

Verifying the reportable range of an analytical method in clinical chemistry

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Verifying the reportable range is not a unique action which is performed by reagent makers or clinical chemists when they start a new reagent. It is a recurrent action necessary to troubleshoot out-of-control situations or bad EQA returns. So verifying the reportable range is an integral part of quality control. The quality control software *MultiQC* (www.multiqc.com) includes an original linearity module to calculate the non-linearity error of all the analytes which it controls. A demo of 33 slides is available at :

- www.multiqc.com/ReportableRange1.htm (Shockwave Flash Demo)
- www.multiqc.com/ReportableRange1.exe (Off-line executable file)

MultiQC does not use the protocol EP6-A by the CLSI / NCCLS [2]. This protocol is not well fit to the needs of clinical chemistry laboratories because it is not based on the very definition of the reportable range. The error of its editors was to think primarily in terms of linearity instead of terms of allowed error. Straight line or not straight line ? That is not the question. Perfect linearity does not exist. Non-linearity depends on the energy we are ready to apply for its demonstration.

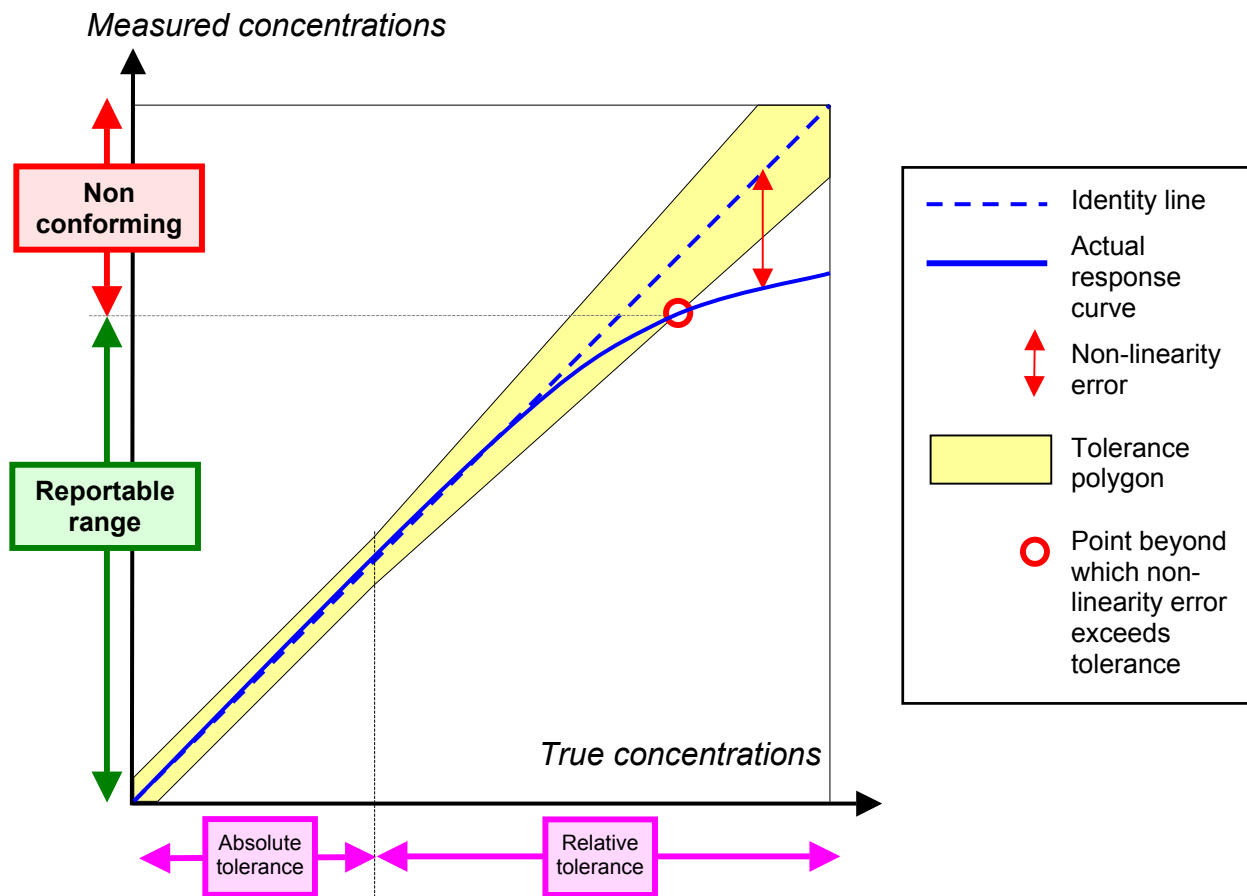
The true subject to deal with is to decide whether the departure of the actual response curve of an analytical method from the ideal straight line is acceptable or not. Stepwise polynomial regression is recommended by the EP6-A protocol. It is an efficient statistical tool to find out a significant non-linearity but verifying linearity is unimportant in a clinical laboratory. What we do need is to establish a reportable range for a given shape of response curve and for the medically allowed tolerance of the analyte.

1. Response curve and tolerance polygon

The *response curve* of an analytical method is a plot of the measured concentration as a function of the true analyte concentration. The ideal response should be the identity “*measured concentration = true concentration*”, whichever these concentrations might be. Graphically, the ideal response curve or identity line is the bisecting line in a plot of measured-versus-true concentrations.

A significant departure from this perfect agreement is nevertheless acceptable because of the error medically tolerated for the analyte. The vertical distance between the identity line and the actual response curve is named *non-linearity error*. It must not exceed the medically allowed *tolerance* of the analyte. This tolerance may be expressed as an absolute or as a relative acceptable error. Most often in clinical chemistry, both are associated so that the absolute error applies to the lower concentrations and the relative error applies to the higher concentrations.

Graphically, the tolerance intervals for each concentration are merged into a polygonal area framing the identity line. The top and bottom edges of this polygon are parallel for an absolute tolerance. The edges are diverging rightwards for a relative tolerance. The polygon may be more complex when setting up different tolerance values for low, mid and high concentrations of the analyte as it is possible in *MultiQC*.



2. Reportable range

A curved response line may partially meet the tolerance of an analytical method provided that the interval of measured concentrations is adequately limited. The *reportable range* is the range of concentrations within which non-linearity error is smaller than the tolerance. Analytical results inside this range are conforming and can be reported. The other ones must be discarded and the sample reprocessed.

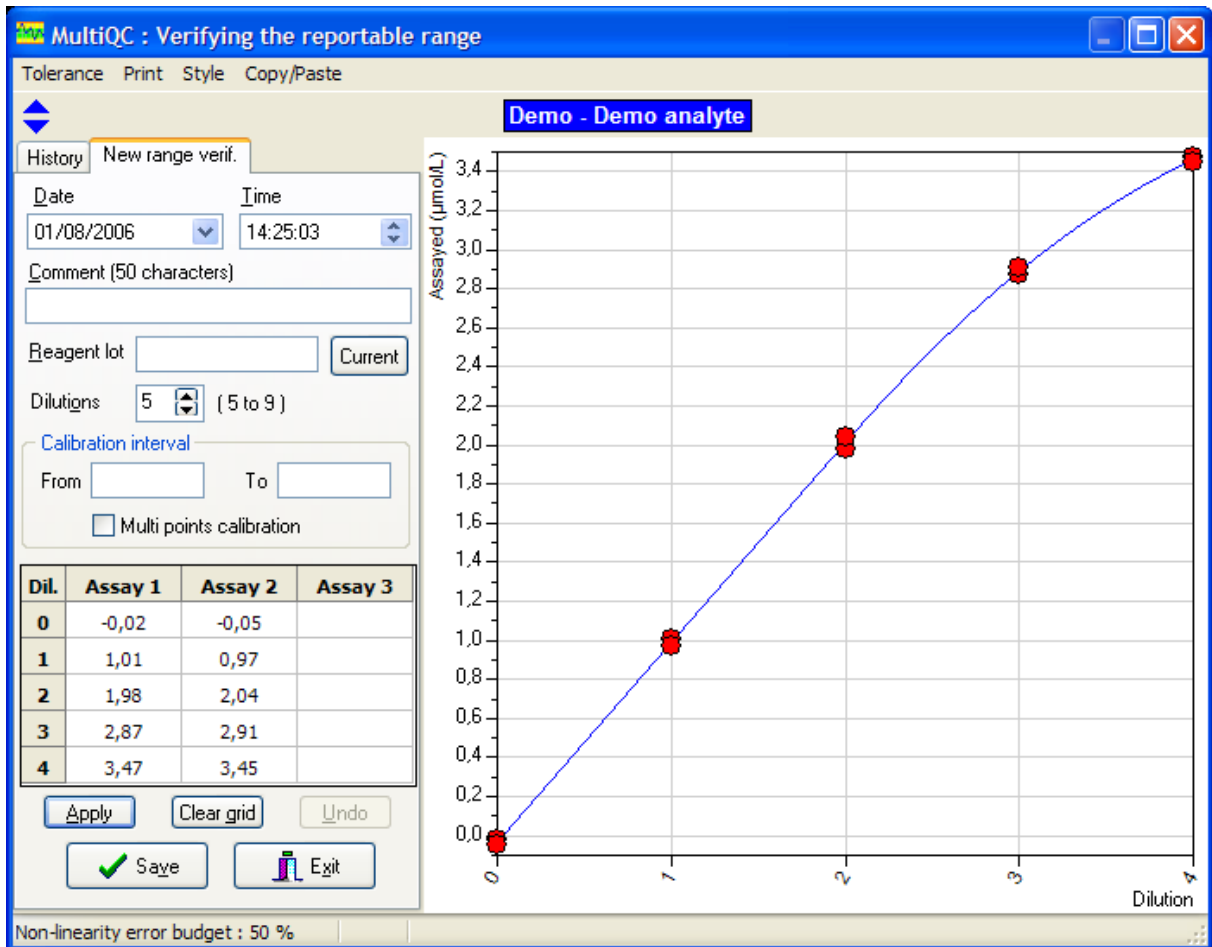
The reportable range of an analytical method is established searching for the segment of the actual response curve which is interior to the tolerance polygon and then projecting it onto the ordinate axis. Why not project onto the X axis? Because of the practical use of the reportable range. Technicians will compare each concentration measured by their instruments to the reportable range to decide whether the assayed values are conforming or not. It is the reason why the reportable range must be also expressed in terms of measured concentrations (ordinates) and not in terms of true concentrations (abscissas).

Practically we have to search for the intersections of the response curve of the method with the top and bottom edges of the tolerance polygon. The number of intersection points depends both upon the shape of the response curve and upon its position relative to the axes of the plot. The former is linked to the principle of the analytical method. The latter is linked to the calibration of the method. Our first step will focus only on the shape.

3. Determining the shape of the actual response curve

Obtain 5 or 9 levels of concentration over a range that is a bit wider than the anticipated reportable range and with equally spaced concentrations. See below how to make intermediate dilutions of two pools through sequential mixing. The exact concentration of each linearity material may be ignored but the dilution ratios between successive samples must be very accurate. Run one, two or three replicate samples according to the degree of precision that you need for the response curve that is going to be estimated and enter the results in the linearity module of *MultiQC*.

MultiQC searches its library of mathematical functions for the one which best fits the experimental points. This function is adopted as the estimated response curve of the analytical method and the relevant curve is drawn. At this step, we have found a shape but the response curve remains uncompleted because the X axis is yet graduated with the number of the dilutions instead of the true concentrations.



4. Relating assayed concentrations to true concentrations

A faulty calibration might shift the response curve out of the tolerance area and alter the reportable range. But this is another problem. We must pull apart non-linearity error and inaccuracy supposing that the method was precisely calibrated before assaying the set of samples with equally spaced concentration.

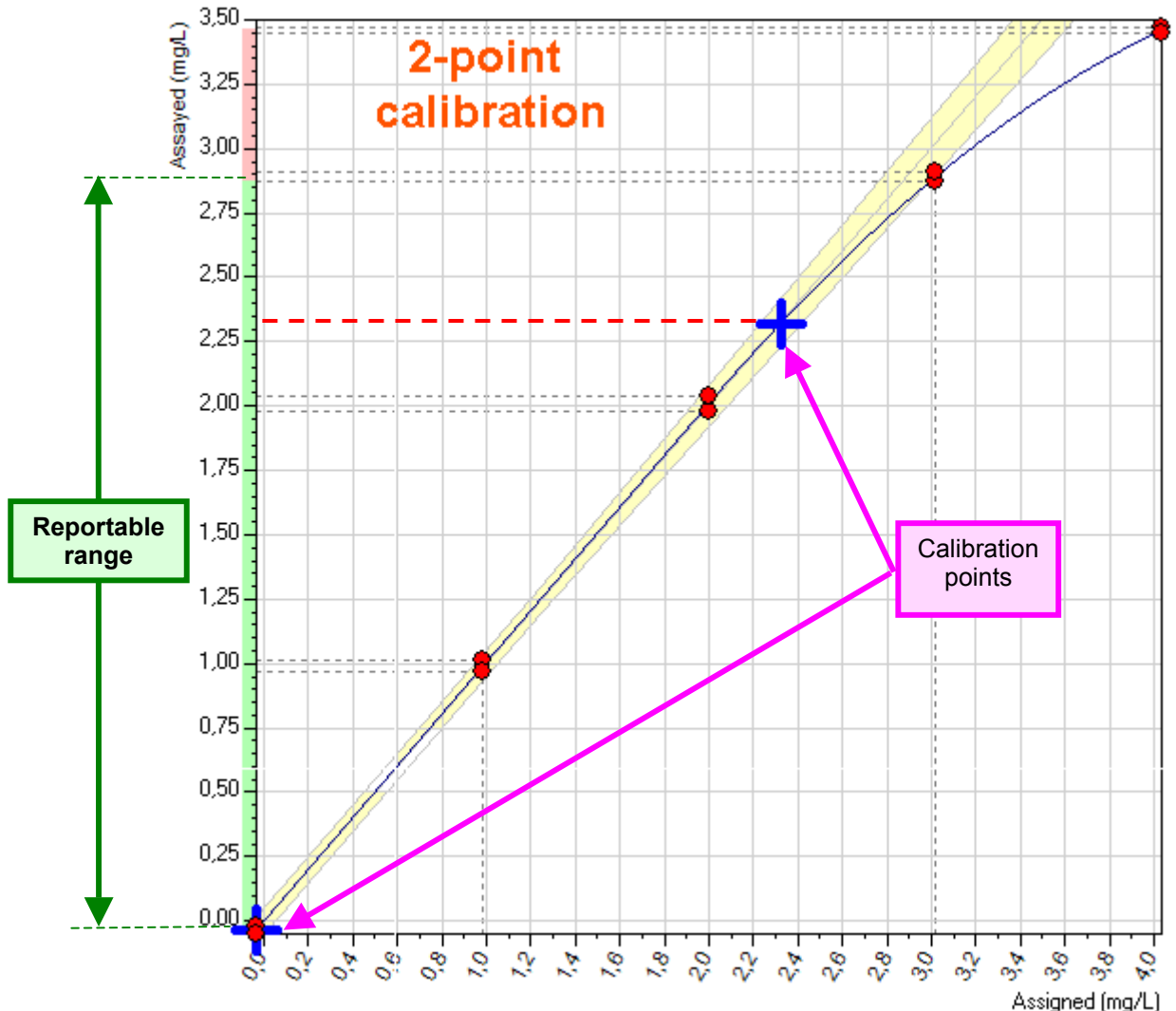
➤ 2-point calibration

Let us assume that the instrument is in-control and that it was calibrated in 2 points: 0 g/l and 2.34 g/l (picture below). This means that, by definition and ignoring the uncertainty of calibration, the actual response curve is true for these two concentrations 0 and 2.34 g/l. Hence we know two points of the identity line and consequently can draw it on the plot.

Enter 0 and 2.34 in the entry fields *Calibration Interval From and To* or move the mouse cursor over the plot and drop two calibration marks (blue crosses) at the ordinates 0 and 2.34. As soon as the two calibration points are set, *MultiQC* updates the plot:

- It draws the identity line that crosses the two calibration marks.
- It draws the tolerance polygon framing the identity line with tolerance intervals.
- It graduates the X axis with a scale of assigned values replacing the scale of dilutions.

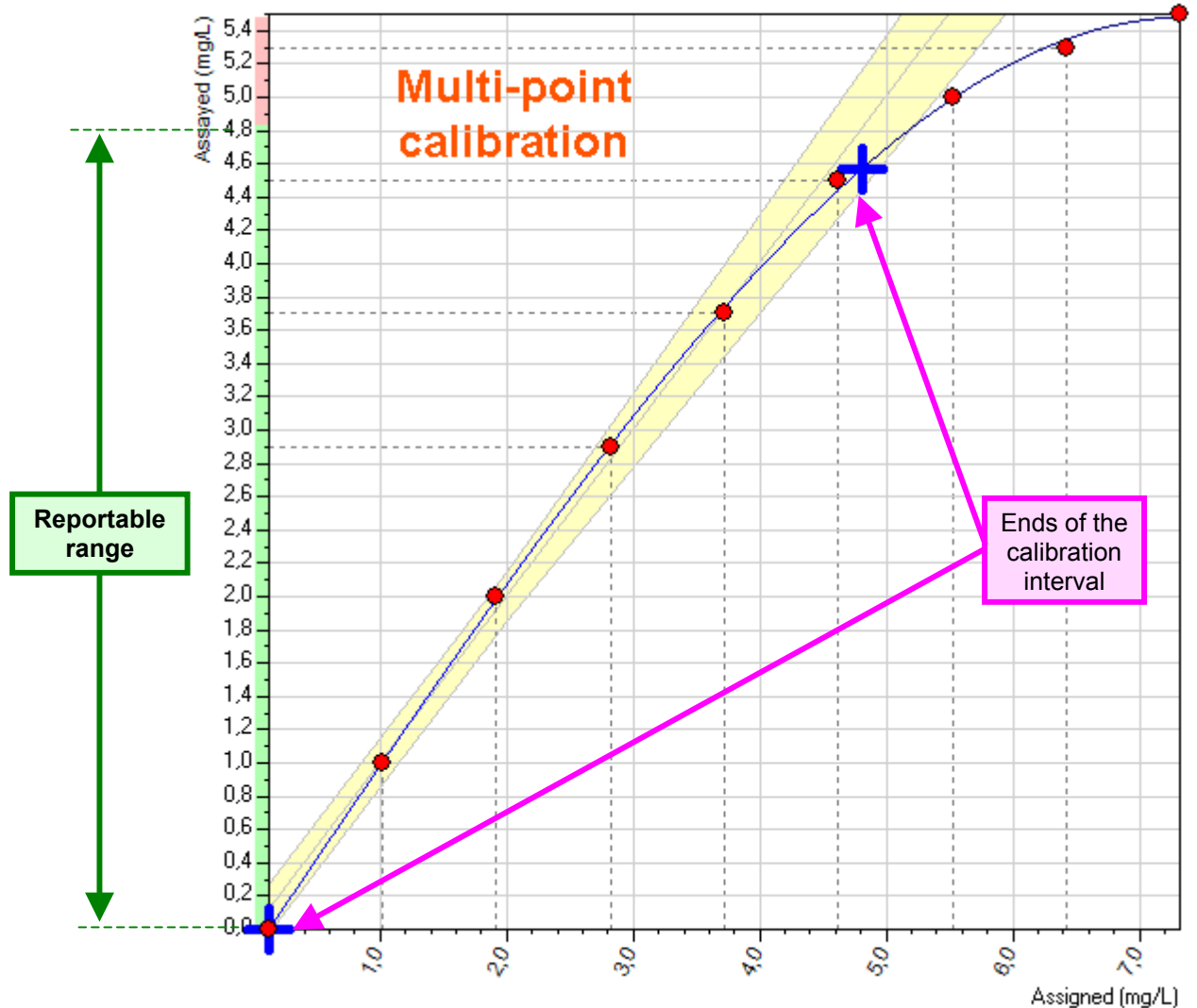
- It searches for the segment of the actual response curve interior to the tolerance polygon and project it onto the Y-axis to graphically show the reportable range as a green background behind the axe.
- It displays the numerical value of the reportable range in the bottom status bar of the Linearity window.



➤ Multi-point calibration

Let us now assume that the instrument was calibrated in 6 points. This would mean, as above, that the actual response curve is true for these six concentrations. Practically because of the calibration uncertainty these six points are never perfectly aligned. In this case you must check the box [Multi point calibration](#) and drop two calibration marks on the curve at the two ends of the calibration interval. So *MultiQC* calculates the identity line as the regression line of all the points of the response curve between the two calibration marks.

The response curve is drawn blue between the two calibration marks to remind that the position of the identity line is based on the whole interval and not only on its two ends.

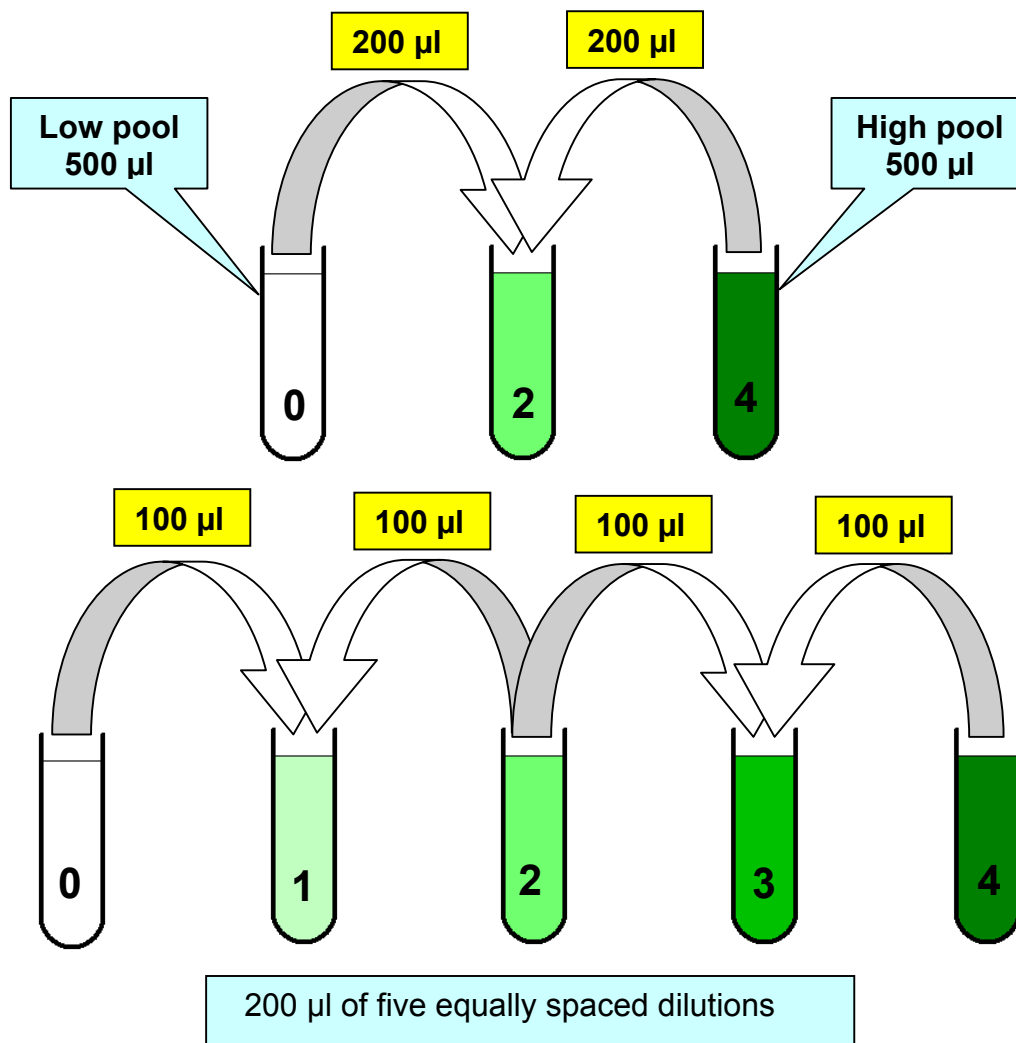


5. Tolerance for non-linearity error

Tolerance for non-linearity error is based on the overall tolerance of each analytical method which is recorded in *MultiQC* to create acceptance charts and to calculate the capability indexes. Non-linearity is a component of total error, but not the only component. So tolerance for non-linearity error must be smaller than the overall tolerance to take into account the other causes of error (imprecision, bias, interferences...). *MultiQC* makes use of a reduction factor named **Non-linearity error budget**. Its default value is 50%. This means that if the overall tolerance for serum glucose is 4%, the reportable range will be the range where non-linearity error does not exceed 2%. *MultiQC* can associate a relative and an absolute tolerance in three different intervals. This may lead to complex tolerance areas and discontinuous reportable ranges.

6. Preparation of samples with equally spaced concentrations

The most precise way to mix low and high concentrations pools to produce samples with equally spaced intermediate concentrations is sequential mixing [3]. A middle pool is obtained by mixing equal volumes of the low and high pools. Then the middle pool is mixed with the high and the low pool, in equal volumes, to produce a mid-high and a mid-low pool. Thus a set of five equally spaced concentrations is made up. A set of nine equally spaced concentrations can be easily prepared mixing again the adjoining samples of the set of five concentrations.



The volumes in the above dilution scheme should not be reduced to keep a good precision. Conversely, it is highly recommended to work with higher volumes if enough pool is available. Increasing the volumes is also required for the preparation of a set of 9 equally spaced concentrations which requires a third dilution step.

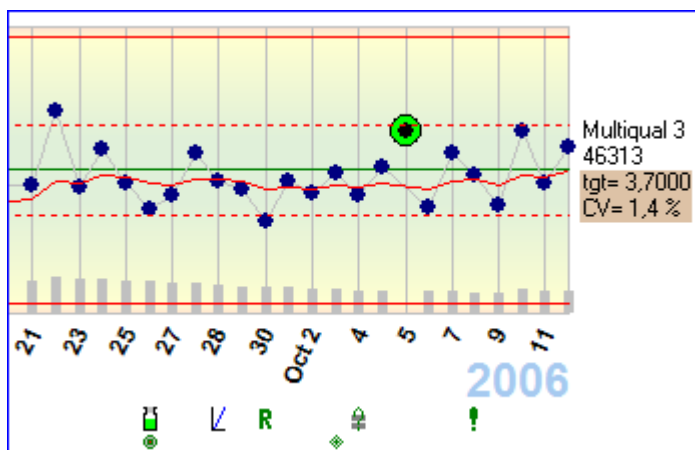
Sequential mixing is very fast, accurate and precise even with volumes as small as 100 µl. Errors might come from the nature of pools materials which generally have a high viscosity and a tendency to foam easily. These issues are overcome by using the reverse pipetting technique to dispense materials and by a careful mixing of tubes. A bias in the calibration of pipettes is not harmful because of the principle of mixing equal volumes from the same pipette.

Saline or even water can be taken as low pool with a nil concentration in many cases. The objection of matrix effect seems to be often overemphasized. A high pool should be easily found among the highest daily samples of the laboratory that need to be re-processed after having been diluted. Considering the accuracy of in-house sequential mixing by trained operators, purchasing commercial linearity verifiers often appears as a waste of money which is not balanced by more reliable samples.

7. Linearity and quality control

The quality control software *MultiQC* (www.multiqc.com) includes a module to verify the reportable range of the analytes that it controls. Data is archived among the particular events of the relevant analyte. These events also include calibrations, changes of reagent lots, method comparisons and repeatability tests.

Analytical events can be shown at any time by clicking the relevant icon in the *events bar* located under the QC charts. So everyone can easily access the information needed to troubleshoot an out-of-control situation of a bad EQA return.



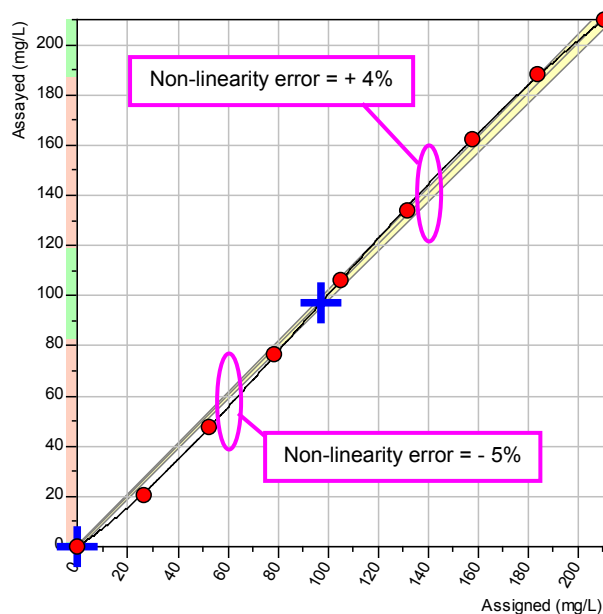
Medically acceptable error is a basic figure whose knowledge is essential to a cost-effective management of quality in a laboratory. MultiQC maintains a table of medical tolerance intervals for every analyte that it controls. Sophisticated tolerance schemes are possible with separately defined relative and/or absolute errors for low, mid and high concentrations. The tolerance table is shared by QC, reportable range verifications, method comparisons, repeatability testings and capability analyses.

8. Case study 1

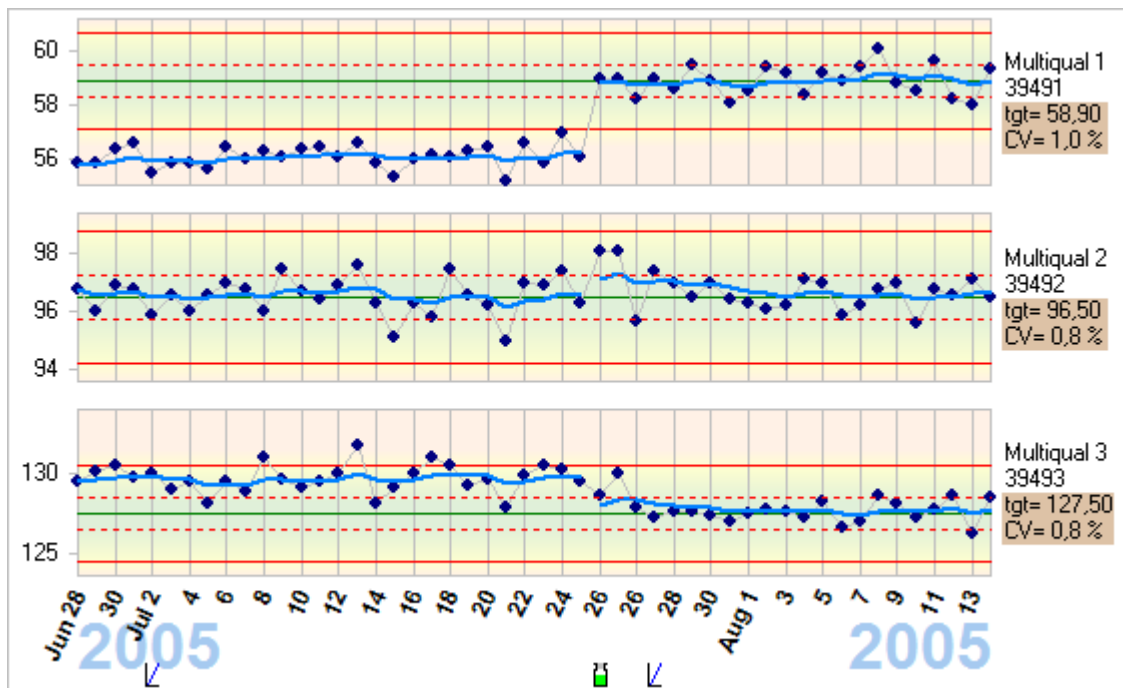
Calcium arsenazo reagent response curve is generally slightly S-shaped. The reagent lot 2454 by Olympus-Ireland was particularly bad in this regard. The linearity plot on the right shows a discontinuous reportable range of [87 - 130] + [174 - 210] mg/l. A large segment of the response curve is outside of the tolerance area.

The tolerance polygon is a thin strip. It is built on the basis of a maximum non-linearity error of 2% (the overall tolerance for the analyte is 4% and the non-linearity error budget is 50%).

Calibration was performed according to the reagent maker (2-point calibration: 0 and 97 mg/l). The two blue crosses are placed on the two calibration points of the observed response curve.

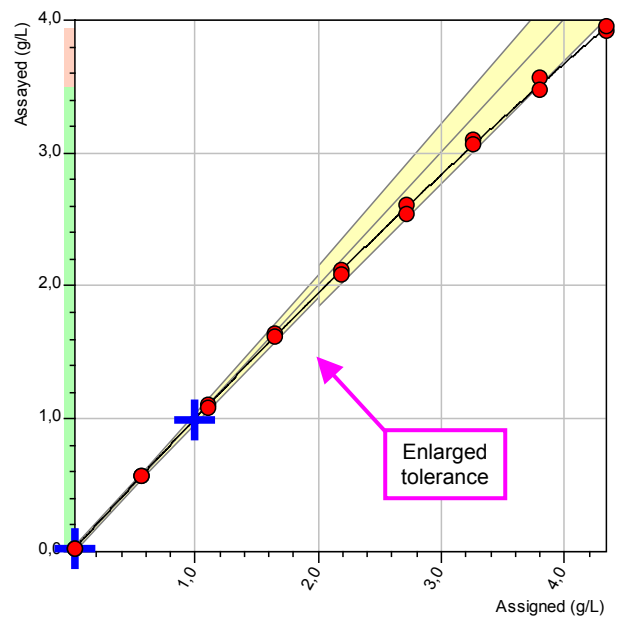


The next reagent lot had a less marked S-shaped response curve. This immediately appeared in the QC plot as a shift in the low and high levels. The mid level was not moved because very near to the calibration point.



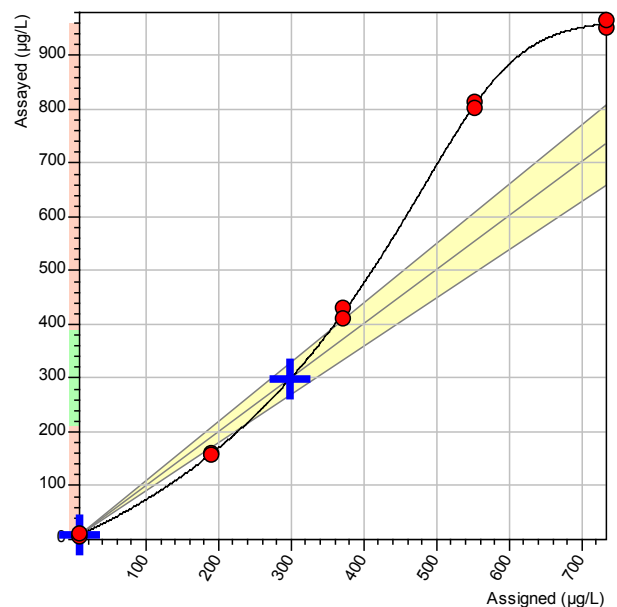
9. Case study 2

Blood ethanol assay: The reagent maker specifies linearity up to 3.5 g/l. Such an assertion is meaningless if no error specification goes with it. It is a lie if the allowed tolerance is 8%. It becomes the truth if the tolerance is enlarged to 15% for concentrations higher than 2 g/l.



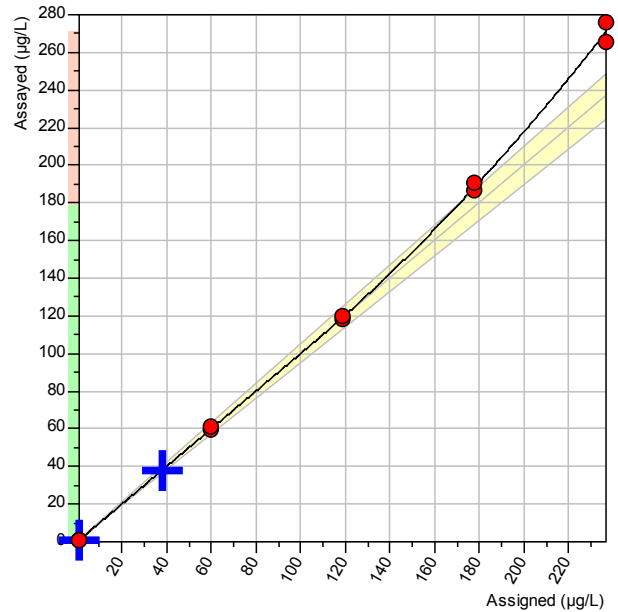
10. Case study 3

Urinary opiates assay (Olympus β -galactosidase reagent): Using a 2-point calibration scheme leads to a very narrow reportable range. This is quite acceptable for a qualitative method. Two positive and negative control materials are provided with the reagent. They have assigned concentrations of 225 and 375 $\mu\text{g/l}$. The response curve shows that they will have average assayed values of 200 and 440 $\mu\text{g/l}$.



11. Case study 4

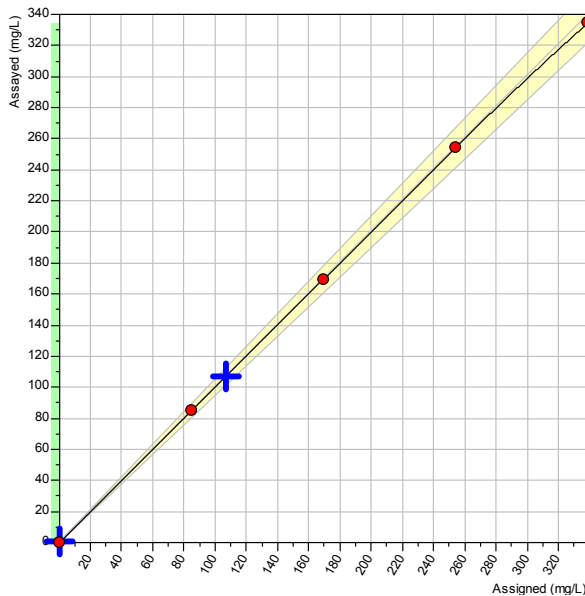
CK-MB assay (Bayer ACS 180 reagent). Calibration is based on a master curve provided by the reagent maker and a 2-point on-site calibration. The master curve defines the shape of the calibration curve which is supposed to be same for all the analyzers working with a given lot of reagent. The 2-point on-site calibration customizes the master curve for the actual analyzer. It also compensates to reagent aging. The reportable range is specified by Bayer as [0 – 300 µg/l]. With a tolerance of 10% and a non-linearity error budget of 50%, the estimated reportable range is much narrower: [0 – 180 µg/l]. The curved response line shows that the master curve is not appropriate for the local conditions.



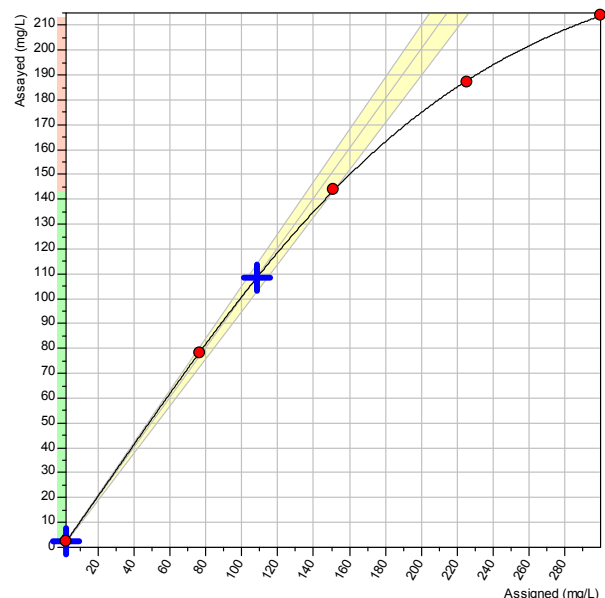
12. Case study 5

Assay of serum acetaminophen/paracetamol (Bayer reagents). Contact of reagents with atmospheric air progressively changes linearity. The reportable range goes beyond 200 mg/l with fresh reagent taken from closed bottles (left picture). After one week in contact with air, bottles open on the reagent tray, the reportable range is reduced to 145 mg/l (right picture).

Fresh reagents



Reagents in contact with air for a week



13. Discussion

The power of *MultiQC* linearity module is based on a library of mathematical functions which can modelize all the analytical response curves which are met in clinical chemistry. The author would be very pleased to receive linearity data, if any, which would not be correctly modeled by *MultiQC*.

14. References

[1] Marquis P. MultiQC, Quality Control Software for Clinical Chemistry Laboratories.
www.multiqc.com

[2] Document EP06-A. Evaluation of the linearity of quantitative measurement procedures: a statistical approach; approved guideline. www.nccls.org

[3] Vaks JE. Preparation of samples with equally spaced concentrations through mixing. Clin Chem 1996; 42: 1074-8