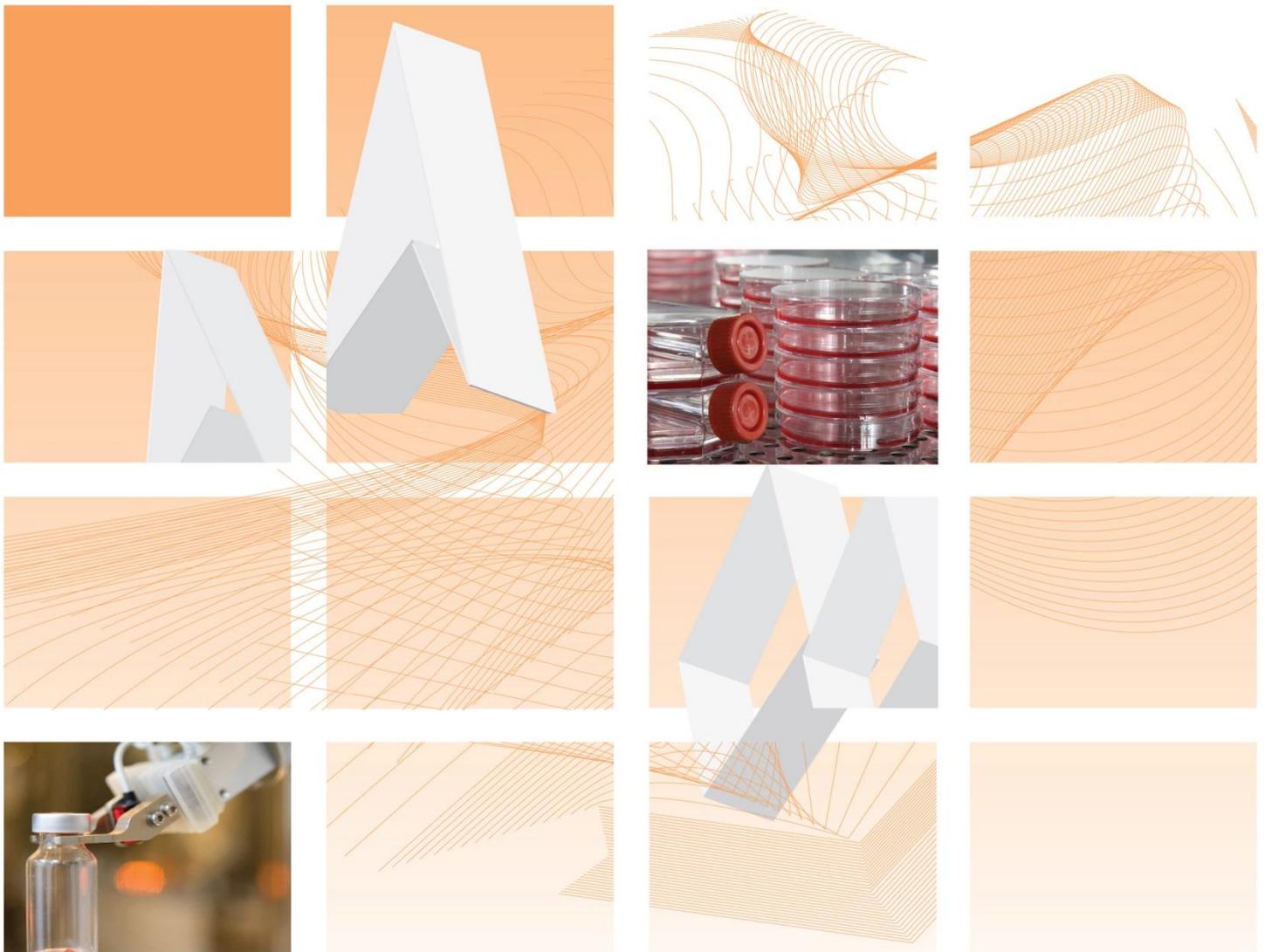


# UK Standards for Microbiology Investigations

Quality assurance in the diagnostic infection sciences laboratory



"NICE has renewed accreditation of the process used by **Public Health England (PHE)** to produce **UK Standards for Microbiology Investigations**. The renewed accreditation is valid until **30 June 2021** and applies to guidance produced using the processes described in **UK standards for microbiology investigations (UKSMIs) Development process, S9365', 2016**. The original accreditation term began in **July 2011**."

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Quality Guidance | Q 2 | Issue no: 8 | Issue date: 23.07.21 | Page: 1 of 23

## Acknowledgments

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## Amendment table

Each UK SMI has an individual record of amendments. The current amendments are listed on this page. The amendment history is available from [standards@phe.gov.uk](mailto:standards@phe.gov.uk).

New or revised documents should be controlled within the laboratory in accordance with the local quality management system.

Amendment no/date.	10/23.07.2021
Issue no. discarded.	7
Insert issue no.	8
Anticipated next review date*	23.07.2024
<b>Section(s) involved</b>	<b>Amendment</b>
Whole document	Document presented in new template, and updated in line with ISO 15189:2012
Figures	All figures updated with newer nomenclature where appropriate
Scope of document	Definitions of EQA, IQA and IQC added
6	Section updated to reference alternative methods to the use of Westgard rules
7	Section updated to note that alternative methods may be used
10	New section on management of non-conforming work
Appendices	Appendix 3 and Appendix 4 have been removed

\*Reviews can be extended up to 5 years subject to resources available.

## 1 General information

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[View](#) general information related to UK SMIs.

## 2 Scientific information

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[View](#) scientific information related to UK SMIs.

## 3 Scope of document

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This UK SMI describes aspects of quality assurance in the infection sciences laboratory, specifically the analytical phase. However, laboratories are urged to pay equal attention to ensuring that pre-examination and post examination phases covered in ISO15189:2012<sup>1</sup> are reviewed and controlled to minimise the risk of errors occurring.

The quality and thus reliability of results is not limited to the analytical phase, and laboratories should provide information to service users to ensure that the most relevant sample, process, and information is available to them. See [UK SMI user manual template](#) for guidance on presenting this information.

Delivering a high quality service requires the selection of the appropriate analytical method and equipment. Practice should, where possible be supported by reference materials such as World Health Organization (WHO) or National Institute for Biological Standards and Control (NIBSC) International Standards reference materials. Standardisation of methods and results is key to the use of pathology data between laboratories and for wider purposes such as surveillance.

ISO 15189:2012 mandates that laboratories establish a quality management system; laboratory managers should ensure the integrity and effectiveness of the quality management system is maintained. The quality management system is used to integrate and document the processes required to fulfil the established quality policy of the laboratory such as external and internal quality assessment, quality control, monitoring of equipment and auditing of processes.

Quality assurance should aim to ensure that tests requested on clinical specimens are processed, analysed and interpreted in agreement with defined professional standards and in accordance with patients' needs for diagnosis, treatment and management. For further information refer to ISO 15189:2012.

**Note:** See the [Pathology quality assurance review report](#) for information on how to harmonise and embed quality assurance in the different disciplines in Pathology.

This UK SMI should be used in conjunction with other UK SMIs.

## 3.1 Definitions

### 3.1.1 External quality assessment (EQA)<sup>1,2</sup>

External quality assessment schemes consist of a programme of inter-laboratory comparison that assesses whether participants meet predetermined performance criteria.

Requirements for participants are described in ISO 15189:2012 clause 5.6.3. Requirements for providers of accredited inter-laboratory comparison programmes are described in ISO 17043.

### 3.1.2 Internal quality assessment (IQA)<sup>3</sup>

Internal quality assessment schemes utilise retesting of routine clinical samples to identify discrepancies, which should then be discussed within the laboratory.

### 3.1.3 Internal quality control / independent quality control (IQC)<sup>4</sup>

IQC consists of statistical and non-statistical techniques for the verification of the quality of results. Quality control materials are developed or obtained from a source independent of the manufacturer of the test kit. These are used to simulate patient samples in the clinically significant range, and results are periodically examined.

## 4 Background

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ISO 9000:2015 defines quality as “the degree to which a set of inherent characteristics of an object fulfils requirements”. In pathology a quality product or service can be defined as the right result on the right specimen from the right patient that is accurate, timely and properly interpreted. The objective of a diagnostic laboratory should be to produce cost-effective, accurate, reproducible and timely results, which are comparable with the results obtained in a similar laboratory elsewhere, and which are promptly, effectively and appropriately communicated to the users of the service. In this way the quality of the product or service can be guaranteed and thus meet the requirements of the user.

Laboratories achieve a quality service through the application of monitoring processes within quality assurance (QA). Continual monitoring comprises all the different measures taken to ensure the reliability of investigations. Relevant measurable items include: the quality of training and education of staff, the quality of reagents, apparatus and specimens and the suitability of the techniques in use.

Therefore, quality assurance relates to the entire process of diagnosis which starts and ends with the patient. If an error has occurred at the pre or post examination phase which might have resulted in the wrong patient being identified, the wrong specimen being taken, the specimen’s storage conditions during transit to the laboratory being incorrect, a data entry error occurring at specimen reception, an incorrect interpretation of the results or the result having been sent to the wrong address, then the accuracy of the test itself becomes irrelevant.

Quality assurance in the examination phase is the collective term for several distinct procedures used to monitor the performance of all aspects of work in the laboratory. Quality assurance should be used to identify procedural and technical issues, check the adequacy of current techniques and calculate the frequency of errors.

A comprehensive quality assurance programme should be an integral part of the procedures of a diagnostic infection sciences laboratory and is necessary for compliance with accreditation standards. In the UK, assessment and accreditation is carried out by the United Kingdom Accreditation Service (UKAS).

The use of these procedures is designed to increase confidence in the overall processing of the sample from receipt to report including handling and testing of a specimen, the validity of the assay results, and the final report. However, a laboratory-based quality assurance scheme may only monitor the procedures over which the laboratory has control. Joint audits should be conducted to monitor all processes associated with the diagnosis of infections.

Quality assurance should be an integrated system, in which results obtained from one of its constituent parts are confirmed by another, for example:

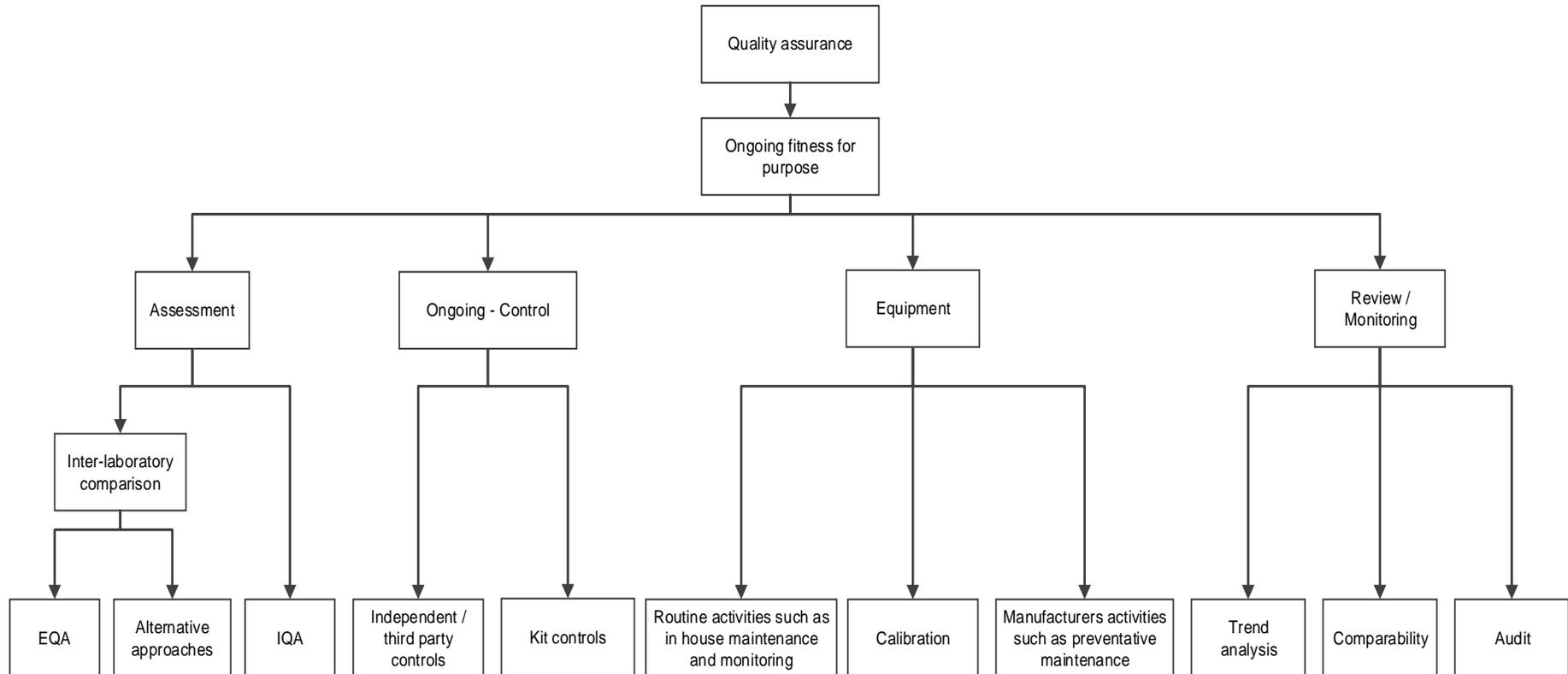
- the coefficient of variation of an assay determined in the QC scheme can be used to determine if there is a significant difference in the results obtained in the IQA scheme
- reciprocally, IQA is a useful procedure when the quality of control material is in doubt
- violations of the Westgard QC  $4_{1SD}$  or  $10_x$  rules may be investigated through equipment monitoring. Reduced incubator temperature would indicate equipment failure, whereas correct temperatures may suggest reagent deterioration

Assay kits, processes and equipment should undergo thorough evaluation before being introduced for routine use in the laboratory. See [UK SMI Q 1: evaluations, validations and verifications of diagnostic tests](#) for further information on this topic. The use of EQA, IQA or IQC procedures will not improve the innate performance of processes, assays or equipment.

Quality assurance can only be undertaken effectively if data obtained in the scheme, comments on that data and remedial actions taken are recorded. This supports the following investigation to determine root cause, and the implementation of the appropriate corrective action to remove or reduce the possibility of further errors. This should be the designated responsibility of a senior and experienced member of staff.

**Figure 1. Overview of quality assurance**

An accessible text description of this figure is provided with this document.



## 5 Inter-laboratory comparison

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ISO 15189:2012 states that “the laboratory shall participate in an inter-laboratory comparison programme (such as external quality assurance or proficiency testing programmes) appropriate to the examination and interpretations of examination results (5.6.3.1).

### 5.1 External quality assessment

External quality assessment (EQA) schemes facilitate comparison of laboratories and detection systems followed by comprehensive discussion of results and discrepancies.

Clinical specimens and artificially “spiked” samples (a sample to which a known concentration of analyte has been added) are distributed from external sources or reference laboratories to assess techniques and assays performed in microbiology. EQA distributions are normally limited in number and clearly identifiable, which could allow laboratories to process them in a special manner, however ISO 15189:2012, clause 5.6.3.3 specifies that “Inter-laboratory comparison samples shall be examined by personnel who routinely examine patient samples using the same procedures as those used for patient samples”. UKAS assessment ensures that this clause is met.

EQA schemes are available for the majority of assays and organisms routinely tested in the majority of diagnostic laboratories, but may not be available for some services provided by specialist centres or referral laboratories. In the latter case, alternative approaches should be explored (refer to section 5.2: Alternative approaches). EQA providers are considered suppliers by UKAS and so must meet suitable methods for acceptance. For more information on participation in EQA testing, refer to [TPS 47: UKAS policy on participation in proficiency testing](#) which also contains a link to [EPTIS website](#) which provides a database of proficiency testing schemes available around the world.

Laboratories should not communicate with other participating laboratories or refer their EQA samples for confirmatory examination elsewhere, until after the closing date for submission of results. Analysis and comparison of results will be performed externally. The findings should be fully investigated and widely disseminated within the laboratory. Laboratory managers should monitor the results of EQA and ensure the implementation of corrective actions when required.

### 5.2 Alternative approaches

Where no EQA is available ISO 15189:2012 states that “the laboratory shall develop alternative approaches”. Section 5.6.3.2 of the ISO standard recommends testing appropriate alternative materials such as: samples previously examined; samples exchanged with other laboratories; or certified reference materials<sup>1</sup>.

## 6 Internal quality control

Quality control procedures should be used in the laboratory so that the attainment of the intended quality of results is verified; materials chosen should provide assurance on the performance of the process and thus should react in a manner as close as possible to a patient sample. Such materials will normally be obtained from external sources as independent third-party controls; however, where these are not available the laboratory may consider alternative methods.

Quality control data should be reviewed at regular intervals to detect performance trends that may indicate issues. Laboratories should determine a suitable frequency for this.

### 6.1 Standards used for QC samples<sup>5</sup>

QC samples include international, regional/national (secondary standard) or local material (tertiary standard) that has been well characterised in previous assays, and which produce values within clinically significant ranges.

Secondary standards are calibrated against an international standard and should be used for the calibration of tertiary standards, and the calibration/validation of assay systems.

Tertiary standards are calibrated against secondary standards and are often used as external control material (in addition to kit controls).

Where material traceable to an international standard is not available, other control material may be produced by commercial and in-house laboratories. The choice of appropriate QC samples is dependent on the assay to be controlled.

### 6.2 Procedure

This section describes an IQC procedure using Shewhart control charts in conjunction with Westgard rules to establish quality control limits. However, other methods available include, but are not limited to, use of national guidelines such as the Richtlinien der Bundesärztekammer (RiliBÄEK) guidelines<sup>6</sup> used in Germany or the use of larger datasets such as the entire historical dataset for a given assay/QC combination<sup>7</sup>.

Procedure:

- select control material which is suitable for assessment of the performance of part of (preferably all of) the process undertaken on a clinical sample
- test the control material in 20 separate assay runs (see Appendix 2)
- determine the mean (target value) and standard deviation (SD) of the control material
- establish a Shewhart control chart with the mean and  $\pm 1SD$ ,  $\pm 2SD$  and  $\pm 3SD$  delineated
- include the control material in each subsequent assay run and plot the result obtained on the control chart
- determine the validity of each assay run by applying the Westgard rules listed in table 1 (page 12)<sup>7,8</sup>

- re-evaluate the mean and standard deviation of the control material periodically and in particular after any violation of any statistical rules applied to monitor assay performance and after changing kit reagent and/or control material lot numbers

### 6.3 Quantitative versus qualitative assays

Monitoring of ongoing performance provides significant quality assurance to the continuing validity of procedures. The use of external material allows independent monitoring and can be performed in both quantitative and qualitative assays. Where the process has a numerical output irrespective of whether the result is reported as a quantitative value or qualitatively the test process can be monitored using control charts and application of statistical acceptance criteria.

### 6.4 Monitoring the testing process and QC results

Where there is a numerical output from QC material, interpretation of QC results can involve both statistical and graphical methods. There are several mechanisms to do this, but the most common is the use of Shewhart plots based on a small dataset (typically 20 results) to establish limits from mean and SD, and the application of Westgard rules to identify error.

#### 6.4.1 Control charts

Shewhart or Levey-Jennings plots should be drawn, with the target value (mean) and the limit values of  $\pm 1SD$ ,  $\pm 2SD$  and  $\pm 3SD$  delineated, for each control used. Subsequent values obtained with the assay controls are plotted and the Westgard rules applied to determine the validity of each assay run. (See Appendix 2 for examples of documentation used with internal quality controls).

The coefficient of variation (CV) is a measure of variability and is expressed as a percentage. Variation may be caused by the assay, the equipment used to perform the assay, the assay operator or by environmental factors.

#### 6.4.2 Westgard rules<sup>8,9</sup>

Westgard rules are a set of statistical rules which can be used individually, or in combination, to detect both random and systematic errors. Such errors create uncertainty of measurement, which must be considered when testing procedures and / or testing results are compared with each other or against specifications. Westgard rules can be used to define specific performance limits for a particular assay and verify the reliability of test results.

A random error is any deviation away from an expected result. Any positive or negative deviation away from the calculated mean of QC results is regarded as random error. Causes include errors in pipetting and changes in incubation period. Random errors can be minimised by training, supervision and adherence to standard operating procedures.

A systematic error is an error which occurs consistently - such as when a number of measurements are made under the same conditions - or varies predictably when conditions change. Systematic errors may occur gradually, as demonstrated by a trend in control values or may be abrupt as demonstrated by a shift in control values. Example causes are variations in incubation temperature, blockage of plate washer,

aging of the reagents, gradual deterioration of control materials, issues with storage conditions and change in the reagent batch or modifications in testing method.

It should be noted that Westgard rules are one possible tool, and other methodologies are available. Laboratories should review carefully what method for managing quality control is used locally to ensure that it is applicable to the process and will provide the most useful information on ongoing performance.

**Table 1: The Westgard rules<sup>8,9</sup>**

<b>A</b>	1 <sub>2SD</sub>	This rule is used as a warning rule to trigger careful inspection of the control data. If one control measure exceeds the mean $\pm 2SD$ , control values in the previous run should be considered to rule out a trend.
<b>B</b>	2 <sub>2SD</sub>	This rule detects systematic errors and is violated when 2 consecutive control values (on the same side of the mean) exceed the same mean $+2SD$ or mean $-2SD$ limit.
<b>C</b>	4 <sub>1SD</sub>	This rule detects systematic error. The rule is violated when 4 consecutive values exceed the same mean $\pm 1SD$ . The run does not need to be rejected if this rule is violated but should trigger recalibration or equipment maintenance.
<b>D</b>	1 <sub>3SD</sub>	This control rule detects random error. Violation of this rule may also point to systematic error. The assay run is considered to be out of control when one control value exceeds the mean $\pm 3SD$ .
<b>E</b>	R <sub>4SD</sub>	This is a range rule which detects random error only. This rule is applied only within the current run. The rule is violated when one control measurement in a group exceeds the mean $+2SD$ and another exceeds the mean $-2SD$ .
<b>F</b>	10 <sub>x</sub>	This rule detects systematic error and it is violated when 10 consecutive values fall on the same side of the mean. Its violation often indicates the deterioration of assay reagents. The 10 <sub>x</sub> rule is usually applied across runs and often across materials.
<b>Note:</b> Often the 4 <sub>1SD</sub> and 10 <sub>x</sub> must be used across runs in order to get the number of control measurements needed to apply the rules. There are some modifications to the 10 <sub>x</sub> rule to make it fit more easily with the 4 <sub>1SD</sub> . They are as follows (see below):		
<b>G</b>	8 <sub>x</sub>	This rule is violated when 8 consecutive values fall on one side of the mean.
<b>H</b>	12 <sub>x</sub>	This rule is violated when 12 consecutive values fall on the same side of the mean.
Some other control rules that fit better and are easier to apply in situations where 3 different control materials are being analysed include:		
<b>I</b>	3 <sub>1SD</sub>	This rule is violated when 3 consecutive control measurements exceed the same mean $\pm 1SD$ control limit and fall on the same side of the mean.
<b>J</b>	6 <sub>x</sub>	This rule is violated when 6 consecutive control measurements fall on one side of the mean.
<b>K</b>	9 <sub>x</sub>	This rule is violated when 9 consecutive control measurements fall on one side of the mean.

In the event of a violation of a Westgard rule, there are 3 potential actions that may be taken:

- accept the test run in its entirety – this usually applies when only a warning rule is violated.
- reject the whole test run – this applies only when a mandatory rule is violated.
- enlarge the grey zone and thus re-test range for that particular assay run – this option can be considered in the event of a violation of either a warning or mandatory rule.

Laboratories should document which rules are applied locally, and justify actions taken in their local SOPs.

The mean or target value, the coefficient of variation and the SD of the proposed control are calculated from the results obtained after testing the control material on 20 separate occasions (see Appendix 2). For example, this process may be accelerated by testing 4 aliquots of the sample on each of 5 occasions, however the mean and SD should be recalculated after 20 assay runs. The values obtained are then used to set acceptable limits for the results obtained subsequently with the assay control.

The concentration of analyte in the control material should be within the clinically significant range and preferably within a region that is highly sensitive to changes in apparent analyte concentration. For example, in an ELISA the OD value of the control should lie within the linear part of the dose response curve. Control material that is strongly positive and therefore saturates the assay should not be used. Control material that is close to the limit of detection (e.g.  $<2SD$ ) should not be used in isolation as this may cause assays to be invalid.

The above approach may not be suitable for all procedures such as those producing qualitative results or utilising reciprocal titres and the laboratory should determine appropriate mechanisms to continually review the performance of quality controls used in these types of procedures.

## 6.5 Comparability

Where laboratories have more than one process or instrument (either different or duplicated) they should employ mechanisms that provide assurance that the testing outcome for all is comparable. The use of quality assurance protocols can aid this:

- testing of EQA samples on 2 different procedures can show that both provide comparable results.
- comparison of uncertainty for 2 instruments of the same type can show that each instrument is producing results which are acceptable for patient samples, irrespective of which instrument is used to perform the testing.

## 7 Equipment monitoring

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Laboratories should verify upon installation, and before use, that equipment can achieve the necessary performance and that it complies with requirements relevant to any examinations concerned. This applies to equipment used in the laboratory, on loan, or in associated facilities when authorised by the laboratory.

The performance of equipment (for example the maintenance of appropriate temperature in water baths, incubators and freezers) should be checked and recorded at regular, predetermined intervals. The laboratory may wish to undertake interim checks of some equipment such as pipettes, spectrophotometers and balances in house but equipment should be recalibrated according to manufacturer recommendations. Adjustable and fixed volume pipettes should be calibrated when new and while in use. The introduction of automated liquid handling devices, capable of preparing or performing assays, may offer increased efficiency and reproducibility.

Calibration should be traceable to an international standard and laboratories should ensure that the service provided by the calibration company meets the requirements of the laboratory. The type of calibration will be determined by the end use of the equipment.

## 8 Audit

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According to ISO 9000:2015, audit is defined as “a systematic, independent and documented process for obtaining objective evidence and evaluating it objectively to determine the extent to which audit criteria are fulfilled”. It can also be defined as the process used to evaluate, amend and improve procedures in a systematic way to enhance quality. It is often used to highlight difficulties in those procedure(s) or to identify bottlenecks.

An audit can be categorised as internal or external. Internal audits are organised and carried out by laboratory staff and management. External audits are carried out by external bodies such as HSE or UKAS, and through participation in external quality assessment schemes.

**Note:** Audits of the quality management system should be conducted by personnel trained to assess the performance of managerial and technical processes. Audits should be:

- planned
- scheduled
- structured – following similar patterns
- independent – independence can be demonstrated by freedom from responsibility for the activity being audited
- reported and recorded on time
- followed-up and acted upon

The documentation required will depend on the procedure being audited. The laboratory may wish to create proformas to ensure that all the appropriate areas are included in the audit.

There are 3 main audit types which can be performed as part of the internal audit process. The laboratory should determine which type is employed based on their requirements and repertoire. An audit schedule should be established which covers the laboratory’s scope and is undertaken over a specified time frame, such as annually.

### **8.1 Horizontal audit**

A horizontal audit assesses one element of the quality system, for example staff training or equipment calibration. This type of audit ensures that individual elements of the quality system are in place and functioning properly. However, it does not assess how the whole system functions.

### **8.2 Examination audit**

Examination audit is also known as “witness” or “observation” audit. In an examination audit, a member of staff undertaking a task is assessed by the auditor. This assesses whether an SOP is being followed and whether work is competent and safe. It provides an opportunity to ascertain whether the staff member is satisfied with their training; has the correct level of supervision; understands all aspects of the procedure they are audited against; and is aware of the impact that their work has.

### **8.3 Vertical audit**

A vertical audit assesses all the activities associated with processing one item or sample, covering the whole sample journey from receipt to report, to ensure that all parts of the system are functioning. This audit may be prospective or retrospective. As well as tracking the sample itself, a vertical audit should include all activities which relate to the testing of the sample; such as training record of personnel involved in testing the sample, records of equipment and reagents used to perform assays, IQA and IQC results relevant for the time the test was performed.

## **9 Internal quality assurance**

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Internal quality assessment (IQA) schemes are used to monitor all activities involved in the passage of specimens through the laboratory, from first receipt to issuing of the final report. In an IQA scheme, a proportion of specimens (for example 0.5 to 1.0%) received in the laboratory are anonymised and resubmitted for testing (Figure 2).

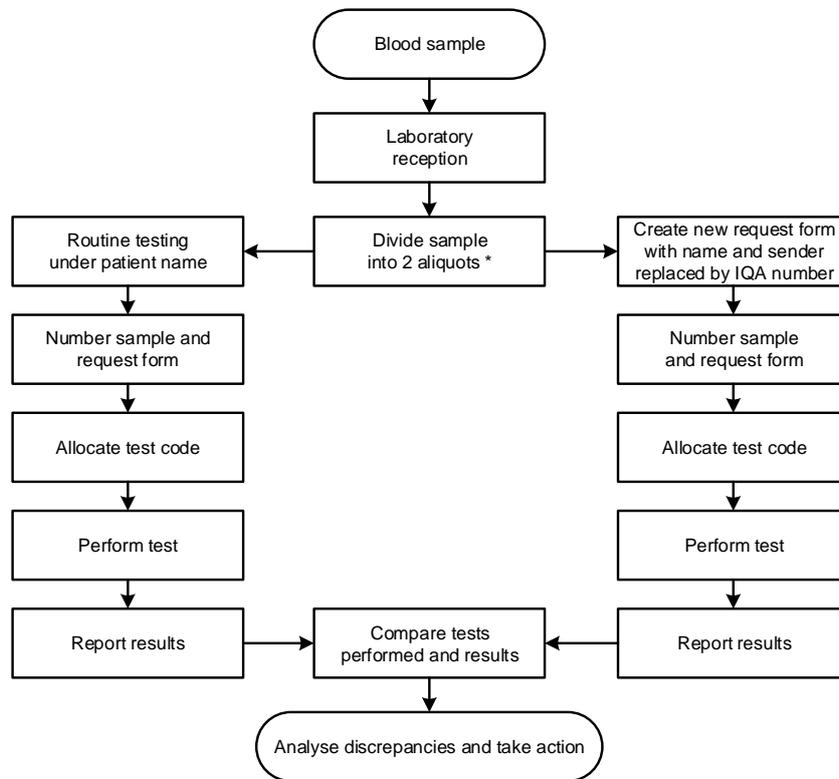
Specimen selection should be random in an IQA scheme for general serology but should reflect the proportion of the total workload submitted for each test.

IQA schemes for monitoring culture, antigen detection, genome detection or electron microscopy may be more difficult to construct. Loss of viability or degradation during storage, inadequate specimen volume, or a low frequency of positive specimens may all complicate the analysis. Such IQA schemes are often supplemented by the use of “spiked” specimens.

Local adaptation of traditional IQA methods may be appropriate based on the methodologies or procedures in use by a laboratory. Laboratories should document and justify the approach taken.

**Figure 2: Internal quality assessment scheme**

An accessible text description of this figure is provided with this document.



\* Paired samples previously tested and stored under optimal conditions can be resubmitted for testing to ensure that samples are not easily paired with the named aliquot during testing and to monitor:

- test variation
- operator variation

Discrepancies between the results obtained for the original named sample and the anonymised sample should be recorded and reviewed by a senior member of staff, independently of the staff member who issued the original result report. This senior independent staff member may comment on the discrepancy and may retest the 2 samples in parallel if appropriate. The results should be circulated widely and staff should be encouraged to discuss any discrepancies found. Results from the IQA scheme should be recorded and reviewed so that recurring problems or trends can be identified. See Appendix 1 for examples.

## 10 Management of non-conforming work

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Irrespective of what the quality assurance process is, where issues are identified and logged the laboratory should investigate the root cause of the issue and implement corrective actions to ensure that repetition is avoided. In addition, when the issue could have affected patient results, appropriate actions should be taken to ensure that reports are updated and relevant clinicians are informed.

## Appendix 1: Documentation used in IQA

### 1 Example record sheet

#### 1.1 Internal quality assessment

	Original	IQA 26	
Laboratory No.	10223	10345	
Date	12/6/21	15/6/21	Possible cause
<b>Serology</b>			
HAV IgM	Negative	Positive	Equipment calibration between results impacts on results
HBsAg	Positive	Equivocal	Testing performed on 2 instruments with non-comparable measurement uncertainty
Anti-HBs	<b>96 mIU/mL</b>	<b>106 mIU/mL</b>	No significant difference – within known measurement uncertainty for procedure
Viral Load test	3.24logs	3.96logs	Assess against measurement uncertainty
MC&S	<b>Significant organism isolated</b>	<b>No significant growth</b>	Stability of sample type impacted on organism recovery
Microscopy	WBC - 11	WBC - 24	Uncertainty associated with manual method

Where the impact is considered significant laboratories should review occurrences as non-conforming work and investigate according to local policies.

## Appendix 2: Documentation used in IQC

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### 1 Example method for determining the mean and SD of a QC sample and setting acceptable limits for its use (see Appendix 3)

Assay run	Antibody concentration (Arbitrary units/mL)
1	68
2	68
3	64
4	72
5	61
6	65
7	66
8	65
9	65
10	70
11	72
12	73
13	63
14	67
15	69
16	66
17	63
18	67
19	70
20	65

QC data:

Number tested = 20

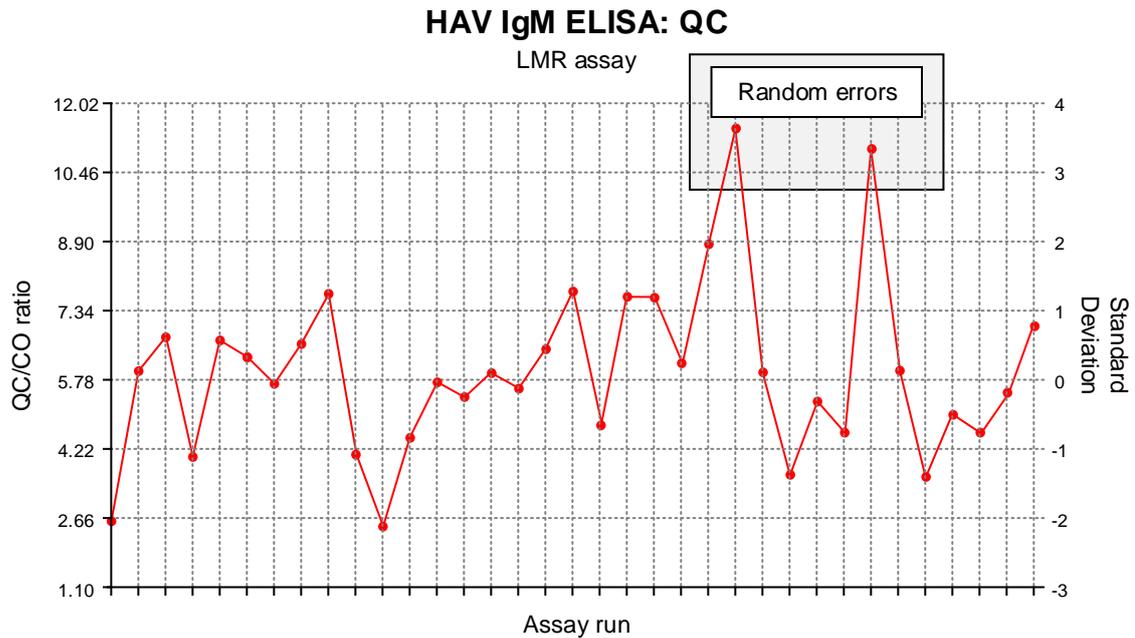
Mean (target value) = 66.95 AU/mL

Standard Deviation (SD) = 3.28 AU/mL

Acceptable range ( $\pm 3SD$ ) = 57.1 - 76.8 AU/mL

A Shewhart plot may now be constructed with the mean,  $\pm 1SD$ ,  $\pm 2SD$  and  $\pm 3SD$  values delineated. The result obtained with the QC sample should be plotted after each assay run and must lie within 57.1 AU/mL and 76.8 AU/mL for the run to be valid.

## 2 Examples of Shewhart control charts



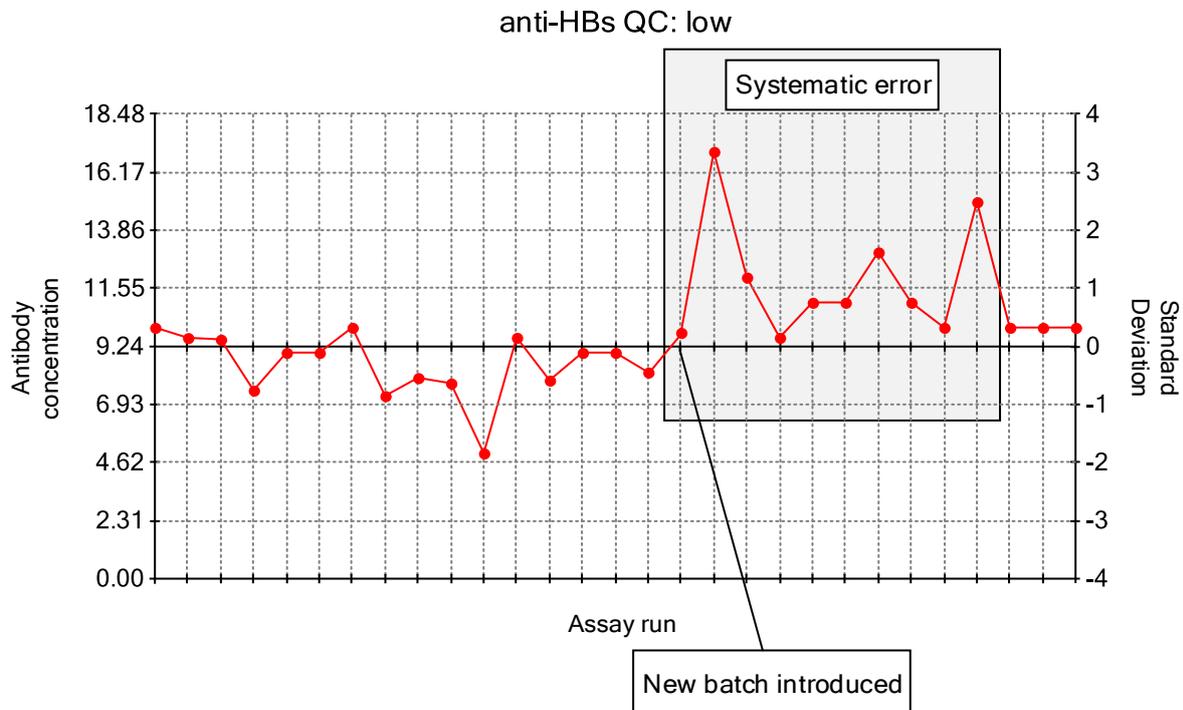
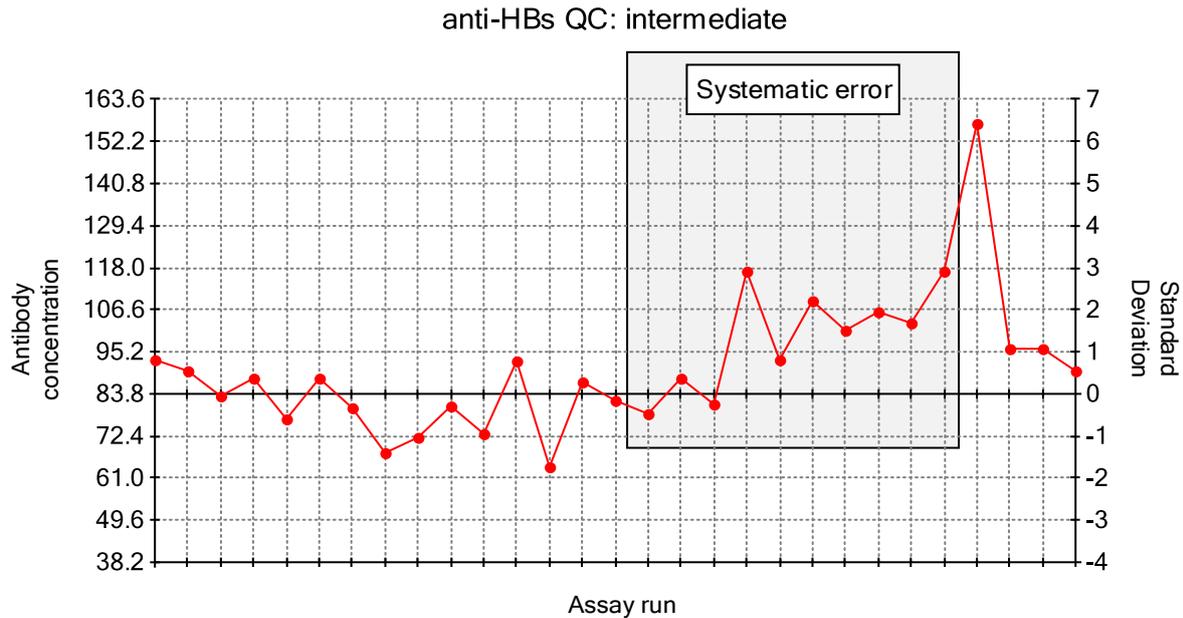
The chart above shows examples of random errors or operator errors. The  $13SD$  rule has been violated on 2 occasions. Therefore, the results obtained in both of these assay runs are invalid.

**Note:** The results of the QC sample are plotted as a ratio of the OD of the QC sample: OD of the assay cut-off value. This compensates for the small differences in assay performance seen day-to-day. Assays incubated at room temperature are particularly susceptible to small, but acceptable, changes in performance.

**Note:** the examples above are one possible method that may be used; however, other methods for monitoring IQC are available.

The QC charts below are an example of systematic errors. A change in assay performance was detected with both controls and was associated with a new batch of reagents. Violations of the 10<sub>x</sub> rule were detected with both controls and the 4<sub>1SD</sub> with the intermediate control.

The QC procedures indicate an increase in the sensitivity of the assay. If this is acceptable then the QC limits should be recalculated using 20 control values obtained with this batch of reagents. This process can be speeded up by testing 4 aliquots of each control in 5 assay runs. A recalculation should be made after the results of 20 runs are available, in order to check the accuracy of the values used.



## Appendix 3: Statistics used with IQC

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These are examples of the most common methods of statistical analysis (below) but it should be noted that other methods are also available.

### 1 Mean

The mean is defined as the arithmetic average of a set of data points. It is expressed as:

$$\text{Mean} = \frac{\sum x_i}{n}$$

where  $x_i$  = each data point

$n$  = the number of data points in the set

The mean identifies the “target value” of a set of QC data points.

### 2 Standard deviation

The standard deviation (SD) is a measure of the distribution of data points above and below the mean. It is used to set acceptable limits for values obtained with IQC samples.

$$SD = \sqrt{\frac{\sum(x^2) - \frac{(\sum x)^2}{n}}{n-1}}$$

Where  $\sum(x^2)$  = the sum of the squares of each value of  $x$

$(\sum x)^2$  = the sum of all data points squared

$n$  = the total number of data points in the set

Quality control data exhibit a normal distribution, therefore:

68.3% of values are within  $\pm 1SD$  from the mean

95.9% of values are within  $\pm 2SD$  from the mean

99.7% of values are within  $\pm 3SD$  from the mean

### 3 Coefficient of variation

The coefficient of variation (CV) is a measure of the variability of an assay and is expressed as a percentage.

$$CV = \frac{SD}{\text{mean}} \times 100$$

The CV is useful for determining whether values, obtained with duplicate samples, which lie either side of an arbitrary cut-off value are within experimental error.

For example, in anti-HBs antibody determination a value of 105mIU/mL would indicate satisfactory immunity whereas a value of 98mIU/ml would signal the need for a booster dose of vaccine. If the assay has a CV of 10%, both values would be acceptable.

#### **4 Uncertainty of measurement**

There are several metrics designed to quantify uncertainty of measurement (UM). One method of estimating UM is the statistical formula below:

$$UM = \pm s.k$$

where

**s** = population standard deviation (combined standard uncertainty)

**k** = coverage factor

Multiplication by a coverage factor gives the confidence interval for the distribution of values which could be derived from the quantities measured. This is known as the expanded uncertainty of measurement.

For  $\geq 30$  samples, using a confidence level of 95% is obtained using  $k = 2$ .

For  $< 30$  samples, a confidence level of 95% is obtained when  $k =$  the 2 tailed value of Student's t-test for those measurements and for the level of confidence required.

## References

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For the information for the evidence grade ratings given, refer to the scientific information link above in section 2.

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