SCIENTIFIC WORKING GROUP ON DNA ANALYSIS METHODS[1]

SWGDAM Modified Rapid DNA Analysis Internal Validation Module for Forensic Samples

Short Title: Forensic *Modified Rapid DNA Module*

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Scope

The SWGDAM Modified Rapid DNA Analysis Internal Validation Module for Forensic Samples specifies best practice guidelines to assist laboratories in designing internal validation studies for modified Rapid DNA analysis. These guidelines are intended to serve as instructions for laboratories validating modified Rapid DNA analysis of forensic samples consistent with the *FBI Director's Quality Assurance Standards for Forensic DNA Testing Laboratories* (QAS), and the SWGDAM Validation Guidelines for DNA Analysis Methods: Overview Document. Examples provided in the Appendix are for informational purposes and are not meant to dictate the types and numbers of samples for every laboratory in developing their internal validation plan for modified Rapid DNA analysis.

[1] The Scientific Working Group on DNA Analysis (SWGDAM; *see* SWGDAM.org) is comprised of forensic science practitioners and other experts who represent government laboratories within the U.S and Canada, as well as intra- and international professional groups and academia. SWGDAM recommends to the FBI Director revisions to the *Quality Assurance Standards for Forensic DNA Testing Laboratories* and the *Quality Assurance Standards for DNA Databasing Laboratories (QAS)*. SWGDAM provides a forum for its members and invited guests to discuss research, technologies, techniques, and training; and conduct or recommend studies to develop, test, and validate methods for use by forensic laboratories. SWGDAM's Guidelines and Recommendations represent best practices within the discipline. The term "should" is used herein to indicate good practices identified by SWGDAM. "Shall" distinguishes mandatory elements, which may be specified in the Quality Assurance Standards for Forensic DNA Testing Laboratories and/or Quality Assurance Standards for DNA Databasing Laboratories.

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Key Concepts:

- While validation studies cannot account for all sample types or scenarios that may arise during Rapid DNA examinations, laboratories should design and conduct experiments to establish and verify the instrument's performance before implementation in forensic casework.
- Purposes, considerations and outcomes are outlined for the following validation studies as applicable to forensic-type samples: contamination, sensitivity and stochastic; precision and accuracy; mixture; and known and non-probative evidence samples or mock evidence samples.
- Following implementation, laboratories are encouraged to assess the results produced from a method to ensure it performs as expected within the scope of the validation. If necessary, the laboratory may conduct supplemental studies to expand the scope of the validation.

1. Introduction

Though the Rapid DNA Systems were originally developed to be a fully automated process of developing a DNA STR profile from a reference sample buccal (cheek) swab without human intervention, enhancements have been made to enable the Rapid DNA instruments to be utilized for forensic samples. The 2025 Forensic QAS requires a laboratory to validate modified Rapid DNA analysis should a Rapid DNA instrument be utilized for forensic samples. The focus of this document is the validation of modified Rapid DNA analysis for forensic Rapid DNA cartridges/chips and does not address the use of fully automated Rapid DNA Systems for reference samples.

Modified Rapid DNA analysis is the semi-automated (hands-free) process of developing a CODIS acceptable STR profile from a casework reference or forensic sample. The "swab in – profile out" process consists of automated extraction, amplification, separation, and detection of DNA STR profiles without human intervention but requires an analyst to perform manual interpretation and technical review.

This document will highlight the primary considerations when validating modified Rapid DNA analysis on forensic-type samples and should be reviewed in conjunction with the SWGDAM Validation Guidelines for DNA Analysis Methods: Overview Document. The studies presented herein are not synchronized to the FBI QAS; instead, they are presented in a suggested order to conserve resources such as time, reagents, samples and consumables and to streamline required testing. Example validation studies are provided in Appendix A.

General considerations:

- Rapid DNA instrument manufacturers provide users different sample cartridge/chip and processing protocol options. There are cartridges/chips designed to process high quality/quantity samples (e.g., reference samples) and cartridges/chips intended to work best with lesser quality/quantity samples (e.g., forensic samples). Validation studies for forensic samples must be performed employing a forensic specific cartridge/chip and processing protocols intended to be used by the laboratory.

- The validation studies shall include representative forensic sample types that are appropriate for the laboratory's Forensic Rapid DNA Program, as defined in the 2025 Forensic QAS and outlined in Standard 18.6.

- Data interpretation parameters shall be empirically determined for each cartridge/chip type and protocol validated by the laboratory in the analysis software of choice.

- The laboratory must evaluate the raw Rapid DNA data (e.g., .fsa files) to validate modified Rapid DNA analysis for forensic samples.

- The laboratory must include evaluation of the analysis software used for interpretation of Rapid DNA data, including parameters affecting allele calling, thresholds, data quality assessment, and profile generation, as part of the validation.

- A laboratory may choose to validate a forensic cartridge/chip for forensic samples or for both forensic and casework reference samples (sub-sampling may be required).

- In accordance with the NDIS Operational Procedures Manual, only NDIS-approved Forensic Rapid DNA cartridge/chip must be used for CODIS upload of forensic samples.

- A laboratory can use data generated from one validation study in another study. However, the laboratory should ensure that the data being used to establish a given parameter differs from the data being used to test such parameter.

- Once an instrument is validated, each additional Rapid DNA instrument of the same make, model, and system software version shall require a performance check upon installation, prior to implementation. At a minimum, a previously characterized sample shall be run in each sample position of the cartridge/chip to ensure the proper operation of the instrument. Generated data should be verified for genotype concordance.

- The validation of a mobile/temporary component must include relocation of the Rapid DNA instrument. In addition, a subset of representative sample types must be processed in a mobile/temporary location as part of the validation.

- A laboratory may choose to perform additional studies or to use additional samples for any of the studies outlined in this document. If a sample fails or if non-expected results are obtained, an increased number of samples for the affected studies is also appropriate.

2. Contamination Study

2.1 *Study Purpose:*

The contamination study is performed to establish there is no detectable exogenous DNA within the Rapid DNA reagents and consumables. It will also characterize the potential for sample-to-sample contamination.

2.2 *Study Considerations:*

Rapid DNA reagent cartridges/chips are single-use/self-contained items; therefore, instances of environmental and/or user-introduced reagent contamination are greatly reduced. Nevertheless, reagent contamination during the manufacturing process and sample-to-sample contamination due to faulty cartridge/chip lots, carryover, or capillary crosstalk (depending on the instrument used) remain a possibility.

- The laboratory should perform a contamination assessment for each lot of cartridges/chips utilized during the validation to ensure that they are free of contaminants.
    - If a 'clean' swab is used in place of an empty well for this study, the user must keep in mind that any potential contamination could be originating from the substrate rather than the Rapid DNA chemistry or process.
- Contamination studies can be performed in combination with other validation studies.
- Laboratories should examine the data for the presence of carryover/crosstalk from one sample to another (e.g., high input swab to blank swab, high input swab to low input swab, etc).
    - Data from all studies should be evaluated for carryover/crosstalk, as it may appear at a low level between samples or across multiple samples.
- Should the laboratory consider the use of Rapid DNA for forensic samples requiring pre-processing (e.g., bone and sexual assault kit evidence), reagent blanks associated with such samples should be included in the contamination assessment.

2.3 *Study Outcome:*

The laboratory should use any instances of contamination that occur during the validation to inform their procedure for the detection and control of contamination during routine Rapid DNA operations.

3. Sensitivity and Stochastic Studies

3.1 *Study Purpose:*

Sensitivity studies for modified Rapid DNA analysis are performed to determine the dynamic range, heterozygote balance (e.g., peak height ratio [PHR]) and the signal-to-noise ratio associated with the assay. Sensitivity studies can also be used to evaluate stochastic effects generally resulting from low quantity and/or low-quality samples, as well as assessing other artifacts (e.g., stutter) or oversaturation effects created by high template samples.

3.2 *Study Considerations:*

Data obtained from the sensitivity study should be used to determine analytical and stochastic thresholds. Depending on the analysis software used, a threshold can be established either per marker (locus), per dye channel, or across all dye channels of the

multiplex kit. These thresholds can be applied to all instruments of the same make, model and system software.

- The laboratory must use unextracted biological fluids for this study.
    - An identical set of samples can be processed via conventional methods to determine the amount of DNA in a sample.
    - The user should keep in mind that conventional laboratory methods are used to quantify the amount of DNA prior to amplification whereas the Rapid DNA instrument estimates the quantity of DNA in the sample that was amplified.

- The use of different Rapid DNA swabs or Rapid DNA instrument protocols affects the amount of DNA being introduced at amplification and should be considered when establishing the subsequent interpretation parameters.

    - If supported by validation results, a laboratory may choose to apply the same modified Rapid DNA interpretation thresholds for the cartridge/chip regardless of the type of swab or extraction protocol being used.

    - The laboratory can validate the use of a separate set of interpretation thresholds specific to a type of swab and or Rapid DNA instrument protocol to maximize the use of the data generated for specific sample types (e.g., samples known to yield low amounts of DNA).

- The assessment of the sensitivity data should consider the instrument generated quantification values or ranges to ensure that they are consistent with expectations based on the sample type and amount (stain size, etc.) being introduced.

3.3 *Study Outcome:*

At the completion of these experiments, the laboratory should be able to characterize the instrument's limitations and establish general performance expectations for modified Rapid DNA analysis.

4. Precision (repeatability and reproducibility) and Accuracy

4.1 *Study Purpose:*

To establish if the Rapid DNA instrument can generate consistent and correct allele calling results.

4.2 *Study Considerations:*

- Accuracy can be measured by evaluating concordance between samples with known genotypes and samples run on a Rapid DNA instrument. Samples from other studies can also be used to demonstrate reproducibility.

- Some Rapid DNA instruments use a pool of virtual ladders to account for environmental variations and ensure the most precise sizing of the allelic data for each run. Because of their 'intentional' size variation, traditional precision studies are not possible using the pool of virtual ladders. Therefore, precision may be evaluated using samples with known genotypes and comparing the sizing in relation to the virtual ladder applied.

4.3 *Study Outcome:*

Concordance assessment should demonstrate the Rapid DNA instrument's ability to consistently generate accurate DNA STR genotypes using modified Rapid DNA.

5. Mixture Study

5.1 *Study Purpose:*

To demonstrate the ability of the Rapid instrument to detect mixed DNA samples and to determine if the laboratory will interpret mixtures of Rapid DNA data.

5.2 *Study Considerations:*

- Mixture samples used for this study should also be run with conventional DNA typing methods to enable a comparison between the conventional and Rapid DNA typing data.
- If a laboratory decides to conduct mixture interpretation on Rapid DNA data, a more thorough mixture assessment must be conducted to establish guidelines for mixture interpretation, in accordance with the SWGDAM Autosomal Multiplex Kit Internal Validation Module.
    - Mixed samples representative of the sample type, number of contributors (NOC), contributor ratios and template quantities expected to be interpreted should be included in the study.

5.3 *Study Outcome:*

The data obtained from this study will provide an understanding of the performance of mixed DNA samples on the Rapid DNA instrument.

When evaluating the data, the laboratory should determine the criteria to be used to identify a mixture (e.g., the number of alleles present, peak height imbalance, etc.), distinguish alleles from potential artifacts (e.g., stutter), estimate the number and relative ratio of contributors and establish the potential for variability between replicate amplifications.

For laboratories that determine not to interpret Rapid DNA mixture data, data from this study will demonstrate the Rapid DNA instrument's ability to detect mixtures that will not be further analyzed.

For laboratories seeking to interpret Rapid DNA mixture data, the information obtained from this study will form the basis of subsequent mixture validation studies to define the limitations of modified Rapid DNA analysis mixture interpretation. Comparison between Rapid DNA data and conventional DNA data is critical for the laboratory to make informed mixture policy decisions due to the decreased sensitivity of Rapid DNA instrumentation. Within these studies, the laboratory may define parameters for discerning a major contributor(s) and may determine a lower limit where a minor contributor component can be detected. The effects of pull-up and stutter on the interpretation of minor contributor(s), and how mixture ratios can vary across the profile at varying levels of DNA template may also be evaluated.

6. Known and Non-probative Samples or Mock Samples Study

6.1 *Study Purpose:*

Known and non-probative samples, and/or mock casework samples, are used to evaluate allele call concordance information using the validated parameters generated from the internal validation studies. This study is intended to assess the overall performance of the Rapid DNA instrument and the modified Rapid DNA analysis parameters being validated.

6.2 *Study Considerations:*

The samples used for this study should be representative of the types of samples/substrates expected to be processed on the Rapid DNA instrument, to include those associated with mobile/temporary deployment of the instrument and those processed as part of an agreement with partner agencies, if applicable.

- In addition to the 'routine' forensic type samples, the following should be assessed, as applicable:
  - Any subsampling of reference or forensic samples
  - Sample preprocessing methods

- Sample types associated with unidentified human remains (UHRs) such as tissue, calcified samples (i.e., bones, charred bones), and/or keratinized samples (i.e., hair and fingernail clippings)

6.3 *Study Outcome:*

The data from this study will provide an evaluation of the performance of the Rapid DNA instrument as it relates to the relevant sample types and the data interpretation parameters determined throughout the validation.

7. Appendix A: Example Validation Studies – Modified Rapid DNA Analysis for Forensic Samples

The following examples are representative of studies conducted by a typical casework laboratory. These examples are informational and are not intended to dictate the types and numbers of samples used to satisfy each study. Each laboratory seeking to evaluate a new method, whether databasing or casework, must determine which validation studies are relevant to the methodology, in the context of its application, and determine the experiments required to satisfy each study. Laboratories are encouraged to consult the published literature (some provided in the references list) for additional examples or information. While validation studies cannot account for all sample types or scenarios that may arise during Rapid DNA processing, laboratories should design and conduct experiments to establish and verify the platform's performance for the intended scope of use before implementation in forensic casework. Following implementation, laboratories are encouraged to assess the results produced from a method to ensure it performs as expected within the scope of the validation. If necessary, the laboratory may conduct supplemental studies to expand the scope of the validation.

Study #1: Contamination Study

A contamination study was performed by alternating empty sample wells/cartridges and known samples within a chip or as consecutive runs.

The data was evaluated for carry-over from an adjacent well or previous run as well as for extraneous peaks in the electropherogram.

Additional runs were performed to address some unexpected signals in the positive/known samples.

Study #2: Sensitivity and Stochastic Studies

A sensitivity study was performed by applying decreasing volumes of a given biological fluid to a swab. The amount of DNA was estimated by performing quantitation using normal laboratory techniques on an identical sample set.

- Blood from two donors was spotted in duplicate onto appropriate swabs in volumes of 0.5 µL, 1 µL, 2 µL, 3 µL, 5 µL, 10 µL.
- The quantification values generated by the Rapid DNA instrument were compared to the sample input volume for each of the samples to establish the correlation between them.
- The DNA profiles obtained from each sample were assessed for allele dropout, heterozygote peak height ratios, intra-dye peak height balance, and stutter. These results were compared to the input volume and quantitation results to establish their correlation.
- Based on the results obtained, additional input amounts (higher and/or lower) were assessed to cover a broader expected range that may be encountered in casework.

Study #3: Precision (repeatability and reproducibility) and Accuracy

Samples used throughout the validation that had been previously characterized using traditional laboratory methods were used to conduct an allele call concordance check.

For instruments that do not use a pool of virtual ladders:

Ladder data from the runs conducted throughout the studies were collated. The standard deviation for each detected allele from the group of ladders was calculated to ensure that no value exceeding 0.167 standard deviation (SD) was obtained.

For instruments that rely on virtual ladders:

Data generated throughout the studies were grouped according to the virtual ladder used. Calculations were conducted to ensure that the differences in allele size between a sample allele and the corresponding ladder allele were ideally less <0.3 bp, but never greater than 0.4 bp.

Study #4: Mixture Study

This laboratory does not expect to interpret mixtures using modified Rapid DNA analysis based on results from the sensitivity study. Appropriate amounts/volume of blood were used to create 2-person mixtures at 10:1, 5:1 and 1:1 ratios.

The results were used to establish the instrument's ability to detect mixed sample data.

Study #5: Known and Non-probative Evidence Samples or Mock Evidence Samples Study

Fifteen samples representative of the substrates and sample types to be processed in the Forensic Rapid DNA Program were analyzed on the Rapid DNA instrument using the analysis/interpretation parameters optimized throughout the validation studies.

Samples that required preprocessing and/or sub-sampling were included in this study and analyzed according to the laboratory's Rapid DNA instrument method.

All samples generated data concordant with expected results. Data from this study supported the sample types best suited for testing in a Forensic Rapid DNA program.

8. References and Suggested Readings

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Validation information on STRBase. Available at https://strbase.nist.gov/Information/Validation

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