HORIBA

QUALITY CONTROL | BASIC PRINCIPLES

HORIBA
Medical

To make laboratory Quality Control accessible to all

Hematology version only

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Foreword

This document presents the basic principles of quality control to support laboratory good practice. The aim being to help them to develop protocols in the daily assessment of results analysis.

1 | QUALITY CONTROL

1.1 | What is Quality Control?

The blood analysis performed in laboratory allows, combined with observed clinical signs, to make a diagnosis and then to be able to set up a therapeutic prescription to improve the patient health.

The quality control is a way to assess the analytical process, which also allows validation of the results of this analysis.
It will enable the identification of possible malfunctions in the analytical process and the laboratory will then have to find the source of these malfunctions, to implement the necessary corrective actions.

The principle is easy: the laboratory is provided with control samples which will be analyzed and compared, either to the target values defined by the provider, or to the results obtained by other laboratories on the same control sample's batch.

There exists several quality control types:

- Internal quality control (precision assessment).
- Externalized Internal Quality Control (bias of trueness assessment).
- External Quality Control (bias of accuracy assessment).

1.2 | Internal Quality Control (IQC)

1.2.1 | Definition

The internal quality control is performed within the laboratory with blood control samples.
Most of the time, the IQC are designed and developed by the manufacturer of the instrument/reagent, coupled with a specific technology, often representing the best compromise between quality/specificity/stability/matrix-effects and, sometimes, is the only control material available.

The IQC corresponds to a "**continuous control**" allowing the assessment of the stability of the process through the **precision** of the control samples used daily in real-time.

Standard excerpt

"The laboratory shall design quality control procedures that verify the attainment of the intended quality of results."

ISO 15189:2012 - § 5.6.2.1 General[2]

1.2.2 | Procedure

The laboratory will receive a control sample batch, provided by the manufacturer of the instrument or by an independent provider, which will be analyzed **daily**, morning and evening time as a minimum, to frame the routine analysis (depending on the laboratory policy).

It's highly recommended to run at least **two samples levels** at minimum (high and low), to frame the measuring field of the laboratory.

***Note**:*

- *the shutdown of the instrument processed at the end of the day corresponds to a maintenance action which can have an impact on the analytical process. It's therefore necessary to run the controls before to validate the results of the day and to close the session.*
- *in case of incorrect results, all the analysis from the previous control will have to be assessed and could have to be repeated (after possible corrective measures and a valid control).*

Standard excerpt

"The laboratory shall have a procedure to prevent the release of patient results in the event of quality control failure."

ISO 15189:2012 - § 5.6.2.3 Quality control data

1.2.3 | Utility

The IQC is used for:

- ensuring the follow-up of the analytical system by a surveillance of the control results over time and to detect any analytical issue.
    - to assess if the **precision** of the results is constant from a day/month to another (CV of the laboratory).
    - to assess the IQC results and then **validate** the daily series of patient results.

1.3 | Externalized Internal Quality Control (eIQC)

1.3.1 | Definition

This is a comparison of IQC processed by several laboratories on the same control sample batch, including comparison of the mean, generally over a monthly period, allowing the estimate of **precision** and **trueness (bias)**.

***Note**:*

- *for more details on precision, see chapter "2.5 Precision Index (PI)".*
- *for more details on trueness, see chapter "2.6 Standard Deviation Index (SDI)".*
- *the IQC does not have to be related to an External Quality Control (EQC).*

It generally means routine control samples used for the IQC (continuous control of precision), from which the monthly mean results are externalized to be compared to a **peer group**.
The comparison to the peer group's mean as a reference value can be more relevant than the target values of the provider. The laboratory can use this externalization to assess the **trueness of the method**.

***Note**: For more details on the true value, see chapter "4.Definitions".*

The peers correspond to all the laboratories contributing to the same quality control program. They are divided into homogenous groups (same instrument type, same control sample batch, same technology or same instrument manufacturer), which are called "peer groups" (considering that there needs to be a minimum between 6 and 10 instruments to establish a peer group).

***Note**: the peer group segmentation can vary according the quality control providers.*

1.3.2 | Procedure

This is the same as the IQC procedure (see previous chapter), except for the externalization of the data required for the comparison to the peer group.

QCP Benefits

HORIBA Medical provides an externalized internal quality control program (eIQC): **the QCP**.

Peers represent the **laboratories all over the world** who possess HORIBA Medical's instrumentation and contribute to this program.

Each peer group represents an homogenous group using the same **type of instrument** and the same **control sample batch**.

***Note**: The laboratory will have to send their results to the QCP website (manually or automatically).*
The final QCP reports will be then sent to each participant between the 15th and the 18th of the month for the results of the previous one.

Every month the laboratory will send the mean of their results for each parameter, which will then allow them to receive the eIQC results (comparing their results to the peer group ones).

The comparison will be carried out between the mean of **several runs** of **several sample tubes** of the laboratory (for a same level, a same batch and over a specific period), with the mean of the **multiples runs** performed by the laboratories of the peer group.

QCP Benefits

With the QCP, the laboratories are able to compare their results at **any time** with the laboratories of the peer group. In addition, it is possible to download some **intermediary reports** throughout the duration of the control, without having to wait to receive the final reports which will be sent to them at the end of the month.
This option allows them to assess their trueness, according to the data already gathered.

To do so, they will have to enter the daily results of the control sample batch, which will then allow them to download **real time reports**.

The final reports will automatically be sent (15 days after the end of the month and the results submission).

***Note**: There are generally enough laboratories with the same instrument configuration to get a minimum one peer group (minimum of 6 instruments).*

1.3.3 | Utility

The eIQC is used:

- to assess if the **results' precision** is constant from one day/month to another (CV of the laboratory) and to **compare this precision** to the one of the peer group (PI).
- to measure the **bias of trueness** (closeness of the laboratory value with that of the peer group, reference value defined by the mean of the multiple runs of the peer group).

***Note**:*

- *the IQC process takes into account the fact that the instrument has been correctly calibrated (at the installation, after a major maintenance intervention, or following bad eQC results).*
- *The controls used in the case of an externalized IQC are the same than the ones used in the case of an IQC. The difference is the comparison of these results with the ones of the peer group.*
- *see chapter 1.5.3 "Use of the target values".*

1.4 | External Quality Control (eQC, eQA)

1.4.1 | Definition

The external Quality Control external Quality Assurance (eQC, eQA) represents the analytical process of a patient sample at a specific time.
The control samples are provided by an **external entity, independent** of the laboratory and of the instrument manufacturer.
Unlike the control samples of an IQC (also used for the eIQC), they will not be delivered with known target values ("**blind tests**").

eQC/eQA Definition

"The external quality assessment (eQA) term is used to describe a method allowing to compare the analysis of laboratories to an external reference […]. This comparison may be lead to compare the performances of a group of similar laboratories.

The eQA term is sometimes used interchangeably with the "Proficiency Testing" term […].

The eQA is defined as a system allowing to objectively assess the performances of the laboratories […]."

World Health Organization[1]

***Note**: an externalized ICQ does not exempt from the use of an EQC, but it becomes mandatory when no EQC exists for the considered method.*

1.4.2 | Procedure

When a laboratory subscribes to an external Quality Control, it will receive one or several control samples.

Each of these **control samples is processed singly** on **only one run** per cycle / event of the program (corresponding to a month or a quarter, dependent on the provider).

Standard Excerpt

"The laboratory shall participate to an interlaboratory comparison program(s) (such as an external quality assessment program or proficiency testing program) appropriate to the examination and interpretations of examination results".

ISO 15189:2012 - § 5.6.3.1 Participation[2]

The results will be then be sent to the eQA provider, and these results will be compared to the mean of the peer group's runs.

The comparison will be made between the **unique run** of a **unique tube** in the laboratory and the mean of the **unique runs** obtained by each laboratory of the peer group.

The laboratory will receive a report at the end of the analysis period (cycle / event, for example a cycle can correspond to one month, a quarter or a semester, dependent on the eQC providers).

1.4.3 | Utility

The eQC is used to measure the **accuracy of the bias** (comparison of the results of the laboratory with the ones of the peer group).

***Note**: the trueness and the accuracy are very close notions. For more details on their differences, please refer to the chapter 2.6 "Standard Deviation Index (SDI)").*

1.5 | The control samples

1.5.1 | Definition

As a full blood count control sample consists of biological material which is limited by the stability of its components over time, manufacturers are stabilizing them with chemical treatments. Even so, it can nevertheless be possible to observe a small variation of the MCV over time (ageing of the control which must be known and documented in order to control the variation).

These samples can be subjected to a wide range of temperature and mechanical agitation, which could damage the cells and, therefore, the strict respect of their open stability and the number of planned runs is crucial.

It means that the blood is specifically designed for a specific quality control program.

- either only **human,**
- or **synthetic**: mix of the different blood types (human and animal).

Most of the control samples are available in three different levels of concentration:

- low,
- normal,
- high.

HORIBA Medical provides several types of controls for hematology (synthetic bloods):

- **ABX Minotrol 16**: for the control of instruments with a screening leukocyte differential (3 Diff).
- **ABX Difftrol**: for the control of instruments with a complete leukocyte differential (5 Diff and 5Diff + nRBC for the Yumizen H1500/2500).
- **Erytrol**: for the control of instruments with a separate nRBC (Erythroblast) counting (only for the Nexus instruments).
- **ABX Minotrol Retic**: for the control of instruments with reticulocyte counting.
- **ABX Minotrol CRP**: for the control of the instruments with a screening differential (3 Diff) combined with C-reactive protein (CRP).

***Note**: the control samples are considered stable, but if assessment of the instrument and of the human process (pre and post analytic) has not led to determine the origins of any issues of a non-correct result, it is then recommended to question their stability).*

1.5.2 | Reminder of the procedure for the use of HORIBA Medical control samples:

1. Remove the control from the refrigerator and allow it to reach ambient temperature for 10 minutes. Roll the tubes between the palms for 30 seconds. Do not shake.
2. Refer to the user manual to enter the control on the instrument (manually or via the bar-code scanner).
3. Gently invert the tube 8 to 10 times (180° rotation) immediately before sampling on the instrument
4. Verify the control results according to the procedure described in the user manual.
5. After use, wipe the threads of the sample tube and tube cap with lint-free gauze.
6. Close the tube and replace it in the refrigerator immediately after use.

These actions have to be followed and standardized between all the laboratory's technicians.

1.5.3 | Using of target values and confidence ranges.

Every control sample is provided with **target values** and **confidence ranges** (tolerance ranges).

***Note**: this only concerns the control samples provided in the frame of an IQC or an eIQC (the eQA being blindly performed).*

It is highly **recommended to not use these target values and confidence ranges over long term period** in the laboratory. They have to be used only at the installation of the instrument or when starting a new

IQC/eIQC batch, to be then quickly replaced by the values **calculated by the laboratory itself** (the mean and the (Standard Deviation) SD obtained on their own results).
When a change of control batch occurs and before the end of the former one, the laboratory will have to run the new and the former one in parallel during a probationary period (recommended 5 days). This is to allow time to calculate the mean and the CV of the new batch and define the new target value of the laboratory.

***Note**: it is recommended to use a mobile mean to define the target value of the laboratory, it means to recalculate the mean after every run and while the all life duration of the batch (only in hematology).*

Indeed, the target values and confidence ranges are provided by the manufacturer taking into account all the variations which can be linked to a same type of instrument (inter-instruments variations).
It is therefore highly recommended for the laboratory to define their own target values and confidence ranges to take into account the variations of the instrument they are using (intra-instrument variations).

***Note**: The confidence ranges, communicated by the provider are specific to the control samples. A value initially found inside these confidence ranges ensure that the control has not been altered before his first use.*

How to define your target value (mean recalculated by the laboratory) and your limits (SD)? :

1. Analyze the control sample 10 to 20 times (over at least 5 days, either 4 runs/day over 5 days minimum).
2. Calculate the mean of these runs for each parameter.
3. This mean has to be between the confidence ranges indicated by the provider.
4. The mean calculated corresponds to the new target value or reference value of the laboratory for the new control batch.
5. Use the standard deviation previously determined in the laboratory via previous control batches or the one from the last QCP report (for the same control sample level).
6. Following the process of these 10/20 runs, it is then possible to establish a new mean (target value).
7. Generally, the acceptable limits are established at ±2SD or ±3SD, following the quality policy of the laboratory.

- 2 SD: 95% of the results are expected to be between ± 2SD. If a value is beyond these limits, it will transgress the 1_{2s} rule of Westgard *(see chapter "Levey-Jennings and Westgard rules" for more details)*.

***Note**: the use of the 2 SD control limits can be a risky practice, because it may generate false alarms, which could then lead to ignoring the true ones. When a control exceeds 3 SD, it is highly likely that it is a relevant alarm, because the probability of false alarms is very low.*

- 3 SD: 99,7% of the results are expected between the mean ± 3SD. If a value is beyond these limits, it will transgress the 1_{3s} rule of Westgard *(see chapter "Levey-Jennings and Westgard rules" for more details)*.

***Note**: ideally, the quality control procedure should be chosen to minimize the false alarms and optimize the true alarms for important analytical errors. It is also crucial that the limits of the controls are correctly set up to characterize correctly the variability observed in the laboratory, if not, the quality control procedure will not behave as expected.*

2 | CALCULATIONS

To assess the quality of the results of the control samples, different elements will be calculated:

Simple elements: directly calculated from the analysis results (from the laboratory and from the peer group participants).

1. A Mean of the laboratory or peer group's results by parameter and levels.
2. A Standard Deviation (SD) by parameter and levels
3. A Coefficient of Variation (CV) by parameter and levels

Complex elements: calculated from the simple elements:

1. The Precision Index (PI) by parameter and levels
2. The Trueness (SDI) by parameter and levels
3. The Uncertainty by parameter and levels
4. The Sigma by parameter and levels

The goal of these complex elements is to compare the simple elements from the laboratory and from the peer group.

QCP report example:

	Laboratory			Group		Comparison		Uncertainty		Sigma	Sigma
	Mean	CV	SD = U1	N	Mean	PI	Z-Score	Uc %	R	Lab.	Obj.
WBC - L ▲	2	1	0	1	2	0	3	7	2.68 ± 0.38	7	8
WBC - N ▲	8	5	0.4	20	7	2	6	11	8.68 ± 2.04	0	10
WBC - H ▲	20.97	1.8	0.379	186	18.04	0.65	5.86	9.54	20.97 ± 4.00	0.9	11.2
RBC - L	2.37	0.6	0.015	184	2.34	0.33	0.58	0.90	2.37 ± 0.04	5.4	4.7
RBC - N ▲	4.74	2.4	0.113	207	4.60	1.48	1.78	2.90	4.74 ± 0.27	0.7	5.6
RBC - H	5.03	1.0	0.051	186	5.09	0.64	-0.71	1.21	5.03 ± 0.12	3.3	5.6
HGB - L	6.8	1.5	0.10	184	6.7	0.89	0.32	1.51	6.8 ± 0.2	2.5	5.1
HGB - N ▲	14.4	1.3	0.19	207	13.8	0.96	3.20	2.85	14.4 ± 0.8	0.0	6.1
HGB - H	16.0	0.4	0.06	186	16.2	0.27	-0.91	0.80	16.0 ± 0.3	8.2	6.2
HCT - L ▲	19.9	0.8	0.15	184	18.9	0.38	2.70	3.18	19.9 ± 1.3	-1.4	4.0
HCT - N ▲	40.3	2.9	1.18	207	37.9	1.72	3.55	4.57	40.3 ± 3.7	-0.6	4.7
HCT - H	44.9	0.8	0.35	186	44.6	0.44	0.33	0.84	44.9 ± 0.8	4.4	4.6
MCV - L ▲	84.0	0.0	0.00	177	80.6	0.00	3.67	2.33	84.0 ± 3.9		4.2
MCV - N ▲	85.8	2.2	1.92	200	82.4	2.20	3.98	3.24	85.8 ± 5.6	-0.7	4.7
MCV - H ▲	89.3	0.6	0.58	179	87.6	0.59	1.77	1.29	89.3 ± 2.3	0.8	4.4
MCH - L ▲	28.6	1.5	0.43	177	28.7	1.01	-0.23	1.54	28.6 ± 0.9	1.4	3.3

- Mean of the laboratory
- CV of the laboratory
- SD of the laboratory
- Nb of instruments part of the peer group
- Mean of the peer group
- Precision Index (PI)
- Trueness (SDI or Z-score)
- Uncertainty (%)
- Result +/- the uncertainty (#)
- Sigma (Ricos)
- Sigma (HORIBA)

2.1 | Mean

The mean corresponds to the best estimate of the **target value of the laboratory** (reference value for the IQC) and the **value** for the peer group (reference value for the eIQC), of a parameter and for a specific control level *(See chapter "4.Definitions").*

Mean formula

$$\bar{X} = \frac{\sum_{i=1}^{N} X_i}{N}$$

Σ : Sum of the measured values
$\bar{X}$: Mean of the measured values
X_i : Set of the measured values
N : Number of the measured values

To calculate the mean of a specific control level, sum all the values gathered for this control (generally over a one month period).
Then, divide the sum of these values by the total number of values.

***Note**: The mean of the peer group corresponds to the global mean of each peer group laboratory mean.*

2.2 | XB

This indicator aggregates patient results (non-flagged ones) in batches of 20. The average of a batch of 20 results will be compared with the **mean** of previous batches to detect gaps in patient data,
all day long.

An XB alarm will be triggered if one or more means are outside the target value.
This indicator therefore allows continuous monitoring of the stability of the analytical process and is then complementary to the framing of the series of results achieved by the use of internal controls.

***Note**:*
- this indicator exists directly on some diagnostic instruments, but can in no way replace the use of internal controls.
- The triggering of the XB alarm will depend on the instrument settings (percentage deviation from the average, target values)

2.3 | Standard Deviation (SD)

2.3.1 | Definition

The standard deviation measures the dispersion of a value's group as a function of its mean.

2.3.2 | Explanations

Standard deviation formula

$$SD = \sqrt{\frac{\sum (X_i - \bar{X})^2}{N - 1}}$$

SD : Standard deviation
$\sum (X_i - \bar{X})^2$: Sum of squares of the difference between the individual measured values and the mean
$\bar{X}$: Mean of measured values
X_i : Values individually measured
N : Number of measured values

To calculate the standard deviation:

1. Calculate the mean of the results series.
2. Calculate the difference of every individual result with the mean of the series.
3. Calculate the square of every previous difference.
4. Calculate the sum of all the squares.
5. Divide the sum by the number of results minus 1.
6. Apply the square root to the previous result.

***Note**: The formula concerns the calculation of the standard deviation of a part/sample of a statistical population. If we want to calculate the standard deviation of the entire population, we only need to divide by N and not by N-1.*

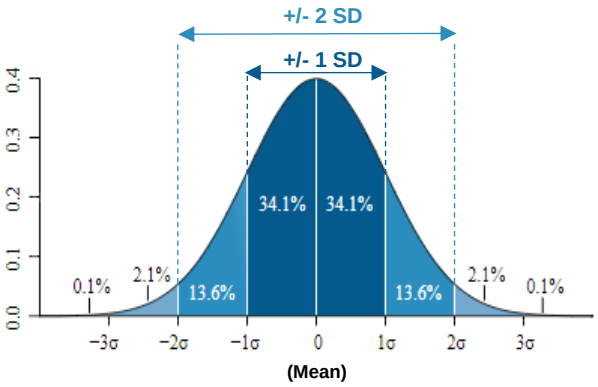
Calculation example of a Standard Deviation

Series: 8, 12, 13, 16, 7, 13, 18.

1. Mean: 12,43
2. Differences: (**8**-12,43= -4,43) ; (**12**-12,43= -0,43) ; (**13**-12,43= 0,57) ; (**16**-12,43= 3,57) ; (**7**-12,43= -5,43) ; (**13**-12,43= 0,57) ; (**18**-12,43= 5,57)
3. Squares: $(-4,43)^2=19,02$; $(-0,43)^2=0,18$; $(0,57)^2=0,32$; $(3,57)^2=12,7$; $(-5,43)^2=29,5$; $(0,57)^2=0,32$; $(5,57)^2 = 31,02$
4. Sum: 93,66
5. Division: 15,61
6. Square root: 3,9

3,9 is equal to **one standard deviation (1 SD)**.

2.3.3 | Interpretation



If the studied statistical series follows a normal law, we will get the following repartition:

- 1 SD: 68,2% of the elements/results of the statistical series are between ($\bar{X} - \sigma$) and ($\bar{X} + \sigma$).
- 2 SD: 95% of the elements/results of the statistical series are between ($\bar{X} - 2\sigma$) and ($\bar{X} + 2\sigma$).
- 3 SD: and 99,6% of the elements/results of the statistical series are between ($\bar{X} - 3\sigma$) and ($\bar{X} + 3\sigma$).
- 4 SD: and 99,8% of the elements/results of the statistical series are between ($\bar{X} - 4\sigma$) and ($\bar{X} + 4\sigma$).

(where $\bar{X}$ represents the mean of the series and a sigma (σ) = 1 SD).

The further the elements/results of the series are from the mean, the higher the standard deviation (and the instrument results are less homogenous).

If the value of a standard deviation (+/- 1 SD) increases over time, it means that it will need to move more away from the mean to encompass 68,2% of the series results.

2.4 | Coefficient of variation (CV)

2.4.1 | Definition

The coefficient of variation also represents the spread of the measures around the mean and assesses the precision of the instrument:

The precision refers to the **reproducibility** of the analyses and the measure of the closeness of the different results of a same sample.

This indicator **only assesses the precision** of the results of several runs and not whether these results are true or accurate.

2.4.2 | Explanations

The coefficient of variation corresponds to the ratio of the standard deviation to the mean and is expressed as a percentage:

Coefficient of variation formula

$$CV\ \% = \frac{SD}{Mean} \times 100$$

CV: Coefficient of variation

SD: Standard deviation of the laboratory or peer group

Mean: Mean of the laboratory or peer group

2.4.3 | Interpretation

The lower the value of the CV, the higher the precision of the instrument. Indeed, it will mean that the different results will be framed in a more contained space around the mean.

These statistics allows the pathologist to more easily compare the global precision of the results and therefore the stability of the analytical process.

The **standard deviation generally increases along with the concentration** of the parameter, but the CV can be considered as a statistical moderator.
If the pathologist or technicians try to compare the precision of two different methods and overall two different levels and are only using the standard deviation, this can easily mislead them.

2.5 | Precision index (PI)

2.5.1 | Definition

The goal of the Precision Index is to compare the precision of the laboratory with the mean of the peer group.

2.5.2 | Explanations

As the precision is measured by the Coefficient of Variation (CV), the precision Index simply divides the CV of the laboratory by the CV of the peer group.

The result will then indicate if the analyses of the laboratory are as reproducible as the ones performed by the peer group.

Precision formula

$$PI = \frac{CV_{lab}}{CV_{peer\ group}}$$

CV_{lab}: CV of the laboratory

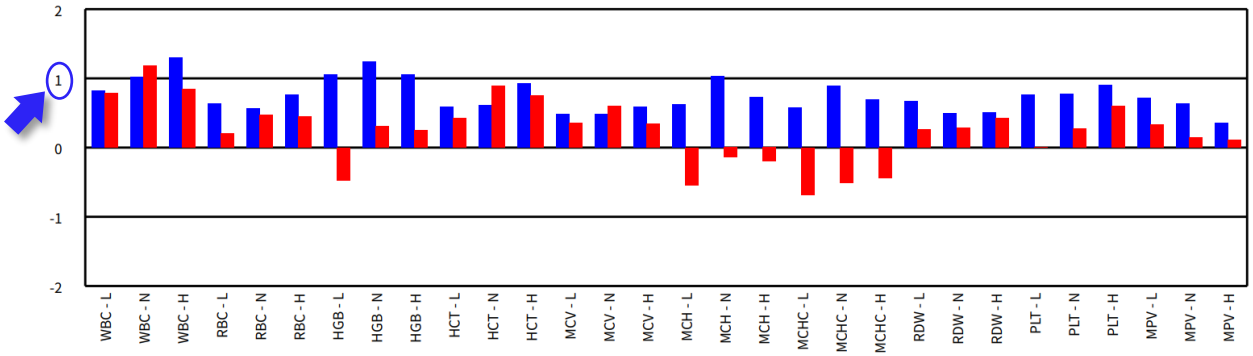
$CV_{peer\ group}$: CV of the peer group (average performance for a considered parameter)

2.5.3 | Interpretation

More the CV of the laboratory will be small (so better), more the value of the PI will be small too.

PI **< 1**: CV of the laboratory better than the CV of the peer group.
PI **= 1**: CV of the laboratory equivalent to the CV of the peer group.
PI **> 1**: CV of the laboratory worse than the CV of the peer group.

QCP report example: graphical representation of the precision (in blue)



The blue bars indicate the precision (ratio of the CV of the laboratory and the CV of the peer group).
The precision is always positive.
The value of **1 corresponds to the average CV of the peer group**.

2.5.5 | Recommended actions

Precision between **0 and 1**: no action required.
Precision between **1 and 2**: the precision could be improved.
Precision above **2**: it is mandatory to have a corrective action.

QCP Benefits

If the **precision is above 1**, a notification (▲) will appears on the report:

WBC - L	
WBC - N	▲
WBC - H	▲

Your local QCP manager will have the possibility to be aware of the precision values which are **above 2** and to call the laboratories to provide a technical support.

If the precision is above 1, but overall above 2, here are the recommended corrective actions:

- First, check if the 3 different levels are given the same bias of trueness:
  - If this is the case, it's probably due to an instrument issue.
  - If it's not the case, it's probably due to a tube issue. You will then have to run a new control to confirm this hypothesis.
- It is necessary to study the Levey-Jennings charts, to know if the incorrect values are isolated and to apply the Westgard rules.
- To check the data entered into the QCP.

Control samples

- Check the shipment conditions: the control sample tubes can be exposed to temperatures above the stated ones (2°C - 8°C).
  Conversely, too low a temperature could generate a lysis of the cells (low level of red blood cells and high level of platelets, beyond the confidence ranges), leading to reject the tube from the first run.
- Check the storage conditions of the control sample (refrigerated between 2 and 8 °C).
- Check if there is progressive deterioration of the control sample (duration date).
- Check if the control sample batch has changed.

Reagents

- Check the reagents (duration date, remaining volume, storage conditions, etc…)
- Check there is no change in the reagent's formulation.

Human process (pre-analytical)

- Check the quality of the technician's actions.
  - Warming control for 10 minutes until reaching room temperature.
  - Homogenization of the tube control.
  - Do not mix until it is at room temperature.
- Rerun the control sample.

Maintenance history

- Check if there was significant recent maintenance/repair.
- Check if there was a new calibration recently performed.

Technical

- Clean / carry out the maintenance of the instrument.
  - Check the status of the sampling system (status of the sampling needle).
  - Check there was no failure in the reagent distribution system.
  - Check the lamps status.
  - Check the tubing and valves status.
  - Check for progressive accumulation of debris in sample/reagent tubing.
  - Check for progressive accumulation of debris on apertures.
  - Check for variation in the incubation temperature.
  - Check for change in the room ambient temperature/humidity.

***Note**: this list of actions is not an exhaustive one and it's a recommendation only given for information. It is the responsibility of the laboratory to master the corrective actions which have to be done.*

2.6 | Standard Deviation index (SDI)

2.6.1 | Definition

The Standard Deviation Index (SDI) indicates the reliability of the results, measuring the **accuracy bias** or the **trueness bias** which corresponds to the discrepancy of the value of the laboratory compared to a reference value (or a true value).

We are discriminating the accuracy and the trueness according to the two following definitions:

Accuracy

Gap (bias) between a **unique value** from the laboratory and the value from the peer group (reference value assimilated to the true value).

Trueness

Gap (bias) between the **mean of the values** measured by the laboratory and a reference value (target value provided by the controls manufacturer, target values defined by the laboratory itself or the peer group mean).

Warning

The mean of the laboratory can get closer to the reference/true value with very scattered values (bad precision).

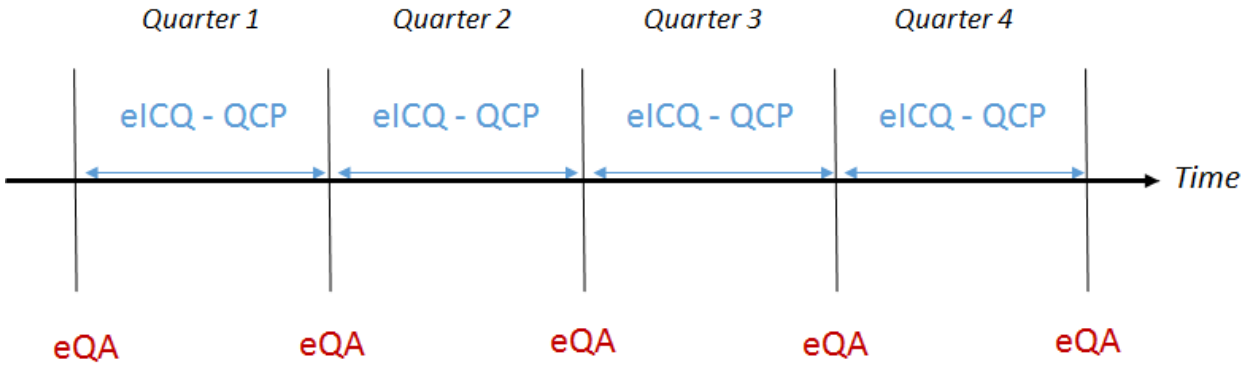
In this case, the accuracy/trueness can appears **wrongly** true.
(there cannot be a good accuracy/trueness without a good precision).

The externalized eIQC and the eQC are **complementary** to each other.

- Indeed, the externalized IQC can be likened to a “continuous control” which allows the assessment of **precision** through the CV in daily time.
  It will also measure the gap (bias) to the reference value through the **trueness bias** (faster detection than with an EQC).

- The eQA can be likened to a “punctual exam” which allows the assessment of the gap (bias) of a result compared to a reference value through the **accuracy bias**.

- The trueness bias being calculated from a mean, it will not be **impacted by the precision**, which is **not the case** for the accuracy bias because it is based on a unique value.



Schema of the eIQC and eQA complementarity over a year.

***Note**: the range of time between two eQA can vary according the provider (monthly, quarterly, bi-annually).*

To sum up:

The results are not **precise** nor **accurate**: no reproducibility.

The **precision is good**: the results are close to one another (reproducible).

But the measured values are **not accurate** (far from the reference/true value).

The results are **precise** (reproducible), and they are **accurate/true**.

***Note**: the results cannot be accurate if they are not precise (not reproducible).*

2.6.2 | Explanations

Accuracy/Trueness formula

$$SDI = \frac{\bar{X}_{lab} - \bar{X}_{peer\ group}}{SD_{peer\ group}}$$

$\bar{X}_{lab}$: Mean of the laboratory

$\bar{X}_{peer\ group}$: Mean of the peer group

$SD_{peer\ group}$: Standard deviation of the peer group

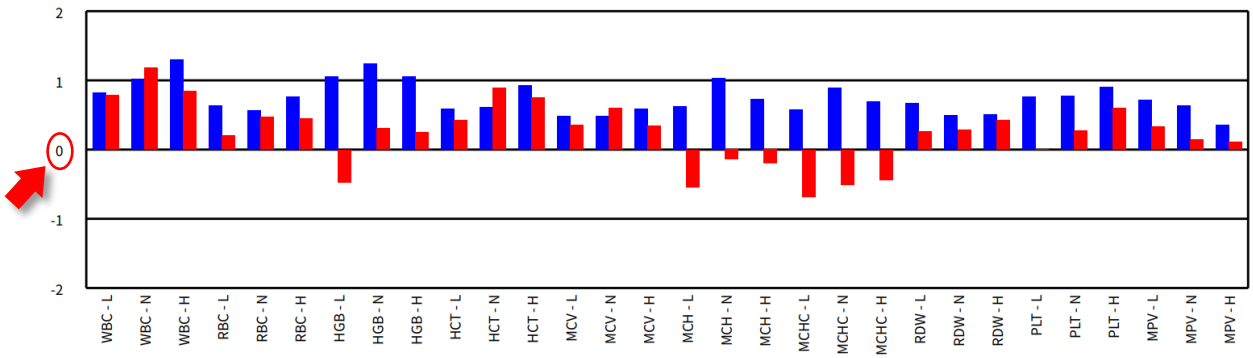
The comparison of the means of the laboratory and the peer group, allows the demonstration of how far the standard deviation of the laboratory is from the true value (peer group mean).

2.6.3 | Interpretation

The further the mean obtained by the laboratory is from the peer group mean, the greater the value of the SDI (and the lower the accuracy of the laboratory).

SDI **= 0**: there is no bias.
SDI **= 0 to 0,5**: the trueness of the laboratory is excellent.
SDI **= 0,5 to 1**: the trueness of the laboratory is satisfactory.
SDI **= 1 to 2**: the trueness of the laboratory is acceptable.
SDI **> 2**: the Trueness of the laboratory is poor.

QCP report example: graphical view of the trueness (in red)



The red bars indicate the trueness (the getting away from the laboratory mean compared to the peer group mean).
The trueness could be positive or negative.
The value of **0 corresponds to the peer group mean**.

2.6.4 | Recommended actions

Trueness between **-1 and 1**: no action required.
Trueness between **-2 and -1** or **1 et 2**: the trueness could be improved.
Trueness **below -2** ou **above 2**: it is mandatory to have a corrective action.

***Note**: the trueness is to put in perspective to the precision of the control level and to the clinical interest of the parameter (example: less relevant for the parameters as MCH, MCHC and HC, which will be calculated from others parameters which already have their own precision).*

QCP Benefits
If the trueness is **below -1** or **above 1**, a notification (▲) will appear on the report:

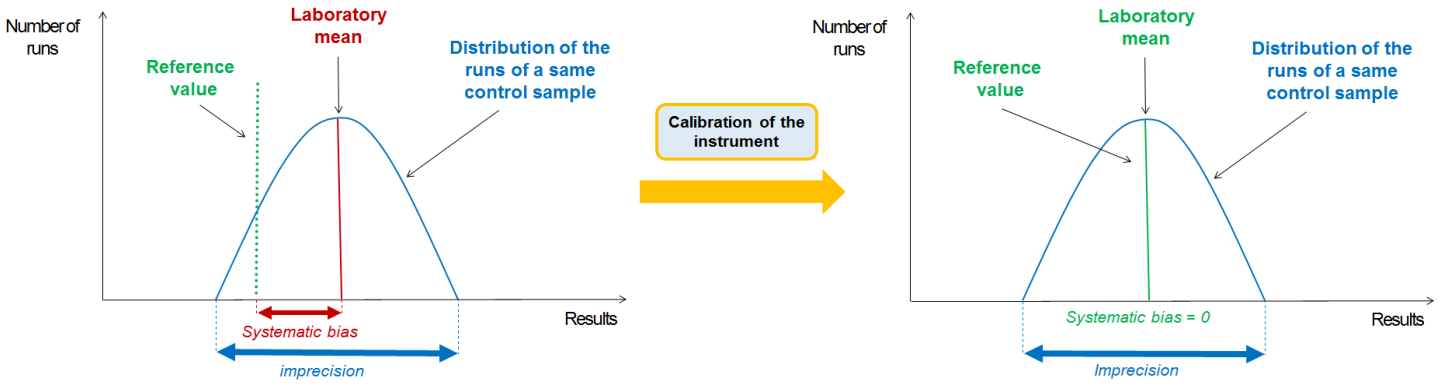
WBC - L	
WBC - N	▲
WBC - H	▲

Your local QCP manager has the possibility to be automatically informed of the values of trueness which are **below -2** and **above 2** and to call the laboratories to provide technical support.

Recommended actions:

- Check the data entered into QCP.
- Calibration of the coefficients

***Note**: this list of actions is not an exhaustive one and is a recommendation only given for information. It is the responsibility of the laboratory to master the corrective actions which have to be done.*



***Note**:*

- *the calibration should only be done in the following cases:*
  - *At the installation*
  - *After a significant maintenance action (spare parts changing…)*
  - *Following bad eQA or eIQC results*
- *it is recommended to run the control multiple times (or a patient sample) to obtain a repeatability and then quickly assess the precision of the method, before carrying out the calibration (never calibrate an imprecise method: it is important to fix any the imprecision issues before any calibration and to correct the accuracy/trueness issue).*
- ***the calibration has absolutely no effect on the imprecision.***

2.7 | Uncertainty

2.7.1 | Definition

The uncertainty of the measure is composed of systematic (linked to the accuracy/trueness) and random effects (linked to the precision).
The uncertainty is an indicator of the result quality and reliability, it is associated with every measuring result.

Example 1

Example 2

Example 1: If the measured value perfectly corresponds to the reality, the result would be equal to the real value of the parameter.
Example 2: However, the real value of the parameter can be inside a wider area (uncertainty area of the measuring), than the result indicated by the instrument.
This is true when we are doing a measure, whatever the parameter and whatever the technology used.

Standard excerpt

"The laboratory shall determine measurement uncertainty for each measurement procedure in the examination phase used to report measured quantity values on patients' samples. "

ISO 15189:2011 - § 5.5.1.4 Measurement uncertainty of measured quantity values[2]

2.7.2 | Explanations

The uncertainty calculation takes into account the **precision** (standard deviation of the laboratory, U_1(IQC) and the one of the **accuracy/trueness bias** (difference between the values or mean of the laboratory and the peer group), U_2(eQA).

Formula for the calculation of uncertainty

$$U_1\,(IQC) = SD$$

U_1: Standard deviation of the laboratory

(Uncertainty linked to precision)

Bias: Difference between the mean of the laboratory and of the peer group

m: Mean of the laboratory

V: Mean of the peer group

$$Bias = (m - V)$$

U_2: Uncertainty linked to the trueness and accuracy (systematic error)

Biais: Trueness or accuracy bias

$$U_2\,(eQA) = \frac{Bias}{\sqrt{3}}$$

$$U_c = \sqrt{U_1^2\,(IQC) + U_2^2(eQA)}$$

U_c: Combined uncertainty

U_1: Uncertainty linked to the precision (random error)

U_2: Uncertainty linked to the trueness and accuracy

U: Measuring uncertainty

U_c: Combined uncertainty (total error)

$$U = 2U_c$$

$$R = m +/- U$$

R: Reportable result of the parameter

m: Measured result

U: Uncertainty

Uncertainty calculation example

	Laboratory			Group		Uncertainty	
	Mean	CV	SD = U1	N	Mean	Uc %	R
WBC - L	2.54	3.1	0.079	96	2.47	3.56	2.54 ± 0.18

QCP Report excerpt

$U_1 = SD_{lab} = 0,079$

$U_2 = (moy_{lab} - Mean_{group}) / \sqrt{3} = (2,54 - 2,47) / \sqrt{3} = 0,07 / \sqrt{3} = 0,0404$

$U_1^2 = 0,006241$
$U_2^2 = 0,001637$

$U_c = \sqrt{(U_1^2 + U_2^2)} = 0,088758$

$U = 2U_c = 0,177 = 0,18$

$U_c\,(\%) = U_c / moy_{labo} = 0,088758 / 2,54 = 3,5\ \%$

QCP Benefits

The uncertainty calculation in the QCP includes an added element: **the SD of the accuracy bias**.

$$U_c = \sqrt{U_1^2(IQC) + U_2^2(eQA) + \mathbf{SD_{eQA\ bias}}}$$

As we have seen it at the chapter "2.5.1 Definition", the accuracy can appear falsely correct.
For example, over a year, if the eQA values are equivalents, but vary between positive and negative values as 2 and -2, the accuracy bias (U_2 (eQA)) can be equal to zero. However, the accuracy will not be correct at all.

$$SD_{bias\ eQA} = SD_E = \sqrt{\frac{\sum (E_i - \bar{E})^2}{N - 1}}$$

The SD_{bias} allows to correct the phenomenon. Indeed, even if the calculation of the bias (U2 (eQA)) is equal to zero, if an alternation of positive and negative values indicate an accuracy falsely correct, the spreading of the values to the mean (SD bias) will be high.
It corresponds to a second accuracy checking.

$$E_i = X_{lab} - X_{gp}$$

Xi: Individual measured values

$$\bar{E} = \frac{\sum (X_{lab} - X_{gp})}{N}$$

$\bar{X}$: Mean of measured values

In the SD calculation, as seen at the chapter "2.2.2 Explanations", we measure the spreading of the **individual values** (X_i) in function of the **mean of these values** ($\bar{X}$) (only internally, without comparison to the peer group).
This SD calculation corresponds to U_1 (IQC).

In the case of the SD_{bias}, we are measuring the spread of the **gaps between the laboratory means and the ones of the peer group** (E_i) in function of the **mean of these gaps** ($\bar{E}$) between these same values.
This SD calculation corresponds to U_2 (eQA).

***Note**: The greater precision of the instrument, the closer the eQA values will be to one another and smaller the* SD_{bias} will be.

2.7.3 | Interpretation

The uncertainty of measure indicates the reliability of the measuring and is used to assess if the observed variation really corresponds to a real **analytical variation** or comes from a **physiological modification**.

- If the uncertainty is small and that the observed variation is high, this would indicate that the variation comes from a physiological modification.
- In the opposite, if the uncertainty of measuring is high, we should not take a variation as a pathological variable and take inappropriate therapeutic decisions.

This uncertainty is important to be taken into account in two situations:

- Comparison of the result of a parameter to a previous one.
- Comparison of a result of a parameter to a clinical or regulatory threshold.

2.7.4 | Recommended actions

As the uncertainty of measuring is calculated taking into account the reproducibility (standard deviation) and the bias, the recommended actions to improve this indicator, are the same than the ones indicated in the case of precisions and accuracy improvements.

***Note**: As reminder, the comparison of the reproducibility corresponds to the precision, and the bias is involved in the accuracy calculation and the trueness.*

2.8 | Sigma

2.8.1 | Definition

The sigma is an **indicator of performance** which is used in the field of various activities (aviation, medical field…).
The only difference between these sigma calculations, will be the performance thresholds which are defined as authorized (**total errors**) for each measured elements.

Dr. Carmen RICOS

Dr. Ricós graduated in Pharmacy at the University of Barcelona in 1969 and earned her Doctorate in Pharmacy at the University of Barcelona in 1973. […]

Besides her normal tasks, she has been involved in establishing external quality assessment schemes organized by the Spanish Society of Clinical Biochemistry and Molecular Pathology since 1981 and has been chairwoman of the Analytical Quality Commission of this Society since 1990.

She has contributed to several European working groups concerning external quality assessment, and now participates in Technical Committee 140 (Laboratory Medicine) of the European Committee for Normalization (CEN) and in Technical Committee 212 (Clinical Laboratory and in vitro laboratory tests) of the International Standardization Organization (ISO) working group on subjects related to External Quality Assessment and Analytical quality Goals.
She is also a member of the Expert Advisory Panel on Health Laboratory Services, World Health Organization (WHO) for the period 1997-2001.

She has directed two doctoral theses, and has written and lectured on a number of works related with internal and external quality control, quality systems and quality goals. She regularly imparts classes on laboratory accreditation, in connection with the ENAC (Spanish Accreditation Body)

Official Westgard website[4]

In hematology, the only thresholds unanimously recognized for the different measured parameters, are the one **defined by RICOS for each parameter** (calculated on mean values from literature and statistics).

2.8.2 | Explanations

Source: Clinical Chemistry Journal[3]

The sigma is an indicator of performance which is comparing the **required performances** (threshold defined as authorized or total errors), to the **actual performances** of the laboratory (bias and CV) or from the **peer group** (bias and CV).

We will first study the calculations to define the required performances (or total errors), the calculation of the sigma of the laboratory, and then the calculation of the sigma of the peer group (taking into account the technology).

2.8.2.1 | Total Error (provided by RICOS)

It exists 3 levels of total errors defined by RICOS (for each parameter):

- Optimal (TE**o**)
- Acceptable (Total Error allowable, TE**a**)
- Minimal (TE**m**)

Total Error formula, TE (Ricos)

TE = Repro. X 1,65 + Bias

TE : Total Error

Repro. : Reproducibility

Bias : Based on the intra and inter individual variations

The 1,65 factor involve that 95% of the results will be under the limit of the total error, following a Gaussian distribution.

These thresholds (total errors) are dependent on two different types of variations:

- **Intra-individual** variation: variation of the parameters for a same person over a determined period (example: from the beginning to the end of the year).
- **Inter-individual** variation: variation between people (example: a smoker will have more white blood cells than a non-smoker).

Acceptable Total Error formula, TEa (Ricos)

TEa = Accept. Repro. X 1,65 + Accept. Bias

TEa : Acceptable Total Error

Accept. Repro. : Acceptable reproducibility

Accept. Bias : Acceptable Bias

Accept. Repro. = 0,5 x CVw

CVw : Intra-individual variation

Accept. Bias. = $0,25 \times (CVw^2 + CVb^2)^{1/2}$

CVw : Intra-individual variation

CVb: Inter-individual variation

The calculations of the **TEm** (Minimal Total Error) and **TEo** (Optimal Total Error), are made following the same way, only the coefficients used for the calculation of the reproducibility and bias are varying.

It means that the Total Error is **purely statistical** and which, following the inter-individual variation and the precision of the instruments currently on the market, can either be not really relevant/achievable, or in the opposite, too easy to achieve.

***Ex** : the WBC parameter has an **high** inter-individual variation (smoker, pregnant women, athletes…) and intra-individual (fasting, resting,…) which mean that the statistics indicate a Tea of 15,49%.*
*This percentage will be **too easily achievable** by the existing instruments of hematology and will not represent a relevant goal.*

***Ex** : the MCHC parameter has an inter-individual (smoker, pregnant women, athletes…) and intra-individual (fasting, resting,…) **extremely low**, which involve a Tea of 1,27%.*
*This percentage will **very hard to achieve** by existing hematology instruments and also does not represent a relevant goal*

2.8.2.2 | Sigma of the laboratory

Sigma calculation formula (by Ricos)

$$Sigma_{lab} = \frac{(TEa\ \% - Bias\ \%)}{CV\ \%}$$

TEa : Total Error allowable *(provided by Ricos for each parameter)*

Bias : Lab Mean – Peer Group Mean

CV : Coefficient of variation of the Laboratory

In this calculation, the laboratory will be able to compare his performances (his CV and his bias) with the statistical ones, coming from the art of state done by RICOS.

2.8.2.3 | Sigma of the peer group

The 6 sigma is equivalent to 6 standard deviations of the laboratory sigma and is corresponding to the best reachable performances. This indicator represents the performances to reach, but only from a **physiological and statistical** point a view, and not from a technological one (it's not taking into account the constraints and limits of the already on the market instruments).

To solve this, HORIBA Medical also provides a **sigma objective** which allows the determination of the performance of the peer group and which therefore becomes the objective to be reached, because it takes into account the technological specificities.

Sigma formula (by HORIBA)

$$Sigma_{Obj} = \frac{(TEa\ \% - Bias\ \%)}{0{,}5 \times CV\ \%}$$

TEa: Total Error allowable *(provided by RICOS).*

Bias: Mean of the peer group – Mean of the peer group = 0.

CV: Coefficient of variation of the peer group.

Knowing that this sigma objective corresponds to the sigma of the peer group, for the bias we are comparing the difference between the mean of the peer group and the reference value (defined by the peer group mean).

The difference is therefore equal to zero and corresponds to a lack of bias.

The CV is multiplied by 0,5 (so divided by 2 to select a more restrictive CV).
This corresponds to taking into account only the best laboratories of the peer group, and therefore the ones which represent the real and best performance of the instruments (objective to achieve with the instruments).

2.8.3 | Interpretation

The greater the variations between the measures in a laboratory (bad precision/reproducibility), the greater the CV of the laboratory.
In this case, the ratio TE/CV will be low, and the performance of the laboratory considered as poor.

2.8.4 | Recommended actions

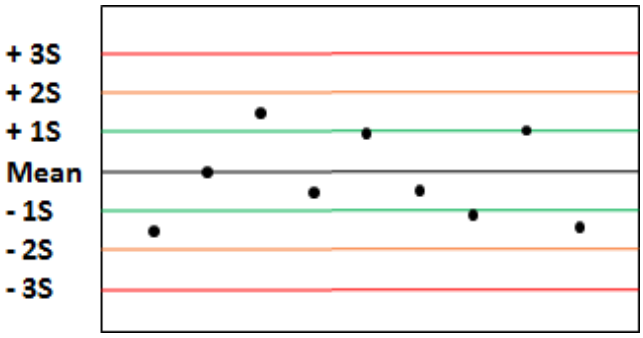
As the sigma is calculated taking into account the precision (CV) and the accuracy (bias), the recommended actions to improve this indicator are the same as the ones indicated in the case of precision and accuracy improvements.

3 | LEVEY-JENNINGS AND WESTGARD RULES

3.1 | Levey-Jennings

3.1.1 | Definition

The Levey-Jennings chart allows the laboratory to visualize if the results of their analysis are reliable and of good quality.
It is by means of a chart representation of the results dispersion from the mean, expressed in standard deviation (SD).



3.2 | Westgard Rules

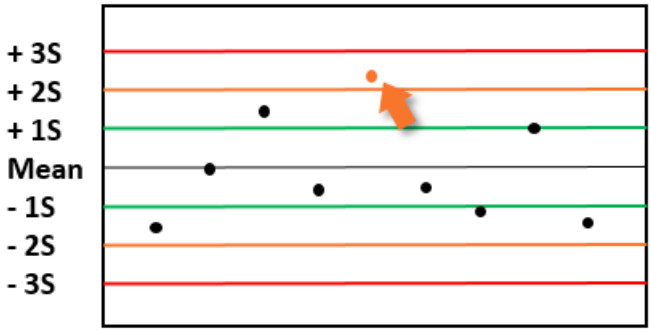
3.2.1 | Definition

These rules are calculated to define if a measured value is considered as acceptable or not.
They give the objective means to technically validate a series.
The results are not corrects if a specific number of measured values are above a specific limit (distance to the mean).

3.2.2 | Nomenclature

By convention, we are writing the rules as the following: A_L (or A:L).
- "**A**" represents the number of measured values which have to be beyond of the limits to reject the results.
- "**L**" represents the specific limit (indicated in number of standard deviation, SD).

Example :
1_{2S} Rule: the results are rejected if there is ONE measured value beyond of TWO standard deviations (2SD).



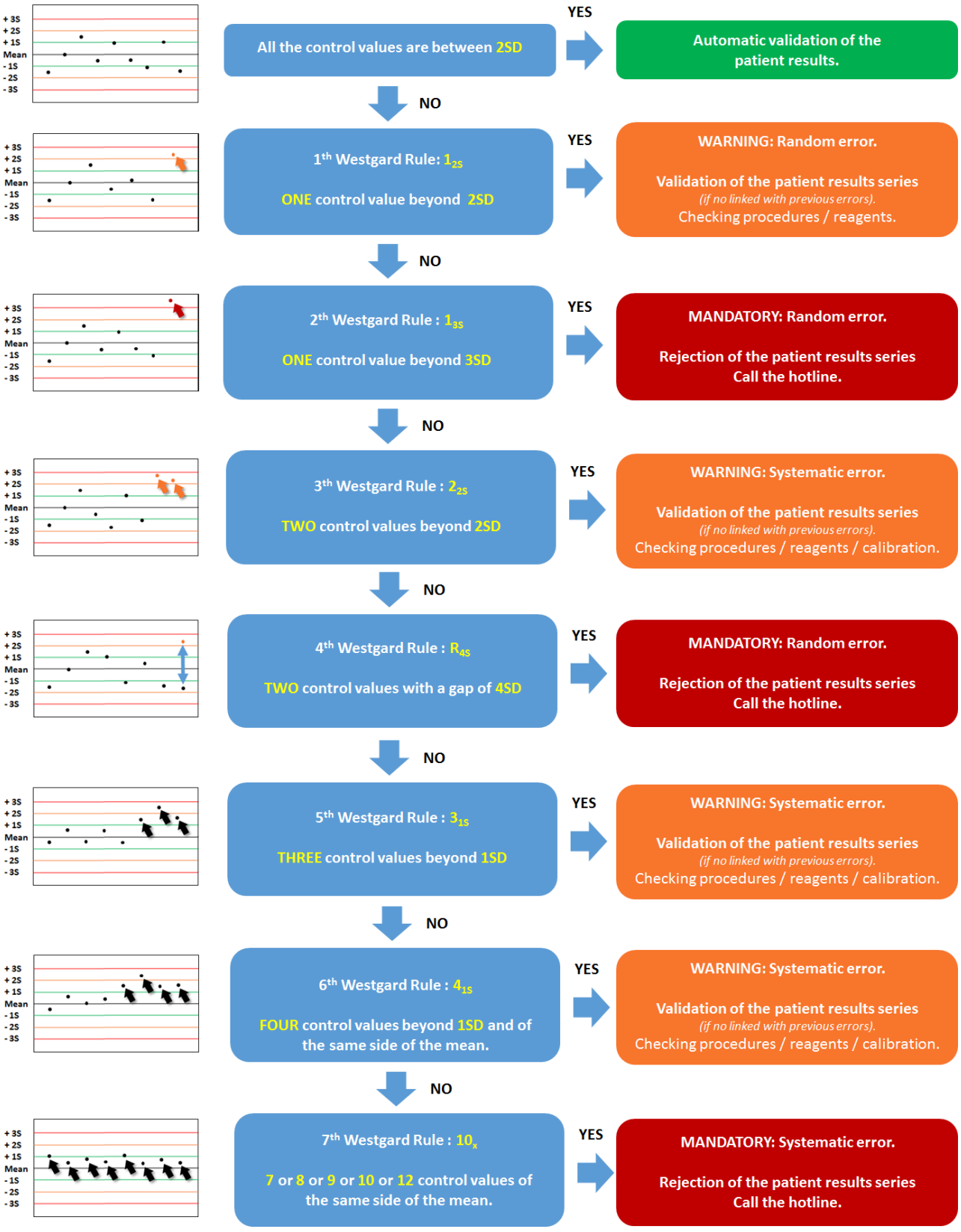
We can split the different Westgard rules into two groups:

- **Warning** rules: 1_{2S}, 2_{2S}, 3_{1S}, 4_{1S}
These rules lead to a review of the analysis procedures, reagents and calibration.

- The **mandatory** rules: 1_{3S}, R_{4S}, 10_X
These rules result in a rejection of the patient result series (from the previous correct quality control).

3.2.2 | Logigram

To know if a series can be validated, it needs to apply the following rules to the control samples results:



***Notes**:*

- *the choice of validation or rejection of a series of results is under the responsibility of the laboratory (according to its quality control policy). The information provided in this document is for guidance only.*
- *for definitions of systematic and random errors, refer to Chapter 4 "Definitions".*

3.2.3 | Recommended actions

If the study of the detailed Levey-Jennings charts allows to show a systematic or random error, it is recommended to put in place corrective actions (please refer to the recommended actions indicated for the improvements of precision and accuracy).

4 | DEFINITIONS

4.1 | Repeatability

The repeatability is obtained by multiple consecutive runs of a same sample, keeping the **same conditions** at every run (operator, time, reagents batches, calibration, etc…).

The repeatability is used to check that the instrument is ready to work correctly.

The repeatability has to be made at:

1. The installation
2. Before a calibration
3. Before and after a significant maintenance action (changing of spare parts, etc…).

It will allow the operator to determine the minimum CV is achieved (optimal reproducibility), and then to quickly check the performances of the method before a calibration.

***Note**: a repeatability is generally done on a patient sample.*

4.2 | Reproducibility

The reproducibility is obtained by the analysis of a same sample in the normal conditions of use **varying** the factors as: operator, time, reagent batches, calibration, etc…).

***Note**: in the hematology field, according to the low stability of the blood parameters over time, a reproducibility is generally done on a control sample.*

4.3 | True value

The true value is a complicated value to achieve in hematology and there is no international standard that can specify this value for the different parameters.

Values as close as possible to the true value are those measured by the reference methods:

- **microscopy with Neubauer, Malassez or Thomas cell** for counting white blood cells (WBC), red blood cells (RBC) and platelets (PLA); selective lysis for WBC and PLA.
- **photometry** for the measurement of hemoglobin (HB); cyanmethemoglobin complex method and lysis with cyanide.
- **measurement of the volume of red blood cells** in relation to the total volume of blood after centrifugation for the measurement of hematocrit (HT).
- **microscopy with Romanowsky staining** (Giemsa, Wright, MGG,...) of a blood smear for leukocyte count.
- **microscopy with supra-vital staining** (B azide, blue methylene, bright blue cresyl) for counting Reticulocytes.

***Note**: although microscopy can be considered as a reference method, it has a precision limit related to the number of counted cells. This inaccuracy is all the more important when the number of cells counted is low, which creates a bias in the comparison of this count with the so-called "true" (or assimilated to) measure. The relationship between imprecision and the number of counted cells is summarized in the "Rumke table".*

4.4 | Target value

Reference values are the values to which the results will be compared without being considered as true values:

- The **manufacturer's** target value corresponds to the target value provided with the control samples.
- The laboratory target value is the mean of the laboratory results for a parameter.
- The value corresponding to the mean of the peer group's results for a parameter.

Notes:

- *The value of the peer group can be assimilated to the true value in the absence of a value measured from a reference method.*
- *The target value is the value to be achieved for each parameter and control sample level.*
- *Each target value is provided with confidence ranges in which 95% of the results of the control samples must be contained.*
- *The median can be considered as equivalent to the mean for a significant number of values.*

4.5 | Systematic errors

A systematic error is detected as soon as there is a change of the control values mean. This change in the mean can be progressive and appears as a **drift** or it can be sudden and appears as a real **shift**

- **Drift**: it appears as soon as there is a progressive and subtle (positive or negative) change of the control mean.
  A drift indicates a progressive loss of reliability in the analytical system.

- **Shift**: it appears as soon as there is a sudden change (positive or negative) in the control mean.
  This indicates an important variation of the analytical performances.

Systematic error creates a **bias of trueness or accuracy** that can be corrected by a calibration of the instrument.

4.6 | Random errors

Error that varies unpredictably, positive or negative from the calculated mean. It will be acceptable or not depending on the defined value of the standard deviation and of the applied Westgard rules (example: a data outside the range ± 3SD).

The random error is related to the precision of the instrument and it is difficult to make it completely disappear.

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