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Review Article

Quality Assurance in the molecular diagnostic laboratory

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Abstract
Molecular diagnostics is currently playing an imperative role in the clinical microbiology laboratory. Quality assurance and quality control issues have often remained underdeveloped in our country and are still critical. To relate patient results to these, molecular diagnostics carry values in clinical diagnosis, therapeutic drug monitoring and remission and those results need to be comparable across time and methods. This may be achieved either by producing the identical value across methods and test versions or by using reliable and stable reference materials in the medical laboratories engaged with molecular tests. The establishment of international standards and reference materials, assessment and maintenance of technical competencies, regulatory oversight and best practices considerations are thus of utmost importance. This review focuses on general and specific issues relevant for quality assurance and quality control in the routine molecular diagnostics laboratory.

Tasks related to validation and verification

"Lab tests guide more than 70% of medical decisions"- this notion in practice requires accreditation that a diagnostic lab perform all its assays following established validation protocols and practices relevant for quality control and quality assurance. At present, the commonest test performed in the Molecular Lab is Polymerase Chain Reaction (PCR), which consists of a series of varied steps- nucleic acid extraction, quantitation, nucleic acid amplification and detection of the amplified products. Each of these steps requires different reagents and tools that demands adequate verification and calibration. It is pertinent to evaluate the adequacy of the specimens for the prevalence of the disease and the mutation of variants.

According to ISO 9001: 2008, validation and verification work is defined as confirmation through the provision of objective evidence that requirements for a specific intended use or application have been fulfilled[1]. Additionally, ISO 15189: 2012 recommends "The laboratory shall use only examination procedures that have been validated or verified by the laboratory for the intended use," in case of introducing a new test or in-house test. In accordance with the terminology of ISO 15189: 2012, a "validated examination procedure" is considered equivalent to the terminology "standard method." Simply put, validation is required to introduce a new technology, for which no performance characteristics is available whereas verification determines whether a test is being performed correctly[2].

Frequent confusion may arise concerning the related terms validation and verification. Consequently, the question arises when to validate or when to verify a test method? For validation and verification purpose, the diagnostic molecular laboratory needs to consider the performance characteristics of a particular test. Performance characteristics include the following parameters: measurement trueness; measurement accuracy; measurement precision; measurement uncertainty; analytical specificity and sensitivity, detection and quantitation limit; and measuring interval, diagnostic specificity and sensitivity of the measurement[2]. The performance characteristics of a reagent for instance, may

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be already established and supplied with the manufacturer's protocol or it may be collected from relevant articles in a peer-reviewd journal or from an accreditated laboratory; where the reagent is already validated. However, a number of tests need to be performed in the laboratory conditions, to verify whether the acceptance criteria have been met. Therefore, if a test procedure, or reagent is validated elsewhere, the laboratory requires verification of the validated data in its own lab environment. On the contrary, if the laboratory requires establishing the performance of a new reagent, or it may require modifying a certain step of a procedure; a series of tests need to be performed, documented and evaluated before setting up a "standard" protocol.

Components of validation[3]

1. Quality control: internal controls, external controls, and reference materials.
2. Proficiency testing participation for comparison of inter-laboratory test results.
3. Employee competency.
4. Instrument maintenance and calibration.
5. Correlation with clinical findings.

1. Quality control

To ensure that a certain nucleic acid sequence is correctly amplified without any interference from inhibitors that may give rise to false negative results, internal control (IC) must be included in a test run alongside sample sequence. An IC is defined as a sequence of nucleic acid that is amplified together with the target nucleic acid in the same reaction, but maybe differentiated from the target amplicon using a sequence specific probe tagged with a reporting dye that fluoresce with a different wavelength from that of the target specific probe[3,4]. Internal controls can be of two types- homologous and heterologous as mentioned in the flow chart below[4]

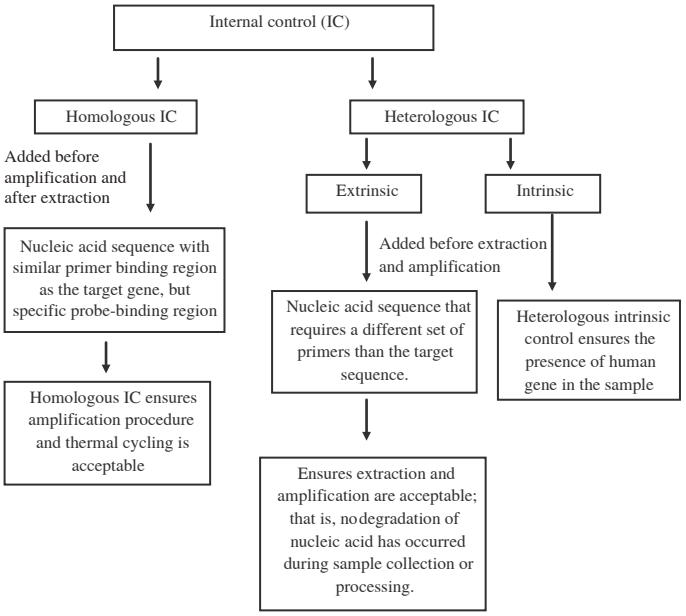

Fig: Work flow chart showing use of internal control in molecular tests.

External control: External controls are known positive or negative clinical specimens that must be run alongside the unknown sample in order to validate the integrity of the reagent[4].

External quality control: In the present scenario, quality control of COVID-19 PCR test is maintained at regular intervals using known positive/ negative samples by the diagnostic laboratories. The Institute of Epidemiology, Disease Control and Research (IEDCR) is a Bangladesh Government research institute, under the Ministry of Health, responsible for researching epidemiological and communicable diseases in Bangladesh as well as disease control. After every 2 months, as per IEDCR requisition and protocol, diagnostic laboratories send reports of demographic data of 10 positive and 5 negative COVID-19 samples with CT-values of the gene detected. The tests are further conducted, evaluated and reported by IEDCR to each of the diagnostic laboratory, thus maintaining external quality surveillance service.

Reference Materials

An essential element of quality assurance in the molecular laboratory is the utilization of well defined, easily comprehensible and accessible reference materials. The Committee on reference materials (REMCO) of the International Organization for Standardization (ISO) has

defined the term "reference material" as "material sufficiently homogenous and stable with respect to one or more specified properties, which has been established to be fit for its intended use in a measurement process"[5]. Well-characterized reference materials play a fundamental role in the implementation of quality control of molecular tests, verification of tests as well as performance characteristics, error detection, proficiency testing and additionally cross-check the performance of IVD/CE labeled or FDA approved molecular tests. However, in comparison to the growth of the molecular diagnostic industry, the quantity and availability of reference materials are still meager. Considering the present and future needs of molecular tests, regulatory bodies should institute and prioritize the establishment of reference materials in order to ensure patient safety and patient outcome. The table below summarizes the sources of available reference materials for molecular diagnostics tests.

Table 1: Sources of reference materials in the context of major molecular tests[6]

Institution	Types of reference materials:	Infectious disease	Heritable genetics	Molecular oncology
NIBSC, WHO[7]	International Standards	✓	✓	✓
NIST, Department of Commerce, National Metrology Institute USA[8]	Standard reference materials	✓	✓	
IRMM, Joint Research Center, National Metrology Institute EU[9]	Certified reference materials		✓	
US FDA, HHS USA[10]	FDA-cleared reference materials and controls (instrument platforms and assays)	✓	✓	
GeT-RM, Center for Disease Control, HHS USA[11]	Information resource GeT-RM program studies	✓	✓	✓

**GeT-RM: Genetic Testing Reference Materials Coordination Program; HSS: Department of Health and Human Services; IRMM: Institute for Reference Materials and Measurements; NIBSC: National Institute for Biological Standards and Control; NIST: National Institute of Standards and Technology.

2. Proficiency Testing

The concept of proficiency testing is relatively new in our country's perspective. Proficiency testing, in broad terms, is the process of inter-laboratory comparison of a certain test to evaluate its testing performance. While participating in proficiency testing, a medical laboratory must provide a series of clinical specimens along with testing results to a second participating laboratory. The second laboratory performs the tests with the provided specimens in their clinical setting and sends a report. A deviation in a single parameter of the testing system does not necessarily mean the abandonment of the entire test, but attention must be given on that particular parameter to overcome this deviation. The proficiency testing report of a certain test must include test results, reports and signature of the lab coordinator. Medical laboratories must be able to submit this report to the National body of accreditation when they are seeking accreditation of a certain test or a number of tests.

In Europe, several proficiency programs for molecular tests are being conducted efficiently such as the European Commission funded Equal-qual[12], commercially available projects QCMD, NEQAS and INSTAND[13,14,15]. In Bangladesh, although no such commercial projects are available for proficiency testing, few medical laboratories have begun to conduct inter-laboratory comparisons (ILC) of molecular tests with Bangabandhu Sheikh Mujib Medical University (BSMMU) in order to be able to submit the report to Bangladesh Accreditation Board (BAB) for accreditation of molecular tests.

Employee Competency

All the staffs working in a molecular laboratory must be sufficiently competent to perform the tests and deliver reports efficiently. The employees have to be aware of the advancement of latest test procedures and technologies. The laboratories shall encourage employees to participate in trainings and workshops on molecular diagnostics to improve their knowledge and skill that will aid in maintaining a constant level of expertise at the work station.

3. Instrument maintenance and calibration

An integral part of quality control and quality assessment of a molecular laboratory is the maintenance and calibration of instruments at a regular interval. The time of the installation of an instrument, calibration according to manufacturer's protocol, any required servicing shall be documented and all records shall be preserved.

4. Correlation with clinical findings

In order to avoid false positive and false negative results in the laboratory test results, the diagnostic sensitivity and specificity of a certain test has to be significantly considered. The diagnostic sensitivity of a clinical test refers to the ability of the test to correctly identify those patients with the disease. Here, the clinical disorder must be characterized by clinical criteria independent of the test system. On the contrary, the diagnostic specificity of a clinical test refers to the ability of the test to correctly identify those patients without the disease, thus avoiding false positive results.

Components of verification

In case of IVD/CE or FDA approved tests, the components of verification are as tabulated below:

Table 2: Components of verification and their assigned value of acceptance[3]

Components	Assigned value of the accepted reference material
Accuracy	Positive[a]
	Low positive[b]
Imprecision	Negative
	Positive[a]
	Low positive[b]
Analytic measuring range	Positive[a]
Recovery	Positive[a]

[a] More than 1 log10 over the limit of detection (LOD) and within the upper limit of linearity of the appropriate reference material.
[b] Upto 1 log10 over the LOD of the appropriate reference material.
[c] In case of quantitative test.

In case of introducing a new test by the laboratory, a few additional components need to be verified that include testing of reproducibility, analytic specificity, determining the limit of detection (LOD) in case of a qualitative molecular test or determining the limit of quantitation (LOQ) in case of a quantitative molecular test. The results need to be validated employing standard statistical methods.

Documentation of data: According to good Laboratory Practises, data should be documented related to completeness, consistency, accuracy and reconstructability.

SOP manual should be in place regarding procedures for reagent receipt, storage and preparation, instrument operation and calibration logs, instrument maintenance and repair logs, freezer logs, inventory logs, biosafety cabinet log, results and report format and protocols. Lab users should be aware of the intended use of the test he is doing in the lab test methods, analytical and clinical validity information, limitation of the tests, whether this test is FDA approved, and sample collection procedures clearly explained in the document and verbally.

Reporting of molecular test results

Treatment of a patient significantly depends on the report obtained from the laboratory. It is of utmost importance that laboratory results be reported accurately, clearly and briefly. Significant effort has been made to develop and refine methods for the detection of trace levels of DNA. Such methods are proving to be immensely useful in the fields of public health and laboratory medicine. However, little has been done for the standardization of data handling from these methods. Standardization will benefit in analysis and interpretation of the generated data.

Qualitative results are rather simple to interpret, indicating the presence or absence of nucleic acid in a clinical specimen, either in terms of "positive/ detected" or "negative/ not detected." The report must always include the LOD of the assay for ease of interpretation.

Quantitative molecular assay results may be of three types: 1) if the value acquired is above the limit of quantitation (LOQ), it is reported as "above the analytic measuring range. 2) If the value is within the analytic measuring range, the actual quantity along with the unit shall be reported. 3) Finally, if the value obtained falls below the LOQ, it is either reported as "not detected" or "not accurately quantifiable." Consequently, the analytic measuring range must be included in the laboratory report[3].

Conclusion

Molecular diagnostics is a thriving industry in Bangladesh, but standardization of assays and quality control assessment procedures requires vast attention. Common standards (ISO 9001:2008 and ISO 15189) for validation work are available but there are no conclusive materials for verification needed for introducing a new or in-house test. Before introducing a molecular test with clinical specimen, all tests require extensive verification. Reporting a molecular test result with interpretive guidelines is of imperative significance as it guides patient diagnosis and treatment outcome.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.

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