Standards in Molecular Diagnostic Lab

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Definition

Quality assurance encompasses all procedures and activities directed toward ensuring that a specified quality of product is achieved and maintained. Quality assurance systems and must address all phases as follows:

1. the preanalytical phase;
2. the analytical phase; and
3. the postanalytical phase

QA investment

The amount of ongoing quality assurance and quality control performed will depend on the **volume and frequency** of the testing, as well as the **type and format of test employed** (i.e. approved vs home-brew" assay).

QA activities

1. SOPs
2. Training
3. Statistical QC
4. Documentation
5. Review

Preanalytical Phase

Sample collection and storage

Special attention must be paid to **specimen collection, transport, and storage.** Since in many situations, the reported result will influence prognostic or treatment decisions, the **value must be reflective of patient status**, not specimen handling.

Reagents: Materials Handling

Components and reagents from manufacturers' assays should be checked for physical integrity upon arrival, receipt date recorded, and then promptly stored in **controlled-temperature** areas according to the package insert.

Instruments/Equipment

The manufacturers' instructions for maintaining, checking, and calibrating the instruments should minimally be followed and documented. However, it is incumbent on laboratory directors to assess new instruments and decide if the manufacturers' directions are adequate.

Analytical Phase

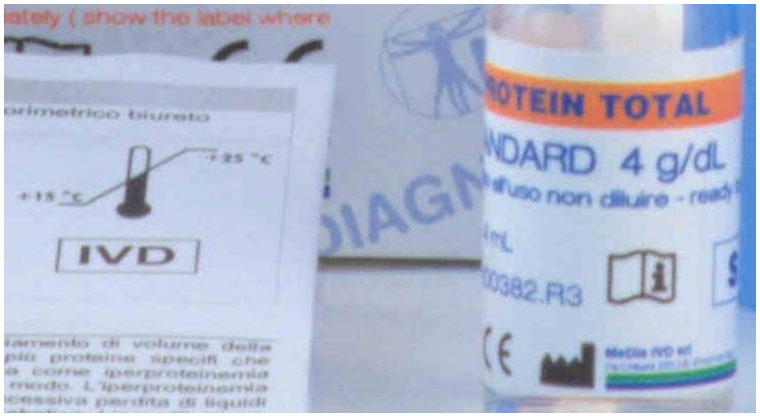
Verification and Validation

Activities that are performed **prior to the routine implementation** of a procedure in the clinical laboratory.

The term **verification** will be used to denote those activities that provide confirmation that specified requirements have been met (i.e., **analytically**). The term **validation** will denote those activities providing confirmation that requirements for specific intended use have been met (i.e., **clinically**).

Analytical Phase Quality Control

1. Regulatory agency-cleared or -approved test, manufacturer's test kit used according to manufacturer's instructions: Analytical quality control of the whole system with **kit controls** and **at least one long-term independent control**.
2. Adapted or changed ("off-label") use of a manufacturer's kit: Quality control on each **new lot of the changed component** may have to be performed (or certificates of analysis received) prior to analytical quality control of the whole system with kit controls and **at least one long-term independent control**.

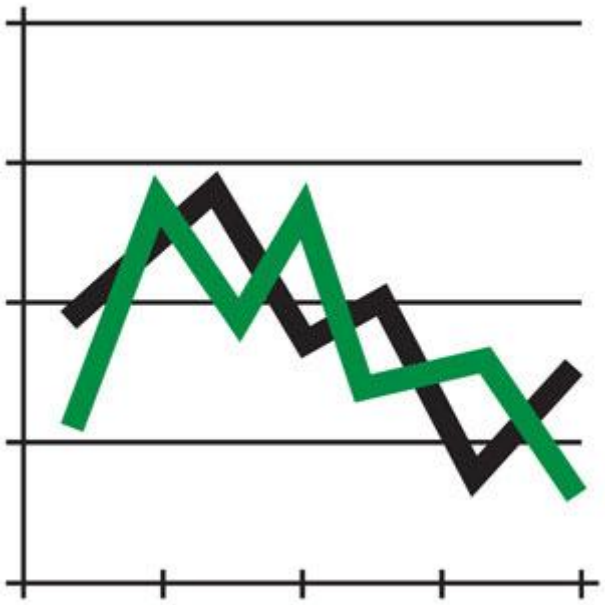


Analytical Phase Quality Control

1. "For research use only" or "For investigational use only" manufacturer's test kit: With each **new lot** of reagents, analytical quality control of the whole system with **kit controls** and **at least one long-term independent control**.
2. Laboratory-developed test/"home-brew" assay: Quality control on each **new lot of the changed component** may have to be performed (or certificates of analysis received) prior to analytical quality control of the **whole system with in-house run controls** and at least one long-term independent control.

Statistical Quality Control

In fact, only by running an independent control can a laboratory track trends and shifts in the manner that clinical chemists have traditionally done.

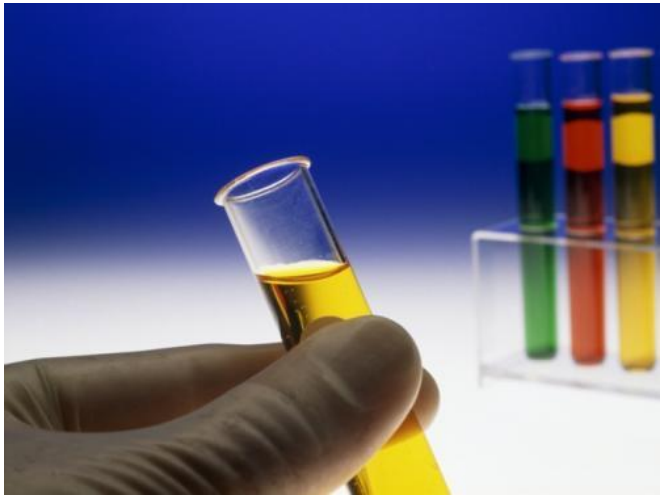


External Controls

- **Reagent Blanks** (Nontemplate Controls): Applicable reagent controls should be interspersed within each amplification batch run. These controls contain all of the necessary components of the reaction without the addition of template nucleic acid.
- **Negative Controls** Negative controls should contain known nontarget nucleic acid rather than only water or buffer. Always dispense and transfer reagents to negative controls last so that they reflect cumulative effects during manipulations.

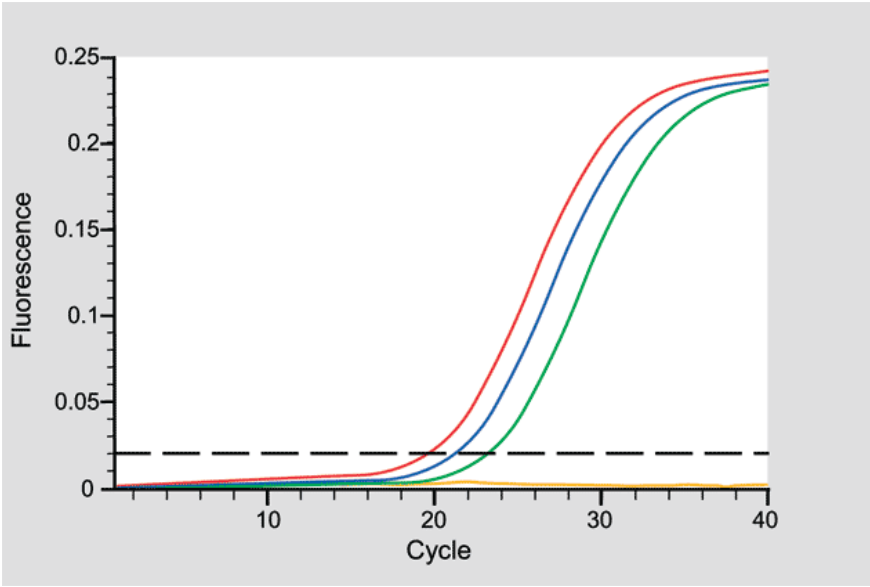
External Controls

Positive Controls: A positive control that has a low concentration of target nucleic acid and amplifies weakly, but consistently should be selected.



Internal Control

An advantage of using an internal calibrator is that it allows for, but generally does not distinguish between, detection of inhibitors and recognition of nucleic loss during extraction. When added **prior to specimen extraction**, internal calibrators will undergo the same assay process as the specimen itself.



Postanalytical Phase

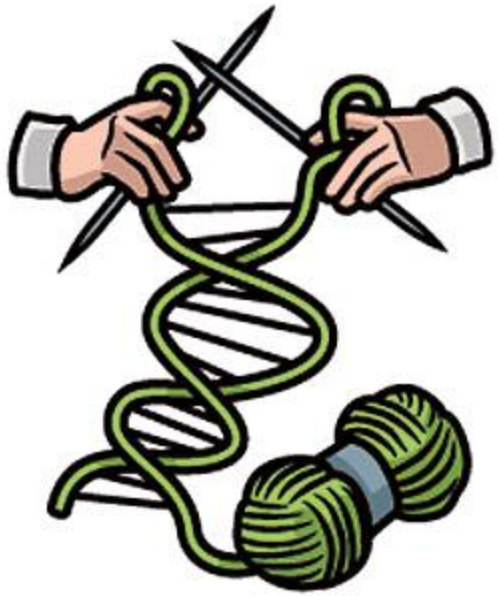
Reporting the Result

To avoid confusion, laboratories may include an appropriate description of the method used, the **nucleic acid target**, **reference range**, and any **limitations of the test** in the final report. Additional clarifying statements are required for in-laboratory-developed methods using analyte-specific reagents

Organism and Nucleic Acid Target

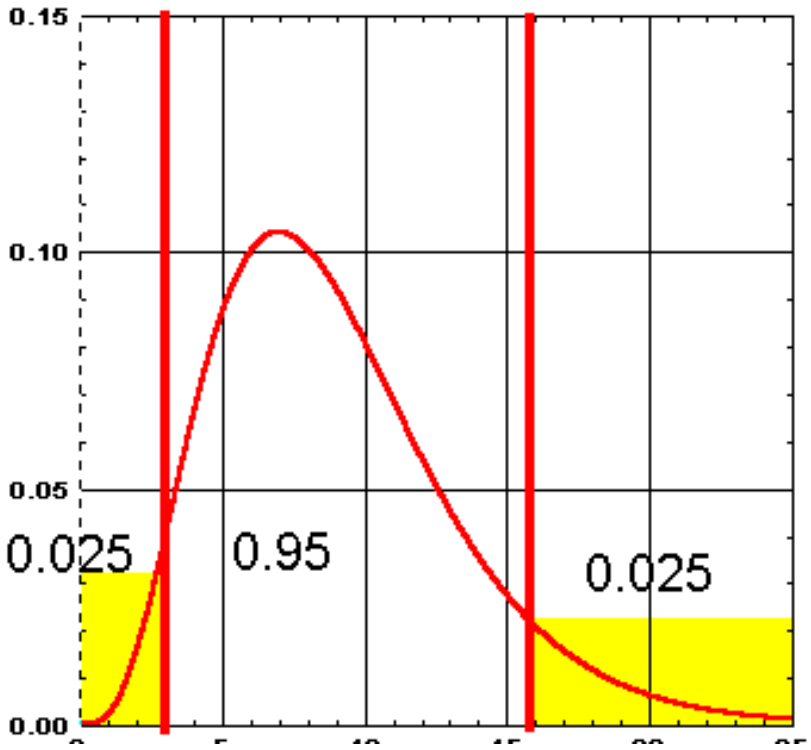
e.g., “HBV DNA detected,” “*Chlamydia trachomatis* plasmid DNA detected,” or “HCV RNA detected”

If a test has an established equivocal range around the cutoff value, then the results falling in that range can be reported as “**equivocal**” or “**indeterminate**.”



Reference Range

A reference range for a qualitative test should be stated as less than the lower limit of detection, or not detected. For example, the reference range for an HCV RNA test with an LoD of 50 IU/ml would be “< 50 IU/ml,” or “not detected.”



Critical Results

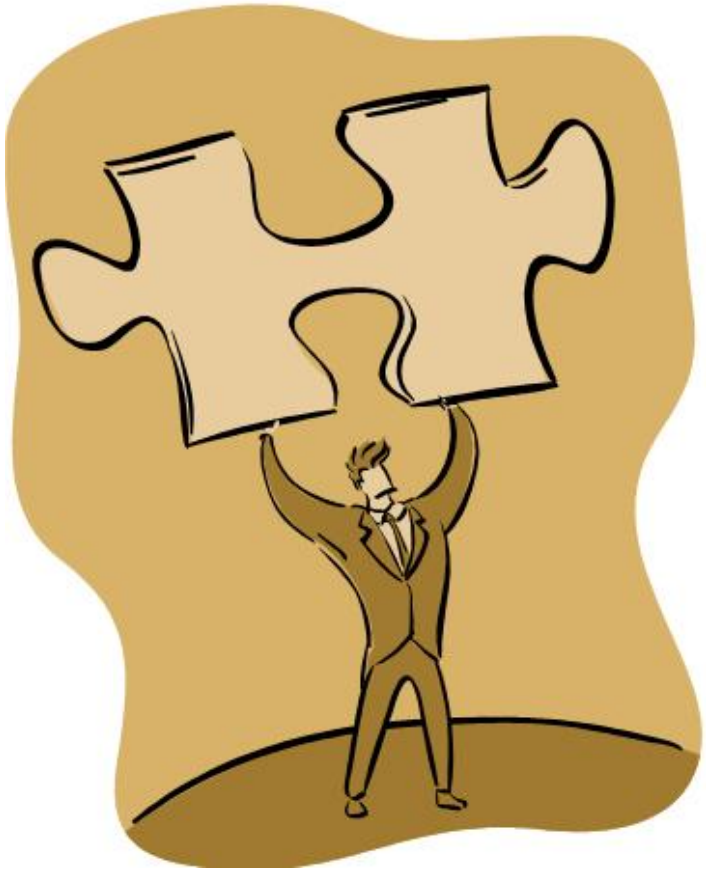
Critical results must be defined for tests that significantly **impact patient management decisions**, and procedures must be in place for prompt notification of a physician or other healthcare provider. Persons performing the tests must be familiar with the critical results for the procedures they perform.

Test Limitations

Any known clinically significant limitations of the test should be indicated in the report. These could include **cross-reactions**, genotype bias, and the presence of **interfering substances**. For FDA-cleared tests, these limitations are given in the package insert. For in-laboratory-developed tests, these should be defined in the procedure manual.

Interpretation

Reports for some tests may require interpretive comments. For these tests, the laboratory must have a procedure that ensures that the report has been reviewed and approved by a **qualified person** before its release



Contamination Control

Sources of Contamination

In a typical PCR reaction, up to 10^{12} identical amplicons can be generated.

Another source of contamination can be the patient sample itself.

Laboratory design

Laboratory design is a key element of QA for molecular assay development and for routine testing inclinical laboratories. Ideally, for amplification-based assays, **three physically separate areas** should be available for reagent preparation (cleanroom), sample preparation and amplification, and product detection (postamplification). **Unidirectional workflow** must be maintained for molecular amplification procedures. The **preamplification laboratories should be under positive air pressure**

Sample Preparation

Even with closed systems, **sample preparation should minimally be performed in a class II biological safety cabinet** or maintained at a positive pressure to other areas.

Topical Inactivation Methods

- A useful and effective method is a 10% (v/v) dilution of sodium hypochlorite
- Ultraviolet lights placed over working surfaces may also be effective in controlling contamination with small amounts of nucleic acid (below 100 pg).

Contamination Control

- Maintain separate equipment and supplies for assay setup in the clean area from that used for handling specimens and extracting DNA.
- Always use aerosol-guarded (filter) pipettor tips to prevent contamination of pipettor barrels by aerosols; never reuse tips.

Contamination Control

- Change gloves between each step, or more often as needed, and when entering or reentering separate areas.
- Change laboratory coats when moving between areas.

Contamination Control

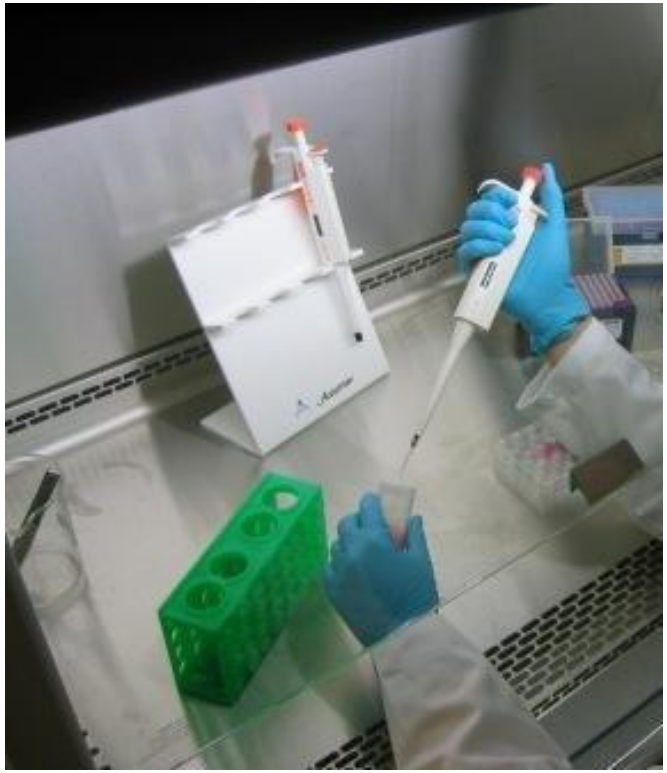
- Decontaminate surfaces daily with 10 to 20% bleach, followed by ethanol or clean distilled water, or as recommended by equipment manufacturers.
- Control airflow within and across work areas.

Contamination Control

- Consolidate all handling of postamplification products and used materials in a defined area.
- Quick-spin tubes to force any liquid down from the sides, before removing caps. Tubes should be uncapped carefully to prevent aerosols and recapped as soon as transfers are completed.

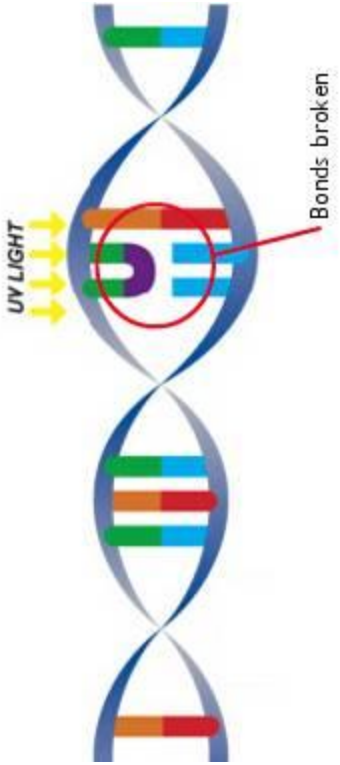
Contamination Control

- Keep reagent tubes capped unless in use.
- Dispense negative controls last so that they reflect the cumulative effects of manipulations.



UV

Ultraviolet treatment of DNA induces crosslinking of the two strands of DNA by forming thymidine dimers .This crosslinked DNA can no longer serve as an effective template .One disadvantage of ultraviolet treatment is that it is most effective in sequences over 700 nucleotides in length .

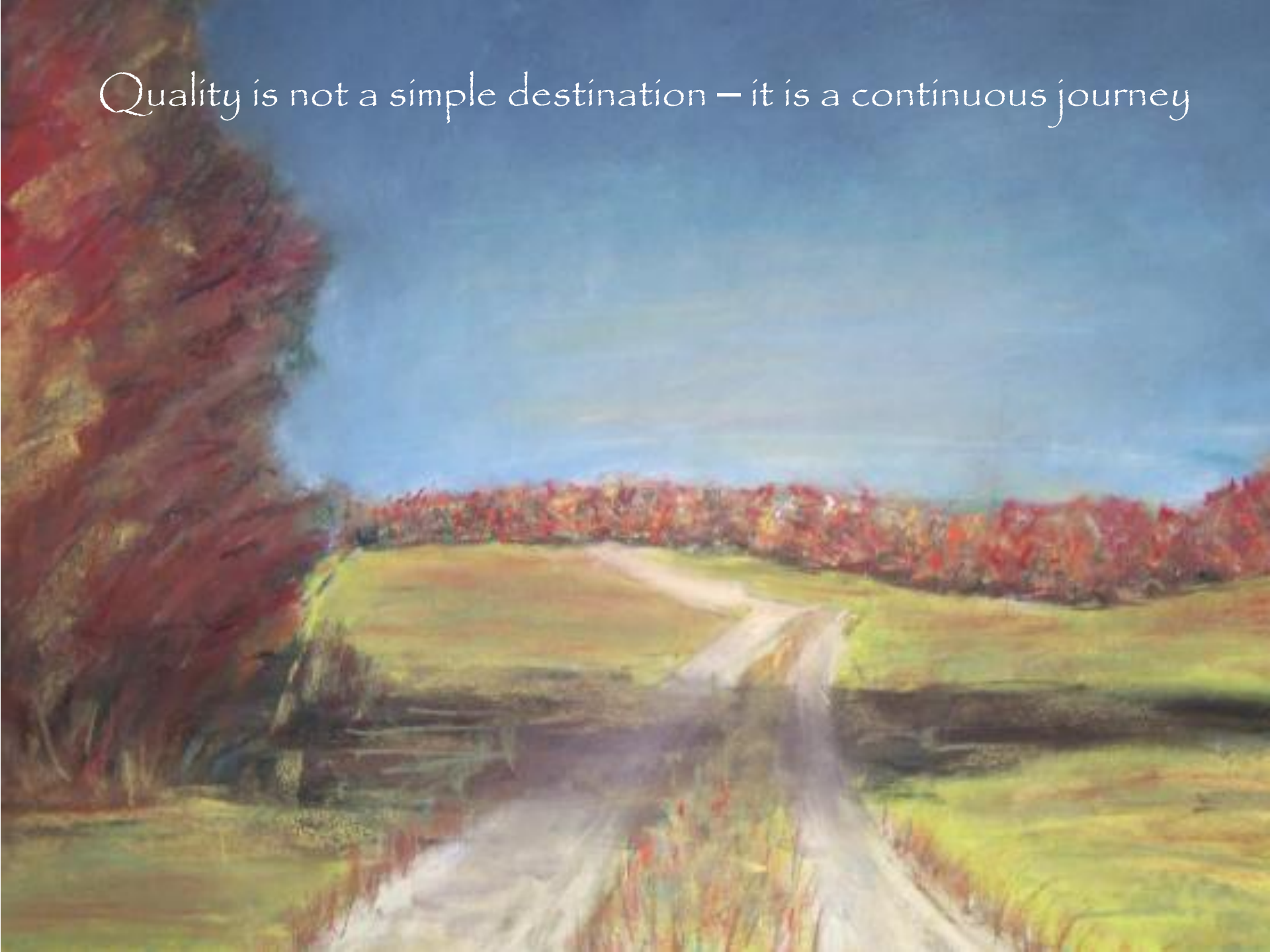


Photochemical Inactivation

Isopsoralen compounds are added to the reaction mixture prior to amplification. Following polymerase chain reaction (PCR) but before the reaction tube is opened, the vessel is exposed to ultraviolet light (300–400 nm), which activates the isopsoralens to form adductors between the pyrimidines on the amplicons. These adductors stop *Taq* polymerase from processing along the amplicons and thus prevent subsequent reamplification of any of these contaminating amplicons

Enzymatic Inactivation

UNG removes uracil residues from the amplicon, still leaving an intact phosphodiester backbone on the amplicon. During the first denaturation step of the amplification procedure, the phosphodiester bonds break at the sites where the uracil residues were located. The fragmented amplicon is no longer able to act as a template. Use of deoxyuridine triphosphate and UNG have been shown to be effective in controlling contamination if amplification of the uracil-containing amplicon does not exceed 106–107 copies per reaction.


Quality is not a simple destination – it is a continuous journey