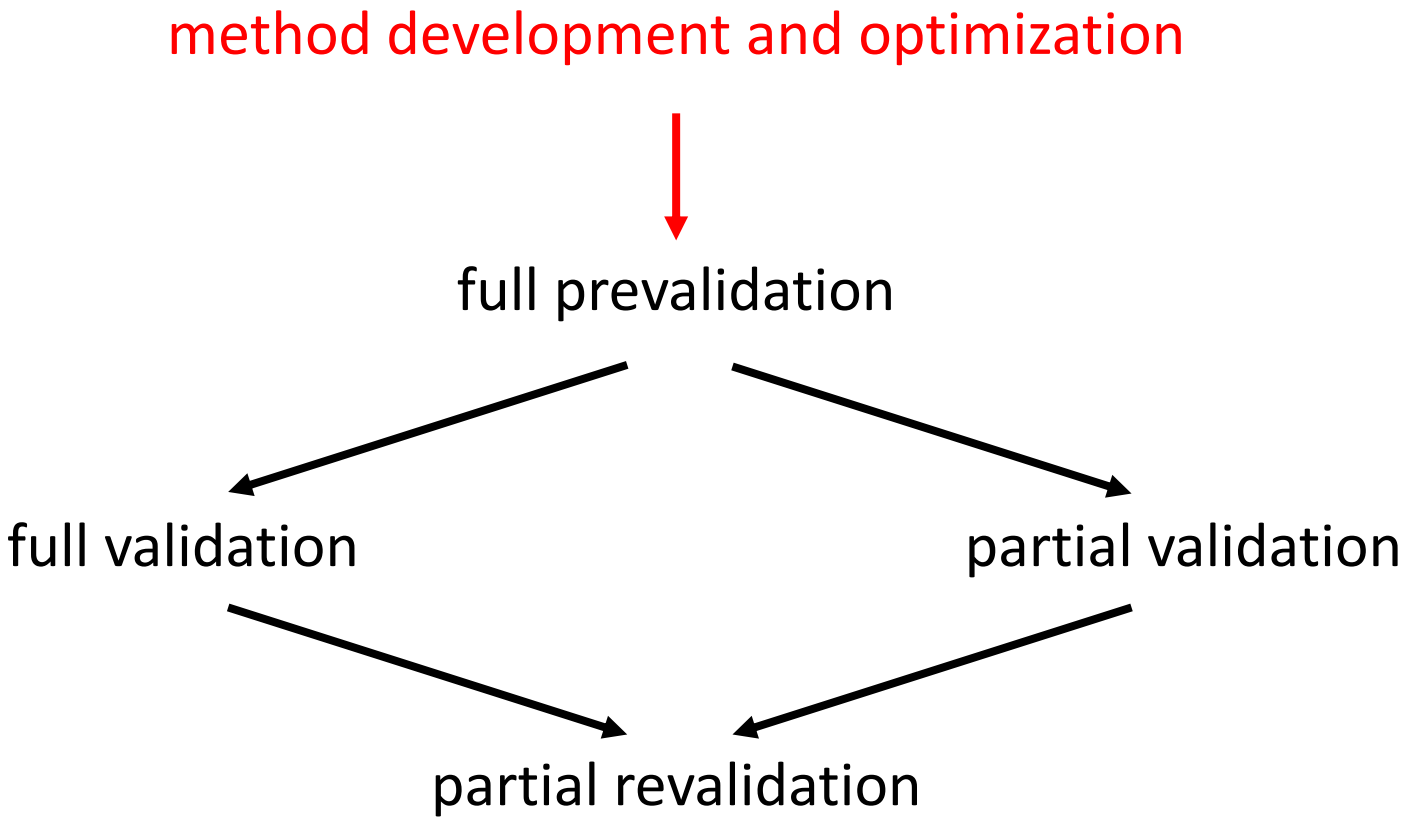
Quality control of assays

Step 1: method validation

Method validation has 3 phases



When should full method validation be performed?

- before method is implemented
- when considerable changes have been introduced to the method (eg. another ion transition is selected for evaluation, change in mobile phase composition or employed stationary phase)
- when new analytical instrumentation is introduced (eg. switch from mass spec brand A to brand B)
- when method is applied to a new type of sample
- when untolerable systematic errors in methodology are identified

When should partial method validation be performed?

- whenever there is a minor change in the assay method which is not expected to affect the method performance characteristics (eg. new internal standard is used)
- whenever there is reason to believe that the method performance may have changed
- when external quality assessment scheme or interlaboratory method comparison results are unacceptable
- periodically, to verify that the method performance has not changed over time

Method validation guidelines

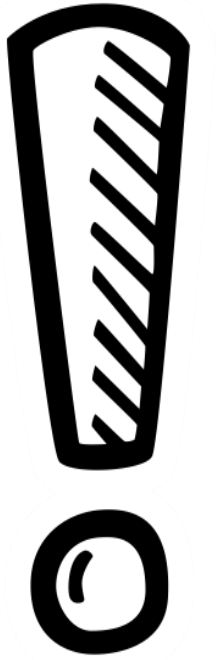
- International Conference of Harmonization (ICH) – Q2(R1), effective November 2005
- Federal Drug Administration (FDA) – effective May 2018
- European Medicines Agency (EMA) – EMEA/CHMP/EWP/192217/2009 Rev. 1 Corr. 2 ** (effective February 2012)
- ICH M10 expected to be published mid-2019

- The bioanalytical method validation guidelines have been introduced to establish clear regulations for generating analytical results which support pre-clinical and clinical pharmacokinetic studies.

- The method validation guidelines have been established for assaying drugs and their metabolites primarily in blood samples.

- No specific method validation guidelines have been introduced for the analysis of endogenous substances using chromatographic and mass spectrometric methods.

- Method validation based on the available guidelines is mandatory for laboratories operating in a GXP environment and providing analytical results for the industry.



Method performance characteristics to be included in the validation process (EMA Guideline)

- Performed in analyte solution:
  - limit of detection (quantity, not concentration!)
  - injection reproducibility (intermediate concentration)
  - linear dynamic range of detector response
  - analyte carry-over (high-end of linear dynamic range)
  - stability in solution (at least 2 levels)

Method performance characteristics to be included in the validation process (EMA Guideline)

- Performed in matrix samples:

  - selectivity
  - calibration curve: mathematical relationship between analyte concentration and detector response
  - lower and upper limits of quantitation
  - within-run reproducibility
  - between-run reproducibility
  - autosampler stability
  - benchtop stability (as suits the lab workflow and the sample preparation process)
  - storage stability in samples (short-term and long-term, various temperatures)
  - storage stability in prepared samples (at least short-term, various temperatures)
  - dilution integrity (if applicable)

Step 2: quality control of batch runs

Quality control of assays – why is it important?

- The MS is not a stable detector → various ion transitions are affected in various manners!

- Autosampler tray stability of analytes and internal standards may not be 100% over the batch cycle.

- QC is a fundamental requirement for interlaboratory comparisons.

- misquantitation may be a result of:
  - chemical degradation of analytes or internal standards
  - contamination of the ion optics
  - appearance of interferences in the ion chromatograms

What sort of quality control do you need?

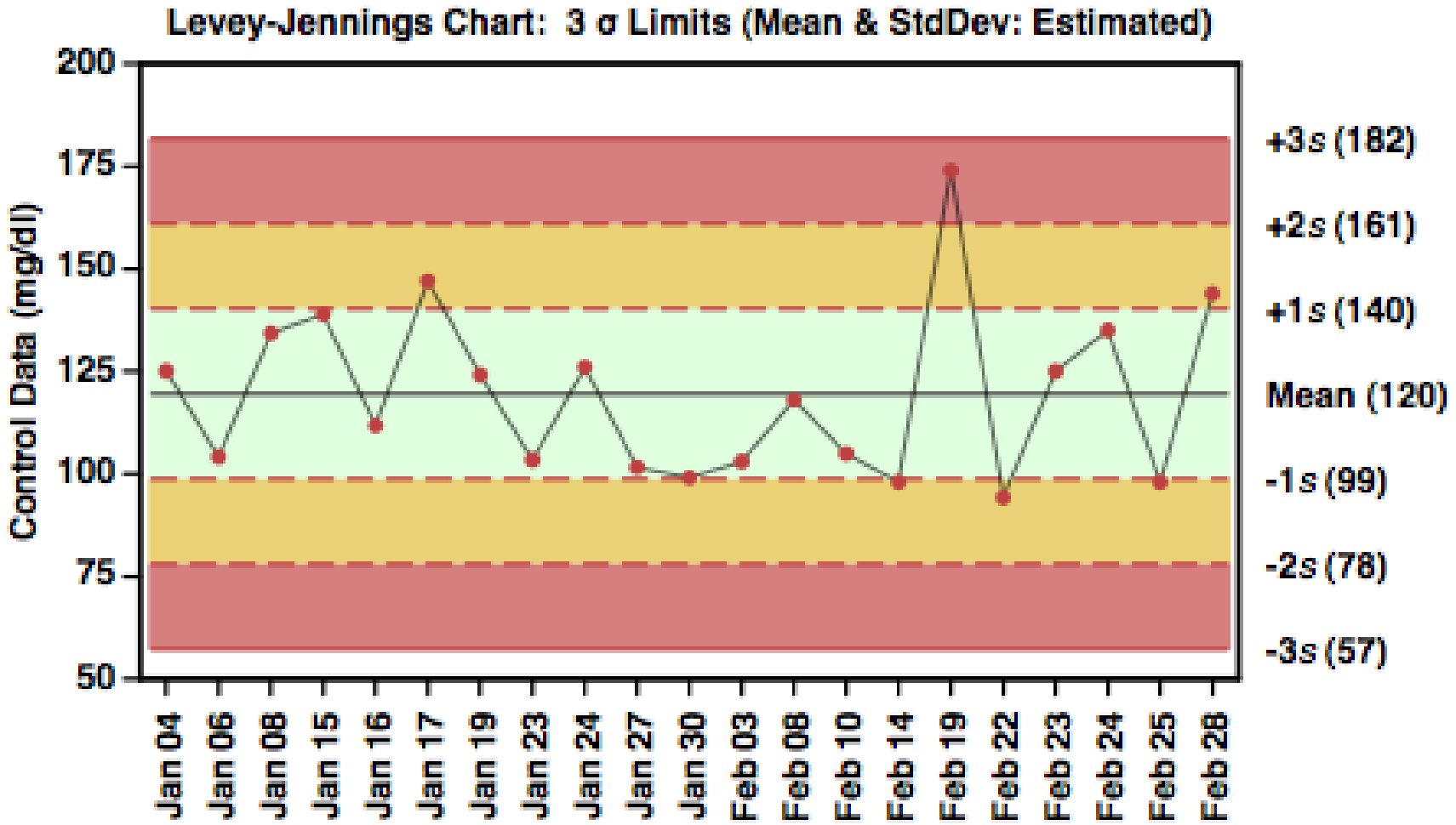
… That depends on the type and quality of information you would like to attain.

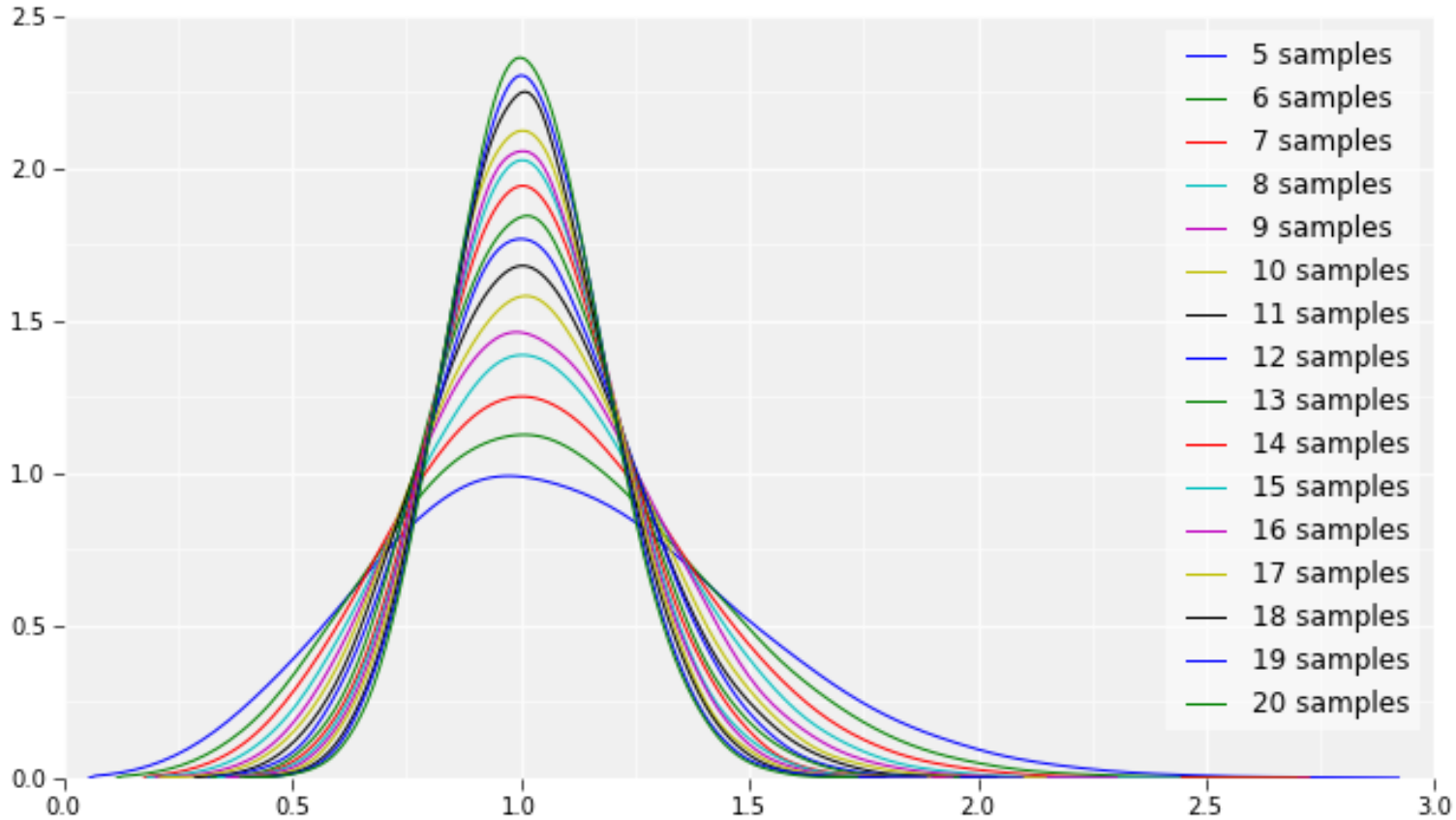
… And the regulations you are required to stick to.

Quality control of assays – approaches

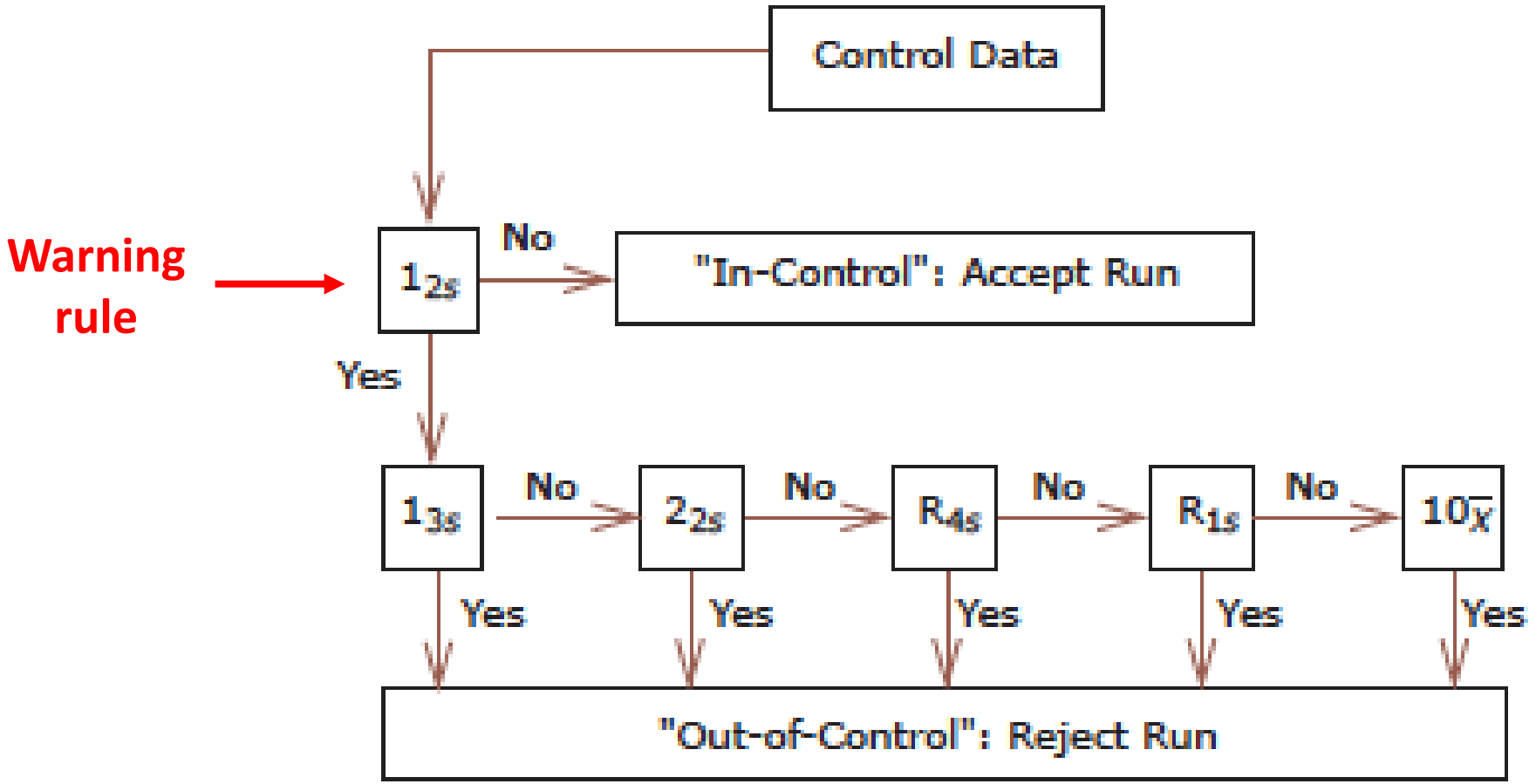
Approach	Identifited assay errors
multilevel matrix controls run at least at the beginning and at the end of the batch	loss of the validity of calibration due to contamination of the ion optics
spiked matrix samples	matrix-specific contamination of the ion optics
repeat analysis	if prepared sample is reassayed: lack of system stability if collected sample is reassayed: lack of reproducibility
incurred sample reanalysis	lack of system stability, inadequate method validation
external quality assessment scheme	suboptimal method performance

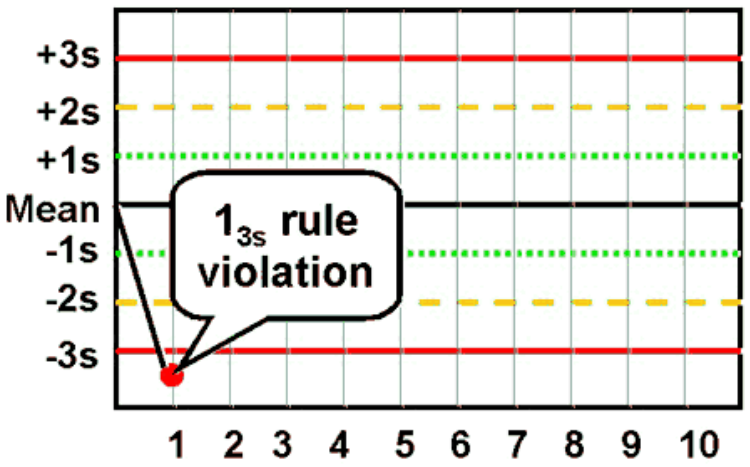
Interpretation of internal QC results: Levey-Jennings curves

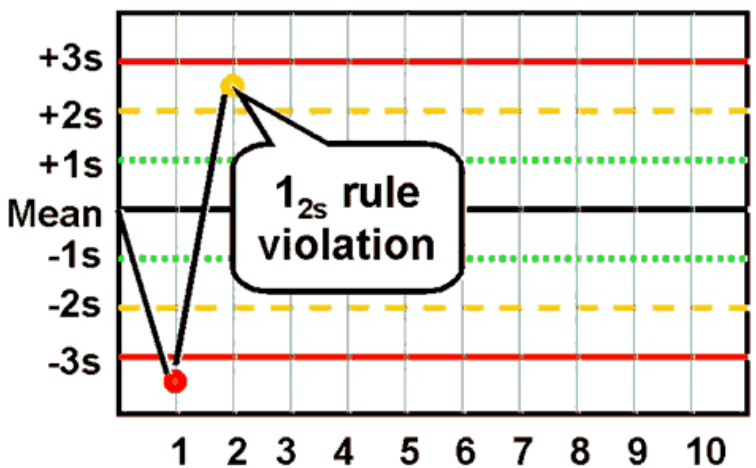


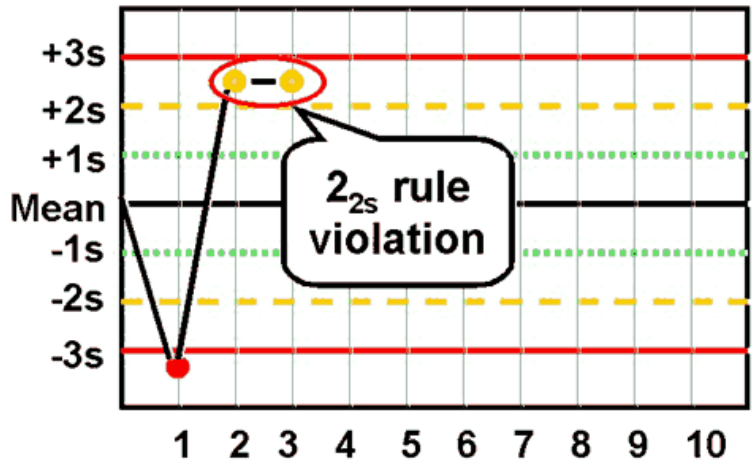

2.5
2.0
1.5
1.0
0.5
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
5 samples
6 samples
7 samples
8 samples
9 samples
10 samples
11 samples
12 samples
13 samples
14 samples
15 samples
16 samples
17 samples
18 samples
19 samples
20 samples

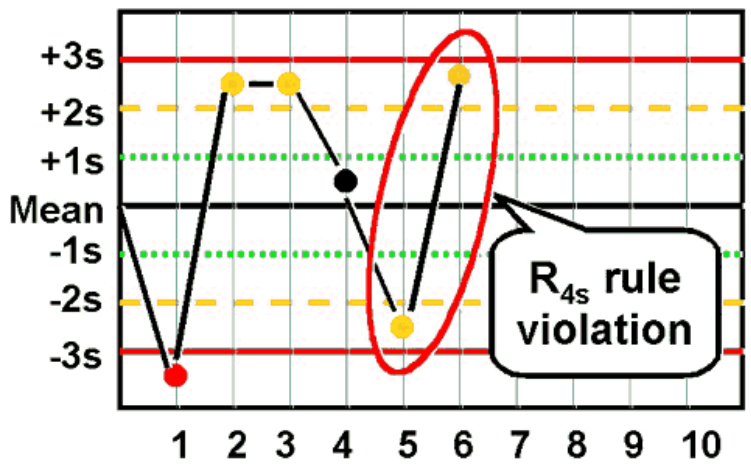
Interpretation of Levey-Jennings charts: the Westgard multirule quality control approach



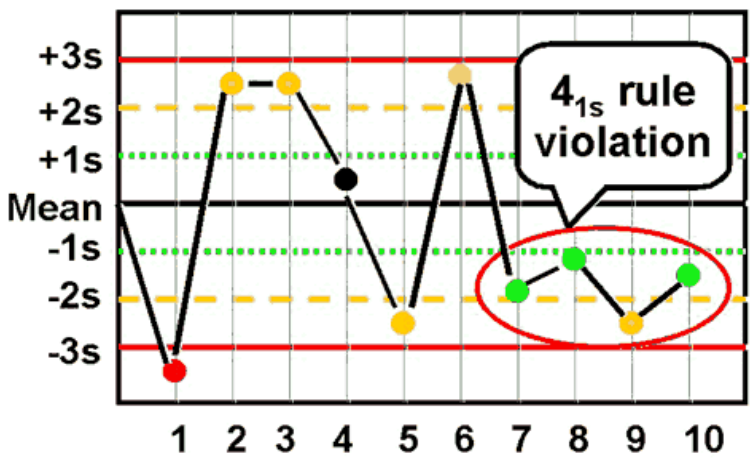


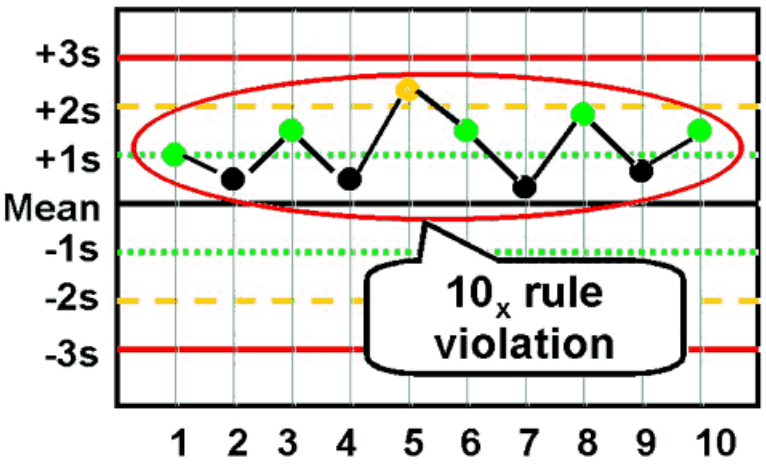




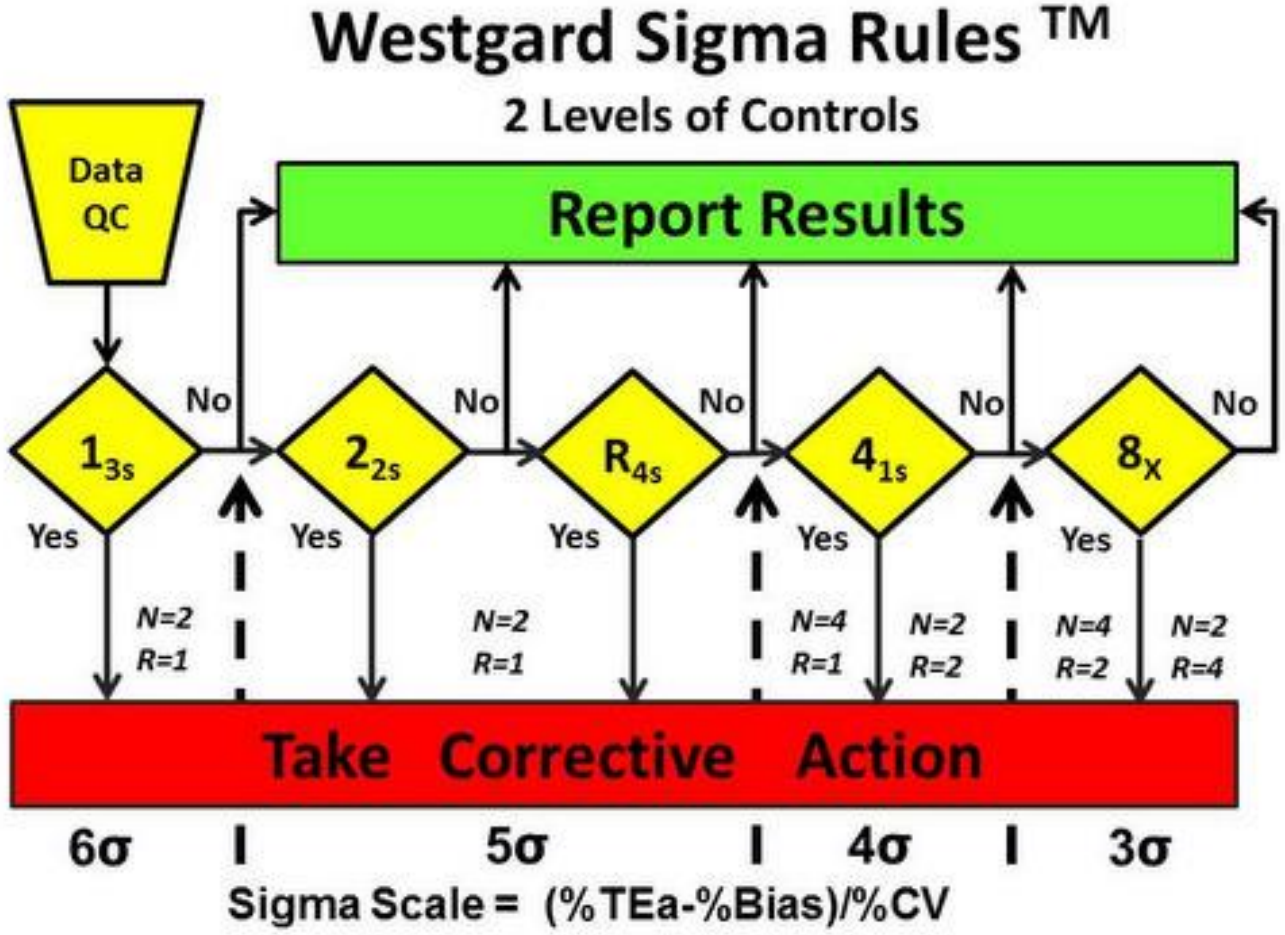


Within-run!





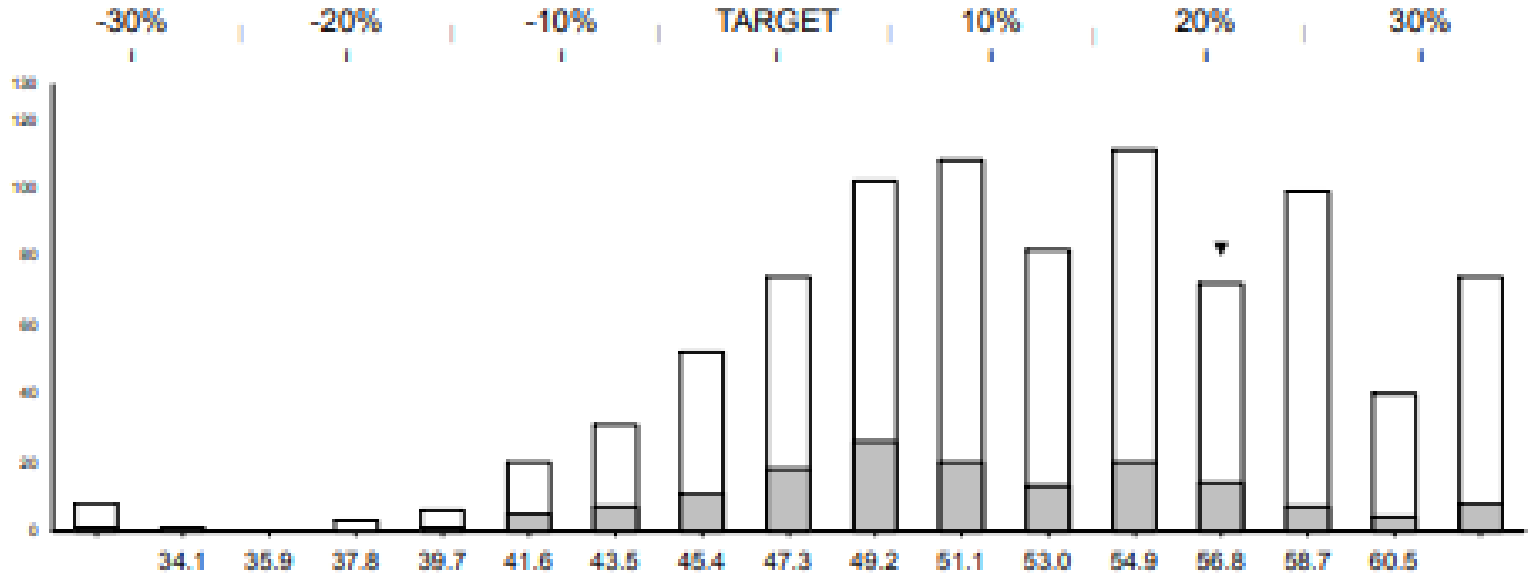
Interpretation of internal QC results: the Westgard Sigma Rules



Interpretation of the reports of external quality assessment schemes

25 Hydroxyvitamin D **January 2018** **Laboratory 2178**

Histograms



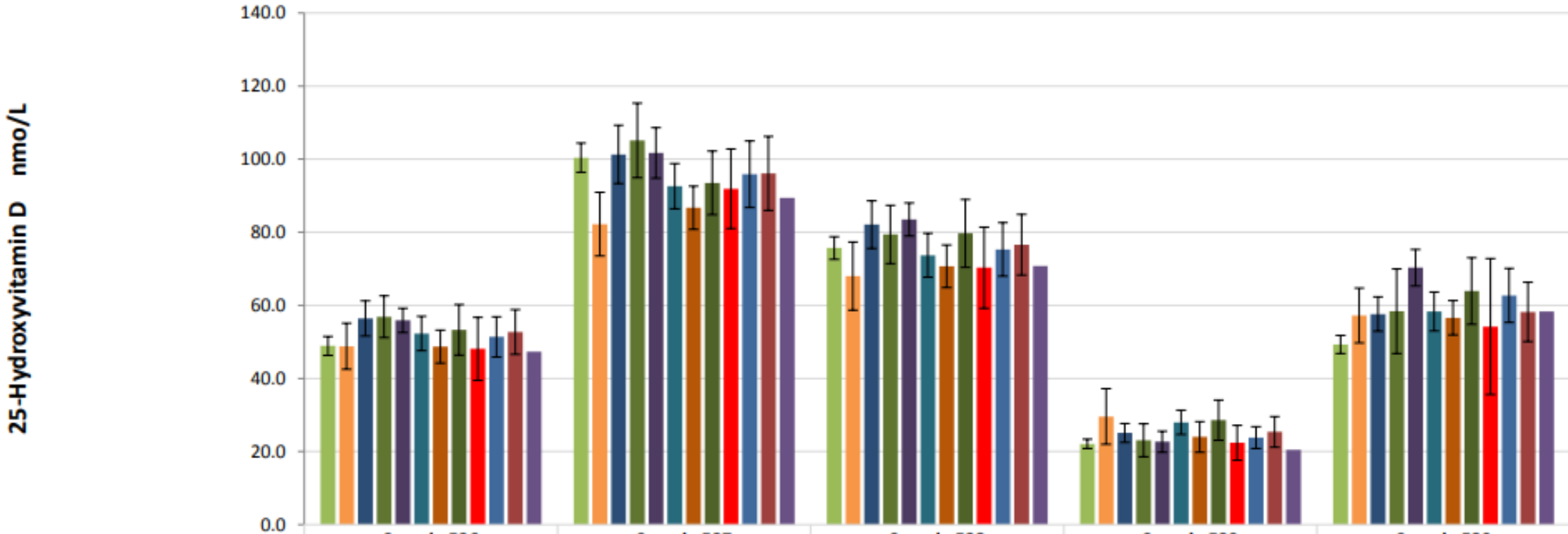
Sample: 526 (n=842)

Target Value :	47.3 nmol/L
ALTM	52.7 nmol/L
Your Method Mean (MM) :	51.4 nmol/L
Your result :	56.3 nmol/L
Bias from Target Value :	19.0 %
Bias from ALTM :	6.8 %
Bias from MM:	9.6 %

All Methods

Your method (LC-MS-MS)

DEQAS January 2018 - 25-OHD Method Means (+/-1SD) for Major Method Groups*



	Sample 526	Sample 527	Sample 528	Sample 529	Sample 530
Abbott Architect New Kit (n=70)	48.9	100.4	75.7	22.1	49.3
Beckman Unicel (n=32)	48.8	82.2	68.0	29.6	57.2
DiaSorin Liaison (n=197)	56.4	101.3	82.1	25.1	57.6
IDS iSYS (n=51)	56.9	105.1	79.4	23.1	58.4
IDS-iSYS New (n=14)	55.9	101.7	83.5	22.7	70.3
Roche Total (n=152)	52.3	92.6	73.7	28.0	58.3
Roche Vitamin D total II (n=25)	48.7	86.7	70.7	24.0	56.6
Siemens (n=65)	53.3	93.5	79.7	28.6	63.9
HPLC (n=16)	48.1	91.9	70.3	22.4	54.2
LC-MS/MS (n=154)	51.4	95.9	75.3	23.8	62.7
ALTM (n=842)	52.7	96.1	76.6	25.4	58.2
TARGET VALUE	47.3	89.4	70.8	20.5	58.3

Results from the NIST Reference Measurement Procedure for the October 2016 to January 2018 25-hydroxyvitamin D EQA Samples

Distribution	Sample No.	NIST 3-epi-25-OHD3 nmo/L	NIST 25-OHD2 nmol/L	NIST 25-OHD3 nmol/L	NIST 'Total' 25-OHD (25-OHD3 + 25-OHD2) nmol/L	DEQAS ALTM nmol/L	% Difference *
October 2016	**501**	6.5	2.8	93.4	96.2	100.4	4.4
	502	1.4	1.2	38.8	40.0	41.5	3.8
	503	5.5	1.4	79.2	80.6	87.1	8.1
	504	2.9	2.0	55.5	57.6	64.1	11.3
	505	0.9	0.7	21.0	21.7	23.5	8.3
January 2017	**506**	2.5	1.3	54.5	55.8	52.4	-6.1
	507	4.1	1.5	73.1	74.6	73.1	-2.1
	508	n/a	1.9	29.5	31.4	29.4	-6.4
	509	n/a	1.1	70.4	71.6	67.9	-5.1
	510	12.1	0.5	134.1	134.6	133.6	-0.8
April 2017	**511**	(4.3)	1.5	65.7	67.2	72.5	7.9
	512	(2.7)	1.9	44.9	46.8	49.9	6.6
	513	(6.8)	0.8	102.8	103.7	104.4	0.7
	514	(1.5)	0.6	27.1	27.7	29.6	6.9
	515* **	(3.0)	18.5	47.7	66.2	66.3	0.2
July 2017	**516**	2.9	1.3	45.2	46.5	47.3	1.7
	517	7.1	0.8	67.5	68.3	70.5	3.4
	518	8.7	1.2	103.7	105.0	110.3	5.1
	519	2.7	1.1	32.1	33.2	33.1	-0.3
	520	8.3	1.2	102.9	104.1	110.0	5.7
October 2017	**521**	2.1	1.0	39.6	40.5	41.2	1.7
	522	9.1	1.0	83.9	84.9	89.3	5.2
	523	1.2	3.5	22.5	25.9	25.7	-0.8
	524	14.1	0.9	107.9	108.8	124.8	14.7
	525	3.8	0.8	55.6	56.3	61.5	9.2
January 2018	**526**	3.0	0.9	46.5	47.3	52.7	11.3
	527	5.5	1.0	88.4	89.4	96.1	7.5
	528	4.5	1.9	68.9	70.8	76.6	8.2
	529	1.4	0.7	19.9	20.5	25.4	23.7
	530* **	2.8	21.5	36.8	58.3	58.2	-0.1

BIO-RAD

Lab 145728

SE LABORATORIUMI MEDICINA INTEZET
NAGYVARAD TER 4.
BUDAPEST 1083
HUNGARY

Sample Summary Report
Immunoassay (Monthly) Program

Cycle 15
Dec 2017 - Dec 2018
Sample No: 4
Sample Date: 09 Apr 18
Lot No: 231400

EQAS
External Quality Assurance Services

Instrument: Shimadzu LC-MS

	Analyte	Unit	Result	Mean	Z-score	RMZ	Comparator
✔	11-Deoxycortisol	ng/mL	0,04	0,158	-0,96	-1,05	Mode

Instrument: Tandem Mass Spectrometer

	Analyte	Unit	Result	Mean	Z-score	RMZ	Comparator
✔	17-a-OH-Progesterone	ng/mL	5,82	5,79	0,04	-0,64	Mode
✔	Aldosterone	pg/mL	253,7	286	-1,07	-0,68	Mode
✔	Androstenedione	ng/mL	1,78	1,89	-0,17	-0,09	Mode
✔	Cortisol	ng/mL	87,7	104	-1,64	-3,16	Mode
✔	DHEA	ng/mL	0,67	2,03	-1,79	-1,93	All
✔	Progesterone	ng/mL	9,68	11,4	-1,19	-1,24	Mode
✔	Testosterone	ng/mL	4,18	3,26	1,77	2,03	Mode

Instrument: Waters Mass Spectrometer

	Analyte	Unit	Result	Mean	Z-score	RMZ	Comparator
✔	DHEA-Sulfate	ng/mL	1815	1969	-0,71	-0,51	Mode

Legend: ✔ No Warnings Missing Result 1 Late Results ▼ 2,0 ≤ |Z-score| < 3,0 ✘ |Z-score| ≥ 3,0

* Amended Result (per participant's request) ¤ Non-robust determination of Mean and SD

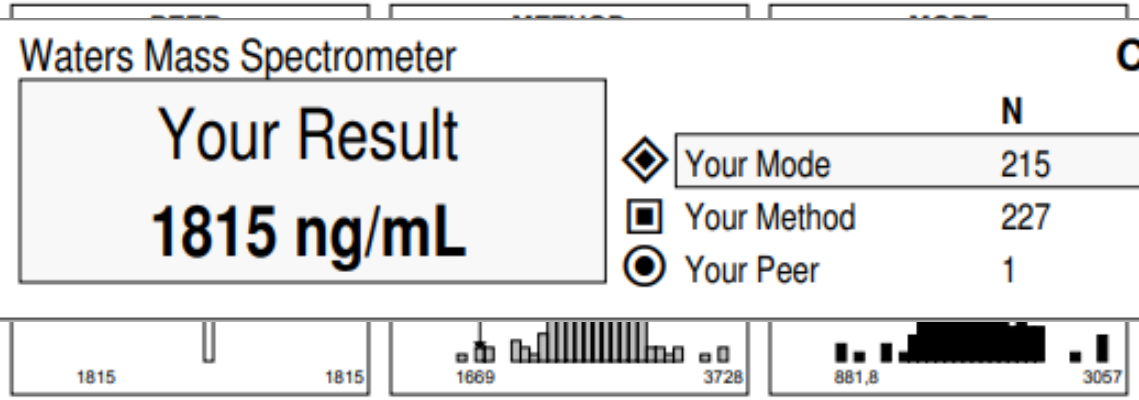
Waters Mass Spectrometer

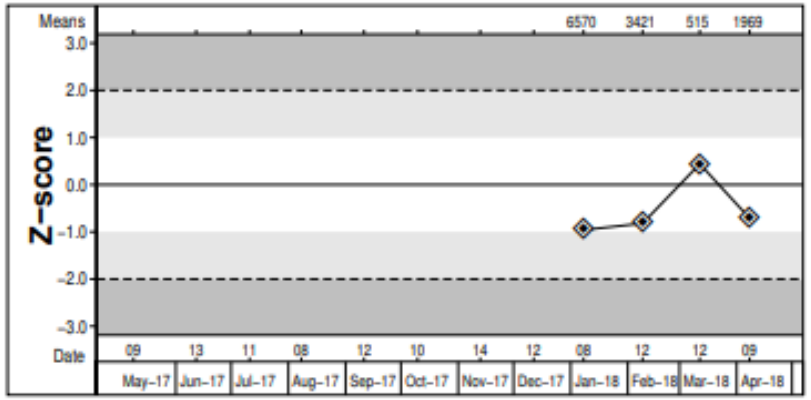
Your Result
1815 ng/mL

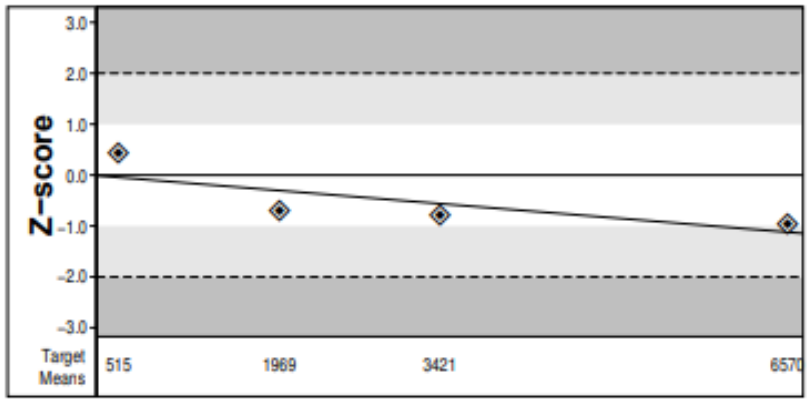
	N	Mean	SD	CV	U¹	Z-score	RMZ	%
Your Mode	215	1969	218	11,0	37,1	−0,71	−0,51	−7,84
Your Method	227	2698	206	7,63	34,2	−4,29	−4,41	−32,7
Your Peer	1	1815	0	0	0	0	0	0

Comparative Statistics

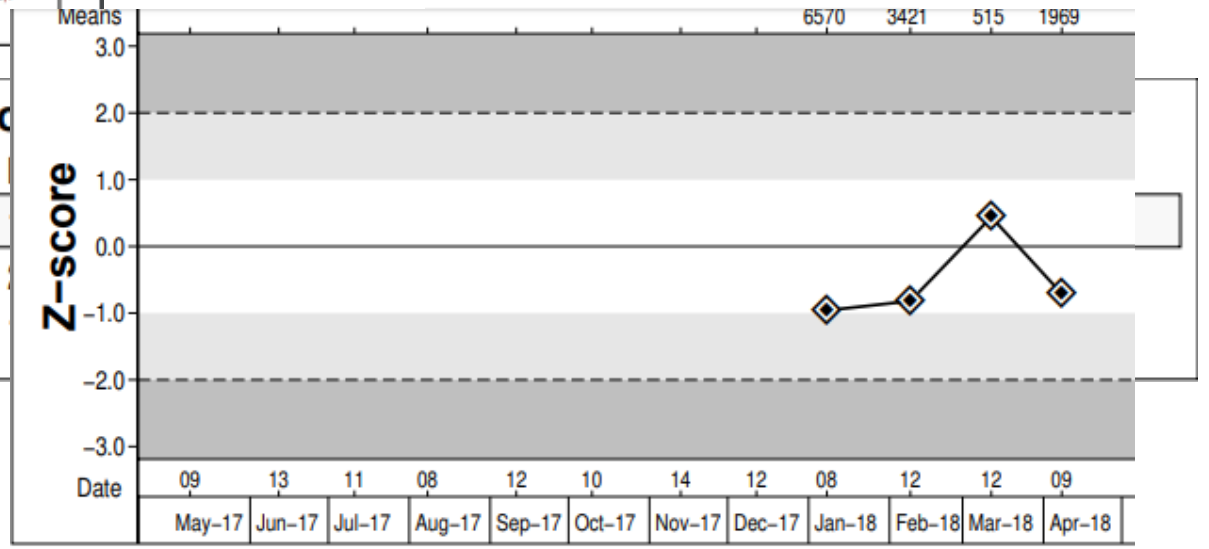
Your Deviation

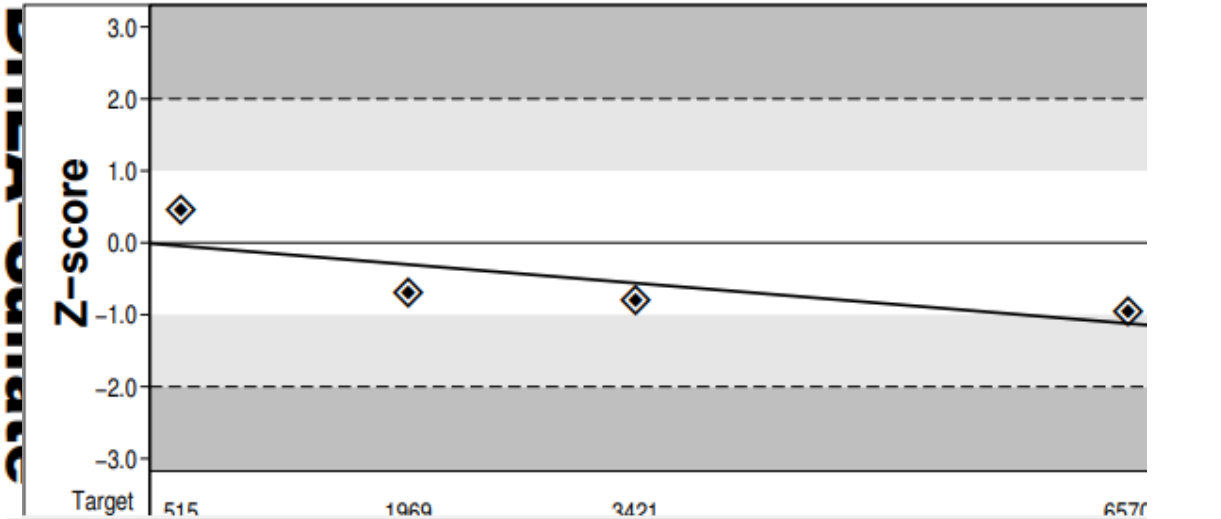






	N	Mean	CV	U¹
Roche cobas 6000/8000/c 311 Electrochemiluminescence (ECL) Dedicated Reagent	123	2702	6,46	39,3
Abbott AEROSET/ARCHITECT (c, i, ci ... Chemiluminescence Dedicated Reagent	110	2520	5,47	32,8
Roche Elecsys / cobas e 411 Series Electrochemiluminescence (ECL) Dedicated Reagent	94	2694	8,65	60,1
Siemens IMMULITE 2000/2500/XPi Chemiluminescence Dedicated Reagent	69	1908	11,7	67,2
Beckman Coulter UniCel DxI Series Chemiluminescence Dedicated Reagent	48	1979	8,96	64,0
Siemens ADVIA Centaur Systems Chemiluminescence Dedicated Reagent	39	2040	8,62	70,3
Beckman Coulter Access, LXi 725, Dx... Chemiluminescence Dedicated Reagent	22	2130	11,3	129
Siemens IMMULITE/1000 Chemiluminescence Dedicated Reagent	17	1927	9,00	105
DiaSorin LIAISON/XL Chemiluminescence Dedicated Reagent	9	1663	10,5	145





	17	1927	9,00	105
DiaSorin LIAISON/XL Chemiluminescence Dedicated Reagent	9	1663	10,5	145

Yundt chart