Quality assurance (QA) is an overall term that may be used to describe all measures taken to ensure the reliability of laboratory testing and reporting. This includes the choice of test, the collection of a valid sample from the patient, analysis of the specimen and the recording of results in a timely and accurate manner, through to interpretation of the results, where appropriate, and communication of these results to the referring clinicians.

Internal quality control (IQC) and external quality assessment (EQA) (sometimes referred to as proficiency testing) are two distinct, yet complementary, components of a laboratory quality assurance program. IQC is used to establish whether a series of techniques and procedures are performing consistently over a period of time. It is therefore deployed to ensure day-to-day laboratory consistency. EQA is used to identify the degree of agreement between one laboratory’s results and those obtained by other centres.

In large EQA schemes, retrospective analysis of results obtained by participating laboratories permits the identification, not only of poor individual laboratory performance, but also of reagents and methods that produce unreliable or misleading results.

The primary function of EQA is proficiency testing of individual laboratory testing. The World Federation of Hemophilia International EQA scheme includes analyses of particular relevance to the diagnosis and management of bleeding disorders (for further information, contact the WFH). Data from this scheme have been published in the following references:


Larger EQA schemes can provide information concerning the relative performance of analytical procedures, including the method principle, reagents, and instruments. Continued participation in EQA schemes has been linked to improved laboratory performance. This has been seen not only in the overall performance, evidenced by a reduction of the variability of results between laboratories, but also in respect of individual laboratories.

The assessment of individual laboratory performance is an essential component of EQA schemes. The WFH EQA scheme compares participants’ results with the results on the same samples when analysed in up to 700 centres from around the world who participate in the U.K. National External Quality Assessment Scheme (UK NEQAS) for Blood Coagulation.

There are many reasons why a laboratory might produce results that are considered unsatisfactory. While the cause for this might be immediately apparent, the identification of the underlying problem is not always simple. Larger schemes are able to identify performance problems that relate specifically to reagent differences or differences of methodology.

Total confidentiality is an important feature of all EQA schemes. In the International EQA referred to above, information regarding individual laboratory performance is divulged to anyone other than the nominated head of the department only with written authorization.

**INTERNAL QUALITY CONTROL**

Internal quality control is used to establish whether a series of techniques and procedures are performing consistently over a period of time. The expression “quality control” is commonly used to describe the set of procedures used to check that the results of laboratory investigations are reliable enough to be released to assist clinical decision making, monitoring of therapy, and diagnosis of hemostatic abnormalities. Quality control procedures should be applied in a way that ensures immediate and constant control of result generation.
Within a laboratory setting, the quality of results obtained is influenced by many factors, including:

- appropriate sample collection and handling
- selection of suitable techniques and maintenance of an up-to-date manual of standard operational procedures
- use of reliable reagents and reference materials
- selection of suitable automation and adequate maintenance
- adequate records
- reporting system for results

In addition, the quality of results obtained in routine practice is highly dependent on the selection, training, and motivation of an appropriate complement of suitable personnel.

Internal quality control is particularly useful to identify the degree of precision of a particular technique — precision being the degree of agreement among repeat measurements on one sample. It is important to recognize that a precise technique is not necessarily accurate, accuracy being a measure of the closeness of an estimated value to the true value.

**QUALITY CONTROL MATERIALS**

To assess the precision of a particular method, it is necessary to perform repeated analyses of aliquots of the same sample. It is important to include quality control (QC) samples with normal and abnormal values to ensure that a method is under control at different levels of a particular analyte, since relatively minor changes in an analytical process may be more apparent when testing an abnormal control.

The control material should be similar in properties to test samples and be analysed concurrently. Quality control materials of human origin are more likely to closely resemble human test samples. All vials or aliquots of the control material should be practically identical, so that any variation in test results is not a consequence of vial-to-vial variation.

The QC material should also be stable for its intended period of use. In respect of hemostatic tests and assays, plasma samples have to be deep frozen (preferably at -35°C or lower) or lyophilized in order ensure adequate stability for use as QC material. For reconstitution of lyophilized samples, it is important to use distilled water with pH 6.8–7.2 and to allow at least five minutes for reconstitution.

If commercial QC material is used, this should be reconstituted according to manufacturer’s instructions using an accurate pipetting system. If deep frozen
QC material is used, this should be thawed rapidly at 37°C for five minutes. In the selection of QC material, the risk of transmission of blood-borne viruses should be considered. High-risk material should not be used.

At least one QC material should be included with each group of screening tests or assays. For screening tests, it may be most appropriate to include a normal QC in this way and to test abnormal QC materials once per day or shift, or when doubt exists about whether a method is under control. For a guide to troubleshooting problems with PT/APTT IQC problems when analyzing two different levels, see Figure 5.1, below.

**Figure 5.1. IQC troubleshooting**

<table>
<thead>
<tr>
<th>PT Level 1 IQC</th>
<th>PT Level 2 IQC</th>
<th>APTT Level 1 IQC</th>
<th>APTT Level 2 IQC</th>
<th>Conclusion/Check</th>
</tr>
</thead>
<tbody>
<tr>
<td>Out</td>
<td>In</td>
<td>In</td>
<td>In</td>
<td>PT Level 1 IQC material</td>
</tr>
<tr>
<td>Out</td>
<td>Out</td>
<td>In</td>
<td>In</td>
<td>PT reagent</td>
</tr>
<tr>
<td>In</td>
<td>In</td>
<td>Out</td>
<td>In</td>
<td>APTT Level 1 IQC material</td>
</tr>
<tr>
<td>In</td>
<td>In</td>
<td>Out</td>
<td>Out</td>
<td>APTT reagent</td>
</tr>
<tr>
<td>Out</td>
<td>Out</td>
<td>Out</td>
<td>Out</td>
<td>Instrument</td>
</tr>
</tbody>
</table>

A QC material with a reduced level should be included with tests used for the diagnosis and monitoring of congenital deficiency states associated with bleeding.

In all cases, the control material must be treated exactly like test samples, if possible. Since some variation will necessarily occur as a result of biological, technical, and analytical variation, each QC result should be recorded and assessed against the range considered to be acceptable, as described below.

**ACCEPTABLE LIMITS OF VARIATION**

For commercial IQC, samples manufacturers often provide a target range of acceptable values. In the case of screening tests and occasional assays, the results obtained will be dependent on the reagents and endpoint detection system used to perform the tests. The target range must take account of these effects. Where a target range is not available for a particular technique, this can be established locally.
The IQC material is tested repeatedly (minimum 10 times) on different days when the method is known to be under control (as indicated, for example, by within target results on an alternative QC material).

The mean and standard deviation (SD) of these results are then calculated. The SD is the square root of the sum of $d^2$ divided by $n-1$, where $d$ is the difference of individual results from the mean and $n$ is the number of determinations. The SD is a measure of the spread of results: the larger the SD, the greater the spread of results. Another important parameter is the coefficient of variation (CV), which is the SD expressed as a percentage of the mean ($CV = \frac{SD}{mean} \times 100\%$). The CV of results on different days for prothrombin time and activated partial thromboplastin times of a QC sample should always be less than 8%, and preferably lower. For assays such as FVIII:C and FIX, CVs of less than 10% should be attainable for tests performed over a number of days.

In most cases, results obtained for an IQC sample will show a normal (Gaussian) distribution. It is common practice to set the target range for IQC results as the mean ± 2 SD, since this should include 95% of values.

Individual results should be recorded on a chart that identifies the target range. An example is shown in Figure 5.2, opposite. Any future values that lie within these limits are considered acceptable.

Results outside this range indicate that the QC material has deteriorated or been handled incorrectly, or that the method is not properly controlled. Repeat testing of further QC material will then differentiate between these two possibilities, further out-of-limit results confirming that the test system is out of control. Medium or long-term drift in QC test results — for example, due to instrument or reagent deterioration or change — will become apparent by scrutiny of cumulative record charts.
Each point is a different assay on the same material. The solid lines represent the mean and two standard deviations of 20 assays on this material, considered to represent the limits of acceptable results.