Queensland Health
Forensic and Scientific Services

Forensic DNA Analysis Validation and Verification Guidelines

1 Purpose and scope

Validation is the developmental process used to acquire the necessary information: to assess the ability of a procedure to obtain a reliable result, to determine the conditions under which such results can be obtained, and to determine the limitations of the procedure (National Association of Testing Authorities, 2020). Method validation and verification provides objective evidence that a method is fit for purpose, meaning that the particular requirement for a specific intended use are fulfilled (National Association of Testing Authorities, 2020). Verification studies are typically smaller than those that are required for validation. For full details refer to National Association of Testing Authorities, 2020 specific documents.

The Forensic DNA Analysis laboratory is certified by the National Association of Testing Authorities (NATA) and is obliged to meet these specifications. ENFSI (2010) states that for DNA based tests, validations/verifications must demonstrate that the profile/s obtained under the new regime will be of the same or better quality than those obtained under the previous regime.

The purpose of this procedure is to describe validation and verification guidelines for use within Forensic DNA Analysis. Test methods, equipment, computer/software systems and information management systems must be shown to be fit for purpose before they are used by the laboratory to generate results. Validations will be required in Forensic DNA Analyses for:

- all new methods developed "in-house";
- methods laboratory/commercial that have been modified;
- methods without validation data adopted from other laboratories or from literature;

Verifications will be required in Forensic DNA Analyses for:

- use of a previously published and validated method
- use of commercial kits

This procedure shall apply to all validation/verification projects conducted within Forensic DNA Analysis. The final decision regarding the extent and scope of the study shall be made by the Managing Scientist.

2 Definitions

Accuracy and Precision

Accuracy (trueness): is the closeness of agreement between the test result and the "true" or accepted value.

Precision: is a measure of closeness (degree of scatter) between independent test results under stipulated conditions (National Association of Testing Authorities, 2020). High precision does not necessarily imply high accuracy.

An example of accuracy and precision measures would be, a determination of the proportion of correct genotypic assignment of samples, and a review of the number of alleles correctly assigning to the expected 0.5bp window/bin.

Repeatability is a measure of the maximum acceptable difference between two test results obtained at the same time by the same analyst under identical conditions on the same material. ENFSI (2010) recommends repeatability studies contain a minimum of five replicates, while NATA (2020) specifies at least six degrees of freedom (e.g. 4 times in a series with 2 samples or 3 times in a series with 3 samples). A repeatability test might be: 12 samples on a plate 7 times with standards and/or controls in an amplification plate and processed by a single operator (suggest that the DNA extract of a defined concentration is prepared in a large volume, and aliquot out to PCR plate or CE plate etc. This will ensure pipetting error is minimised in the preparation of multiple samples to an equivalent concentration).

Reproducibility

- Within laboratory (in-house) reproducibility - A measure of the maximum acceptable difference between two test results obtained on the same material by different analysts at different times.
- Between-laboratory reproducibility - A measure of the maximum acceptable difference between two test results obtained on the same material by different laboratories. It is most conveniently determined in collaborative trials.

Reproducibility in Forensic DNA Analysis could be assessed by: several DNA samples being prepared on an amplification plate by one operator, and the same DNA samples prepared on an amplification plate by a second (different) operator.

Sensitivity is the rate of change of the measured response with change in the concentration of analyte National Association of Testing Authorities, 2020). For PCR-based assays, validation studies must consider the stochastic effects of PCR; particularly as it relates to DNA concentration. ENFSI (2010) recommends sensitivity tests have a minimum of 5 dilutions tested.

3 Principle

Validation provides objective evidence that the particular requirements for a specific intended use are met. There is no one method of validation that is universally agreed upon, however the validation guidelines below are consistent with NATA criteria (National Association of Testing Authorities, 2020), and are consistent with Scientific Working Group on DNA Analysis Methods (SWGDAM 2020) recommendations for the minimum criteria for the validation of DNA profiling processes (ENFSI, 2010).

4 Actions

The planning and implementation of a validation/verification project in Forensic DNA Analysis should occur as follows:

a. Determine if it is a verification or a validation that is required. For example - if a standard published method, with full validation data, and a commercially available kits is to be implemented within the laboratory - a verification not validation would be required (prior to its introduction). If a new methodology is developed - a validation would be necessary.

b. Using the 'Procedure for Change Management in DNA Analysis' standard operating procedure QIS 22871, a project proposal must be prepared. In the planning the work consider the following:

- Validation studies require an assessment of reproducibility, repeatability, sensitivity, accuracy and precision (ENFSI, 2010). Refer to definitions section 2 for details.
- Qualifying Test - For validation studies the use of known samples and where possible authentic case samples should be used. This may be accomplished through the use of proficiency test samples, or samples that the laboratory routinely analyses (e.g. controls). Where previous typing results are available concordance of genotypes should be assessed.
- Mixture Studies – Forensic casework laboratories must define and mimic the range of detectable mixture ratios. Studies should be conducted using samples that mimic those typically encountered in casework (e.g. postcoital vaginal swabs)
- The laboratory must ensure that the procedure/s minimise contamination that would compromise the integrity of the results (QIS 22857). The laboratory should employ appropriate controls and implement quality practices to assess contamination and demonstrate that its procedure minimises contamination.
- Manufacturer's information and previous published validation studies should be used to inform the laboratories validation process.
- Refer to all NATA and ENFSI documentation listed in the reference list section 6 for specific and detailed validation study requirements
- Refer to QIS 10662 for additional resources.
- The project proposal must then be submitted to the Forensic DNA Analysis Management Team for approval prior to the initiation of experiment work.
- On completion of the experimental component of the validation, a final report will need to be written using the final report template QIS 23402. The final report is to be submitted to the Forensic DNA Analysis Management Team for consideration

5 Records

Minimum records required for a validation are:

Project Risk Assessment for Change Management in Forensic DNA Analysis 22872.
Project Proposal document. (see Writing Guidelines for Validation and Change Management Reports QIS 22871 & 23402).
Implementation Plan (Refer QIS 22871)
Final Report (Refer QIS 22871 & 23402).

Additional requirements (as applicable):

Ethics approval (Refer QIS 32177)
Technical review (Refer QIS 22871)
Forensic DNA Analysis - Change Management Budget (Refer QIS 31052).

6 References

National Association of Testing Authorities. (2020). NATA – National Association of Testing Authorities, Australia. Available at: https://nata.com.au/nata/ [Accessed 27 Aug. 2020].

ENFSI (2010) Recommended Minimum Criteria for the Validation of Various Aspects of the DNA Profiling Process. ENFSI DNA Working QA/QC subgroup. Issue No 1.

ENFSI (2020) European Network of Forensic Science Institutes. Available at: http://enfsi.eu/ [Accessed 27 Aug. 2020].

Scientific Working Group on DNA Analysis Methods (SWGDAM). (2020). Available at https://www.swgdam.org/ [Accessed 27 Aug. 2020].

7 Associated documents

QIS: 10662 - FSS – Guidelines for Method Validation

QIS: 22871 - Procedure for Change Management in Forensic DNA Analysis

QIS: 22872 - Project Risk Assessment for Change Management in Forensic DNA Analysis

QIS: 23402 - Writing Guidelines for Validation and Change Management Reports

QIS: 31052 - Forensic DNA Analysis - Change Management Budget

8 Amendment history

Version	Date	Author/s	Amendments
0	06 Sep 2005	Mary Gardam	First Issue
1	April 2008	QIS2 Migration Project	Headers and Footers changed to new CaSS format. Amended Business references from QHSS to FSS, QHPSS to CaSS and QHPS to Pathology Queensland
2	25 July 2008	C Revera	New Title, Changed Forensic Biology to DNA Analysis, authorised by C Allen, Chief scientist to Managing scientist. Purpose and scope combined, hyperlinks updated, definition of verification included.
3	4 Dec 2012	K Scott	New header. Complete rewrite to fit with new change management procedures in DNA Analysis
4	18 June 2014	K Scott	Update organisational name, document names and hyperlinks
5	20 Nov 2015	K Scott	Update header/template, references and minor text updates
6	8 Aug 2017	K Scott	Update references
7	27 Aug 2020	K Scott	Minor updates all areas
8	15 March 2022	K Scott	Template update, document names and references updated

9 Appendices

1 Appendix A Additional terms used in validation studies

9.1

Appendix A

Additional terms used in validation studies

Functional Specification: Defines how it is expected to function - these functions are typically outlined by the manufacturer of equipment/software.

Installation Qualification: Verifies design specification, the physical components of the system have been designed/constructed/supplied/installed in compliance with the design specifications. This is usually completed by the company performing the installation.

Lower limit of detection (LOD) - The lowest concentration or amount of analyte that can be reliably distinguished from zero, but not necessarily quantified, by the test method.

Limit of reporting/quantitation (LOR) - The lowest concentration of analyte that can be determined with acceptable repeatability and accuracy by the test method.

Operational Qualification: Verifies the functional specification, that the system functions as intended throughout anticipated operating ranges.

Performance Qualification: Verifies that the system will consistently produce results meeting user requirement specifications and quality attributes under both normal and worst-case conditions.

Uncertainty - The spread of values within which the true value would be expected to lie, with the stated degree of confidence (usually 95%).

User Requirement Specification: Defines how the system is expected to perform - this is usually set out in the tender document requirements.