Writing Guidelines for Validation and Change Management Reports.

1 Purpose

Change management and validation projects in Forensic DNA Analysis are planned using the procedure for change management QIS 22871 and the Forensic DNA Analysis validation guidelines QIS 23401. The purpose of this document is to provide Forensic DNA Analysis staff with guidelines for writing the final report - after completion of either a validation or change management project. This guide applies to all Forensic DNA Analysis staff.

2 Scope

This procedure applies to all validation and change management project reports within Forensic DNA Analysis.

3 Actions

Final reports within Forensic DNA Analysis are to be written using the template located at:

https://qheps.health.qld.gov.au/fss/staff/corporate-identity/templates

General guidelines on the content and style of each of these report subsections are provided below. The quality team is able to provide previous reports – to use as exemplars (on request).

- The suggested major headings to be included in the report are:
    - Abstract
    - Introduction
    - Materials (and/or Resources)
    - Methods
    - Experimental Design (suggested - for large projects)
    - Results
    - Discussion
    - Conclusion/Recommendations
    - Abbreviations (suggested - for large projects)
    - References.
- Authors – must be listed under the report title. All major contributors to the work should be listed as authors. As a minimum this must include: the Project Leader, Project Leader's Line Manager and the Managing Scientist Police Services Stream. The staff member that writes the report is usually listed as the first author, and the Managing

Scientist is usually listed as the last author in the list. Smaller contributions to a project (that are not sufficient for authorship) should be noted within the Acknowledgments section of the report.

Abstract

Abstracts are a single paragraph (200-300 words) written in past tense. The abstract is a summary of the paper and should briefly state:

- Why the project was undertaken (~1-2 sentences)
- What methodology was used (~2-3 sentences)
- What the key findings/trends/results were (~2-3 sentences)
- Implications of project including the interpretation and conclusion/s (~1-2 sentences)

Due to the required content of an abstract, most authors find that the abstract is most easily written last (after the remaining components of the report are complete).

Introduction

The introduction is usually several paragraphs written in present tense. The introduction should outline all relevant primary research literature, and detail how the literature relates to the issue/s under investigation in the project/study. It should clearly state the studies purpose and rationale.

Scope

A statement of the extent/limits of the project and to which area/s it applies.

Governance

A list of the project staff, the roles of the staff, and a statement about how the project decisions will be managed. Example as follows

The Management Team and the Senior Project Officer, are the decision making group for this project and may use the defined acceptance criteria in this project to cease part or all of the experimentation at any stage. The Decision Making Group may also make modifications to this Experimental Design as required, however this must be documented and retained with the original approved Experimental Design.

Materials and/or Resources

- Materials are listed with item (chemical, consumable or equipment), manufacturer and location. For example:
    - Promega PowerPlex®21 Allelic Ladder (Promega Corp., Madison, WI, US)
    - Promega WEN Internal Lane Standard (Promega Corp., Madison, WI, US)
    - Promega PowerPlex 5 Dye Matrix Standard (Promega Corp., Madison, WI, US)
    - 5804 Centrifuge (Eppendorf, Germany)
    - 2800M Control DNA, 10ng/µl (Promega Corp., Madison, WI, USA)
- International Standard (SI) Units are to be used (e.g. µL)

- A description of the organism/biological materials studied should be included (e.g. human, blood, cells)

Methods and/or Experimental Design

Methods are written in past tense (do not use first person). The use of sub-headings may be required in this section of the report. Methods should explain in detail the materials that were used, the experimental design and full methodology. It should be written with sufficient detail to enable an experienced scientist to replicate the work (i.e. temperatures, times, concentrations must be described). Ensure the following:

- Materials are adequately described
- International Standard (SI) Units are to be used (e.g. μL)
- For reporting: numbers less than ten are written in words and not numerals (e.g. two minutes). When writing numbers >10 use numerals, and do not write in words (e.g. 12 minutes).
- Experimental or sampling design is to be described (e.g. structure of the experiments, selection of samples, use of controls, sample numbers, sample duplicates etc.). Refer to Appendix A for guidelines.
- Detail how the procedure was carried out (e.g. DNA extractions details, amplification conditions).
- Explain how the data was analysed (e.g. statistical methodology). Refer to appendix A for recommendations.
- The acceptance criteria for the results is clearly defined.

For Materials and/or Resources and Methods and/or Experimental Design, it is acceptable for the Final Report not to reproduce the content from the Experimental Design, but to reference it and include any changes by exception.

Results

Results are written in past tense. The purpose of this section is to objectively present the key results without interpretation. It should always begin with text presenting the key findings (that address the questions being investigated), and statistical evaluation (Refer to Appendix A for recommendation). Tables and Figures can be included within this section to provide clarifying information.

Tables and Figures

Tables and Figures are included within the results section of a report. Table and Figure presentation guidelines are as follows:

- Tables and Figures are numbered consecutively. Table and Figures are assigned numbers separately e.g. Table 1, Table 2, Table 3 and Figure 1, Figure 2, Figure 3 etc.
- Legends are to be a brief description of the result/information being presented.
- Table legends go above the table and are left aligned.
- Figure legends go below the figure and are left aligned.
- In the text of the report, figures can be abbreviated to “Fig” (i.e. Fig 1). Table is never abbreviated.
- SI units should be specified in the column headings wherever required.

- Footnotes are used to clarify points in the table, denote statistical differences among groups or to convey repetitive information about entries.

Table exemplar:

Table 1 Student's t-testP-values for comparison of QS5-A and Qs5-B with 7500-A

Standard	Instruments compared	SAT	LAT	Y-Target
NIST A	QS5-A & 7500-A	0.70050	0.06813	0.42519
	QS5-B & 7500-A	0.44247	0.77529	0.19765
NIST C	QS5-A & 7500-A	0.23834	0.09180	0.39582
	QS5-B & 7500-A	0.52538	0.45386	0.32165

Note: P-values < 0.05 indicate a significant difference between results produced by the two instruments.

Figure exemplar:

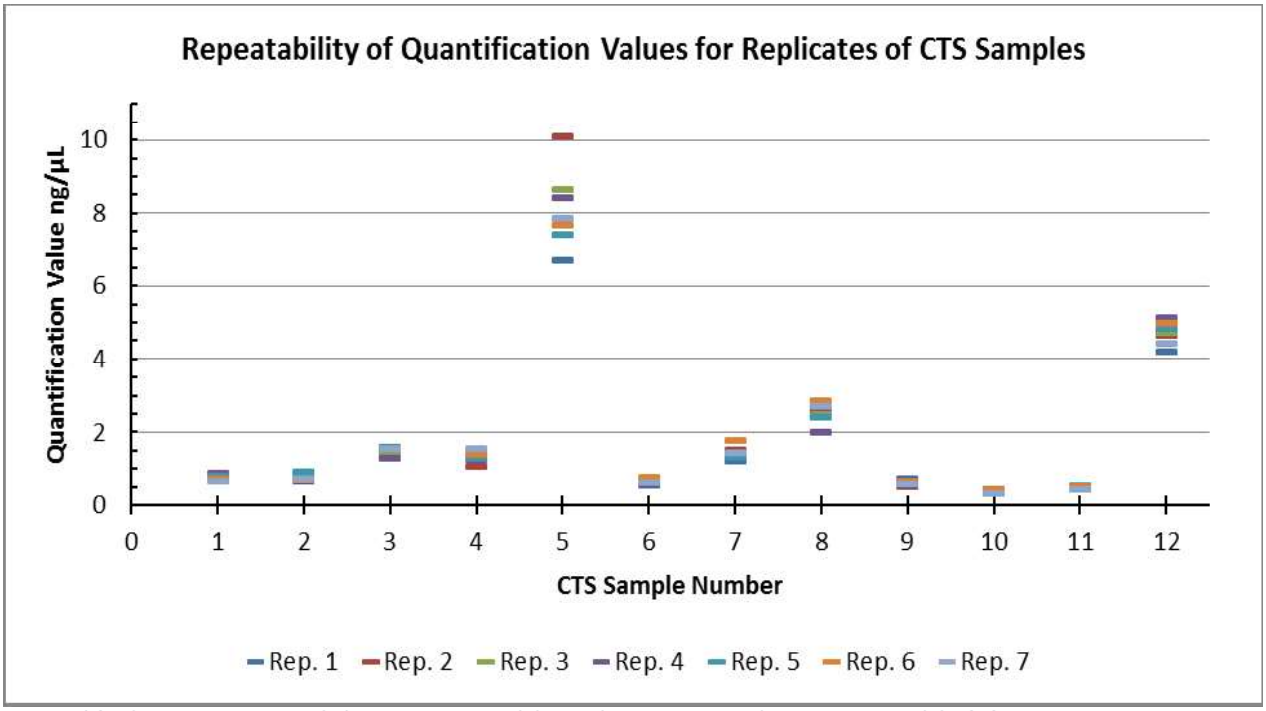

*Figure { SEQ Figure * ARABIC } Repeatability of Quantification Values for Replicates of CTS Samples*

Discussion

A discussion is written in past tense and will usually consist of multiple paragraphs. The purpose of the discussion is to explain/interpret the results with reference to the acceptance criteria and to relate the results back to current understandings in the field, and in the published literature. There should be links/connections of ideas/concepts between the introduction and the discussion; explaining how the project/validation has moved current understandings forward. Questions that should be considered when writing the discussion may include:

- Do the results support the projects hypotheses? If not, why not – try to provide reasons (if it is possible)?

- Do the findings agree with current literature/publications? If not, why not – try to provide reasons (if it is possible)?
- What are the implications of the findings to the laboratory, and to the scientific community?

Note: If appropriate, the results and discussion can be combined under one heading. If the project contains more than one experiment it may be necessary to have a combined result/discussion section for each experiment.

Conclusions/Recommendations

A conclusion and/or recommendation section can either be written as a separate section/s (each under its own heading), or it can be incorporated into the end of the discussion section without a separate heading.

A conclusion is usually one paragraph written in past tense. The conclusion should summarise the most significant finding, the implication of the finding/s, and may indicate what direction – additional projects should take.

Recommendations are usually written as several statements, or dot points that outline what actions are required. This may include recommendations on the implementation (or not) of a procedure, what type of further work that is required, and/or recommendations on how data should be utilised and interpreted.

Acknowledgements

The purpose of acknowledgements is to note the contributions from others (that are not listed authors). This may include acknowledgments of:

- Funding source/s
- Staff that completed laboratory work
- Reviewers/Collaborators.

References

Referencing should be used wherever a report refers to another's work. It is usual for there to be extensive referencing with the introduction section of the report, with referencing also commonly used within the methods and discussion sections of the report. References can be managed with programs such as EndNote.

Requirements for referencing:

- Place quotation marks on either side of text " " when quoting directly.
- A reference list is arranged alphabetically by author (If an item has no author, it is cited by title, and included in the alphabetical list using the first significant word of the title).
- If you have more than one item with the same author, list them in chronological order (starting with the earliest publication).

There are several acceptable methods of referencing including ACS, AGPS/AGIMO, AMA and the Harvard Style. In the Harvard Style referencing: within the text reference by author and date e.g. (Smith, 2012). Referencing format with the Harvard Style as below:

Harvard Style:

Referencing a book:

Smith, JB, Scott, KD & Jones, LM 2012, *Forensics: A molecular approach,* 2nd edn, McGraw Hill, London.

Referencing a chapter in a book:

Martin, F 2012, 'DNA Profiling', in Lee CW (ed.), *Forensics: A molecular approach,* 2nd edn, McGraw Hill, London, pp. 35-61.

Referencing a journal article:

Holden, LM 2011, 'Validation of Powerplex21', *International Forensics*, vol. 50, no. 2, pp. 49-52

Referencing an on-line journal article:

Holden, LM 2011, 'Validation of Powerplex21', *International Forensics*, vol. 50, no. 2, viewed 31 December 2012, <insert website address>.

Appendices

Appendices can be used if required and are numbered consecutively. The appendices contain information that supports the content of the report but is not essential within the body of the report.

4 Records

Nil

5 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 23401	Forensic DNA Analysis Validation Guidelines

6 References

Nil

7 Amendment history

Version	Date	Updated By	Amendments
1	13 March 2006	R Smith	First Issue
2	Sep 2008	T Nurthen	Minor update
3	07 Jan 2013	K Scott	Some content from this document transferred into QIS 23401. Complete re-write of remaining document – focusing on the reporting of validations and projects. Update header
4	17 July 2014	K Lancaster	Changed references to DNA Analysis to Forensic DNA Analysis. Included extra detail for experimental design. Updated titles for hyperlinked documents. Updated title of Managing Scientist.

			Updated report template hyperlink. Included a figure exemplar.
5	03 Feb 2016	K Scott	Template update, separate materials and methods, minor text edits and correction of amendment history table
6	09 Aug 2017	K Scott	Update names of kits used as exemplars
7	21 March 2019	K Lancaster	Updated hyperlink to report templates
8	08 May 2019	K Scott	Inclusion of Appendix A – recommendations for statistics. Minor updates throughout
9	14 Nov 2019	K Scott	Addition of sections on scope and governance. Addition of Appendix B – Resources for Statistics. Minor text edits
10	20/07/2021	A Ryan	Amended header to remove HSQ. Updated hyperlink to report templates. Amended document title for QIS 22872.Captioned table 1 and figure 1 correctly. Added how the acceptance criteria are mentioned and referred back to.

8 Appendices

1 Appendix A Recommendations for Statistics
2 Appendix B Resources for Statistics

8.1 Appendix A: Recommendations for Statistics

The following recommendations have been drawn from a review of literature, NATA guidelines, advice from senior quality staff at Forensic and Scientific Services and from National Forensic Statisticians.

For definitions of accuracy (trueness), precision, repeatability, reproducibility (within laboratory and between laboratory), blank. linearity, limit of detection (LOD), limit of (LOR), sensitivity, uncertainty and verification refer to QIS 10662 and QIS 23849, and NATA guidelines (https://nata.com.au/).

Please also refer to the FSS Guidelines for Method Validation QIS 10662.

Considerations in the design and approach to a validation study or research project:

Are statistics necessary given the experiment or analysis being considered:

- For strong statements "significant difference", "linear trend" etc. a statistic will be required to support the statement. For comparative statements it may not always be informative, or operationally appropriate to complete a statistic i.e. "differences were seen", or "appears to be a trend" statements do not require a statistic.
- Where a statistical test is not informative, and/or particularly where the difference between the experimental groups will not have an operational meaning - use of box plots are recommended. Box plots display the variation present in a system. Generally if the box plots overlap the difference between the groups is functionally non-significant.

Sample numbers:

When deciding how many samples are require for an individual experiment the following should be evaluated:

- Consider the amount of variation you are expecting to see. Where little variation is expected (e.g. number of alleles obtained from blood samples) small experimental sample numbers are needed. Where variation is higher (e.g. peak heights from low DNA quantification samples) sample numbers should be much higher. Where the amount of expected variation is unknown it is possible to run one set of samples, assess the results and then run additional samples if required.
- The experimental design is always aiming to include enough samples to model the expected variation in the relevant experiment (given the experimental factors under consideration). Thereby producing sufficient information (via sample numbers) for the development of methods/thresholds to cover "most situations". It is not possible for a study/validation to cover all possible situations.
- In cases were a project/validation is assessing locus amplification efficiency, and inter-locus peak height balance larger sample sizes may be required (suggest use of population samples ~200-250); this is particularly relevant for Y kits where a linear relationship may not be seen.

Which statistics might be most appropriate:

- ANOVA – to compare independent groups of samples
  Example: Dziak, R, Peneder, A, Buetter, A & Hageman, C. 2018 'Trace DNA Sampling Success from Evidence Items Commonly Encountered in Forensic Casework,' *J Forensic Sci*, vol 63, pp 835-841. doi:10.1111/1556-4029.13622

- Kruskal-Wallis – to compare independent groups of samples
  Example: Henry, J & Scandrett, L. 2019 'Assessment of the Yfiler® Plus PCR amplification kit for the detection of male DNA in semen-negative sexual assault cases,' *Science & Justice,* in press 2019,

- Paired T-test – to compare repeated samples i.e. same samples run through two different methods.
  Example: Tsai, L, Lee, C, Chen, C, Lee, J.C, Wang, S, Huang, N, Linacre, A. & Hsieh, H. 2016, 'The Influence of Selected Fingerprint Enhancement Techniques on Forensic DNA Typing of Epithelial Cells Deposited on Porous Surfaces.' *J Forensic Sci*, vol 61: S221-S225
- Chi-square test – may be applied to demonstrate the average peak heights between loci (or dye layer) may differ
  Example: Montpetit, S & O'Donnell, P, 2015, 'An optimized procedure for obtaining DNA from fired and unfired ammunition,' *Forensic Science International: Genetics*, vol 17, pp 70-74,

Tools for statistics:

- Excel – basic statistics
- R software program
- SPSS Software – commercially available software for statistics and graphing

Practical guidelines and suggestions:

It is not possible for a "procedure" to be written that will cover all possible approaches/analysis for studies that may be required within the Forensic DNA Analysis Laboratory. However, some key principles and guidelines are provided below that may assist with the development of an experimental design.

Instrument validations:

- For instruments that perform pipetting tasks, assessment of %inaccuracy and %CV are generally assessed on the Artel MVS instrument, and must meet laboratory guidelines +/- 5% (10% for volumes ≤10µL)
- Contamination checks can be important in many studies, particularly those that involve pipetting or liquid movement steps. This may include soccer-ball plates for 96-well formats.

Software validations

- Ensure the computer on which the software is installed meets the specifications of the software
- The software must have a version number (this must be referenced in the validation)
- Settings/configurations must be consistent with the software specifications, and only able to be accessed by authorised users
- Software should have pre-existing developmental validation (i.e. publication, or manufacturers validation). This validation should ensure that calculations and parameters meet requirements.

Sensitivity and Limit of Detection:

- Sensitivity studies will often be conducted prior to repeatability/reproducibility assessments.
- Concordance assessments may be incorporated with sensitivity studies. Concordance is usually an assessment of ~100 samples.

DNA Extraction

- Serial dilutions of cell suspensions (where a cell count has been done) are useful for DNA extraction sensitivity studies (Refer project #168 final report for further details)
- Dilutions should result in range of cell concentrations~10-500 cells (per extraction), such that the capacity of extraction technology is assessed at ranges suitable for forensic analysis.

DNA Quantification

- Serial dilutions of NIST standards are useful for LOD and sensitivity assessments where DNA quantifications methods are to be evaluated. Percentage change (inaccuracy) may be calculated from the expected and observed results.

- Dilutions should extend both below and above expected functional range as defined by the manufacturer. For example in validating Quant Studio 5 the dilutions utilised were: 0.0001, 0.001, 0.002, 0.003, 0.004, 0.005, 0.006, 0.007, 0.008, 0.009, 0.01, 0.03, 0.05, 0.07, 0.09, 0.1, 0.5, 1.0 and 5.0 ng/µL (Refer project #185 final report for further details)

DNA Amplification

- Optimal PCR cycle number to be evaluated by the laboratory: this should include 7-12 samples, plus controls over at least 3 different PCR cycle numbers.

Capillary Electrophoresis

- Baseline should utilise 20-100 samples, with samples analysed at 1RFU. Stutter, pull-up, carry-over and artefacts should be removed. Average peak height RFU (μ_{Pk}) for each dye channel calculated using the AVERAGE function (Arithmetic mean) in Excel. The standard deviation (σ_{Pk}) will be calculated using the STDEV function in Microsoft Excel (Refer project #196 for further details)
    - Limit of Detection (LOD)= $\mu_{Pk} + 3\ \sigma_{Pk}$
    - Limit of Reporting (LOR)= $\mu_{Pk} + 10\ \sigma_{Pk}$

Repeatability:

- Run a set of samples multiple times on a plate. Ideally each sample should be run at least 7 times.
    - On a standard PCR plate 12 samples can usually be run on a plate 7 times with standards and/or controls – this is considered statistically sound.
    - Scatter plots or box plots can be a way to display the data and evaluate the variability between replicates.

Reproducibility:

- Run a plate over multiple days (as many as is practicable e.g. over 3-5 days), with different operators.
- The “plate” of samples used for reproducibility may include the same samples used for repeatability. It is suggested that ~12 samples (min 7 samples), plus controls are included in the reproducibility plate.
- Scatter plots or box plots can be a way to display the data to evaluate reproducibility within the system.

Performance Study:

For some projects a performance study is worthwhile. This is generally a set of “Typical” samples received within the laboratory. Generally, a larger number of routine samples are processed.

Exemplar publications of forensic validation studies:

Coble, MD, Buckleton, J, Butler, JM, Egeland, T, Fimmers, R, Gill, P, Gusmão, L, Guttman, B, Krawczak, M, Morling, N, Parson, W, Pinto, N, Schneider, PM, Sherry, ST, Willuweit, S & Prinz, M 2016 ‘DNA Commission of the International Society for Forensic Genetics: Recommendations on the validation of software programs performing biostatistical calculations for forensic genetics applications,’ *Forensic Science International: Genetics*, vol 25, pp 191-197

Hollard, C, Ausset, L, Chantrel, Y, Jullien, S, Clot, M, Faivre, M, Suzanne, E, Pène, L & Laurent, F-X. 2019, ‘Automation and developmental validation of the ForenSeq™ DNA Signature Preparation kit for high-throughput analysis in forensic laboratories’, *Forensic Science International: Genetics,* vol. 40, pp. 37-45

Meuwly, D, Ramos, D & Haraksim, R 2017, ‘A guideline for the validation of likelihood ratio methods used for forensic evidence evaluation’, *Forensic Science International*, vol. 276, pp. 142-15

8.2 Appendix B: Resources for Statistics

QFAB Biostats Sites Sheet

A list of useful online Biostatistics sites.

Introductory Theory

Discovering Statistics by Andy Field
https://www.discoveringstatistics.com/

Study Design

Phases of Clinical Trials by Australian Clinical Trials
https://www.australianclinicaltrials.gov.au/what-clinical-trial/phases-clinical-trials

Study Designs by The Centre for Evidence-Based Medicine
https://www.cebm.net/2014/04/study-designs/

Choosing the Right Statistical Test

Numerical Methods for Biosciences Students
https://web.anglia.ac.uk/numbers/biostatistics/biostatistics.html

Statistical Decision Tree
https://www.anzmtg.org/stats/

Sample Size and Power

Calculating an optimal samples size or identifying the power of a sample size
https://www.anzmtg.org/stats/Guides/PowerOfSampleSize

Sample Size and Power Calculators

G*Power, MedCalc, EpiTools, StatsToDo, StatsPages

Sample size estimates need to be inflated to take into account estimated drop outs and missing values. QFAB has developed a Study Length Calculator for this.

Randomisation

Directory of randomisation software and services
https://www-users.york.ac.uk/~mb55/guide/randsery.htm

Surveys & Questionnaires

Measurement Tools/Research Instruments
http://guides.lib.uw.edu/c.php?g=99174&p=641942

Prepared by the QFAB Biostatistics Team
https://qfab.org/biostatistics

QFAB recommends using REDcap, especially for longitudinal studies, and provides training in this. Please check if your employer supports this software.

Data Management

Good Data Guidelines
https://qfab.org/good-data-guidelines
Numerical conversion is not required is using the software R.

Online Calculators

MedCalc, Vassar Stats, EpiTools, StatSciCalc

Software

Software support
https://stats.idre.ucla.edu/#

We recommend against using Excel for data analysis. In increasing sophistication but decreasing ease of use: GraphPad Prism, SPSS, STATA, SAS, R. Each software has its own online help documentation. QFAB provides training in R, SPSS, and STATA.

Data Analysis

Choosing the right distribution
https://blog.cloudera.com/blog/2015/12/common-probability-distributions-the-data-scientists-crib-sheet/

Everything you need to complete your data analysis
https://statistics.laerd.com/features-overview.php

Statistical Help
https://www.statsdirect.com/help/default.htm

References

Handbook of Biological Statistics
http://www.biostathandbook.com/analysissteps.html

Biostatistics Resource for Medical, Health and Allied Research
http://www.medicalbiostatistics.com/

Statistics for Biologists
http://www.nature.com/collections/qghhqm/pointsofsignificance

Prepared by the QFAB Biostatistics Team
https://qfab.org/biostatistics