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BIOLOGY VALIDATION PROJECT WORKING GROUP

Requirements of Validation Parameters: Thermal Cyclers

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ANZPAA NIFS Document Position Statement

Australia New Zealand Forensic Executive Committee

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Review Date: 3 years from release date. The recommendations in this document are applicable until the review date. However, forensic service providers may continue using the document as guidance if deemed appropriate.

Purpose

Developed collaboratively by a cross-agency working group, this document outlines a shared view of accepted practices in the relevant forensic science field.

This document has been prepared with the aim of enhancing practice via continuous quality improvement and supporting greater consistency and standardisation within disciplines.

Recommendation

ANZFEC members recommend adopting the outlined practices wherever possible, as they enhance shared knowledge and experience, foster collaborative improvement, and support cross-organisational technical reviews, including laboratory accreditation assessments.

Application

The document serves as recommendations only and does not compel forensic service providers to adhere strictly to its contents. Where appropriately justified, applying alternative practices may also be considered effective and acceptable.

It is acknowledged that variations in legislation, organisational policies, infrastructure, equipment, resources and customer requirements may necessitate deviations in the adoption and application of the document's content.

The document's content is intended to guide the development and implementation of organisational practices. The contents are not intended to apply retroactively to organisational practices that were implemented and validated before the document's release date.

Requirements of Validation Parameters: Thermal Cyclers

1.1. Scope

This document describes the experimental work required for the validation of new thermal cyclers for use in forensic laboratories. It outlines the acceptance criteria, fundamental studies to be performed, and the validation requirements, including guidelines for sample selection and the experimental approach. While the following sections are covered within an amplification kit validation, this guide will also assist with the validation of new/additional thermal cycler instruments.

1.2. Introduction

Thermal cyclers are essential instruments in forensic DNA analysis, precisely controlling the temperature cycles required for the polymerase chain reaction (PCR). Forensic laboratories rely heavily on PCR to generate DNA profiles, and the reliability of these instruments directly impacts the quality and reproducibility of these results. This document provides recommended procedures for validating new thermal cyclers in forensic laboratories, including considerations for implementing multiple instruments concurrently.

Forensic laboratories must validate new thermal cyclers before use to ensure reliable and consistent DNA amplification. When validating a new instrument model, or introducing additional units of an existing model, laboratories should consider the potential for variations among instruments. Calibration should ensure that all critical characteristics, such as temperature accuracy, uniformity, and ramping rates are consistent before validation studies are performed. This type of calibration is typically performed by the service technician. These factors influence the efficiency and specificity of PCR, ultimately affecting the generation of DNA profiles.

1.2.1. Relevant Guiding and Critical Documents

Reference should be made to documentation which has informed the experimental design, including but not limited to:

- manufacturer user guide(s)
- published developmental validation data
- accreditation standards
- Thermal cycler installation check and well-specific temperature homogeneity assessment
- *STRmix™ Technical guide: How can Model Maker be used to validate new thermal cyclers?, and*
- *ANZPAA NIFS Guideline for the Validation of Forensic Science Methods.*

1.3. Experimental Design

1.3.1. Sample Collection

Samples must be extracted and quantified using previously validated laboratory methods. The laboratory should evaluate the appropriate sample number and type, based on the methodology and/or application necessary to demonstrate the potential limitations and reliability. Methods intended for casework samples

should be evaluated and tested using known samples and mock casework samples (or non-probative evidence samples), reflective of the range of samples commonly encountered within the laboratory.

1.3.2. Testing

The attached document does not explicitly use all terms from the ANZPAA NIFS *Guideline for the Validation of Forensic Science Methods*; however, the work outlined in this document addresses the relevant requirements. Accuracy is demonstrated through replicate analysis using samples of known composition, sometimes referred to as concordance studies. Linearity is demonstrated through sensitivity studies using dilutions across the method's working range. Ruggedness is addressed through reproducibility studies were applicable.

1.3.2.1. Sensitivity Studies

This experiment should test a range of template input amounts that are reflective of the DNA concentrations typically encountered in casework.

A minimum of 50 samples with known DNA profiles encompassing the dynamic range of DNA concentrations expected in the laboratory is required. As an example, 50 samples could be achieved by amplifying 10 DNA template amounts (e.g., 0.005, 0.01, 0.025, 0.05, 0.075, 0.1, 0.125, 0.25, 0.5 and 1 ng) for five donors. Consider the requirements of the laboratory when selecting template input amounts.

The sensitivity sample set must be amplified across all new thermal cyclers using the same set of amplification reagents (refer to **1.4 Verification**). Additionally, a subset of existing validated thermal cyclers should be used to amplify these samples enabling a comparative analysis across instruments.

The above should be repeated for all PCR amplification kits routinely used in the laboratory. Where STRmix™ is not a viable tool for analysing the validation data (4), for example, the Yfiler™ Plus PCR Amplification Kit, sensitivity study set should be amplified in triplicate.

1.3.2.2. Repeatability and Reproducibility Studies

These experiments assess the thermal cycler's ability to consistently produce the same results when running the same PCR protocol on the same instrument, within and across wells. Repeatability is assessed by testing at least three samples of different concentration inputs in replicates of five (2,3) within the same instrument run that are evenly distributed across the heating block(s).

Reproducibility is assessed by amplifying the same repeatability sample set under a different condition, such as a different thermal cycler, or by a different operator. Following amplification, all samples should be analysed on the same capillary electrophoresis (CE) instrument.

1.3.2.3. Optional Mixture Studies (if required for STRmix™ analysis)

These experiments evaluate the performance of the thermal cycler(s) when amplifying mixed contributor samples.

Prepare sets of mixture samples consisting of two contributors at different ratios, such as 1:1 and 1:10, as well as mixtures with three contributors at defined ratios, for example 1:1:1, 1:5:10 & 1:10:10. These ratio configurations will support a mixture interpretation across a range of contributor imbalances. Amplify the mixtures at five different DNA input amounts to cover the range of inputs encountered in casework sample processing, for example, > 1 ng and < 100 pg.

1.3.3. Data Analysis

1.3.3.1. Genotype Concordance and Peak Height Variance

Review DNA profiles for all samples amplified to ensure the expected genotypes were produced using each thermal cyclers.

Perform a peak height comparison using statistical analysis, such as the ANOVA, to determine whether the peak heights are significantly different between replicate samples within and between runs, and between thermal cyclers.

1.3.3.2. Peak Height Variance (STRmix™: Single source profiles only)

Run each thermal cyclers dataset through Model Maker using a single set of Model Maker parameters. Plot peak height variance distributions and overlay each thermal cyclers plot to assess whether the plots are comparable. This approach allows for an assessment on whether the distributions are comparable in terms of shape, scale, and mode.

1.3.3.3. Additional Model Maker Analysis

Review data from Model Maker for progression, correlation plots and percentage coverage.

1.3.4. Assessment Against Acceptance Criteria

Criteria for assessing the amplification results may be available via comparison to established in-house or published performance data for the instruments. Following the production of comparable peak height variance plots or statistics, datasets can be combined and run through Model Maker to assess whether there are any differences to the currently utilised Model Maker parameters. Model Maker performance should be reviewed by checking progression, correlation plots and percentage coverage as recommended in the STRmix™ Implementation and Validation guide (3).

If overlaid distributions are similar in shape, scale and mode, a similar level of peak height variation across instruments is indicated. If the distributions are dissimilar or the comparison is unclear, a further test on 10 mixed profiles through STRmix™ using each of the respective thermal cyclers specific Model Maker parameters can be undertaken to check the impact on final results, i.e., to the likelihood ratio (LR) (4).

For mixed DNA profiles, review the LRs generated from STRmix™ to assess whether variation is within one order of magnitude. If the LRs are not within one order, perform an investigation to determine if this is instrument specific or sample specific.

1.4. Verification

All additional thermal cyclers of the same make and model will require verification to confirm that the results obtained from the validation of the initial instrument can be reproduced on each additional instrument. This includes a sensitivity series and a temperature check of the heating block, or in the absence of a temperature check, the same sample template must be assessed across different wells of the plate.

These samples should also be amplified on a subset of the existing validated thermal cyclers and run on the same CE instrument for comparison.

1.5. References

1. European Network of Forensic Science Institutes (ENFSI). Recommended Minimum Criteria for the Validation of Various Aspects of the DNA Profiling Process. Document No. ENFSI DNA Working Group, Issue No. 001, November 2010.
2. Butler JM, Tomsey CS, Kline MC. Can the Validation Process in Forensic DNA Typing Be Standardized? In. Proceedings of the 15th International Symposium on Human Identification; 2004 October 6; Phoenix, AZ. Available from: <https://www.promega.com.au/products/pm/genetic-identity/ishi-conference-proceedings/15th-ishi-oral-presentations/>.
3. Institute of Environmental Science & Research (ESR). STRmix™ 2.8 Implementation and Validation Guide; 2023. Available from: <https://support.strmix.com/support/solutions/articles/1000294780-strmix-v2-8-updated-implementation-and-validation-guide-and-user-s-manual->.
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ANZPAA NIFS is responsible for the management and co-ordination of the Specialist Advisory Groups and has reporting accountability to the Australia New Zealand Forensic Executive Committee (ANZFEC).

The Biology Validation Project Working Group members involved in the preparation of this report were:

- Sarah Cockerton (NZ Institute for Public Health and Forensic Science Limited) (Biology SAG Mentor)
- Dr Jeremy Watherston (Forensic Science Queensland) (Chair)
- Karina Muharam (ANZPAA National Institute of Forensic Science)
- April Connolly (NT Police)
- Penny Cooper (PathWest Laboratory Medicine WA)
- Alicia Gagliardi (Australian Federal Police)
- Chris Hefford (Forensic Science SA)
- Dr Julianne Henry (Forensic Science SA)
- Dr Catherine Hitchcock (NSW Health Pathology Forensic & Analytical Science Service)
- Adam Poy (Victoria Police Forensic Services Department)
- Dr Marie Rye (PathWest Laboratory Medicine WA)
- Kate Stevenson (NZ Institute for Public Health and Forensic Science Limited)
- Lisa Wedervang (NSW Health Pathology Forensic & Analytical Science Service)

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References in this notice to ANZPAA are references to the Members of ANZPAA.

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