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Quality Assurance in the Clinical Virology Laboratory

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Glossary

AMR Analytical measurement range.

Assay Drift.

LoD Limit of detection.

LoQ Limit of quantitation.

Probit Analysis.

Reportable Range.

The Principles of Quality Assurance and Quality Management

The principles of quality assurance (QA) and quality management (QM) have their origins in manufacturing and in particular the automotive industry. They were primarily used to support the stream-lining of processes and procedures, with the aim of increasing productivity, while reducing costs but maintaining the quality of the product delivered to the customer. Within the clinical laboratory, the concepts of QA and QM were first introduced in hematology and clinical chemistry by Belk and Sunderman in the late 1940s/early 1950s in an effort help reduce the high number of diagnostic errors observed in relation to the handling and testing of patient specimens which ultimately could have an impact on patient care. These concepts and general principles formed the QA and QM foundation commonly used within the clinical virology laboratory which continue to evolve in order to meet present regulatory demands and assure the safety and effectiveness of the virology testing service.

The key components of quality assurance within a laboratory's quality management system (QMS) are shown diagrammatically in [Fig. 1](#).

As well as supporting the whole diagnostic process (pre-analytical to post-analytical) to delivery of patient result, the QMS also support the verification, validation and implementation phases of the diagnostic assays used within the virology laboratory.

With the implementation of quality principles from other industry sectors comes the adoption of the associated terms and definitions used and one such quality term which has found its way into clinical virology in recent years is Total Quality Management (TQM). In the context of the clinical virology laboratory, TQM provides an alternative description for the integration of all quality parameters and the nurturing of a quality culture followed by all laboratory staff. So that the aim of the laboratory is not only to provide correct results, they also aim to ensure that the correct test is performed on the appropriate specimen, the correct result is obtained and interpreted, and provided to the right patient within a meaningful time frame and using the correct procedures. The goal of the laboratory is, to ensure confidentiality, the safety of the patient, as well as provide a mechanism for continuously monitoring and improving of the diagnostic service.

In many countries, clinical virology laboratories and the testing services they provide are required to be accredited and in some regions accreditation is a pre-requisite to regional reimbursement policy. Accreditation also helps the clinical laboratory demonstrate the competence and reliability of its services to its immediate peers within the healthcare environment, the Clinicians who routinely utilize the testing service, and those external to the healthcare environment such as the legal profession. Increasingly, clinical virology laboratories are gaining accreditation to the Internationally recognized standard ISO15189 which was developed specifically for the medical laboratories involved in laboratory testing and examination, although in some country's laboratories are accredited to the national regulatory framework. The quality management requirements of ISO15189 are also aligned to those in ISO 9001 which provides a basic standard and quality language across diverse industry sectors.

Quality Management System (QMS)

In the context of the clinical virology laboratory, the QMS covers all the policies, documented processes, procedures, and records used in order to deliver the diagnostic testing service to the patient under its defined scope of accreditation. This also incorporates the operational environment in which the testing is performed, the equipment, specialist reagents and essential supplies required, as well as the qualification and competence of the staff tasked with conducting the testing service. The complexity of the quality management system depends on the scope of the service and in many larger Hospitals the QMS is managed centrally and covers the whole of the pathology services, Hematology, Blood Transfusion, Biochemistry, Microbiology including virology.

Quality Management Documentation

The documentation used within the QMS is usually organized and managed through a clear document hierarchy. The top-level documents include the Quality Manual or Services Manual which stipulate the laboratory's quality policy and objectives. These are monitored against defined quality performance indicators, such as the monitoring of the time taken to report a test result from receipt of specimen, and ensure that the service is achieving its stated quality intentions. The Quality Manual also sets out the

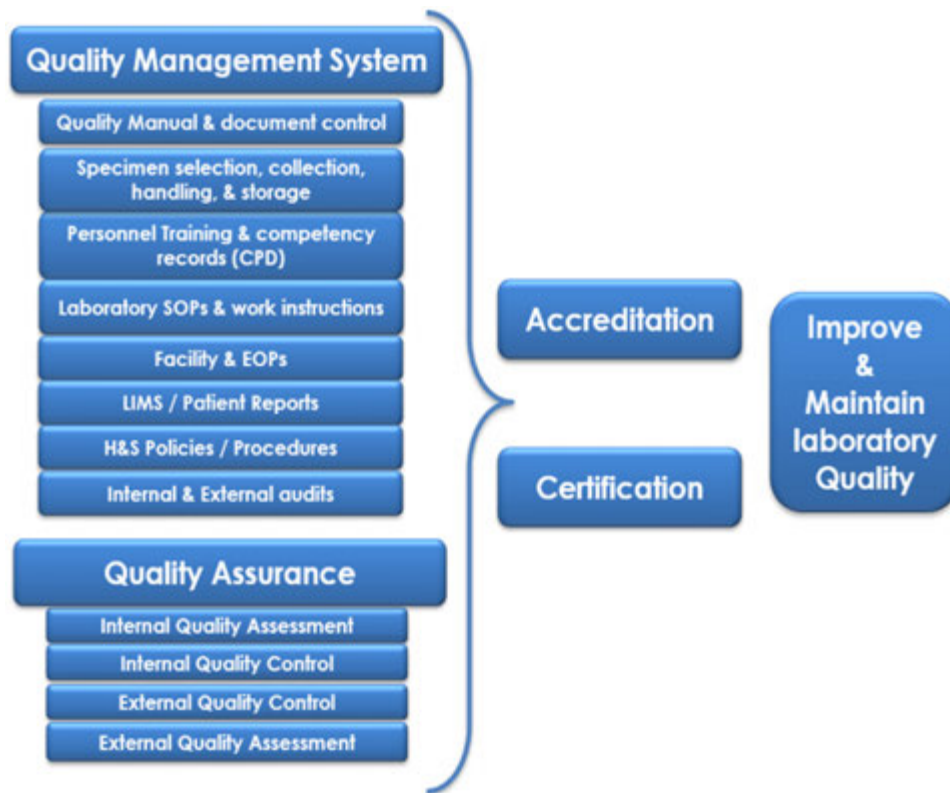


Fig. 1 QMS/QA component.

laboratory organization, management roles and responsibilities in relation to the scope of the services provided. This will include the range and type of tests performed, the specimen types handled, the quality control measures and internal quality assurance procedures the laboratory undertakes, as well as the external quality assessment (EQA) schemes it subscribes to. Practical information on how to order the test, referral of tests and contact details for additional clinical and technical advice are also included within the quality manual or service manual.

The procedural documents including standard operating procedures (SOPs), equipment operating procedures (EOPs), describe in detail how a specific laboratory activity is to be conducted or how a piece of equipment is to be used, who is authorized to do so, as well as when and where that activity is to be performed or equipment used. The procedural documents also describe the inputs and outputs of the procedure and where this information should be recorded. In addition to operating procedures some laboratories may also have separate specific work instruction documents. These detail the exact steps and methods to be employed when conducting a laboratory activity such as the consecutive tasks required in order to set up for the detection of a particular viral pathogen or the operation of a specific piece of equipment, for example the calibration and maintenance of a real-time Thermal Cycler. In some clinical virology laboratories, having separate work instructions means that they can be held directly within the laboratory for easy access and reference where the activity is taking place.

The inputs and outputs of a procedure or specific work instruction are recorded within a Standard Operation Record (SOR). These include paper-based records as well as electronic outputs from equipment or devices within the laboratory such as a thermal cyclor or serological autoanalyzer. In these cases, the devices are interfaced with the laboratory and the records are managed through a middleware software such as FlowG (See Relevant Websites section) which collects, collates, and stores the data which can include calibration and control performance data across a range of laboratory platforms. The middleware is also linked to the general Laboratory Information Management System (LIMS) which provides secure storage and management of all the laboratory data and patient results.

Document Control

All documents within the clinical virology laboratory QMS need to undergo regular review at the specified intervals on when a particular policy, process, or procedure is changed in order to ensure that they remain fit for purpose and compliant. This is managed through a document control policy which also includes an index of all the documents within the laboratories QMS, who is responsible for each one, and when it is scheduled for review.

Many clinical laboratories have moved away from a paper-based Quality Management and document control systems towards having a commercially available QMS software management solution such as Q-pulse (See Relevant Websites section) which is able to integrate directly into LIMS and makes the management of laboratory quality assurance information more efficient and compliant with regulatory standards such as ISO15189.

Specimen Management

The ability of the clinical virology laboratory to carry out a test and to support a diagnosis depends extensively on the quality of the specimen the laboratory receives and the time it takes to get to the laboratory. Pre-analytical errors such as wrong labeling of the specimen, the incorrect specimen collection device, incorrect specimen type are the most frequently reported sources of error (between 48% and 62%) within the clinical laboratory.

In some hospitals or institutes, it is the responsibility of specific clinical virology laboratory staff to provide training on appropriate sample collection, handling, transportation and storage of the clinical specimens to be used. As a result, the clinical virology laboratory details the quality requirements of the specimen types (i.e., serum, plasma, urine, stool, etc) the laboratory is capable of processing in relation to the test repertoire offered under the services it provides. This includes specific instructions to clinicians, general practitioners, and nursing staff on how and when to take the patient specimen as well as the collection device to be used and the minimum volumes required for the test requested.

For example, most common serological tests are performed on either serum or plasma and are best tested within 48–72 h after collection in order to prevent degradation of the analytes, unless they are stored and shipped to the laboratory at between 2–8°C. In comparison, for the molecular viral load determination of blood borne viruses such as HIV, HCV, and HBV, either EDTA plasma or sodium citrated plasma is preferable to serum as the degradation of nucleic acids has been observed within serum/clotted specimens and may result in the under-reporting of the viral load result. Heparinised specimens are also not recommended for molecular testing as the heparin has been shown to inhibit techniques such as PCR, although in most modern molecular technologies this does not appear to be a problem.

Although all clinical specimens must be regarded as potentially infectious, most laboratories will also have a separate policy for the handling of potentially high-risk samples, such as those suspected of having a viral hemorrhagic fever (VHF). The policy covers the way that these specimens are handled and the labeling to be used. This will depend on risk factors identified and any regional regulatory and health and safety requirements the laboratory must adhere to.

However, the sample selection process has been improved significantly through the implementation of electronic test requisitions which allow authorized healthcare professionals such as general practitioners to request a test directly through an internet-based portal. This has helped make the process more efficient and standardized, as well as reduces both transcription and translation errors associated with manual processing. Many of the electronic test requisition systems also enable the generation of unique specimen-Patient barcodes which also help reduce the incidences of under labeling (i.e., not providing sufficient patient information in order to support a test requisition) or mislabelling as the additional information is a required field when completing the 'on-line' form and in many cases is incorporated within the barcode generated.

Transportation and Storage of Specimens

Transportation and storage can have a significant impact on the quality of the clinical specimen and hence the accuracy and outcome of the laboratory result. For example, sputum specimens should ideally be tested within 2 h of collection and stool samples within 12 h in order to prevent background bacterial flora growth masking the virologic examination. If this is not possible then the specimens can be stored at 4–8°C but only for 48 h. So, it is essential that staff are appropriately trained and follow the correct procedures which are usually defined within the laboratory's policy on the handling and delivery of laboratory specimens and in line with the requirements of any national Health and Safety at work legislation.

The transportation of samples within the hospital or institute where the clinical virology laboratory is located is usually conducted through a trained internal porter service using containers dedicated to the movement of potentially biohazardous specimens. An approved specialist courier service will usually be contracted in order to transport specimens from external sites such as clinics, General Practitioner sites to the clinical virology laboratory. Transportation of specimens by road must comply with the carriage of dangerous goods regulations within that region (See Relevant Websites section), which state the specific packaging conditions that need to be followed dependant on the risk the viral pathogen poses. In general, most specimens such as blood, excreta, secreta, for diagnostic purposes will be transported under the category B classification unless they contain live virus cultures that are known to be life threatening to humans, under these circumstances the specimens should be transported under category A. Exempt patient specimens are those with a minimal likelihood that a viral pathogen is present and include blood transfusion products, and most dried blood spot applications.

Where specimens need to be transported by air, the regulations of the International Air Transport Association (IATA) regulations are followed. In addition, where viral specimens are being transported from one country to another for reference testing, assay verification, or quality assurance purposes, etc it is important to verify whether the pathogen is regulated under any regional Human Pathogens and Toxins Regulations which could restrict its exportation and importation. Regulations vary from

country to country dependant the apparent risk associated with the activities performed and also take into consideration whether the virus is a security sensitive biological agent.

Laboratory Specimen Retention Times

Specimen retention defines the length of time clinical specimens will be retained by a clinical virology laboratory. Retained specimens are extremely important to the laboratory for confirmatory testing where required, infection control, and public health investigations, as well as for quality control and new assay evaluation. The specimen retention time will depend on the type and origin of the specimen, the needs of the patient, and also the storage capacity and capability of the laboratory. Monitoring retained specimens with regards to events such as the number of freeze/thaw cycles, viral load over time in storage, is important in assuring the quality of the specimens, particularly if they are going to be used for quality control purposes. Many clinical virology laboratories will utilize software options in order to create an inventory to track the use of important retained clinical specimens.

Personnel Training, Competency, and Continuous Professional Development (CPD)

The clinical virology laboratory operates within a highly regulated environment and in many countries the laboratories can only perform their testing service under the appropriate legal licenses.

An important aspect of human resource management particularly within the clinical virology laboratory is to ensure that all staff have defined roles and responsibilities, and they are clear on what is expected of them within the daily operation of the clinical laboratory. The structure and organization of the clinical virology human resource is dependant on the size of the laboratory, the range and complexity of the testing service provided, the volume of annual clinical specimens, and whether it is a separate independent entity or it is affiliated to a hospital or institute as part of the total pathology services provided. An organogram for the clinical virology laboratory with supporting narrative outlining the structure and reporting relationships is usually detailed within the Quality Manual and is an essential quality assurance requirement.

The head of the laboratory (laboratory Director) will usually be a licensed Medical Virologist either with a Medical Doctorate (MD) or a Senior Clinical Scientist with a MSc, PhD, or equivalent professional qualification specialising in virology. They must ensure that the laboratory has sufficiently trained and competent staff in order to provide the scope of virology testing services specified to the appropriate regulatory standards within their jurisdiction, and where required, in line with their accreditation for example to ISO15189. The laboratory director is usually supported by a consultant clinical medical microbiologist and/or virologist responsible for the overall clinical practice and decision-making process. In line with the requirements of International Standards for Laboratory Accreditation, most laboratories will have a dedicated quality manager and nominated deputy this helps avoid potential conflicts of interest between roles as the quality manager does not have any laboratory operational responsibilities and can therefore assess and monitor the quality aspects of the laboratory impartially. Larger organizations may have a separate quality manager responsible for the whole of the pathology services provided by the hospital. The advantage of this is that they are independent of the clinical virology laboratory and can inspect and advise on quality issues. The disadvantage is they may not be familiar with the technicalities of the virology testing service offered which can potentially lead to misunderstandings.

Under the direction of the laboratory director, the clinical virology laboratory will consist of technical supervisors (or technical section leaders) who lead specific laboratory sections such as the molecular, serology, or viral isolation & culture section and are responsible for the daily management of scientific and technical staff within their respective sections. The scientific and technical staff will be responsible for specimen processing, carry out the various testing procedures and other laboratory activities such as equipment maintenance and calibration. The laboratory will also have general non-laboratory based clerical support staff performing administrative tasks including medical record keeping. Other laboratory support activities such as stock management, the procurement of laboratory supplies, and diagnostic kits would may be the responsibility of a dedicated laboratory manager or may be shared responsibilities in smaller organizations or hospitals.

The human resource (HR) files for each staff member usually contains the most up to date job description, curriculum vitae, qualifications including copies of any certificates/diplomas, and laboratory licenses in regions where this is required. In addition to this training records, and details of any continuous education programs/events the staff member has undertaken are also included within the HR or personnel file, along with any relevant publications.

Each laboratory employee should be provided with a clear training plan. This should cover the preliminary laboratory training requirements, initial duties, and responsibilities, for when the individual joins the laboratory. This should be assessed regularly and if necessary, amended in order to accurately reflect the current status of the individual followed by a further personal training and development plan which supports the employee's continued professional development (CPD) requirements in line with the objectives of the clinical virology laboratory.

The effectiveness of training and the competency of employees must also be reviewed regularly in accordance with the duties the employee is expected to perform. This can be achieved through practical laboratory assessments where the test results obtained by the employee on known QC samples or residual material from an external quality assessment cycle, are introduced into the routine workload and used as a way of checking and monitoring the employee's ability over a defined period of time. Other aspects include monitoring the adherence to laboratory policies and procedures, observing performance when carrying out instrument maintenance and calibration checks, as well as the evaluation of problem-solving and data analysis skills through set

technical questionnaires. The outcome of any competency assessment exercises are recorded within the employees training matrix and competency assessment records. If a performance issue is identified further training may be required followed by a reassessment of the employees' competency. Staff training and competency assessment are key elements within a laboratory's accreditation and some regulatory organizations such as the College of American Pathologists (CAP) in the United States specify the frequency that competency assessments must take place in relation to retraining requirements and test complexity. For example, for employees within the first year of their laboratory duties, competency must be assessed at least twice a year when engaged in moderate to high complexity testing (i.e., molecular testing).

Most clinical laboratories will also have an employee handbook which is provided to each staff member when they commence work within the clinical laboratory, this usually includes guidance to the employee on human resource policies such as performance reviews, absence and holiday policies, information technology and security, as well as health and safety aspects related to the clinical virology laboratory working environment.

Laboratory Facilities and Equipment

The quality of the test results a clinical virology laboratory provides are largely dependant on its facilities, organizational set-up, and equipment used. In general, the laboratory will be organized into functional areas designed to provide a safe and effective environment that ensures the delivery of the testing services to the appropriate quality and regulatory standards.

For example, most clinical virology laboratories will have separate functional areas along the following lines:

- specimen collection and preparation area,
- general laboratory area, stores, plant room, etc.,
- molecular virology,
- serology,
- virus culture and isolation unit (Note: in some specialist virology laboratories this could be further split dependant on the type and risk level of the viral pathogen),
- non-laboratory office area.

Each area will have its own set of operational specifications which may include temperature and cleanroom air-handling conditions. These are monitored as part of the laboratories QA measures against the specifications and defined control limits. In many laboratories the monitoring is 24/7 by way of an integrated electronic system which raises an alarm directly to laboratory management 24/7 in "real-time" should a control limit be broken, who can then take immediate action. In addition, the separation of functional activities and equipment helps prevent cross-contamination which could eventually lead to erroneous test results.

Particular attention is paid to the set-up and management of the molecular virology area as molecular amplification technics such as PCR are extremely sensitive as well as pose a significant risk of introducing amplicon-based contamination if they are not properly controlled. In order to minimize the risk, most clinical virology laboratories will use rooms with a unidirectional workflow with relative air pressures with which to separate and manage the Pre-molecular amplification activities from the Post-molecular amplification activities. The number of separate rooms or areas the laboratory implement largely depends on the facilities and space available.

Four designated areas or rooms provide the laboratory with the ability to separate the molecular activities into the reagent preparation area, the specimen extraction/processing area, the assay set-up room (template addition stage), and the molecular amplification room.

Each room has its own equipment such as biological safety hoods, pipettes, centrifuges, fridge and freezers, etc as well as a dedicated consumable/reagent store which is restricted for use within that room. Staff movement follows the direction of the workflow, and returning to previous rooms in the workflow is not allowed. Personal protective equipment (PPE) such as glove and laboratory coats are required for different rooms and is often color coded for easier visual awareness. The management of waste from the molecular facilities should be properly segregated and disposed according to the institute's disposal and infection control procedures. Amplicon contamination has been a serious problem in the past and has resulted in the shutdown of some laboratory facilities. The use of periodic wipe tests helps to monitor the laboratory environment for potential amplicon contamination. The frequency can be adjusted if any contamination is identified and thorough decontamination measures can then be put in place to rapidly address any issues found. It is also standard practice to include no template control (NTC)/negative controls in order check for potential contamination in the reagents, consumables, and general laboratory environment. Monitoring the positivity rate of the NTC over time can act as an indicator of potential contamination issues.

Equipment Qualification, Calibration, Maintenance and Monitoring

From a quality assurance perspective equipment covers instruments and software including laboratory information management systems (LIMS). Regulatory agencies (ISO15189 or equivalent) place a great deal of emphasis on the quality assurance of

equipment from initial purchase through qualification, calibration, maintenance and monitoring, to its eventual disposal at the end of its intended use and life cycle. Not only is equipment QA an important regulatory requirement, as specialist laboratory equipment is expensive the QA procedures employed can also help to increase the equipment lifespan and therefore provide an additional cost benefit to the laboratory.

Equipment Qualification

The clinical virology laboratory will have an equipment validation and/or verification plan and the initial phase of this will be equipment qualification. The laboratory will evaluate the item of equipment in consultation with the equipment manufacturer in order to define both the functional and operational specifications of the equipment as well as the cost and any preliminary installation, calibration, and training requirements. This step is often called design qualification (DQ) and is followed by installation qualification (IQ) in which the laboratory establishes that the equipment is suitable for operation and meets the manufacturers specifications as well as any regulatory claims. Operational qualification (OQ) is the third step in equipment qualification and it involves proving the equipment's performance within the clinical setting usually against a "gold standard" or predicate device. The final phase is performance qualification (PQ) where the performance of the equipment is monitored over time in the clinical setting against a defined set of performance criteria. Within industry this four-step approach has become known as the 4Qs and within clinical virology the steps are generally covered through assay verification and validation.

For the most generic equipment used within the virology laboratory such as pipettes, vortex's and microfuges the recommendations provided by the equipment manufacturer are usually sufficient for qualification for routine use. However, specialist virology equipment used for molecular and serological testing (e.g., thermal cyclers and autoanalyzer's) will require specific qualification for the equipment's intended use within the clinical context. These instruments can be fully integrated platform or cartridge based commercial assay systems which are essentially 'sample in and result out'. Alternatively, they can be component-based workflows where a combination of equipment is required to create a virology workflow which can be used to generate a test result.

This is largely the case in molecular virology, particularly for laboratory developed assays, where the extraction platform, kit, molecular amplification platform, and detection kit are combined in a molecular workflow. The virology laboratory will define the equipment qualification criterion in accordance with the manufacturers' equipment recommendations and the laboratories intended clinical use. The aim being at qualifying the laboratory equipment and monitoring performance to ensure the validity of data/result generated for the individual items of equipment as well as the whole test workflow.

Equipment Calibration

When using laboratory equipment for measurement purposes whether monitoring viral load or the temperature of the laboratory environment maintaining the quality assurance of the equipment through calibration is essential. Calibration minimizes measurement uncertainty. It also helps establish operational performance criteria which is used to monitor and control errors by ensuring that measurements remain both appropriate and acceptable.

Within the clinical virology laboratory, equipment calibration covers both the fixed plant and machinery used to control the different working environments within the clinical virology laboratory as well as the specialist instrumentation used within each of the designated laboratory areas.

Fixed plant and machinery includes the laboratory's heating, ventilation, and air conditioning (HVAC) systems which are designed to contain any potentially infectious material dependant on the biosafety classification level (1–4, with 4 being the highest risk based on the type of virus and work to be undertaken) as well as any fitted walk-in cold-rooms and specialist culture facilities. The calibration of specialist instrumentation including biosafety hoods, –80°C freezers, thermal cyclers, autopipettes, etc. assures the user that the instrument is working within the manufacturers defined specifications. The calibration of pipettes can readily be performed by the laboratory themselves using gravimetric calibration which is an accepted standard for pipette calibration. However, for most accredited laboratories the calibration of fixed plant and machinery and some specialist instruments (such as thermal cyclers) is usually conducted by the equipment manufacturer or an approved external contractor to an international standard such as ISO17025 or equivalent. If a laboratory decides to change the manufacturers calibration requirements this could result in the manufacturer's warrant for use being invalidated. It is therefore important that the laboratory discusses and agrees any proposed changes with the manufacturer before implementing them.

The frequency of calibration depends on the specific requirements of the equipment, maintenance and safety record, annual service plan, as well as the extent of its use. Calibration events are recorded within a "equipment/calibration log" which identifies the equipment by its assigned asset number.

Equipment Maintenance and Monitoring

The ongoing calibration, service, and preventive maintenance of laboratory equipment throughout its lifespan supports reliability and confidence in the performance of the equipment. Maintenance and monitoring should include a register of all equipment indicating serial numbers, assigned asset numbers as well a location within the laboratory. This is particularly important for

equipment that is designated for use within a single laboratory location. Within the equipment log, details of the validation and outcome of any safety inspections are recorded along with any equipment failures and servicing events.

Most laboratories will assign the responsibility for specific laboratory equipment to an operator or group of operators. It is their job to keep the equipment in good working order and to ensure that calibration and maintenance events are up to date as well as report any reoccurring problems to laboratory management.

Routine preventive maintenance ensures the equipment warranty in line with the manufacturer's recommendations and specified annual schedule. For example, the air filters within the HVAC system should be monitored and cleaned regularly and failure to do this could lead to potential laboratory contamination issues or safety issues as the filters become blocked and cause overheating. In addition, this can also lead to mechanical issues which shortened the equipment life span and become an additional cost burden to the laboratory.

Most of the environmental monitoring within the laboratory (such as temperature and pressure) is managed through wireless monitoring systems. These systems consist of operating software, specific probes/sensors, and receivers/transmitters for picking up the data and enable real-time performance monitoring throughout the different laboratory environments. If the central power fails, the equipment is covered by a back-up power system which ensures the integrity of the laboratory environments is maintained and monitored 24/7. The metrics from the wireless monitoring system usually have operational tolerances set which provide an automatic alarm which is set electronically to the designated operator when the metric is broken. This enables the laboratory to take immediate action as well as helps with the identification of trends over time.

Internal and External Audits

Internal and external audits are an essential part of the clinical virology laboratory's quality assurance armory. The purpose of the internal audit is to provide an objective overview of the laboratory's control and functional effectiveness over its policies, processes, and procedures in relation to the testing service it provides and the regulatory standard and environment it operates within. The quality manager in consultation with the laboratory management team will prepare an annual internal audit plan which sets out the areas to audited throughout the year in relation to the relevant clauses of the standard to which the laboratory is accredited, the date and time of the audit, and those responsible for each audit area. The depth, breadth and scope of each internal audit will vary depending on the technical complexity of the area, any findings from previous audit reports, as well as any open quality activities such as corrective actions and preventative actions (CAPA). Internal audits should only be conducted by those staff members who are trained and shown to be competent auditors. In addition, those staff members being audited need to ensure that they are fully prepared prior to the audit with a full understanding of the policies and procedures they are responsible for and a list of items that could be reviewed during the audit. This helps improve the efficiency of the audit and helps ensure that internal audits are completed within the allotted timeframe. The audit should be non-directive and allow the auditees responsible for their designated areas within the laboratory to explain their processes and procedures. The auditor is then able to gain an understanding of the staff competence, identify areas that may require improvements such as additional training, as well as potential risks or non-conformances within the written policy and procedures that could ultimately lead to a non-compliance with the regulatory standard. A documented plan can then be agreed with management outlining how any findings are to be addressed before any scheduled external regulatory audit takes place.

The external audit is an independently conducted inspection usually by a national accreditation body (NAB) which assesses the laboratory's technical competence and conformity to applicable international standard such as ISO15189 or ISO17025. The audits typically follow a four-year cycle with an annual surveillance audit over a three-year period and a re-accreditation audit every fourth year. Many countries have officially appointed National Accreditation Bodies that carry out conformity assessment and accreditation activities in particular for calibration and testing. The NAB's are subject to oversight through regional co-operative bodies such as the EA in Europe, APAC in the Asia-Pacific, IAAC in the Americas, AFRAC in Africa, SADCA in Southern Africa, and ARAC in the Arabian region, all under the umbrella of the International Accreditation Forum (IAF) which aims to promote multilateral recognition arrangements (MLA) and common recognition and harmonization of the accreditation standards and practice across geographical regions. The International Laboratory Accreditation Cooperation (ILAC) supports the interests of those accreditation bodies that deal specifically with laboratory testing (ISO17025, ISO15189), measurement and calibration (ISO17511) as well as EQA or PT provider accreditation (ISO17043).

Assay Verification and Validation

The implementation of a new assay method or the amendment of an existing assay method needs to be supported by an appropriate verification and/or validation study prior to routine use within the clinical virology laboratory. Assay verification is the process of testing and reviewing an assay's performance in relation to its known or reported performance such as against the assay manufacturers' defined performance specifications as they are stated within the package insert provided. Whereas, assay validation is the process of evaluating the assay method, establishing its performance characteristics and determining its fitness for use within the clinical virology laboratory. The verification and validation requirements depend on the type of assay, its intended use, and the amount of performance data available such as from previous clinical studies. There are numerous national and International regulatory guidelines which outline the verification, validation process as in many countries this is governed by the regulatory

Table 1 Key verification and validation parameters

Assay parameter	Description/Definition	EQA monitoring	IQC monitoring
Accuracy	The closeness between values obtained from a series of test results. Where possible within the assay verification phase the test results are compared to “known” and/or an accepted reference value	+	+++
		Based on consensus	“run to run”
Precision/imprecision	The ability to consistently produce the same result for a given test sample	+	+++
	<i>Repeatability:</i> Repeat testing under unchanged conditions for example within a single assay run (inter-assay) <i>Reproducibility:</i> Repeat testing of the same test sample in different runs across a time period, possibly using different operators (intra-assay)	Duplicate sample/lab peer review	Inter-assay (Intra-assay)
Linear/ Reportable range	This is the ability of the assay to obtain test results which are directly proportional to the concentration of the analyte within a given range	++	-
	The reportable range (also <i>Analytical Measurement Range – AMR</i>) is the interval between the upper and lower concentrations of an analyte for which the assay has acceptable levels of <i>precision, accuracy</i> and <i>linearity</i>	Paired/samples	Only if more than 1 control used
The limit of detection (LOD)	The lowest quantity of a substance that can be distinguished from the absence of that substance (a blank value) within a stated confidence limit. The limit of detection can also be determined using a serial dilution of prequalified standard or reference material spiked into an appropriate clinical matrix. The dilutions are replicate tested and the results analyzed using PROBIT analysis	+++	-
		“Educational” samples	
Limit of quantification (LOQ)	The lowest concentration at which the analyte can be reliably detected within established imprecision (and where appropriate bias) criterion	+++	+
		“Educational” samples	95% CI of +ve result
Sensitivity (analytical and clinical)	The lowest amount of target nucleic acid that still returns a true positive result.	+++	-
	<i>Qualitative assays:</i> The number of true positive samples correctly identified by the assay divided by the total number of true positive samples <i>Quantitative assays:</i> Defined from the limit of detection and where appropriate confidence interval (CI) can be calculated in order to support the chance of obtaining a true positive result	Clinically relevant/ educational samples	
Specificity (analytical and clinical)	The ability to return a “true negative” result when the target nucleic acid is not present	+++	- / +
	Consider any potential interfering or cross-reacting substances that may be present in the specimen or matrix	Clinically relevant/ educational samples	If applicable

requirement within that country and the regulatory category of the assay method. However, the general parameters covered are the same and include accuracy, precision, reportable range, linearity (for quantitative assays), reference interval, analytical sensitivity, and analytical specificity as outlined in [Table 1](#).

Where a clinical virology laboratory uses a commercially developed assay such as one regulated and approved by the Food Drug Administration (FDA) in the US or a CE marked assay in Europe, the manufacturer is responsible for providing the clinical laboratory with the analytical performance characteristics as well as its expected clinical performance and supporting clinical data. The clinical laboratory verifies the assay performance against the manufacturer's performance claims for the its intended use as defined within the instruction manual provided with the assay. Importantly, if the laboratory modifies the commercial assay and/or instructions for intended use, the assay is no longer considered a regulatory approved assay and the laboratory would be expected to fully validate the impact of these changes on the assay's performance. It is important to remember that any changes to the manufacturer's assay or protocol without prior approval could result in the manufacturer withdrawing its support for the assay. In comparison, where the assay has been developed in house by the clinical virology laboratory often referred to as a Laboratory Developed Test (LDT), the process of validating and establishing the performance of a laboratory developed assay would be more extensive and will also include comparative testing of the new test against a previous assay for the same targets as well as reference to clinical performance within the current literature if available.

In both cases, commercial assay or LDT there is a requirement for well characterized reference material and, where available, International standards in order to support the verification and validation process as well as on-going Quality Control (see also section on Internal Quality Control Procedures and Monitoring Assay Performance Over Time). The patient samples, controls, or reference material used for verification and validation should be in the appropriate matrix and at clinically appropriate levels (if known). Where there is limited reference material available or a lack of suitably characterized patient material, the laboratory can use residual material from proficiency testing (PT) or external quality assessment (EQA) schemes. The EQA materials are generally highly characterized as well as have the advantage of comparative peer group data which can help facilitate the verification and validation process. It is also good practice to use the same assay reagent lots when conducting the verification and validation exercise in order to reduce the potential impact of variation introduced through different lots/batches of assay reagents. Finally, before undertaking any verification or validation study it is important that the laboratory ensures that the assay method is calibrated to the manufacturers requirements and there are no recalibration/maintenance events scheduled for the duration of the study.

For the verification of a regulatory approved qualitative assay, the following approach and principles would apply.

Accuracy

In order to verify accuracy a comparison of methods study can be carried out. Known patient samples which cover the assay range, and have previously been tested on the current assay method the clinical virology laboratory uses, are tested on the proposed new method over a defined period of time and the results compared. If insufficient in-house patient samples are available, the laboratory may obtain specimens from other laboratories. However, when using patient samples, it is important to ensure that the specimens have been treated the same prior to testing (i.e., storage conditions, number of freeze-thaw cycles etc). An alternative is to use commercially available control or characterized reference material containing the target of interest across the assay range. The number of samples to be tested and the frequency of testing depends on the type, complexity, and intended use of the assay method. Although some regulatory authorities do provide minimum requirements or recommendations. For example, 20 patient specimens over 2–5 testing events. The qualitative data (positive, negative) obtained is analyzed statistically using Cohen's κ analysis.

Precision

The precision of the assay method would be determined by testing one or two controls at clinically relevant levels (if known) repeatedly over a specified period of time in order to establish the between run precision. The recommended minimum time period for testing is 20 days in many guidelines. However, the testing interval should be sufficient to cover the routine operation within the laboratory, such as rotation of operators. The within-run precision can also be established by running duplicate control samples over a specified time period, usually 10 days. The results are analyzed using standard deviation and coefficient of variance and compared to the claims of the assay manufacturer or the results obtained for the current laboratory method being used.

Reportable range and limit of detection (LoD)

The Reportable range of the assay is verified using known characterized positive clinical samples at clinically relevant levels or by using dilutions of commercially available standards or reference materials tested over a period of time in comparison to the current laboratory method. The LoD of the assay is verified by testing replicate samples at known levels above, at and below the determined LoD (e.g., $\text{LoD} \pm 20\%$) or as specified within the manufacturers Instruction manual in duplicate over a defined period of time (usually 20 days). The percentage detected at each level is then calculated and Probit analysis used to estimate the LoD, which can then be defined as a titer of the target analyte detected at a 95% Confidence Interval (CI).

For the verification of a regulatory approved quantitative assay, such as a molecular assay used for the determination of viral load, the same basic principles used for verifying a qualitative assay apply for accuracy and precision although they need to be verified across the analytical measurement range (AMR) of the assay and in particular at known clinically relevant or clinical decision making concentrations.

Linearity/analytical measurement range (AMR)

In the context of a molecular viral load assay, the AMR or reportable range can be defined as the linear range of test values over which the laboratory can accurately detect the target viral nucleic acid within acceptable degrees of variation. Beyond the manufacturers stated limit of

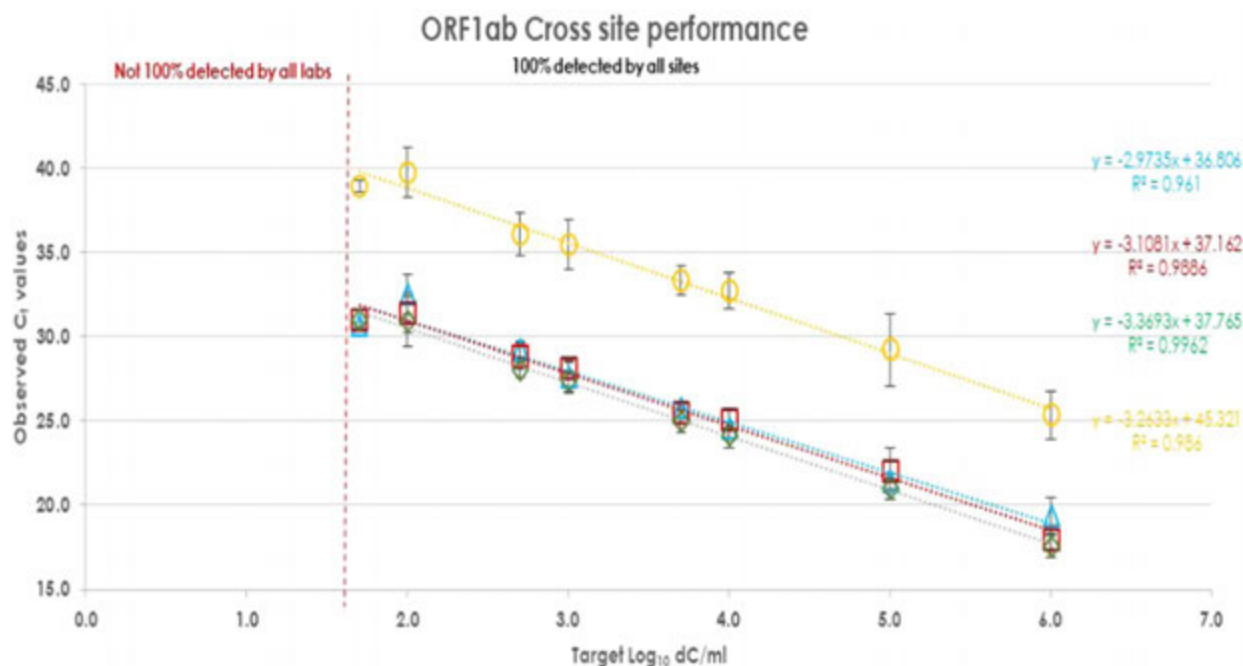


Fig. 2 Graph showing the linear relationship of test results across multiple clinical laboratories for a dilution series of SARS-CoV2.

quantitation (LoQ) the relationship between the measured and actual viral load concentration may not be linear and test results become unreliable. It is therefore important that the laboratory verify the AMR in the clinical setting they intend to use the assay method and in line with the manufacturer's instructions and claims. In some cases, the manufacturer may only have performed an analytical assessment of AMR on contrived or synthesized control materials such as target viral nucleic acid in a plasmid construct and not true clinical samples at relevant titers. As a result, it may not be possible to establish the full analytical AMR equivalence with the manufacturer's specifications. In these circumstances the laboratory should ensure that they assess the AMR within the known clinical context and in line with the laboratory's pre-defined AMR acceptance criteria. At a minimum this should include samples at the low, medium, and high points of the AMR. Polynomial Regression analysis is used to assess the linearity of the assay across all relevant concentrations and if the relationship is found to be non-linear (not first order) those concentrations are removed from the analysis at the high and/or low end and the regression analysis repeated. The testing is usually performed in replicates (2 – 4) at each concentration and precision assessed across the linear range. The highest and lowest concentrations that produce a linear relationship are defined as the upper and lower limits of quantification. (Fig. 2) Where the AMR results are compared against the manufacturer's claims or against the AMR of the comparative method the laboratory is currently using the proportional bias, as measured by the slope of the regression curve, and the constant or systemic bias, as measured by the y-intercept, are determined. A Bland-Altman difference plot is used to measure bias between assays.

Most regulatory standards (such as ISO15189) require the laboratory to have a written policy on verification and validation. This defined the methods, frequency and limits of verification for an assay method. In general, the AMR should be verified in line with the following criteria:

- at changes of reagent lots, unless the laboratory can demonstrate that the use of different lots does not affect the accuracy of patient test results and the range used to report patient test data,
- if QC materials reflect an unusual trend or shift or are outside of the laboratory's acceptable limits, and other means of assessing and correcting unacceptable control values fail to identify and correct the problem,
- after major equipment maintenance or service,
- when recommended by the assay manufacturer.

In addition to this, some regulatory organizations may also specify the frequency of AMR verification, such as every 6 months (e.g., CAP). In most cases the laboratory will only test a smaller sub-set of linearity samples (3 – 4) at the low, mid, and high points within the AMR and only conduct further more extensive testing if these samples fall outside the acceptable criteria.

Sensitivity, Specificity, and Clinical Utility

Key factors that can impact on the accuracy of a test to deliver a result are sensitivity and specificity as well as the prevalence of the disease in the population targeted by the assay method. In the context of a serology assay either targeted at detecting the viral antigen directly or through the indirect detection of antibodies to the viral pathogen, the diagnostic sensitivity and specificity are derived from test results on clinical samples obtained from selected reference patient groups within the population. The degree to

which the reference groups represent all of the host and environmental factors within the total population targeted by the assay method can have a major impact on the accuracy of test result and its interpretation in the clinical setting. Therefore, the clinical virology laboratory also needs to consider this during the verification and validation phase, which is an ongoing process which continues for as long as the assay method is being used within the laboratory and requires constant vigilance, review, and where required reassessment. Specificity also includes any potential interfering or cross-reacting substances that may be present in the specimen or matrix. Interference is basically any non-target organism or substance that can cause a false negative result. Whereas, cross reactivity is a non-target organism or substance that can cause a false positive result. With molecular based assays this can be a problem, particularly when the assays are aimed at differentiating related viral strains. The assay manufacturer or laboratory who developed the assay should have chosen their primers and probes cautiously as well as made an initial "in silico" assessment of the available databases before testing in practice with known samples that are related taxonomically and also epidemiologically. The list of potential cross-reacting and interfering substances is usually documented within the manufacturer's instruction manual. An assessment of analytical specificity should include testing the potential cross-reacting or interfering substance at the known highest concentration that could be expected to be observed within the patient sample. A common approach, in the absence of sufficiently available clinical samples, is to use spike samples in negative matrix and also weakly positive samples (e.g., LoD + 10%) for each of the analytes of interest. From this the minimum inhibitory concentrations can be determined. For quantitative assays any potential cross-reacting targets should be assessed near the Lower Limit of Quantitation (LLOQ) and the quantified target values from spiked and unspiked samples compared and determined to be within the defined precision limits of assay.

Many variables can influence the performance of the assay method, such as the prevalence of the viral disease, the clinical setting, and type of test being performed. Therefore during the clinical evaluation of the assay it is important to consider the testing purpose such as whether it is for diagnosis, screening, or therapeutic monitoring purposes; the environment the testing is likely to take place in such as the central clinical laboratory, out-reach clinic, or point of care setting; the specimen type (e.g., whole blood, plasma, sputum etc); type of result such as quantitative or qualitative result. Two other important measures of assay performance are positive predictive value (PPV), the probability that those testing positive by the test have the viral disease, and the negative predictive value (NPV), the probability that those tested negative by the test are do not have the viral disease. Both PPV and NPV depend on the sensitivity and specificity of the test and also the prevalence of the viral disease within the population which in turn gives the assay its clinical utility. In terms of the number of specimens that should be tested during clinical evaluation in order to assess PPV and NPV, some guidelines recommend 100 negative clinical samples for the evaluation of false positive rates, and 50 clinical samples known to be positive for the target viral pathogen are considered appropriate for determining the false positive rates. The CLSI guidelines (CLSI EP12-A2) recommend a minimum 50 positive and 50 negative clinical specimens. In low prevalent diseases, it can be difficult to obtain sufficient clinical samples to conduct a full clinical evaluation. In these circumstances the clinical laboratory has to consider alternative approaches such as including materials used in previous proficiency testing/EQA scheme challenges.

Internal Quality Assessment (IQA)

"Split sampling" is an effective additional IQA measure for monitoring the whole laboratory testing process. This is where between 0.5%–1.0% of a laboratories clinical patient sample workload are subjected to internal quality assessment. The randomly selected patient samples are split and a new internal testing request generated with a unique laboratory IQA code. This IQA sample is processed through the laboratory in parallel to the original clinical patient sample. At the end of the testing process, results are generated for both original clinical patient sample as well as the IQA sample. These can then be compared internally by the laboratory quality team and any inconsistencies within the results and the report are investigated and corrective action taken through the laboratories quality system. The split sample approach is not always practice or appropriate particularly when the volume of the patient sample is small.

Internal Quality Control Procedures and Monitoring Assay Performance Over Time

Assay variation is the result of systematic and random error which is inherent to the assay method. Common sources of error or variation can be attributed to operator, instrument, reagent lot, calibrator and calibration cycle. The amount and type of variation associated with an assay method is determined during assay verification and/or validation. Having established the extent and where known the clinical relevance of the variation it is important that the laboratory continues to monitor the variation to ensure that it remains within acceptable levels. This is done through Internal Quality Control on a daily run to run basis and is often a requirement of many regulatory bodies (including CLIA and ISO). In general, a commercial assay will be supplied with a set of controls. The intended use of these controls is to monitor the assay in line with the manufacturers defined performance criteria. As such, the manufacturers kit controls are intended for use only with the manufacturers assay and in addition are often specific to a particular kit batch or reagent lot. This can mean that the manufacturers kit controls do not support the monitoring of different reagent lots over time and would not necessarily identify a drift in the overall assay performance over time (assay drift). So, it is important that the operator is aware of the potential limitation of the manufacturers kit controls. For example, many of the controls provided with the manufacturers kit controls only monitor as specific part of the process such as with molecular tests where the manufacturers kit controls may just be free nucleic acid and would therefore only be suitable for controlling the

amplification/detection steps of the assay and not the whole testing process from sample pre-treatment through the extraction phase, amplification and detection, to end result.

Consequently, many regulatory and standards organizations either recommend as “best practice” or in some cases insist on the use of independent third-party controls either instead of, or in addition to those supplied by the assay manufacturer in order to demonstrate compliance. Such recommendations are made at the International level (ISO15189, CLSI) as well as the national level (e.g., RiliBak in Germany). Independent third-party controls are also often referred to as external quality controls or run controls. They aim to monitor the complete testing process and provide an accurate, unbiased measure of quality performance independent of the manufacturers specific control or calibrator.

In general, for qualitative tests a minimum of a positive and negative control is included in each assay as specified in the laboratory procedure. This should also take into consideration the assay manufacturer’s instructions. For multiplex assays covering several different viral targets in a single assay, the laboratory may choose to rotate different positive controls for different targets within the multiplex assay over a given time period at a frequency defined within their laboratory procedure.

For quantitative tests including molecular viral load assays, the laboratory should ensure that the controls it uses are at or near clinically relevant decision levels, where known and/or as specified within the current clinical guidelines. For viral load assays it is common practice to determine performance characteristics for the control under routine laboratory conditions by testing the control over a period of time (usually a minimum of 20 times) in order to obtain sufficient data points to determine the mean and standard deviation from which suitable control limits can be established. Some laboratories calculate a rolling mean based on the last set of observations reported in order to reduce the initial amount of testing and re-evaluate the mean and control limits after sufficient datasets are obtained. Where a Laboratory is using more than one of the same instruments/platforms, they would generally perform the study on one instrument and then perform a comparison testing to confirm performance on the other instruments. Alternatively, the laboratory could opt to run alternate QC runs across platform instruments. Alternative approaches have also been suggested for establishing and monitoring the performance of serology assays based on extended historical data and peer group assessment. This is covered within article by Sally Baylis *et al.*

The most frequently used control rules applied within the clinical laboratory are those defined by Westgard (See Relevant Websites section). The performance can be monitored graphically by plotting the measurements in Levey-Jennings control charts and any deviations from the expected performance can be investigated and the appropriate corrective action taken if required. There are numerous web-based software packages available for the reporting and monitoring quality control data. Some such as Acusera 24.7 (See Relevant Websites section) offer LIMS connectivity, interlaboratory peer group assessment, as well as the ability for the laboratory to further interrogate parameters such as specific operator, and instrument associated with a particular such set of QC data. This enables the laboratory to proactively monitor QC performance and identify important trends or shifts in expected results over time which may impact on the assay performance and ultimately the patient test results.

Suitable Standards, Reference Materials and Controls to Support Quality Assurance in Virology

International standards are essential to quality assurance in virology as they provide the means for the calibration of quality control and EQA materials which in turn help support the comparability of results across assay methods and provide confidence in the ability of the laboratory to deliver accurate and reproducible patient test results. In particular, the utility of viral load assays has improved significantly through the calibration of assays to the available International Standards particularly where patient management depends on the ability to relate patient results to prior results or to defined viral load values which support clinical decision making in line clinical practice, such as in the monitoring of post-transplant CMV infections.

The development of WHO International standards is covered in chapter (Sally Baylis), however in the context of clinical virology, and in particular the quantitation of viral nucleic acid, the concepts of metrological traceability as defined in ISO17511 (In vitro diagnostic medical devices – Requirements for establishing metrological traceability of values assigned to calibrators, trueness control materials and human samples) which are widely used in other areas of laboratory medicine such as clinical chemistry can generally be applied to molecular viral load assays. Metrological traceability describes the steps through which a patient test result can be traced back to a relevant higher order standard and the uncertainty of measurement in relation to each step estimated in a common unit of measurement. The highest order of standard would be one which is ultimately traceable to the metric system/international system of units (SI). WHO International standards are derived by consensus and are not ultimately traceable back to an SI based value and do not have an assigned uncertainty of measurement nevertheless their utility and importance in driving standardization in molecular virology continues to be demonstrated.

Additional to the WHO initiatives, other international organization such as NIST (National Institute of Standards and Technologies) and LGC (Laboratory of the Government Chemist) have supported the development of methods such as Digital PCR (dPCR) which enables the counting of individual target viral nucleic acid molecules. Recent interlaboratory studies have demonstrated the accuracy and reproducibility of dPCR for the absolute quantification of DNA copy number concentration in comparison to techniques such as flow cytometry and isotope dilution-mass spectrometry. Thus, the inclusion of dPCR within the latest version of ISO17511 means it has the potential to become a recognized SI-traceable primary reference measurement procedure. However, dPCR still has limitation in the same context as any other PCR based methodology such as assay bias. Therefore, understanding the relationship between International Units (IU) and digital copies in a clinical context and across different non-PCR based molecular technologies such as Transcription Mediated Activation (TMA) used by Hologic, Strand

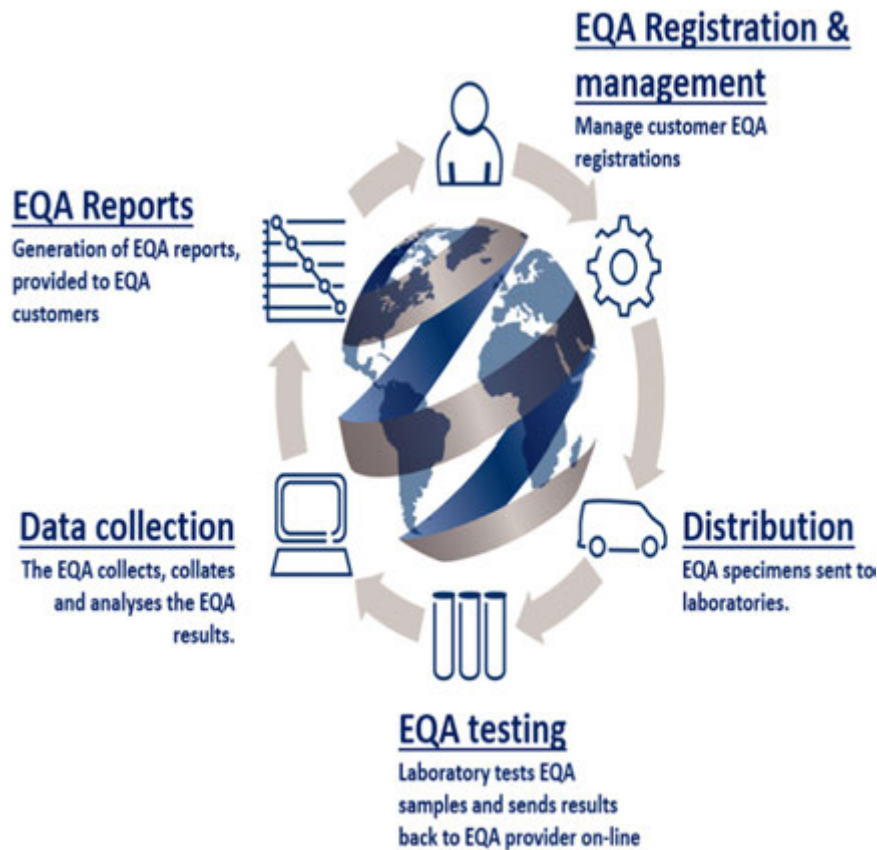


Fig. 3 Schematic of the EQA cycle.

Displacement Amplification (SDA) employed by BD ProbeTec, etc is essential in further supporting standardization. In addition, the commutability of quality control or reference materials needs to be demonstrated relative to the patient specimen and assay technology it is tested on in order to further support comparability of results.

The development of an International standard is primarily based on public health need. However relative to the number of viral pathogens routinely tested in the clinical laboratory there are very few International standards and certified reference materials (CRM) available with which to calibrate secondary standards and commercially available controls. Therefore, technologies such as dPCR and Next Generation Sequencing (NGS) enable commercial control manufacturers to molecularly characterized the materials in the absence of an International standard. When an International standard becomes available these materials can then be back calibrated to the standard to achieve further traceability.

External Quality Assessment and Performance Criteria

External quality assessment (EQA) or proficiency testing (PT) is integral to quality assurance within the clinical virology laboratory. It provides assurance and confidence in the laboratory's procedures and service provision. It also enables the laboratory to compare its performance to other laboratories carrying out similar tests, as well as provides an indication of areas for quality improvements. The participation in EQA is often an accreditation requirement as defined in ISO15189 and more recently ISO22870 which covers the specific use of point of care testing within the clinical laboratory setting. In many countries it can be a mandatory requirement with laboratories required to report their EQA performance to the national body within that region. In these circumstances, EQA is often linked to the test reimbursement policy within specific countries. The EQA provider, is also required to ensure that the EQA schemes it provides fulfill the requirements of ISO17043 (Conformity assessment – General requirements for proficiency testing) (Fig. 3).

The EQA cycle starts with the EQA provider, supplying registered laboratories with "blinded" clinically represented samples at predetermined intervals throughout the year. The laboratory is required to test the EQA samples using its routine laboratory method and return their results through a web-based portal to the EQA provider within a defined period of time. Data analysis is carried out by the EQA provider and an EQA report which details the laboratories individual results and peer group performance is provided to the clinical laboratory along with a certificate of participation. In regions where there are defined national EQA performance criteria for specific target analytes, such as in Germany for the viral load determination of HIV, HBV, and HCV, the EQA provider will also report whether the laboratory has achieved the required level of proficiency.

Individual Report		QCMD 2020 Human Immunodeficiency Virus RNA EQA Programme					
Catalogue Code: QAV994108	Ref Code: HIVRNA20	Challenge: C1	Analysis Type: Qualitative and Quantitative	Dataset:	Report UID:	Laboratory	

Intended Results / Panel Composition

Sample Code	Sample Content	Matrix	Sample Relationships ^[1]	Detection Frequency ^[2]	Sample Status ^[3]	Consensus (Copies/ml) ^[4]		Range ^[5]
						(Log ₁₀)	(n)	
HIVRNA20C1-01	HIV Type 1B	Plasma	D1	Frequently Detected	CORE	2.721	225	1.886 - 3.793
HIVRNA20C1-02	HIV Type 1B	Plasma	D1	Frequently Detected	CORE	2.731	226	0.602 - 3.811
HIVRNA20C1-03	HIV Type 1C	Plasma		Frequently Detected	CORE	3.744	225	0.602 - 4.342
HIVRNA20C1-04	HIV Negative	Plasma		Negative	CORE	N/A	N/A	N/A

Fig. 4 Example of an HIV viral load EQA panel composition.

EQA Design and Objectives

As molecular diagnostics account for the majority of the test output of the clinical virology laboratory, EQA design has progressed in order to meet increasing demand. Specialist EQA providers such as QCMD (See "Relevant Websites section") which focus specifically on the provision of EQA within the area of molecular infectious disease offer a growing range of schemes covering viral quantitation, as well as the genotypic analysis for the detection and typing of drug resistance variants based on nucleic acid sequencing using techniques such as NGS.

The design and objectives of a clinical virology EQA scheme is dependant on many factors such as the type of method (e.g., qualitative, quantitative, sequencing, typing), its intended use, the matrix. In addition to this, in some countries where there are mandatory EQA schemes the regional regulatory bodies will often define the number and type of EQA samples that can be included within the EQA panel as well as the frequency of distribution to the laboratory (e.g., once every 6 months in the US and in Germany).

Within the example shown in [Fig. 4](#), the EQA panel consisted of two different viral strains of HIV, at a range of different clinically appropriate viral loads with the lower titer sample included to assess the sensitivity of the participating laboratory tests. In addition to this the panel also contained a negative matrix only sample in order to assess absolute specificity/false positivity. The inclusion of duplicate samples within an EQA challenge and across EQA distributions also supports the evaluation of homogeneity and reproducibility of the EQA panel members.

For many quantitative EQA schemes, the assessment of performance is based on the consensus mean of results returned by all laboratories which tested the EQA sample, once any outlying values have been identified statistically and removed from the initial analysis. Alternatively, the consensus mean of the assigned method or technology peer group can also be used, such as in the example in [Fig. 5\(a\)](#) and [\(b\)](#).

In both approaches the mean and standard deviation are calculated accordingly and a sample performance score based on the distance the laboratories result is from the consensus mean is established. In the example provided, zero points if the result is within one standard deviation from the mean. One penalty point if the result is between one and two standard deviations, two penalty points within two and three standard deviations, and three penalty points if the result is more than three standard deviations from the mean.

The regional regulatory bodies have oversight of viral diagnostic testing for regulated analytes such as the blood borne viruses HIV, HBV, and HCV in some countries and define the minimum performance criteria for laboratories participating in these EQA schemes. For qualitative tests, failure of the laboratory to obtain results within 80% of the overall consensus is considered unsatisfactory and laboratories which fall outside this range require investigation, the outcome of which may involve escalation to the regulatory body.

a**Your Summary Results**

Units	Copies/ml
EQA Assessment Group ^[1]	Abbott Real Time PCR
Core Panel Detection (Qualitative) Score ^[2]	0
Core Panel Estimation (Quantitative) Score ^[3]	0

Core Panel Members Results

Sample Code	Unitage	EQA Assessment Group Consensus ^[4]	SD ^[5]	Quantitative Result		Qualitative Result		
				Your Result ^[6]	Estimation Score ^[7]	Percentage Correct (All) ^[8]	Your Result ^[9]	Detection Score ^[10]
HIVRNA20C1-01	Copies/ml	2.742	0.114	2.760	0	99.3	Positive	0
HIVRNA20C1-02	Copies/ml	2.720	0.151	2.610	0	100.0	Positive	0
HIVRNA20C1-03	Copies/ml	3.546	0.149	3.525	0	98.5	Positive	0
HIVRNA20C1-04	Copies/ml	N/A	-	LOD/NR	N/A	99.3	Negative	0

Fig. 5 Example of performance scoring within the HIV viral load EQA scheme. (a) Qualitative and quantitative panel score. (b) Individual sample analysis (quantitative).

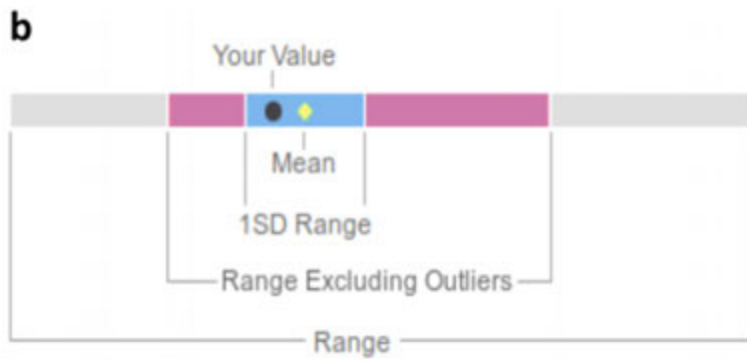
It is important that clinical virology laboratory management and operational staff evaluate the outcome of the EQA schemes through regular quality meetings. A preliminary step is ensuring there are no administrative transcription errors which could inadvertently affect performance. The laboratory should also compare their results to previous EQA distributions and in line with known performance characteristics for the assay as defined through verification/validation and IQC monitoring. Where the IQC indicates that the performance of the assay is acceptable but the EQA indicates a possible quality issue, the laboratory should investigate further and if required obtain further information from the EQA provider as well as the assay manufacturer (if the assay used is commercially available).

As well as meeting regional regulatory requirements, EQA schemes also provide the laboratory with educational feedback in relation to the test coverage for current clinically relevant viral strains, the incidence of false positive results, and differences in laboratory performance with a specific assay or technology. This also includes the monitoring of observed trends in laboratory EQA reporting over time, such as the move from reporting results in copies/ml and the gradual increase in the reporting of EQA results in IU/ml following the introduction of the first WHO International Standard for CMV viral quantitation and more recently the range of gene targets in assays targeting SARS-CoV-2 (Fig. 6(a) and (b)).

The aim is to ensure optimal quality performance and compliance with the accreditation standard and regulatory authority. In addition, participation in EQA schemes also helps identify possible areas for improvement and ensure that the assay is clinically fit for purpose.

Summary and Future Aspects of QA to Virology Laboratory

Viral tests are fundamental for patient management in diagnosis, infection control, and disease outbreaks where the rapid assessments of disease burden and progression is essential for evaluating the effectiveness of interventions and the verification of disease status. Technologies such as Next Generation Sequencing (NGS) and metagenomics allow the whole genome characterization at the nucleotide



Sample Code	Sample Content	Matrix	Sample Relationships	Detection Frequency	Sample Status	Consensus (Copies/ml)		Range
						(Log ₁₀)	(n)	
HIVRNA20C1-01	HIV Type 1B	Plasma	D1	Frequently Detected	CORE	2.721	225	1.886 - 3.793

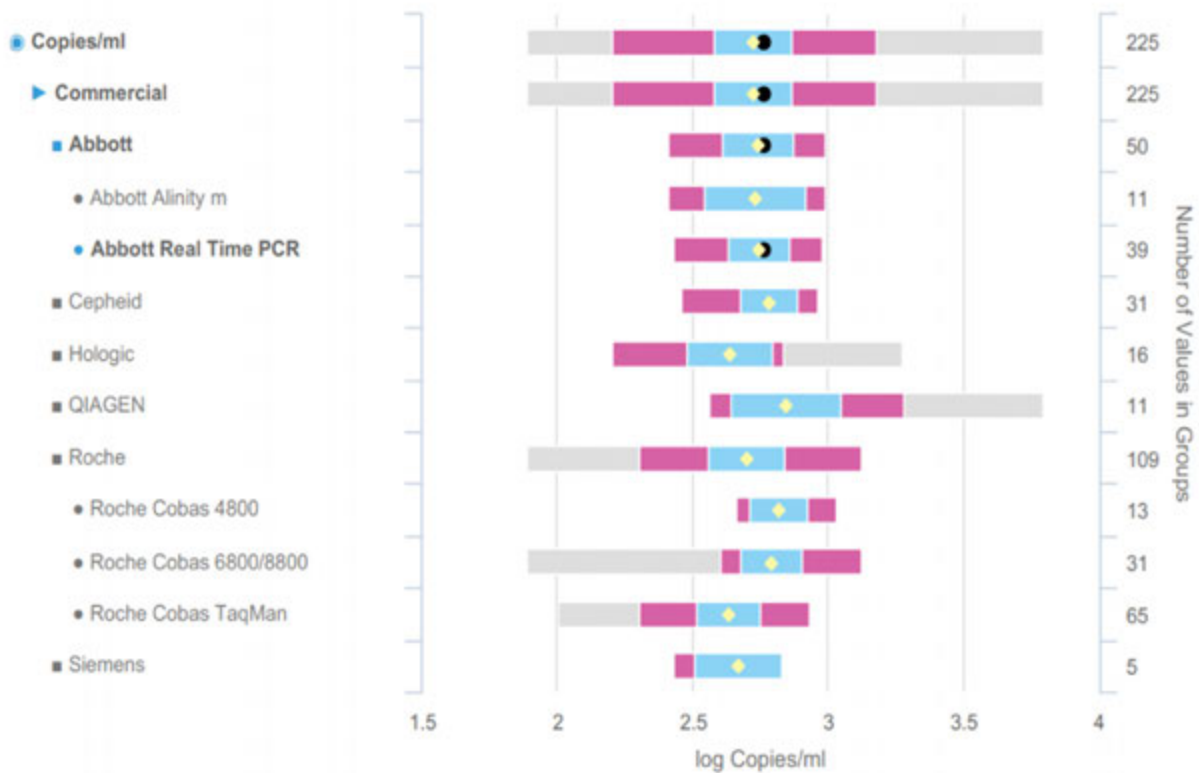


Fig. 5 Continue.

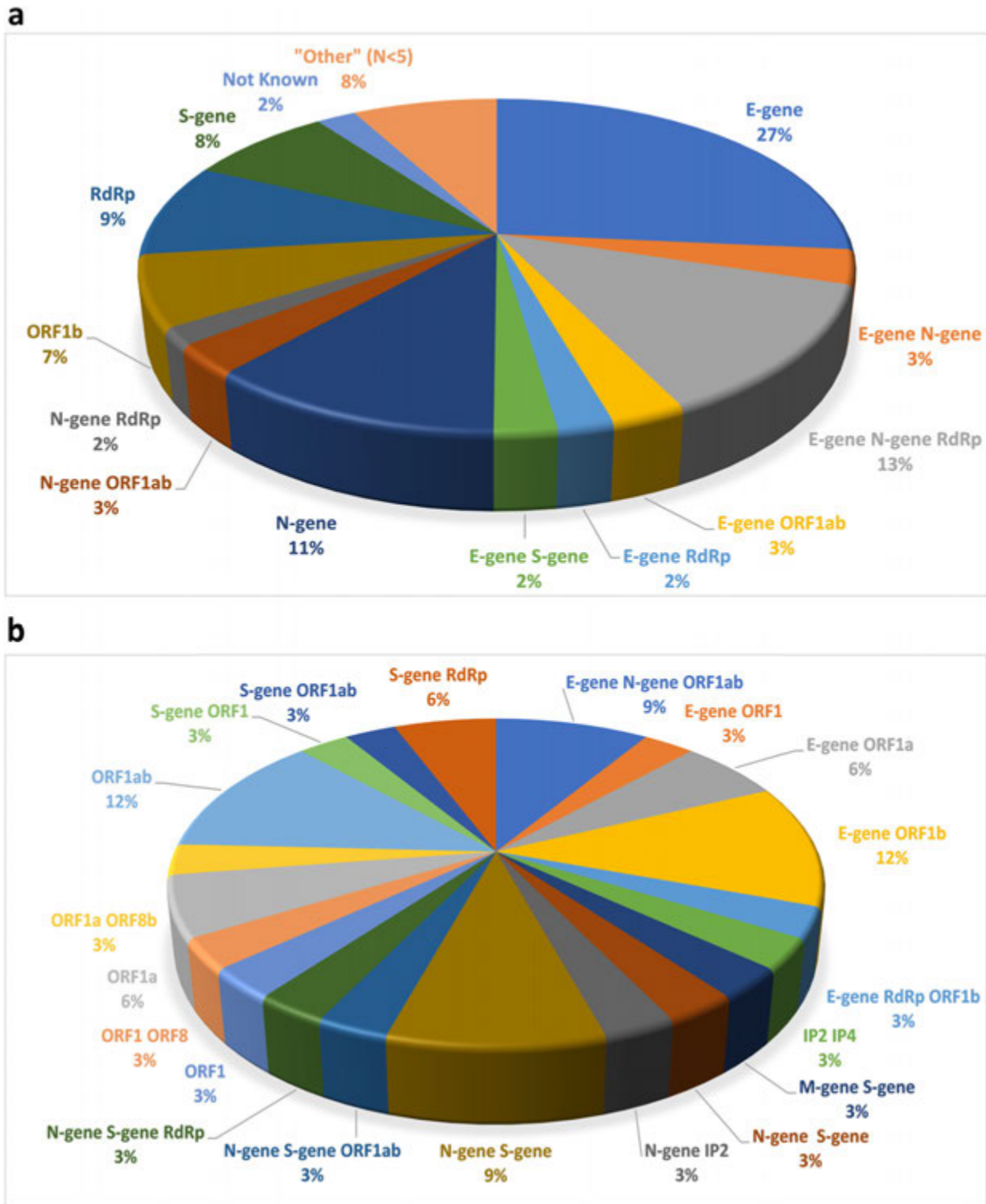


Fig. 6 The range of different target gene loci reportedly used by laboratories for the detection of SARS-CoV2 within the QCMD EQA distribution (n=1011 laboratories). (a) Major gene targets/gene combinations used. (b) Less commonly reported gene targets.

level which as well as enhancing diagnostics also aids rapid epidemiology and infection control which is particularly important in relation to the recent Coronavirus pandemic. These technologies require advanced analytical and artificial intelligence software algorithms which support the extraction of diagnostic information. This poses additional challenges in quality assurance which will need to be addressed before such technologies can reach full routine potential and further drive diagnostic improvements as they become more accurate, simple, and affordable for use across a wide range of intended clinical settings.

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Relevant Websites

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Bureau International des Poids et Mesures.
- <https://doi.org/10.1093/clinchem/hvaa125>
Clinical Chemistry,
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College of American Pathologists.
- <https://clsi.org/>
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- www.idealab.com
IdeaGen.
- <https://www.iso.org/>
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- www.westgard.com
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- <https://www.who.int/>
World Health Organization.