Validation & Continues Monitoring of Analytical Result

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Validation

Vs

Verification

Verification (ISO 15189:2022 Clause 3.32)

Confirmation of **truthfulness** , through the provision of objective evidence that specified requirements have been fulfilled

Objective Evidence to Confirm

Performance specification

Measurement uncertainty

Claimed by Manufacturer / Company,

Is it matching with laboratory requirement ?

Validation (ISO 15189:2022 Clause 3.31)

Confirmation of **plausibility for specific** intended **use** of application through the provision of objective evidence that specified requirements have been fulfilled

Objective Evidence

Perform Exercise to Confirm

Accuracy - Bias

Precision - CV%

Linearity

Limit of Blank (LOB)

Limit of Detection (LOD)

Limit of Quantitation (LOQ)

Diagnostic specificity

Diagnostic sensitivity

Plausibility = Being True

Theory

Check that a Reagent / Equipment meets Designed Specification

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Practical

Ensure Output of The Reagent / Equipment meets Designed Specification

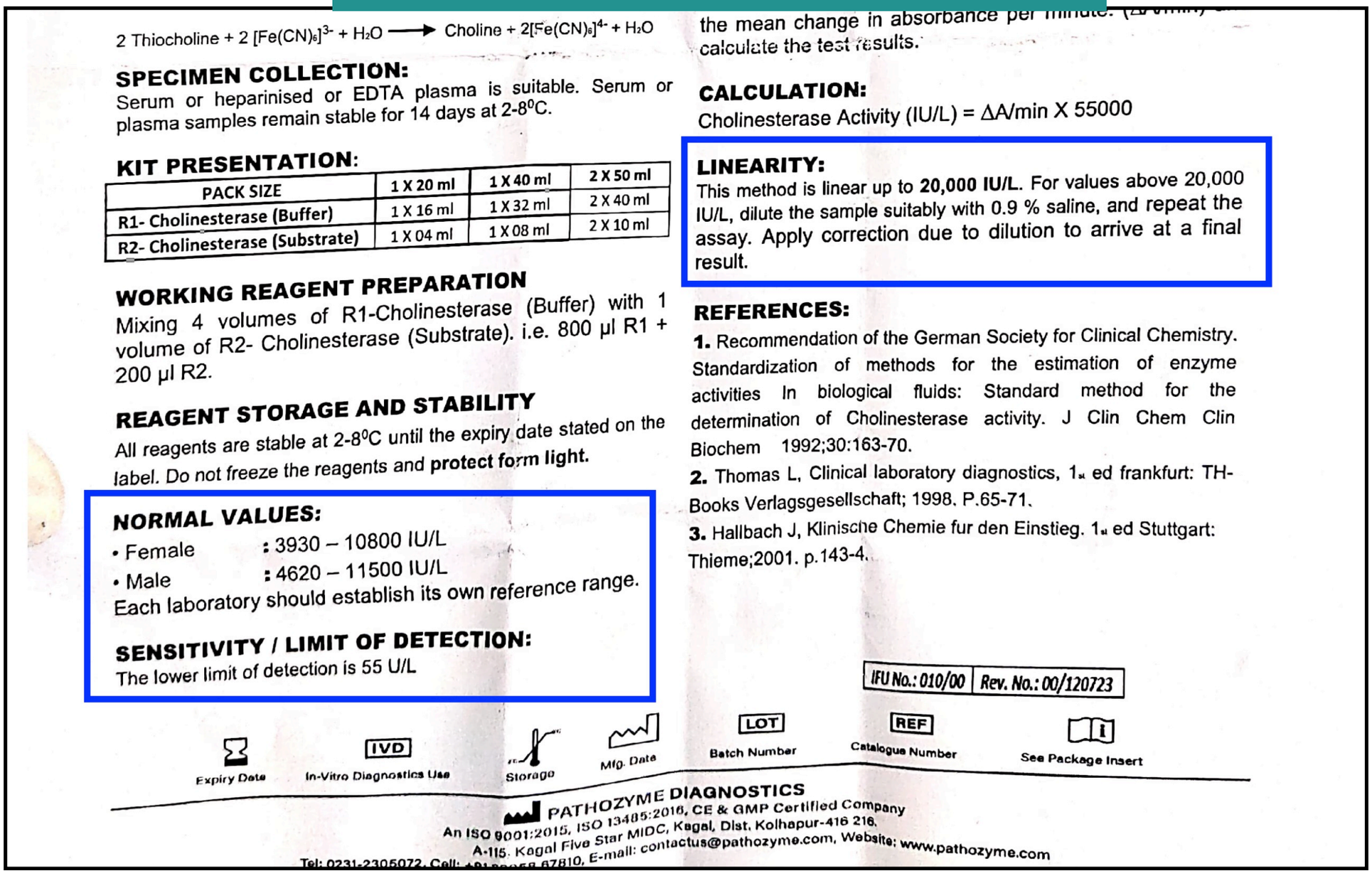
Validation of Report

Pre-analytical Validation

Analytical Validation

Post-analytical Validation

Cholinesterase Kit Literature



2 Thiocholine + 2 $[Fe(CN)_6]^{3-}$ + H_2O ⟶ Choline + 2$[Fe(CN)_6]^{4-}$ + H_2O

SPECIMEN COLLECTION:
Serum or heparinised or EDTA plasma is suitable. Serum or plasma samples remain stable for 14 days at 2-8^{0}C.

KIT PRESENTATION:

PACK SIZE	1 X 20 ml	1 X 40 ml	2 X 50 ml
R1- Cholinesterase (Buffer)	1 X 16 ml	1 X 32 ml	2 X 40 ml
R2- Cholinesterase (Substrate)	1 X 04 ml	1 X 08 ml	2 X 10 ml

WORKING REAGENT PREPARATION
Mixing 4 volumes of R1-Cholinesterase (Buffer) with 1 volume of R2- Cholinesterase (Substrate). i.e. 800 µl R1 + 200 µl R2.

REAGENT STORAGE AND STABILITY
All reagents are stable at 2-8^{0}C until the expiry date stated on the label. Do not freeze the reagents and **protect form light.**

NORMAL VALUES:
- Female : 3930 – 10800 IU/L
- Male : 4620 – 11500 IU/L

Each laboratory should establish its own reference range.

SENSITIVITY / LIMIT OF DETECTION:
The lower limit of detection is 55 U/L

the mean change in absorbance per minute. (ΔA/min) ... calculate the test results.

CALCULATION:
Cholinesterase Activity (IU/L) = ΔA/min X 55000

LINEARITY:
This method is linear up to **20,000 IU/L**. For values above 20,000 IU/L, dilute the sample suitably with 0.9 % saline, and repeat the assay. Apply correction due to dilution to arrive at a final result.

REFERENCES:

1. Recommendation of the German Society for Clinical Chemistry. Standardization of methods for the estimation of enzyme activities In biological fluids: Standard method for the determination of Cholinesterase activity. J Clin Chem Clin Biochem 1992;30:163-70.

2. Thomas L, Clinical laboratory diagnostics, 1st ed frankfurt: TH-Books Verlagsgesellschaft; 1998. P.65-71.

3. Hallbach J, Klinische Chemie fur den Einstieg. 1st ed Stuttgart: Thieme;2001. p.143-4.

IFU No.: 010/00 | Rev. No.: 00/120723

Expiry Date | IVD In-Vitro Diagnostics Use | Storage | Mfg. Date | LOT Batch Number | REF Catalogue Number | See Package Insert

PATHOZYME DIAGNOSTICS
An ISO 9001:2015, ISO 13485:2016, CE & GMP Certified Company
A-115, Kagal Five Star MIDC, Kagal, Dist. Kolhapur-416 216.
Tel: 0231-2305072, Cell: +91 99758 67810, E-mail: contactus@pathozyme.com, Website: www.pathozyme.com

Intended Use of Cholinesterase - Should have Lower limit of Detection 100 IU/L

Dr Shubhankar Jha

Does Performance Check - Output Check

LOD - 1000 IU / L

Pre - Analytical Process - Validation

Drafting Specification for Reagent - Equipment

- **For Analysis of TSH - What should be preferred**
- **What Could be Specification for Equipment - According to**
  - Laboratory Size - Work Load
  - ELISA - CLIA - ELFA

Drug & Medical History

- **pO2 - 300 mmHg**
- **Plasma Glucose Report - Highly Lipemic**
- **TSH - 0.0006 IU/L With Normal T3 &T4**

Pre- requisite of patient & collection

- **ABG Sample - Sodium 180 mmol/L**
- **K/C/O Diabetic**
  - **FBS - 100 mg%**
  - **PP2BS - 300 mg%**
  - **HbA1c - 6.0 %**

Analytical Process - Validation

Validation of Claimed specification

- Accuracy , Precision ,Linearity
- Limit of Blank (LOB)
- Limit of Detection (LOD)
- Limit of Quantitation (LOQ)
- Measuring Interval

For Better Understanding of Validation

Blood Urea - 300 mg % (GLDH Method) -

Potassium - 9.0 mmol/L (ISE Method) -

Serum Protein - 0.5 gm% (Biuret Method) -

S. Creatinine - 8.0 mg% >>>Repeat Analysis - 8.6 mg% -

RMSDI = (- 1.5%) >>>RMDev% = (-7%) >>>

Analytical Process - Validation

Validation of Claimed specification

- Accuracy , Precision ,Linearity
- Limit of Blank (LOB)
- Limit of Detection (LOD)
- Limit of Quantitation (LOQ)
- Measuring Interval

For Better Understanding of Validation

Blood Urea - 300 mg % (GLDH Method) - *Check Linearity*

Potassium - 9.0 mmol/L (ISE Method) - *Check Reportable Range*

Serum Protein - 0.5 gm% (Biuret Method) - *Check LOD*

S. Creatinine - 8.0 mg% >>>Repeat Analysis - 8.6 mg% - *Check Precision*

RMSDI = (- 1.5%) >>>RMDev% = (-7%) >>> *Check Accuracy*

Analytical Process - Validation

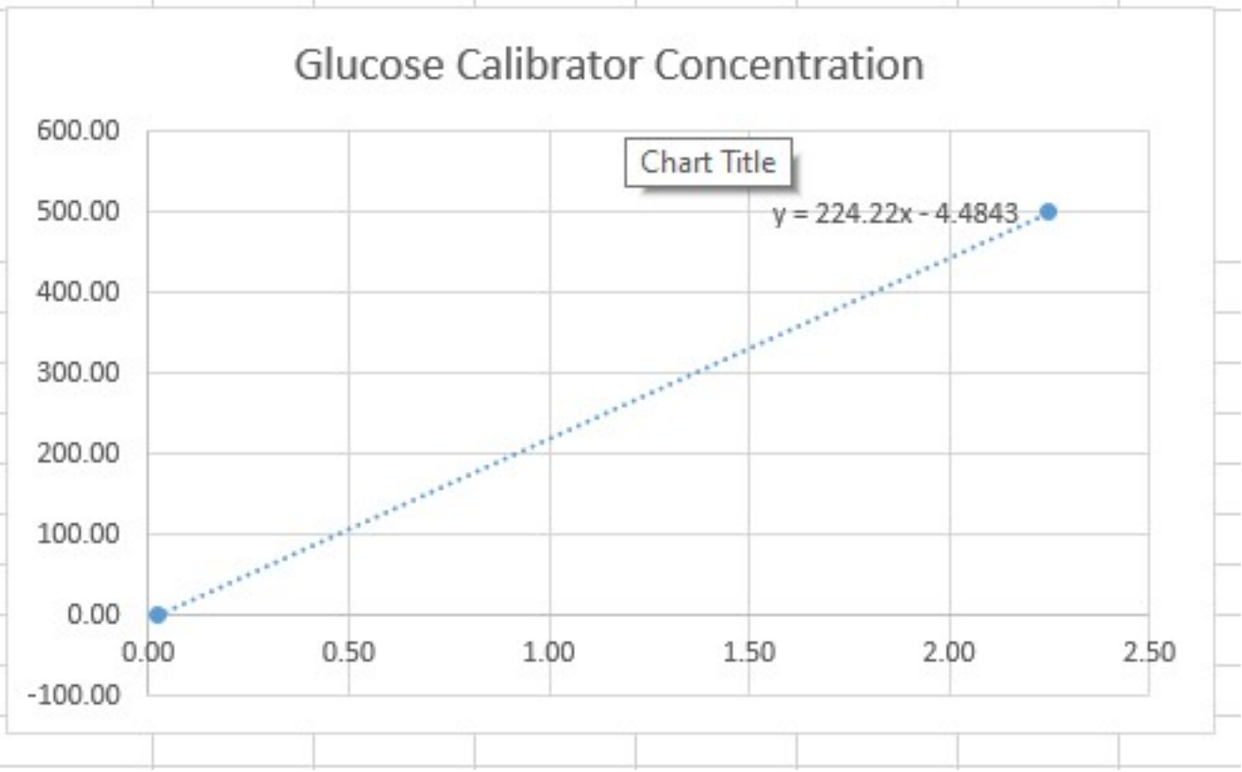
Methodology Validation

Procedure - Protocol of Analysis

- **Is it there any change in manufacturer guideline ????**
- **Is there any change in Sample / Reagent Volume ????**
- Than What to Do ???

Analyte Calibration - Validation

O.D.	Glucose Calibrator Concentration
0.02	0.00
2.25	500.00



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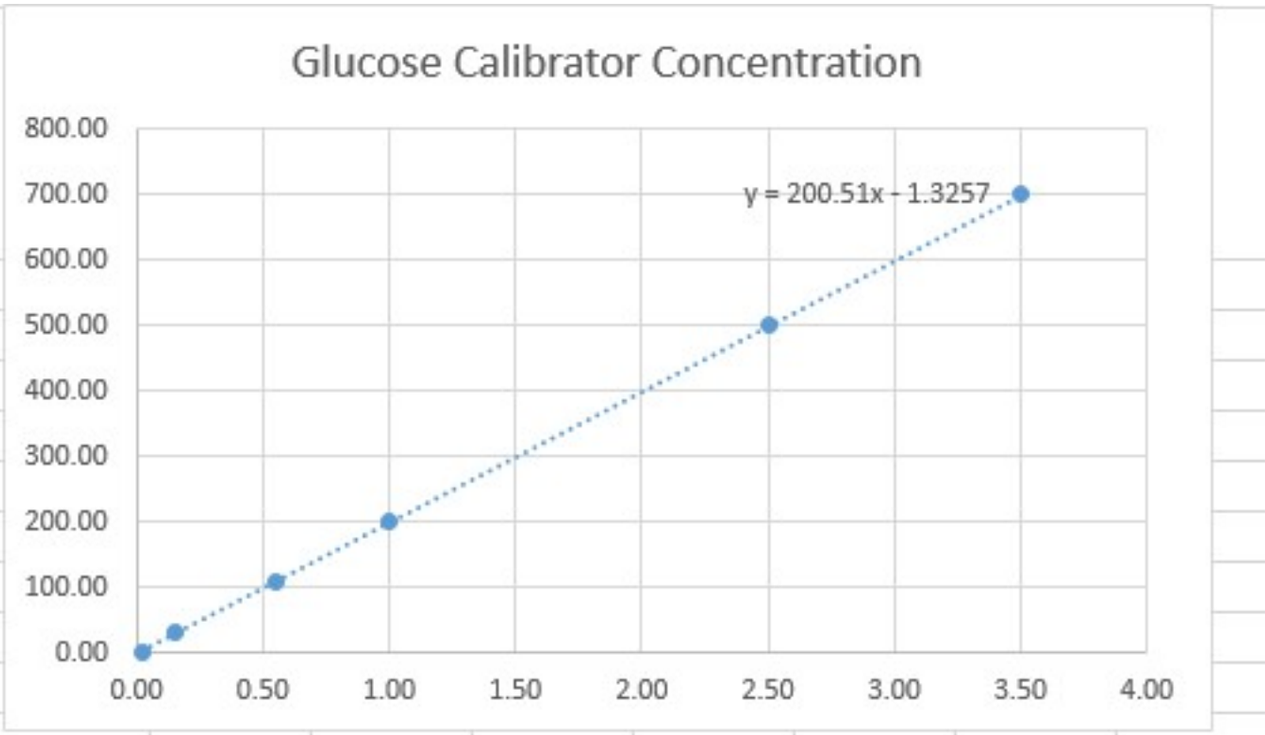
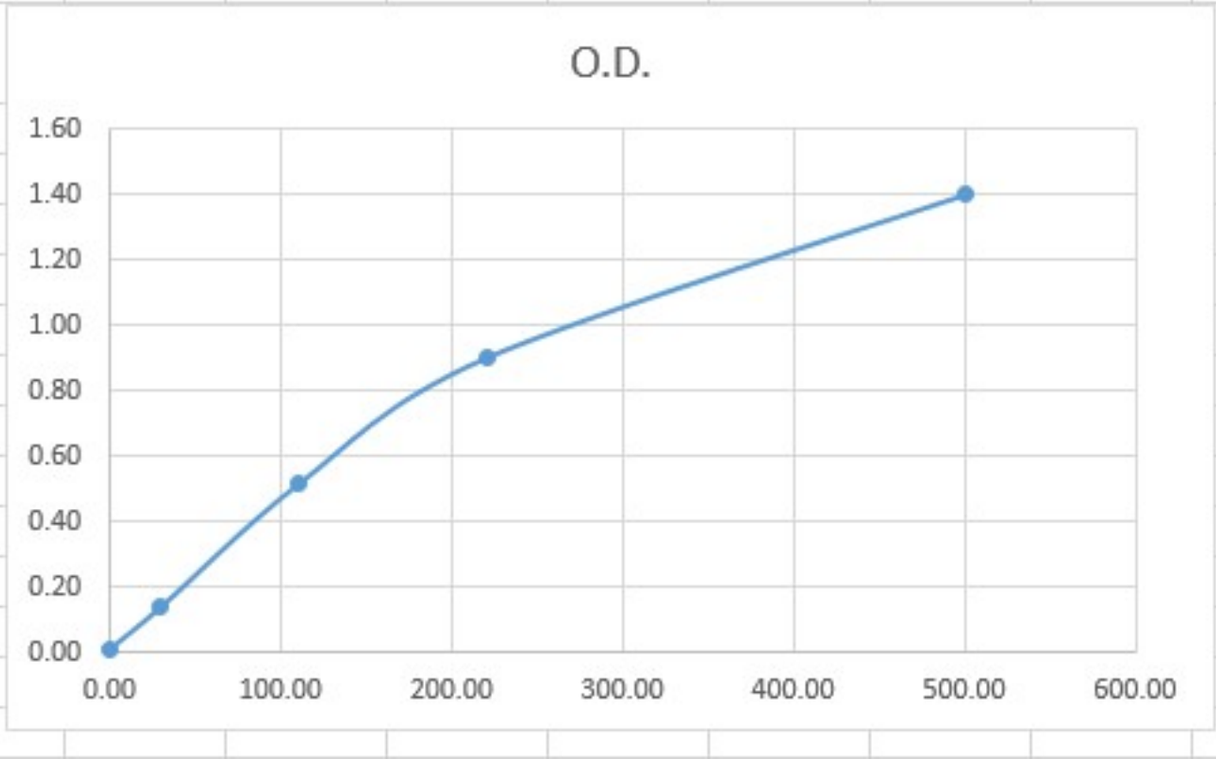
O.D.	Glucose Calibrator Concentration
0.02	0.00
0.15	30.00
0.55	110.00
1.00	200.00
2.50	500.00
3.50	700.00



Analyte Calibration - Validation

Glucose Calibrator Concentration	O.D.
0.00	0.01
30.00	0.14
110.00	0.51
220.00	0.90
500.00	1.40



Analytical Process - Validation

Calibration of Analyse

- 1st Point - Lower range
- 2nd Point - Reference range
- 3rd Point - Clinical decision range
- 4th Point - Higher range

Analytical Process - Validation

Quality Control Validation - IQC , EQAS , Comparability

Internal Quality Control

- **RCA for IQC Outlier**
  - Hidden Error in Sample result
  - Define Frequency & Time for IQC
  - Select Level of IQC

- **Mean - SD comparison with peer group**
- **IQC Trend / Shift Analysis**
  - Prediction of Bias

- **Analysis of IQC CV%**
  - Prediction of Imprecision

Analytical Process - Validation

Quality Control Validation - IQC , EQAS , Comparability

External Quality Assurance Scheme

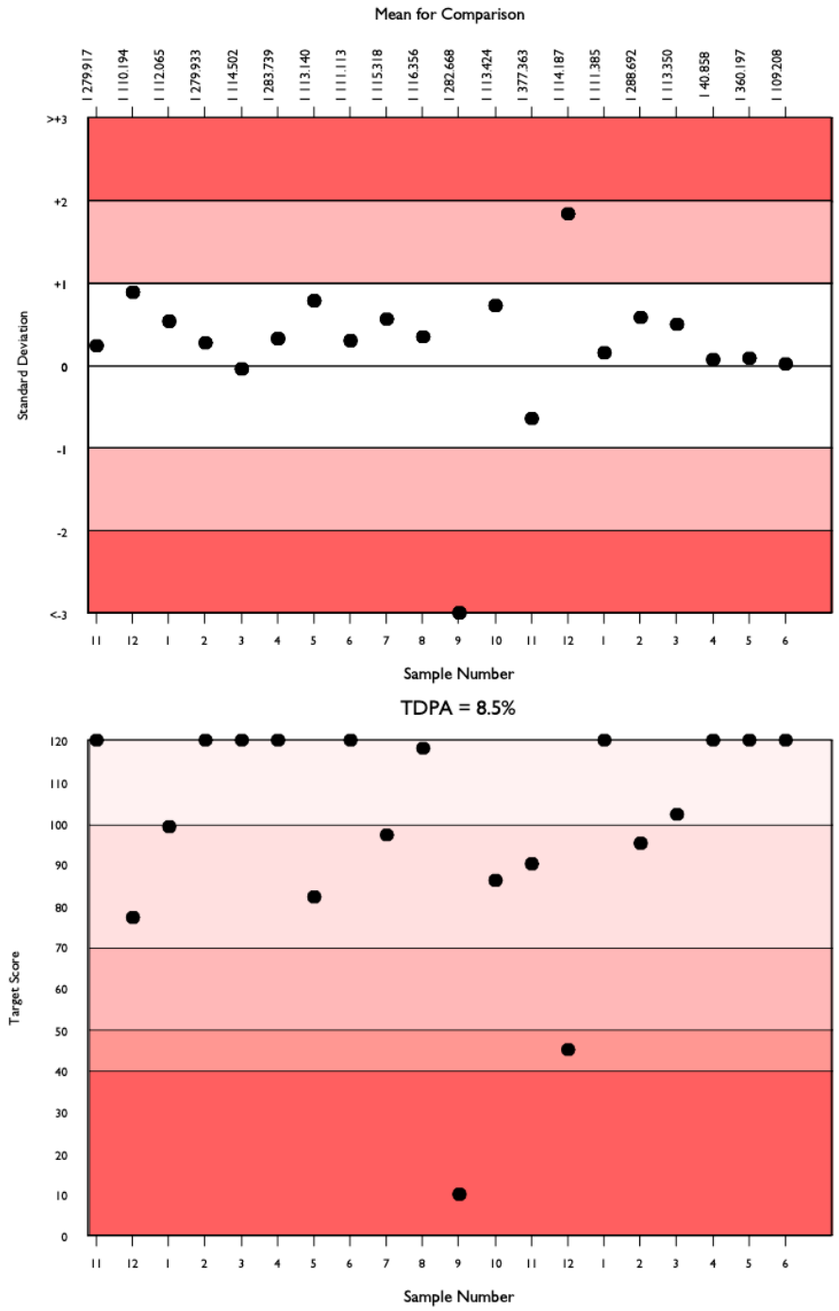
- SDI vs RMSDI
- Dev% vs RMDev%

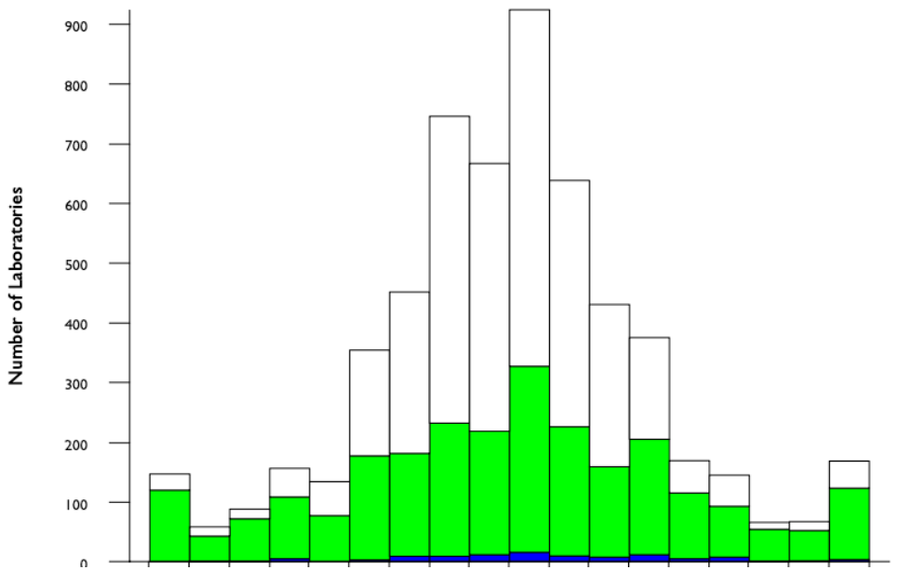
Glucose, mg/dl

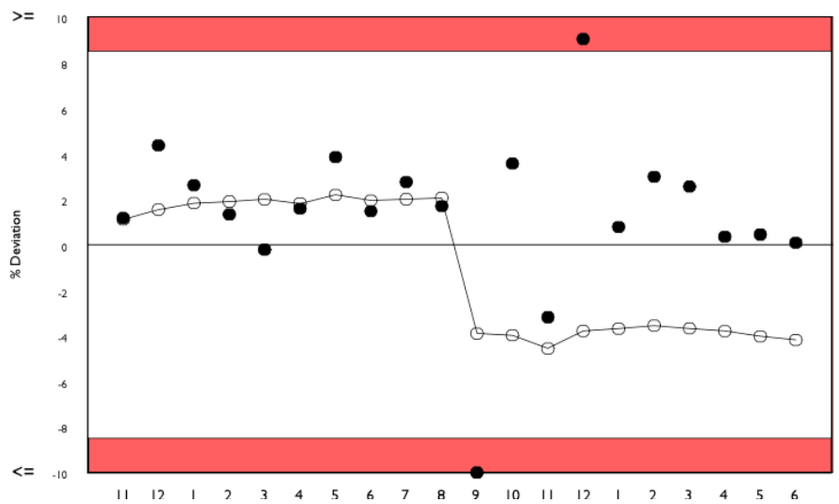
	N	Mean	CV%	U_m	SDPA	Exc.
All Methods	5320	107.817	3.9	0.07	5.57	463
Glucose oxidase	2399	107.898	5.3	0.14	5.58	185
Erba XL Series	98	109.208	4.0	0.55	5.64	8

▲ Your Result	109.300	SDI	0.02
		RMSDI	-0.86
Mean for Comparison	109.208	TS	120
		RMTS	90
		%DEV	0.1
		RM%DEV	-4.2

Acceptable limits derived from Biological Variation	6.96%
Acceptable limits of performance for RIQAS	8.50%







LD (LDH), U/l @ 37°C

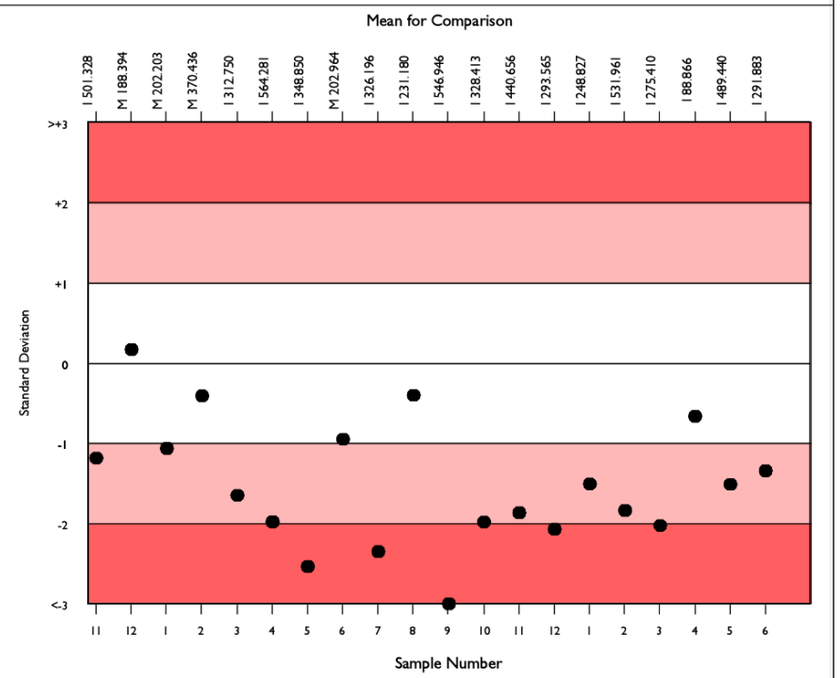
	N	Mean	CV%	U_m	SDPA	Exc.
All Methods	2482	221.031	31.8	1.76	17.60	170
L to P, IFCC	1430	185.344	4.4	0.27	14.76	136
Erba XL Series	6	291.883	43.9	65.43	69.44a	0

▲ Your Result	198.500	SDI	-1.34
		RMSDI	-1.80
Mean for Comparison	291.883	TS	11
		RMTS	12
		%DEV	-32.0
		RM%DEV	-38.1

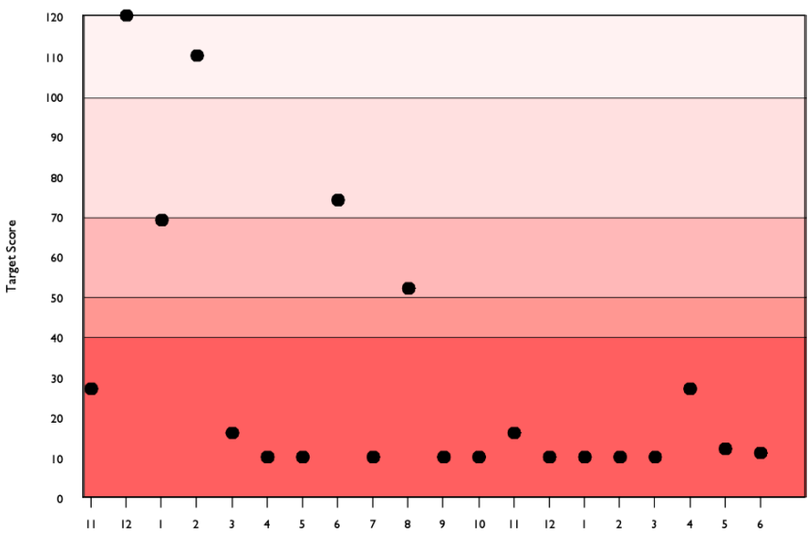
Acceptable limits derived from Biological Variation 11.4%

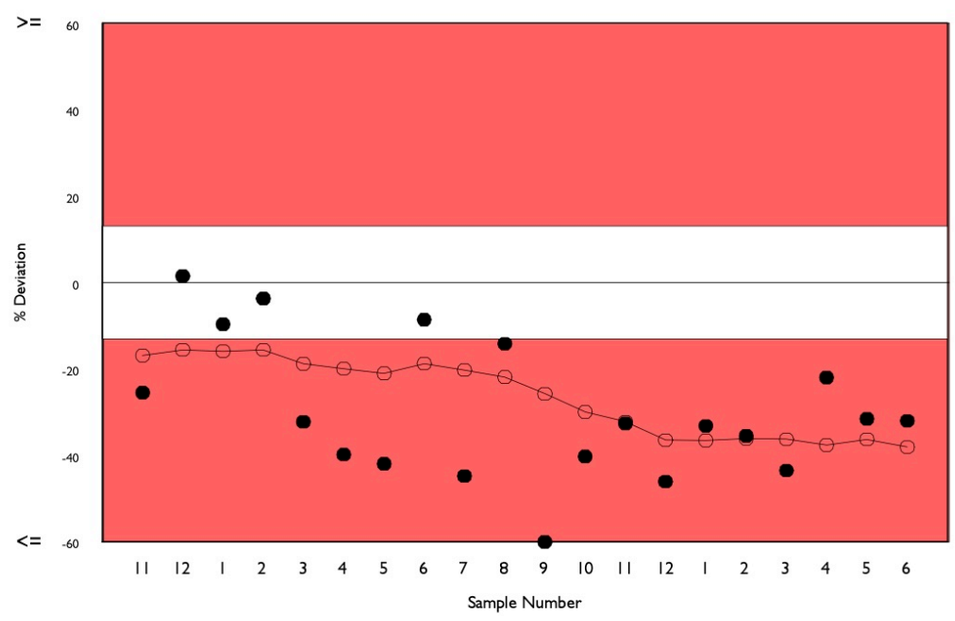
Acceptable limits of performance for RIQAS 13.10%

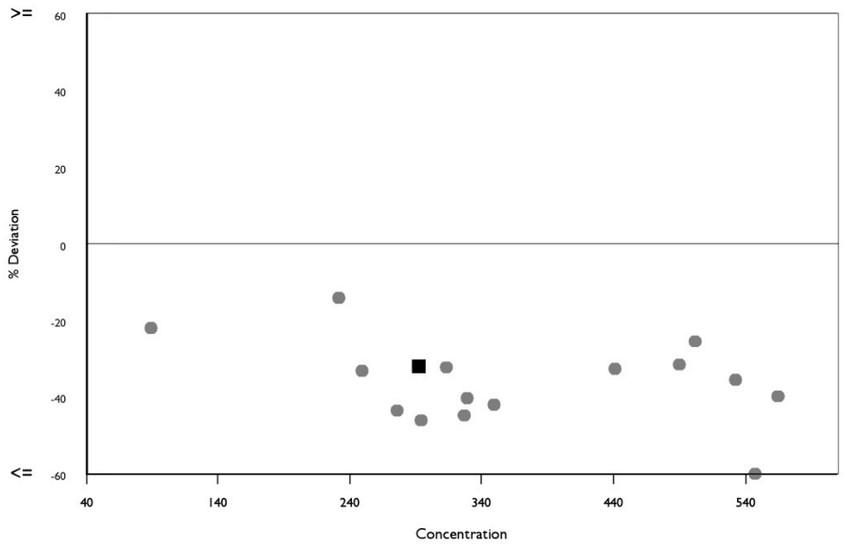
TS & %DEV outside limits



TDPA = 13.1%







Analytical Process - Validation

Quality Control Validation - IQC , EQAS , Comparability

- **Comparability - Harmonisation**
  - ✓ Different Measurement System
  - ✓ To Validate
    - Reference range
    - Diagnostic range
    - Variability

Post Analytical Report - Validation

Laboratory Information System

- Delta Check
- Interphase
- Validation
- Report Format Validation
  - ✓ Demographic data
  - ✓ Reference Range
- Clinical Co-relation

Human Resources

Training

- Calibration
- Quality control training
- Sampling process
- LIS
- Recording data
    - Lot verification

Validation Wants Highest Attitude With Depth of Vision Of Biochemist.

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Appreciating You For Investing Your Presence & Precious Time

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