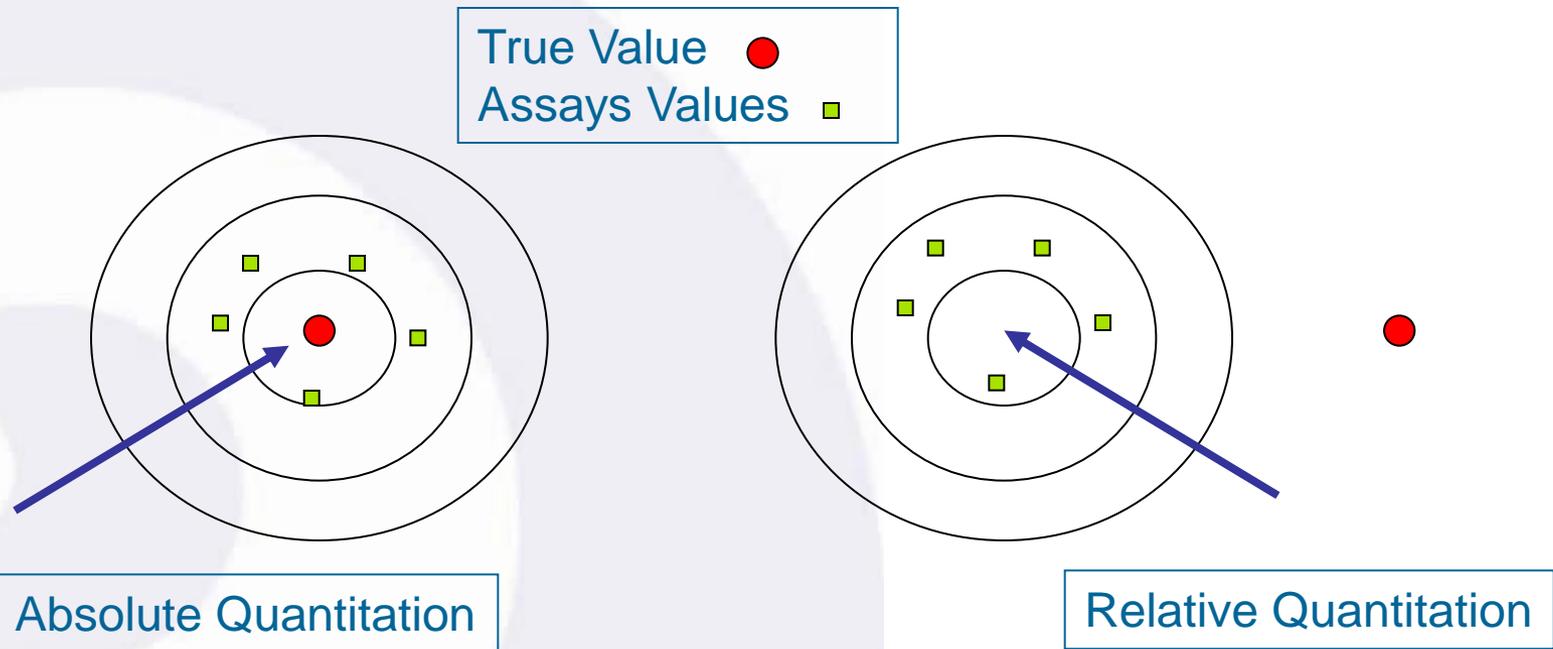
β-Expectation Tolerance Intervals Produce a Plot Called the Accuracy Profile

The β-expectation tolerance interval (BETI) calculates the upper and lower boundaries where each future measurement of patient samples are expected to has a defined probability (β) to fall within and thus informs on analytical accuracy
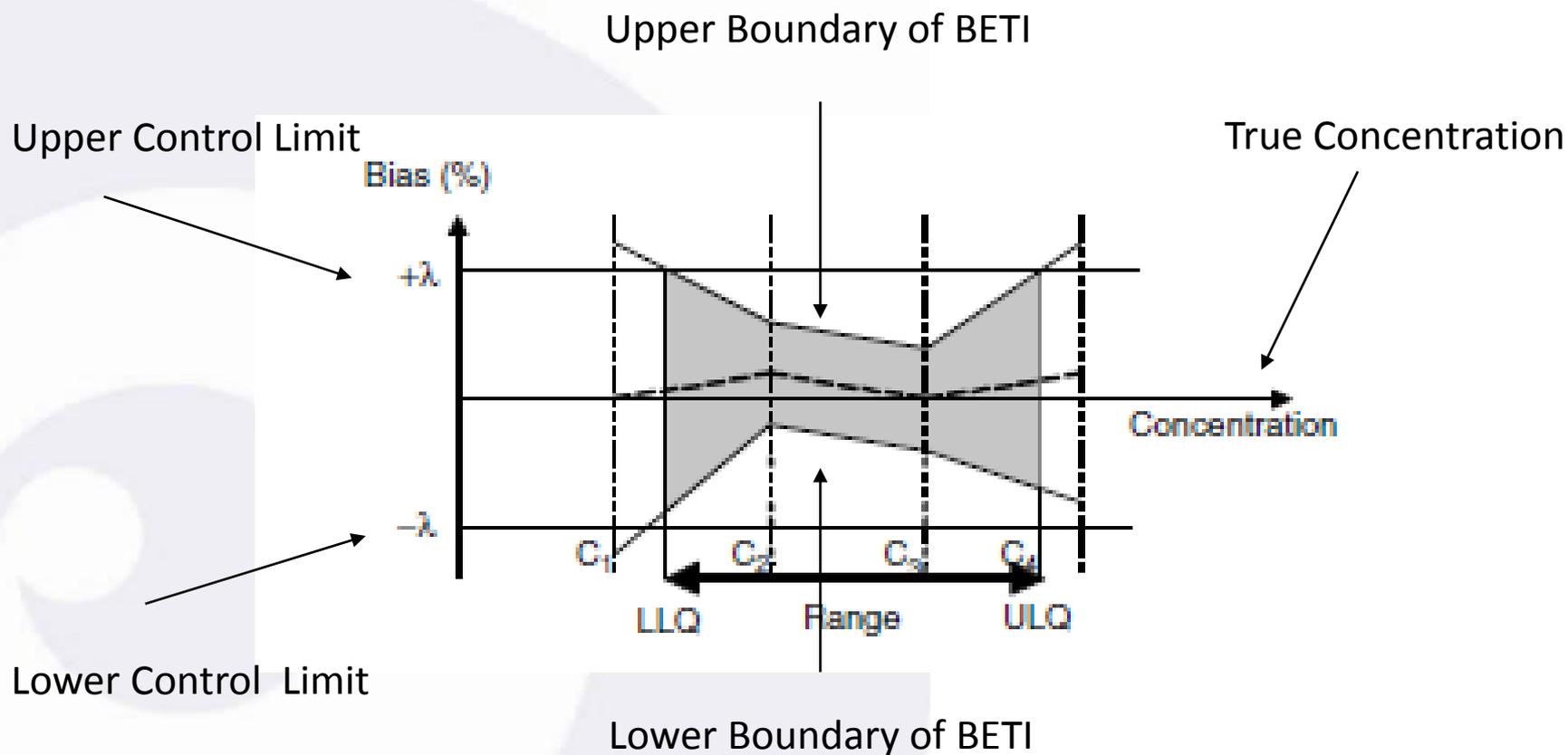
Upper Boundary of BETI

Upper Control Limit

True Concentration

Lower Control Limit

Lower Boundary of BETI

Fit-for-Purpose Quality Control Decision Matrix
Example 1 – IHC as a PD Assays
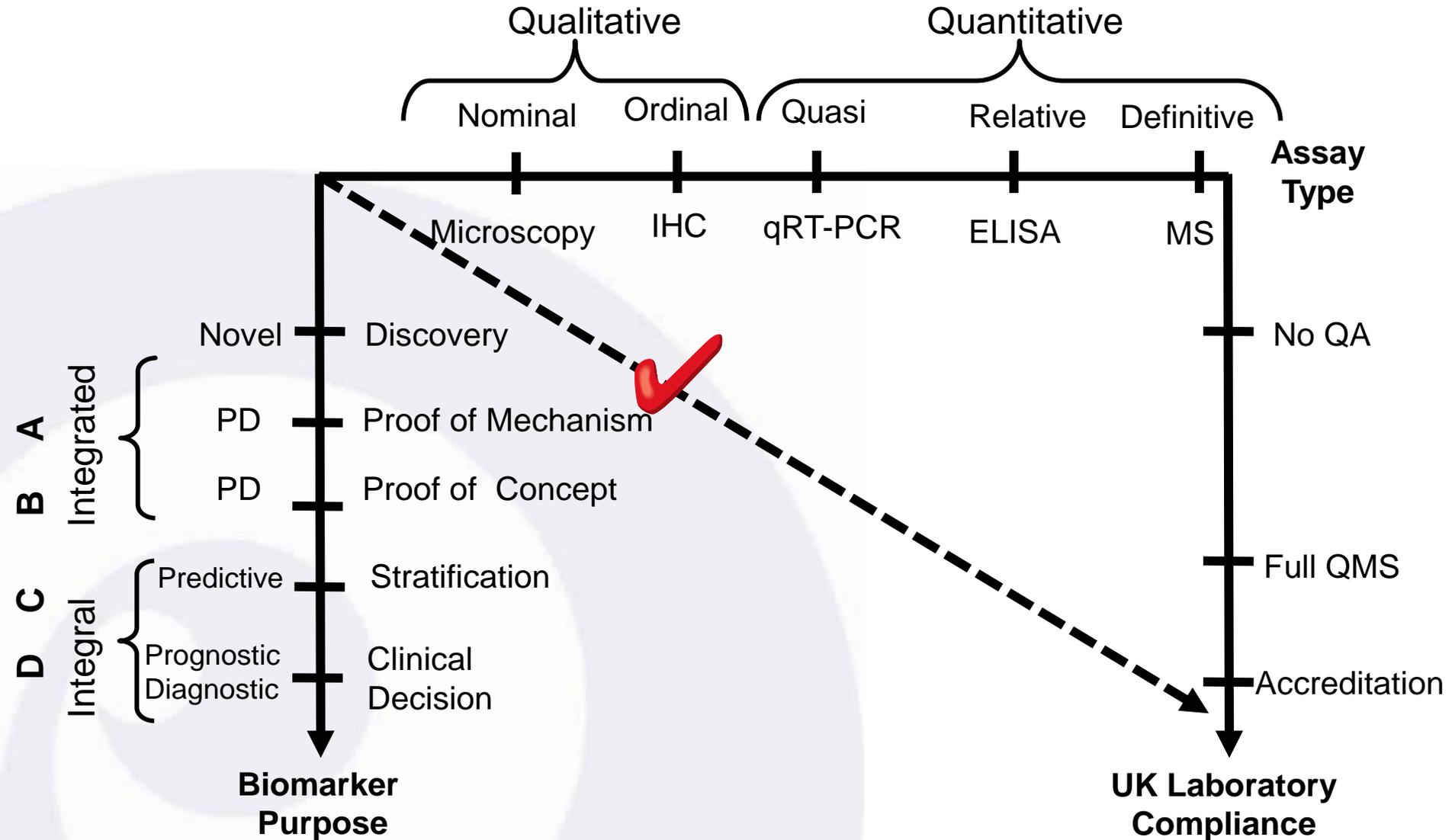
Performance Characteristic	Qualitative
Accuracy	
Trueness (Bias)	
Precision	
Reproducibility	✓
Sensitivity	✓
Specificity	✓
Dilution Linearity	
Parallelism	
Assay Range	
Reagent Stability	✓
Sample Stability	✓
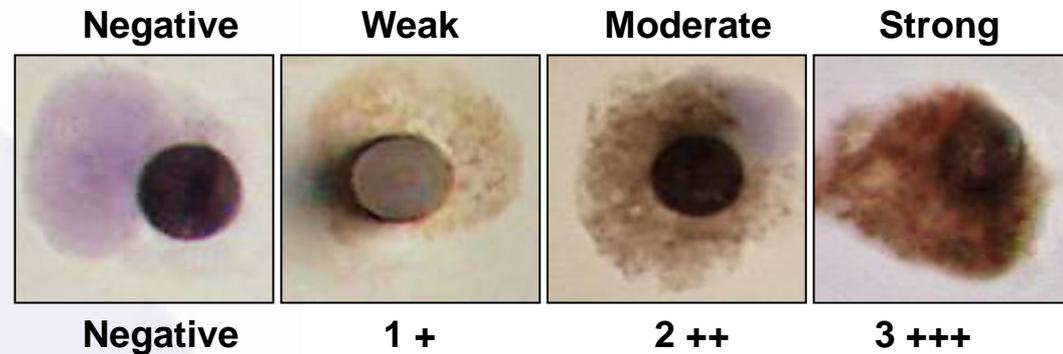
Negative **Weak** **Moderate** **Strong**

Negative **1 +** **2 ++** **3 +++**

Modification of ISR
Substitution with 2 Analysts

Y_i^O = the original measurements (i.e. analyst 1).
Y_i^R = the repeat measurements (i.e. analyst 2).

$$\Delta_i = \log\left(Y_i^R\right) - \log\left(Y_i^O\right)$$

$$\bar{\Delta} = \frac{1}{N}\sum_{i=1}^{N}\Delta_i$$

$$\hat{\sigma}_\Delta^2 = \frac{1}{N-1}\sum_{i=1}^{N}\left(\Delta i - \bar{\Delta}\right)^2$$

Calculation of BCTI:
β-content γ-confidence tolerance intervals

The two tailed β-content γ-confidence tolerance interval is therefore defined as:

$$\bar{\Delta} \pm Z_{(1+\beta)/2}\sqrt{1+N^{-1}}\sqrt{(N-1)\hat{\sigma}_\Delta^2/x_{N-1,1-\gamma}^2}$$

$Z_{(1+\beta)/2}$ is the upper $(1+\beta)/2$ quantile of the standard distribution and $x_{N-1,1-\gamma}^2$ is the lower γ quantile of the chi-squared distribution (N-1 degrees of freedom). Calculation of BCTI was performed utilising MATLAB (as above) at β = 67% and 95% [26]. A plot of BCTI (y-axis) against the operator pair (x-axis) represents a modified form of the 'accuracy profile'. All code developed in MATLAB was validated against previously published data sets as reported previously [26].

Summary: QC in Biomarker Analysis of Clinical Trial Samples

1. The MHRA place great emphasis in Quality Control (QC) in the analysis of trial samples, at each stage in the analytical cycle

2. However, the GCP Guide 2012 gives little indication of the level of QC required

3. A fit-for-purpose approach has been presented based on a decision matrix which takes account of the nature of analytical procedure and the purpose of the biomarker

4. As examples, QC on three different types of assays (ranging from definitive quantitation to categorical) employed as biomarkers with very different purposes (ranging from PD to stratification) are presented

5. These examples were be discussed in more detail at the Workshop on Data Quality