



SWGDM Clarification on Proficiency Testing of qPCR workflows including Y-screening

Scope

This document is intended to clarify the proficiency testing requirements in the FBI Director's *Quality Assurance Standards for Forensic DNA Testing Laboratories* for analysts, technicians or other qualified individuals who perform and/or interpret quantitative (qPCR) results for the purposes of making decisions about whether a sample will be amplified and/or which amplification kit will be used based on quantitative thresholds and/or quantitative autosomal/Y DNA ratios resulting from internal validation data.

Introduction

The use of any DNA method, irrespective of the outcome (e.g., screening, typing), must meet all FBI Quality Assurance Standards to include proficiency testing. Proficiency testing serves several purposes, including evaluating an individual's performance, to ensure compliance with laboratory procedures and to verify the ability to achieve correct DNA typing results using laboratory methods. Standard 13 states that analysts, technical reviewers, technicians, and other personnel designated by the technical leader undergo semiannual external proficiency testing in each technology performed to the full extent in which they participate in casework. In recent years, laboratories have begun to make workflow decisions utilizing quantitative PCR results to selectively process samples. The following are some examples:

A. Y-screening:

Multiple assault samples are extracted and quantified. The scenario supports that only the male DNA is probative; therefore, only those samples in which male DNA is detected are taken forward for amplification and typing. The amount of male DNA may also determine which amplification and typing kit is used. No further testing is conducted on samples lacking male DNA.

B. Quantification cutoff:

A "touch/trace" sample is extracted and quantified. Because the autosomal DNA quantification does not exceed the laboratory's validated quantification threshold, no further testing is conducted on the sample.



Regardless of the workflow, proficiency test samples are expected to be processed in the same manner as evidence samples and are therefore expected to undergo the same qPCR DNA screening as evidence samples. However, current commercial proficiency tests will rarely contain sexual assault evidence without a male component or contain trace samples of limited quantity which are the types of samples which may be discontinued based on qPCR DNA results. Depending on the proficiency test scenario and “evidence”, it may be necessary to consider exceptions to a laboratory’s qPCR screening workflow to ensure all proficiency test samples are amplified and typed for reporting purposes.

For example, an exception to the Y-screening workflow above may be necessary if the proficiency scenario indicated a female assault victim with a male assailant and evidence from the victim indicates samples lacking male DNA. Per the Y-screening workflow, that sample would not be amplified or typed; however, in order to report DNA typing results for all proficiency samples, this sample would be amplified, typed and reported. Documentation of this exception would be noted as part of the laboratory’s standard operating procedures for completing proficiency tests or retained with the laboratory’s proficiency test file.

Below are some examples of how qPCR workflows could be proficiency tested to the full extent of participation:

1. A laboratory has the same analyst performing extractions, quantifications, amplifications, DNA typing, and reporting their own cases that includes workflow decisions based on qPCR male DNA results (Y-screening). As long as the analyst completes a proficiency test to the full extent as he/she conducts analysis of evidentiary samples, then the laboratory’s qPCR workflow satisfies the QAS proficiency testing requirements.
2. A laboratory uses a technician to perform a screening extraction and quantification after which all the data is turned over to the analyst. The analyst is responsible for workflow decisions based on qPCR male DNA results (Y-screening), and that analyst subsequently performs an extraction and quantification followed by amplification, DNA typing, and reporting. As long as the technician performs the screening extraction and quantification methodologies with a proficiency test and the analyst performs the workflow decisions and subsequent extraction, quantification, amplification, typing and reporting with a proficiency test, then the laboratory’s qPCR workflow satisfies the QAS proficiency testing requirements.



3. A laboratory uses several different analysts/technicians to do each of the following steps: extractions, quantifications, workflow decisions based on the qPCR DNA results (either Y-screening or quantification cutoff), amplifications, DNA typing and reporting. As long as the analysts/technicians complete a proficiency to the full extent as he/she conducts case analysis, then the laboratory's qPCR workflow satisfies the QAS proficiency testing requirements.
4. A laboratory uses a technician to perform an extraction and quantification after which all the data is turned over to the analyst. The analyst is responsible for workflow decisions based on validated qPCR quantification thresholds (quantification cutoff) and the analyst subsequently performs amplification, DNA typing and reporting of the selected sample(s). As long as the technician performs the extraction and quantification methodologies with a proficiency test and the analyst performs the workflow decisions and subsequent amplification, typing, and reporting with a proficiency test, then the laboratory's qPCR workflow satisfies the QAS proficiency testing requirements.