Quality assurance of molecular methods in diagnosis of Human papillomavirus

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Quality Control vs. Quality Assurance

Quality control emphasizes testing of products to uncover defects and reporting to management who decides whether to allow or deny product release.

Quality assurance attempts to improve and stabilize production and its associated processes to avoid, or at least minimize, issues which led to the defect(s) in the first place.

Quality Assurance and Quality Management

- Establish, verify, maintain performance specifications
- Total testing process, including the pre-analytical, analytical, and post-analytic phases of testing

Laboratory quality assurance

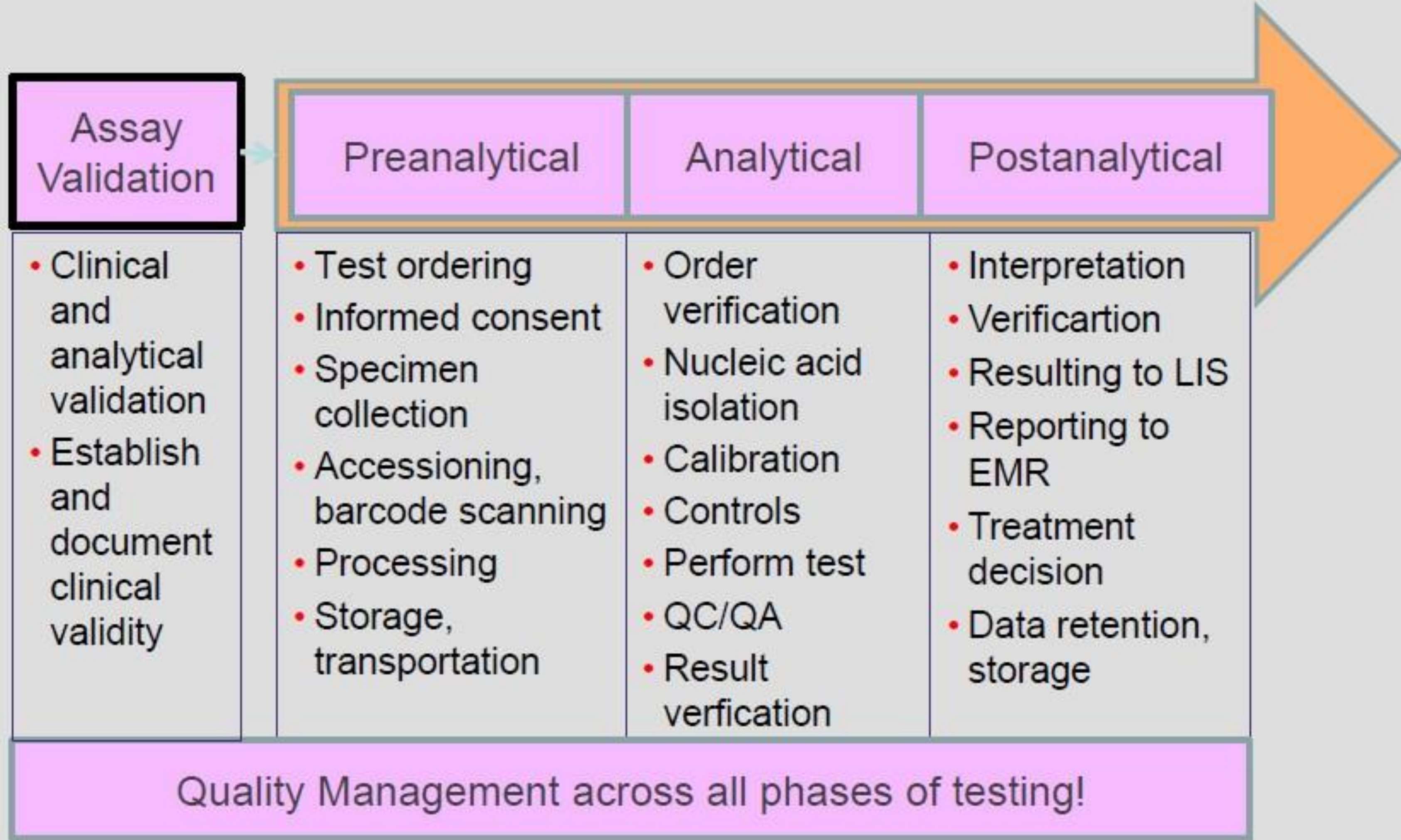
Laboratory quality assurance (LQA) is concerned with the organizational processes and the conditions under which laboratory activities are planned, performed, monitored, recorded and reported. Adherence by laboratories to the principles of LQA ensures the proper planning of activities and the provision of adequate means to carry them out.

It promotes full and accurate reporting, and provides the means whereby the integrity of activities can be verified.

Setting up a LQA system in a laboratory means defining the organizational structure, responsibilities, procedures, processes and resources necessary to achieve the following objectives:

- prevent risks;
- detect deviations;
- correct errors;
- improve efficiency;
- ensure data quality and integrity.

It is the responsibility of the head of the laboratory to establish, implement and ensure compliance with LQA. However, LQA is the responsibility of all laboratory personnel.



Total Laboratory Testing Process
Assay Validation
Preanalytical
Analytical
Postanalytical
• Clinical and analytical validation
• Establish and document clinical validity
• Test ordering
• Informed consent
• Specimen collection
• Accessioning, barcode scanning
• Processing
• Storage, transportation
• Order verification
• Nucleic acid isolation
• Calibration
• Controls
• Perform test
• QC/QA
• Result verfication
• Interpretation
• Verificartion
• Resulting to LIS
• Reporting to EMR
• Treatment decision
• Data retention, storage
Quality Management across all phases of testing!

QUALITY MANAGEMENT AND IMPROVEMENT

Quality Systems Assessment

An ongoing review process encompassing all facets of the laboratory's technical and nontechnical functions

Laboratory directors must show evidence that they actively participate in this process.

Preanalytical systems quality assessment

- Test request forms
- Appropriate specimen collection and handling
- Appropriate criteria for rejection of specimens
- Informed consent forms

Test requisition form – by CLIA

- Name and other suitable identifiers of the authorized person requesting the test
- Patient name and any other unique identifiers
- Indication for testing and relevant clinical information
- Patient's gender and date of birth
- Patient racial/ethnic information, if applicable to test methods and result interpretation
- Information on patient family history or pedigree, or both, that is pertinent to the disease or condition being evaluated or the testing to be performed
- The tests to be performed
- Source of the specimen
- Date and time of specimen collection
- International classification of diseases (ICD code)

Analytical systems quality assessment

- Procedure manual
- Nucleic acid extraction and specimen storage
- Laboratory design
- Laboratory practices
- Controls (positive, negative, amplification, sensitivity; external)
- Test validation
- Maintenance of equipment
- Competency of personnel
- Proficiency testing and accreditation

The analytical testing phase

- CLIA requires laboratories to have procedures in place to monitor and minimize contamination during the testing process and to ensure a unidirectional workflow for amplification procedures that are not contained in closed systems

- SEPARATE AREAS FOR:

- Reagent preparation

- Sample preparation

- Amplification area (neg. pressure, if PCR)

- Post-PCR area (if amplicons are manipulated)

- Use no-template control to detect contamination

Post-analytical systems quality assessment

- Laboratory test reports
- Timeliness of reporting
- Correction of errors
- Patient confidentiality

The post-analytical testing phase

- Molecular diagnostics test reports: by CLIA Language should be understandable by nongeneticist health professionals and other specific users of the test results.

The reports should contain:

- Patient's name and identification number or a unique patient identifier and identification number
- Name and address of laboratory where the test was performed
- Indication for testing
- Test performed Specimen source (when appropriate) Test results and (if applicable) units of measurement or interpretation
- Result interpretation, recommendation or guidance

Assay Validation for detection of HPV DNA

Assays for the detection of HPV DNA must be fit for their intended purpose and be validated for use with the specified samples, i.e. cervical swabs and/or biopsies.

The analytical procedure is defined as the complete procedure from start of sample collection through extraction of nucleic acid to completed HPV typing.

Validation is the process of establishing documented evidence to provide a high degree of assurance that a procedure will consistently perform as intended, that is, to demonstrate that a procedure is suitable for its intended purpose.

Separate validations are required for different elements of a procedure (e.g. the equipment and reagents used in a test may need validation as well as the test as a whole).

Assay Validation for detection of HPV DNA

For HPV DNA detection assays all steps of the process must be validated, including sample preparation, DNA isolation, and amplification and detection.

Assay validation must be demonstrated for each sample type tested, e.g. cervical swabs, biopsies, urine.

The method must also demonstrate lack of interference/ PCR inhibition for all types of samples being tested.

The inclusion of an internal control in the material to be extracted will validate assays on individual samples.

Assay Validation for detection of HPV DNA

In-house tests need full validation. Appropriate parameters must be assessed as not all are relevant to every test procedure.

Once a procedure has been validated, it is expected that it will continue to perform as validated, but this needs to be checked by monitoring test performance.

If modifications are made to the method, or problems occur or equipment is changed or relocated, revalidation needs to be performed.

Assay Validation for detection of HPV DNA

Parameters to be examined will depend on whether the assay is qualitative or quantitative. These may include the following:

- **Sensitivity/limit of detection:** the proportion of samples that contain a specific HPV type that are reported to contain that type or lowest detectable IU (genome equivalents) of virus/PCR.

- **Specificity**: the proportion of reported detections of an HPV type where the reported HPV type was truly present in the sample.

- **Accuracy**: the ability to estimate the correct amount of a substance in a sample.

Assay Validation for detection of HPV DNA

- **Reproducibility**: the ability to obtain the same results on repeat analysis. Reproducibility may be assessed for intra- or inter-lot changes of reagents within the same, or between different techniques or laboratories.

- **Robustness**: a measure of the ability of an assay to tolerate changes in methodology without affecting results, e.g. whether allowed deviations in incubation time will affect results.

- **Interference**: a measure of whether some substances in clinical samples interfere with analysis, e.g. by PCR inhibition. Interference may be assessed by spiking samples with potentially interfering substances, such as crude cell lysate, urine, or transport medium.

Quality assurance specific for HPV DNA testing

Documentation

For biological tests, such as nucleic acid testing, specific problems may arise that may influence both the validation and the interpretation of results.

All procedures should be documented including methods and reporting forms. These should be assigned a version number and be authorized before use.

If any changes are made to the document, a new version must be created and the previous version archived.

The SOPs should cover:

- mode of sampling (type of container, etc.);
- preparation of mini-pools (where appropriate);
- conditions of storage before analysis;
- an exact description of the test conditions, including precautions taken to prevent cross- contamination or destruction of the viral DNA, reagents and reference preparations used;
- an exact description of the apparatus used;
- the detailed formulae for calculation of results, including statistical evaluation.

Standard Operating Procedures

- A brief summary of the assay and its purpose. The agents or genes and/or mutations tested, reference sequences, primer sequences, probe sequences, etc.
- Scope
- Responsibility
- Definitions and acronyms
- Policy
- Material and equipment
- Specimen
- Standard safety precautions
- Quality-control procedures and control materials
- Interfering substances
- Reagents and consumables
- Step-by-step procedures for performing the assay
- Guidance on result interpretation
- Method limitations
- Procedure notes
- Appendix: forms
- List of related internal documents
- References

Operator training and qualification

An appropriate operator qualification programme should be implemented for each operator involved in the testing.

To confirm successful training, each operator should test at least eight replicate samples of diluents/matrix spiked with HPV DNA to a final concentration of three times the assay detection limit.

This test (eight replicate samples) should be repeated twice on two separate days, i.e. a total of 24 tests performed on three different days.

The tests should be performed using a properly calibrated and controlled assay. All replicate results should be positive.

Equipment qualification

An appropriate installation and operation qualification programme should be implemented for each critical piece of the equipment used.

Confirmation of analytical procedure performance after change of critical equipment (e.g. thermocyclers) should be documented by conducting a parallel test on eight replicate samples of diluents/matrix or negative clinical sample spiked with HPV DNA to a final concentration of three times the assay detection limit.

All results should be positive.

Issues to be considered during performance of an assay

Prevention of contamination

The risk of contamination requires a strict segregation of the work areas for the different steps in the procedure. Points to consider include: movement of personnel; gowning, cleaning, material flow and air supply, and decontamination procedures.

The work area should be sub-divided into compartments such as:

- master mix area or "clean" (area where exclusively template-free material is handled, e.g. primers, buffers, etc.);
- pre-PCR or "extraction" (area where reagents, samples and controls are handled);
- PCR amplification or "apparatus" (amplified material is handled in a closed system);
- post-PCR or "detection" (the only area where the amplified material is handled in an open system).

Quality control of reagents

All reagents crucial for the methodology used have to be controlled prior to use in routine applications. Their acceptance/withdrawal is based on pre-defined quality criteria.

Primers are a crucial component of the PCR assay and, as such, their design, purity and the validation of their use in a PCR assay require careful attention.

Primers may be modified (for example, by conjugation with a fluorophore or antigen) in order to permit a specific method of detection of the amplicon, provided such modifications do not affect accurate and efficient amplification of the target sequence.

Inclusion of run controls

The use of a suitable run control (for example, an appropriate dilution of an HPV working standard/run control or an HPV sample calibrated against the appropriate WHO HPV international standard (IS), NIBSC code 06/202 (HPV16 DNA) or 06/206 (HPV18 DNA) can be considered a satisfactory system for continuous monitoring of performance.

Each run should contain:

• positive controls; these contain a defined amount of the HPV types to be tested.

The amount of plasmid to include should correspond to the required detection limit for approval of a test run as a valid test; typically 50 IU of HPV16 DNA, 50 IU of HPV18 DNA and 500 GE, per 5 µL of other HPV types tested for.

— Inclusion of a second "low positive" control that contains even lower amounts of virus and does not affect classification of a test as valid, can be useful for sensitive monitoring of fluctuations in assay sensitivity.

• Negative control: a sample of a suitable matrix already proven to be free of the target sequences.

Determination of the validity of an individual assay

A valid result is obtained within a test only if the positive control(s) included in the same test run is unambiguously positive, and the negative control(s) is unambiguously negative. The data review should also include a "common sense" evaluation for signs of contamination, such as a weak positive in well adjacent to strong positive, or unusual run of the same result.

A strategy for handling an ambiguous test result should be documented. This may include re-extraction and assay of the sample, re-amplification of the extracted DNA using another assay, DNA sequencing, or submission of the sample to a reference laboratory.

External quality assessment

Participation in external quality-assessment programmes (proficiency panel testing and/or organized referral of part of the analysed samples to a reference laboratory), is an important PCR quality-assurance procedure for each laboratory and each operator.

Test material

Because of the high sensitivity of PCR, the samples must be protected against external contamination with target sequences. Sampling, storage and transport of the test material are performed under conditions that minimize degradation of the target sequence.

When preparing samples, the target sequence to be amplified needs to be efficiently extracted in a reproducible manner. A variety of physico-chemical extraction procedures and/or enrichment procedures may be employed. The selected extraction procedure should have minimal loss of viral DNA (that is, have high recovery) and result in samples with sufficiently pure DNA to not cause inhibition of PCR reactions.

PCR tests for a common housekeeping gene (typically the betaglobin gene), may be used as a control for extraction of amplifiable material without significant inhibitors originating from the test material.

Validation of an assay

This will include for both *qualitative* and *quantitative* assays:

- specificity;
- detection limit;
- robustness;
- precision.

Validation parameters

If the assay is to be used on different types of samples (e.g. human cells, tissue, blood, plasma, or urine) each type of sample should be validated for the following criteria.

Specificity

Specificity is the proportion of reported detections of an HPV type where the reported HPV type was truly present in the sample. Specificity may depend on components that can be present in the sample, e.g. cross-reacting HPV types, bacterial infections and human DNA.

The specificity of nucleic-acid amplification analytical procedures is dependent on the choice of primers, the choice of probe for analysis of the final product, and the stringency of the test conditions (for both the amplification and detection steps).

In order to validate the specificity of the analytical procedure, at least 100 samples negative for the HPV type in question should be tested and shown to be non-reactive.

The use of plasmid preparations containing potentially cross-reacting HPV types, added to human HPV-negative cell lines and/or to clinical samples known to be HPV-negative, is recommended for assessing specificity.

Proficiency panels, such as those prepared by, and available to, the WHO HPV LabNet, may be used to assess specificity.

Specificity assessments should also consider that subtypes and variants of HPV may be present in clinical samples.

Detection limit

The detection limit of an individual analytical procedure is the lowest amount of nucleic acid in a sample which can be detected (but not necessarily quantified as an exact value).

The detection limit is, for practical purposes, estimated as the minimum amount of HPV DNA per sample which can be detected in 95% of test runs. To determine an assay's detection limit, which may vary in individual analytical test runs, a dilution series of a working reagent, which has been calibrated against the WHO HPV DNA IS, should be tested on at least three different days.

At least 24 test results for each dilution is required for statistical analysis of the results. For example, a laboratory could test three dilution series on different days with eight replicates for each dilution, four dilution series on different days with six replicates for each dilution, or six dilution series on different days with four replicates for each dilution.

In order to minimize the number of required dilutions, a preliminary test (using, for example, log dilutions of a standard spiked in a diluent/matrix or in a negative clinical sample) could be done in order to obtain a preliminary value for the detection limit (simply the highest dilution giving a positive signal). The range of dilutions can then be chosen around the preliminary detection limit (using, for example, a dilution factor of 0.5 log or less).

The concentration of HPV DNA which can be detected in 95% of test runs can then be calculated using standard statistical methods.

The same set of experiments may also be used to demonstrate the intra-assay variation and the day-to-day variation of the analytical procedure.

Robustness

The robustness of an analytical procedure is a measure of its capacity to remain unaffected by allowed variations in method parameters, and provides an indication of its reliability during normal usage.

Evaluation of robustness should be considered during the development phase.

For nucleic-acid amplification technique (NAT), small variations in the method parameters can be crucial. Examples of critical parameters include the exact concentrations of reagents (e.g. $MgCl_2$, primers or dNTP) and incubation times and temperatures. When data estimating how small variations in such parameters affect detection limit are known, they should be explicitly stated in the SOP. To estimate robustness, the detection limit is estimated (as above) using the maximum and minimum range of allowable variations in method parameters.

Precision

Precision expresses the closeness of agreement between a series of measurements obtained from multiple sampling of the same homogenous sample.

Intra-assay precision expresses the precision under the same operating conditions over a short interval of time; it is assessed by using one assay and testing three replicates of appropriate dilutions of an HPV DNA-positive sample suitably calibrated in IU and covering the whole quantitative range of the assay; the coefficient of variation for the individual samples is calculated.

با تشکر از توجه شما