After confirming that an assay is in control, it may be possible to resume sample analysis. However, attention must be given to any sample analysis that may be affected by the initial IQC fail (ISO1589:2012 standard 5.6.2.3; Technical Committee ISO/TC 212, 2012), which requires that when rules are violated, where patient samples are likely to contain clinically significant errors, a procedure should re-examine relevant patient samples, once error correction and performance has been verified. This includes re-analysing patient samples after the last successful quality control event. Although the literature agrees about the need for such a procedure (Westgard et al., 2006; Kinns et al., 2013), practical guidance and recommendation therein is lacking, as detailed in the following section.

Appropriate responses to rule violations – part 2

Re-analysing samples related to rule violations

Considering the need for re-analysis. Irrespective of whether or not results have been reported to the user, a decision may be taken to re-analyse either all samples or a selection (Figure 1, IQC Flow Diagram 2). This decision depends (in part) on whether all results from the first analysis are erroneous, irrespective of concentration or analysis time. We propose pragmatically that repeat analysis can be avoided when a single IQC level fail has occurred for either the high or low-level IQC but all analysed samples have analyte concentrations that are in each respective situation either less than or greater than the intermediate IQC level. However, for failures at either an intermediate level or at more than one level, repeat analysis is unavoidable.

Identifying affected samples: subjective or objective approach?

Where repeat analysis is required, the time from the last successful IQC up to the rule violation must be considered, to identify when the assay lost control; i.e., the failure point (Figure 1, IQC Flow Diagram 2). By also establishing the concentration range potentially affected by the rule violation, re-analysing only relevant samples can be achieved. For a level one or three IQC fail, samples should be selected and analysed mainly with analyte concentrations below and above each level, respectively, but also towards level two. For a level two IQC fail, or fail at two or all levels, samples at all analyte concentrations should be selected initially for analysis. In our experience with large automated clinical biochemistry and diagnostic endocrinology laboratories, and particularly when rule violations occur for high volume tests; e.g., electrolytes, such a pragmatic approach is necessary to help retain service continuity. Following repeat analysis, analysts could subjectively evaluate any differences between paired measurements; however, this approach would likely lead to variation between analysts, especially when we consider staff grade and experience known to be involved in reviewing IQC (Housley et al., 2008). Such eye balling may at best only serve as an initial guide to identifying the failure point. Instead, we recommend comparing first and second analysis against the critical difference (CD per cent), to objectively identify the failure point. The CD's application is exemplified in Table I. Defining CD. CD (per cent) is usually defined by a formula that incorporates both analytical (CV_a) and within-individual biological coefficient of variation (CV_w) (Fraser, 2012).

$$CD = \sqrt{2} \times Z \times \sqrt{CV_a^2 + CV_w^2}, \text{ where } Z \text{ (total SDs)} = 1.65$$

(for a 95% probability, one sided)

Perhaps a more familiar use for this formula is for evaluating the observed difference's significance in serial results, where variation sources are considered. However, for defining the CD, since only one sample is being used for evaluating paired measurements, the CV_b is not applicable. A truncated CD version, based only on CV_a is therefore calculated. Intra-assay imprecision (IQC) data, reflecting minimal changes to the analytical system, may be a more relevant CV_a estimate, particularly when analysis and re-analysis have occurred within the same analytical run (e.g. day or batch). The CV_a should also represent the reportable range, using data from all available IQC levels, particularly where rule violations have occurred at more than one IQC level and imprecision varies considerably with analyte concentration. In this way, analyte sample concentration may be evaluated against the most relevant CD. The main assumption when applying CD in the current context is that sample storage has had negligible effect on analyte stability in the time between first and second analysis and that the difference between paired results is attributable (mainly) to change in assay performance, following corrective action. For some analytes; e.g., bicarbonate, comparisons may be confounded by the analyte's inherent stability in certain storage conditions, affecting repeat analysis (Oddoze et al., 2012).Analysts would be advised to refer to analyte stability studies where available; e.g.,published data, kit inserts, internal verification studies, etc.

Applying the CD. Identifying (and refining) failure point and affected concentration range. A failure point is identified when the difference (per cent) between paired measurements is greater than the CD (Table I). The CD can also be used to refine this Time point and to ascertain more accurately the affected concentration range. For a level one IQC failure, samples with concentrations between IQC levels one and two may be unaffected; i.e.,

per cent difference (CD, thereby directing subsequent consideration only to those samples with concentrations less than or equal to the level one IQC.

Re-analysis and evaluation

Relevant samples, as defined above, from the refined failure point up to the IQC failure and beyond, should be re-analysed and differences between repeat and initial analysis again evaluated against the CD (per cent). When differences exceed the CD (per cent) and where paired results are either clinically different; e.g., by evaluation against quoted reference ranges or decision limits, and/or show opposing trends to a patient's previous results, amended reports are essential. All amended reports should be communicated promptly to the clinical user.