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Standard for Interpreting, Comparing and Reporting DNA Test Results Associated with Failed Controls and Contamination Events

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Foreword

When a control, mandated by laboratory or discipline policy, incorporated during forensic DNA testing fails, or the profile data indicate a handling error or the presence of contaminating DNA, it may be possible to interpret, compare, and report data without any re-testing of the sample or DNA extract in some situations. This is imperative when the evaluation and reporting of the data may provide critical and valid information in a criminal case, especially when a person of interest can be excluded from the DNA profile.

Upon discovery of a failed control or a contamination event, it is intended that this standard be used in conjunction with the laboratory's documented quality assurance program to conduct a comprehensive documented root cause analysis to determine the likely procedural or scientific explanation for the event and to institute appropriate corrective actions, as needed. It is also intended that the laboratory performs the requirements of this standard using documented protocols for data interpretation, comparison and reporting with appropriate accompanying validation and protocol verification studies developed with adherence to other available standards for forensic DNA testing (e.g., FBI Quality Assurance Standards for DNA Testing Laboratories, ANSI/ASB Standards 018, 020, 040, and 139; see Bibliography) and with decision-makers shielded from irrelevant information to avoid bias; this is critical for evaluating the quality of the DNA profile to determine if it can be interpreted and compared given the root cause of the event. This document is not intended to support the reporting of data associated with failed controls and/or contamination events without the associated prerequisite for thorough evaluation of the possible cause, assessment of the scientific integrity of the associated DNA test results and impact of the events on the data obtained, nor to support the elimination of the use of vital and laboratory or discipline required DNA test controls.

The American Academy of Forensic Sciences established the Academy Standards Board (ASB) in 2015 with a vision of safeguarding Justice, Integrity and Fairness through Consensus Based American National Standards. To that end, the ASB develops consensus based forensic standards within a framework accredited by the American National Standards Institute (ANSI), and provides training to support those standards. ASB values integrity, scientific rigor, openness, due process, collaboration, excellence, diversity and inclusion. ASB is dedicated to developing and making freely accessible the highest quality documentary forensic science consensus Standards, Guidelines, Best Practices, and Technical Reports in a wide range of forensic science disciplines as a service to forensic practitioners and the legal system.

This document was revised, prepared, and finalized as a standard by the DNA Consensus Body of the AAFS Standards Board. The draft of this standard was developed by the Human Forensic Biology Subcommittee of the Organization of Scientific Area Committees for Forensic Science.

Questions, comments, and suggestions for the improvement of this document can be sent to AAFS-ASB Secretariat, asb@aafs.org or 401 N 21st Street, Colorado Springs, CO 80904.

All hyperlinks and web addresses shown in this document are current as of the publication date of this standard. ASB procedures are publicly available, free of cost, at www.aafs.org/academy-standards-board.

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Standard for Interpreting, Comparing and Reporting DNA Test Results Associated with Failed Controls and Contamination Events

1 Scope

This standard provides requirements for the interpretation, comparison, and reporting of DNA data associated with control failures or contamination where re-testing is not performed. These requirements may be applied to any type of forensic DNA testing technology and methodology used in forensic laboratories.

2 Normative References

There are no normative reference documents, Annex C, Bibliography, contains informative references.

3 Terms and Definitions

For purposes of this document, the following definitions apply.

3.1
comparison
The process of examining two or more DNA data sets to assess the degree of similarity or difference.

3.2
contamination
Exogenous DNA in an extract, polymerase chain reaction, or item of evidence, which may be present before the sample is collected or introduced during collection or testing of the sample.

3.3
failed control
A positive control (see 3.7) or negative control (see 3.6) that produces an unexpected result.

3.4
forensic sample
Biological sample recovered from a crime scene or collected from persons or objects associated with a crime.

3.5
interpretation
The process of evaluating DNA data for purposes including, but not limited to, defining assumptions related to mixtures and single source profiles, distinguishing between alleles and artifacts, assessing the possibility of degradation, inhibition, and stochastic effects, and determining whether the data are suitable for comparison.

3.6
negative control
An analytical control that consists of the reagents used in various stages of testing without the introduction of sample; no results are expected from a negative control.

NOTE For DNA testing, negative controls include extraction blanks/reagent blanks and amplification blanks. A negative control in DNA testing is used to detect contamination introduced into the assay during the testing process via reagents, disposables, or handling errors (which may impact the results observed from samples tested at the same time).

3.7
positive control
An analytical control sample that is used to determine if a test performed properly; this control consists of the test reagents and a known sample that will provide an expected positive response with the test.

NOTE For DNA testing, positive controls include positive amplification controls and may include extraction positive controls.

3.8
reference sample
Biological material obtained from a known individual and collected for the purpose of comparison to evidentiary sample(s).

3.9
suitable for interpretation/comparison
Data deemed appropriate for interpretation/comparison (see 3.1 and 3.5) based on developmental validation studies, the laboratory's internal validation studies, and the laboratory's documented and verified interpretation and comparison protocol.

3.10
unsuitable for interpretation/comparison
Data that cannot be used for interpretation/comparison for reasons including, but not limited to, poor or limited data quality, mixture complexity, or a failure to meet quality assurance requirements based on developmental validation studies, the laboratory's internal validation studies, and the laboratory's documented and verified interpretation and comparison protocol.

4 Requirements

4.1 The laboratory protocol shall define what constitutes:

a) contamination in a negative control;

b) contamination in a positive control;

c) contamination in forensic or reference sample DNA test results;

d) contamination in database sample DNA test results;

e) a failed positive control;

f) a failed negative control.

4.2 The laboratory shall perform and document the assessment of the integrity of the associated DNA test results to determine the impact of the failed control or contamination.

NOTE It is intended that this is performed and documented in conjunction with the laboratory's documented quality assurance program.

4.2.1 The assessment shall be based on the laboratory's validation studies and protocols, including but not limited to interpretation and comparison protocol(s) and quality assurance protocols. This assessment shall include a determination of the possible cause and effect of the failed control or contamination, and an assessment of the risks associated with moving forward with data interpretation vs. those associated with re-testing.

NOTE Re-testing the forensic sample prior to the step in which the problem was identified may be performed; however, there are circumstances where this may not be feasible or necessary. Reasons for not conducting retesting include, but are not limited to: the sample was consumed during the initial analysis so re-testing is not possible; additional testing would exhaust the remaining portion of the sample or DNA extract eliminating the possibility of future testing; re-testing will likely not generate a different DNA profile; or the associated profile(s) would not be suitable for comparison even if the controls produced the expected results. Some questions that might be asked to determine whether the data should be reported without re-testing include: 1) would retesting consume or limit the sample such that it would preclude any future testing with another current or future method? 2) based on the profile observed, is there an expectation that the concern will be resolved with re-testing or would similar results be expected (e.g., same number of contributors, contamination still would be present)?

4.2.2 If the DNA test results are determined to be suitable for interpretation/comparison within the constraints of the laboratory's internal validation studies and documented interpretation and comparison protocols and the laboratory does not retest, the laboratory shall perform and report the interpretation and comparison(s) with applicable statistical analysis.

4.2.3 If the DNA test results are determined to be unsuitable for interpretation/comparison and retesting is not conducted, the results shall be reported as not suitable for interpretation/ comparison according to laboratory policy.

NOTE If the DNA test results are determined to be unsuitable for interpretation and retesting is conducted, it may be necessary to report results, interpretations and comparisons from the original test and subsequent test(s).

4.3 When reporting interpretations and comparisons associated with a failed control or contamination event, the report shall identify the associated DNA test results and describe the nature of the event.

NOTE Examples of scenarios where the data are or are not impacted are provided in Annex A.

4.4 The laboratory shall have a written protocol for the release of identifying information for the source of the contamination.

4.5 The case record for each sample associated with a failed control or contamination event must contain documentation of the required assessment detailed in 4.2 including the following for the affected sample(s), as applicable:

4.5.1 The forensic sample, reference, or control DNA test result that failed or was contaminated.

4.5.2 The likely or known source of contamination.

NOTE If an individual is determined to be the source, that individual may be identified by name, employment position or other descriptor as permitted by law and agency policies.

4.5.3 The likely or known cause of the failed control or contamination.

4.5.4 The impact of the failed control or contaminant on the integrity of the DNA test results.

4.5.5 The determination of whether an affected DNA test result is suitable, or unsuitable, for interpretation/comparison.

Annex A
(informative)

Supplemental Information – Foundational Principles

When polymerase chain reaction (PCR) testing was introduced into crime laboratories in the early 1990s, many of the initial issues encountered by researchers using the highly sensitive PCR testing methods had been recognized. As a result, standard procedures for preventing contamination along with quality control and assurance measures were established in forensic DNA testing laboratories. Even with these critical measures in place, occasional problems occur during DNA testing. Forensic DNA testing and databasing laboratories typically have a number of processes in place for monitoring and evaluating the integrity of the DNA testing results obtained from samples received and processed by the laboratory. When the laboratory identifies instances where the DNA test results may be compromised, the laboratory follows required procedures for establishing the likely cause of the event and for assessing its impact on the data obtained. This impact assessment step is critical in that the DNA test results may still be valid and further interpretation may provide valuable information, such as exculpatory evidence. This type of evaluation has been routinely and historically performed for mitochondrial DNA (mtDNA) testing.

When performing PCR testing, forensic DNA testing laboratories are required to have a positive amplification control associated with each set of DNA extracts amplified together [3]. This control monitors the DNA testing process performed through all steps commencing at the amplification step. Some laboratories require an additional positive control to be associated with the DNA extraction batch that then follows the samples through the entire DNA testing process. At the end of testing, DNA test results from the positive controls(s) should be consistent with the expected reference single source profile(s). The presence of the correct DNA test results in the positive control indicates the testing process(es) monitored by the control(s) performed correctly.

There are several possible causes for a positive control failure (as defined by the laboratory), including a technical issue (e.g., problem with an instrument or reagent that precludes the test from working correctly). Similar issues may have occurred with the associated samples. When it is not possible to use the results due to a concern of accuracy, then retesting starting from a point before the instrument or reagent issue is necessary to generate test results that can be reliably interpreted, compared, and reported. If retesting is not possible and the integrity of the DNA test results cannot be confirmed, the results may be reported as “insufficient/unsuitable for comparison” or “inconclusive” due to the control failure.

In some cases, the positive control failure may be determined to be specific to only that sample, with the other DNA test results processed with the control seemingly unaffected. This may occur, for example, if DNA or reagents were inadvertently not added to the control but added correctly for the other DNA extracts. In this case, it may be possible to verify that the other results associated with the failed control can be interpreted, compared, and reported after fulfilling the requirements of this standard without retesting all of the samples involved.

In addition, when performing PCR testing, forensic DNA testing laboratories are required to have two negative controls associated with each set of DNA samples tested [6,7]. One negative control, typically referred to as a reagent blank or extraction blank control, is started with each set or batch of samples extracted together; the second negative control is the negative amplification control started at the amplification step for each set of samples undergoing amplification together. These two negative controls are processed throughout each step of the DNA test alongside the associated

samples. These two controls consist of all reagents, solutions, consumable materials, etc. used during the DNA testing process, and it is expected that the negative controls meet the laboratory's definition for suitable performance when evaluated at the end of the testing. When contamination is identified, the laboratory is responsible for evaluating the likely biological source of the contamination and assessing when and how the event most likely occurred.

In some situations, the contaminating DNA is only detected in a negative control with no apparent presence in or effect on any of the other samples tested. This single contamination event may be due to any number of reasons where DNA could be introduced only into a single sample, for example, its presence in or on a consumable material used in the laboratory during testing (e.g., pipet tip, tube). In other situations, the contaminating DNA may be detected in the profiles from other samples tested along with the control(s) but be present at such a low level that it has minimal to no impact on the quality of the DNA test results obtained from the tested sample (e.g., DNA profile from a high quality single source or two person mixed DNA profile with a very low level minor component consistent with the profile in the negative control and possibly other samples). In these cases, the DNA test results may be reasonably determined, interpreted, and used for comparison according to established laboratory protocols in accordance with the requirements listed in this document.

DNA contamination may also be present in one or more of the forensic or reference samples. Many laboratories have internal DNA databases comprised of DNA data from laboratory or other personnel who may routinely come into contact with samples or be present in the environment where forensic samples are handled or processed (e.g., law enforcement, evidence technicians, crime scene investigators, maintenance staff). These DNA databases may be used as a screening mechanism for the detection of possible DNA contamination events (also see ANSI/ASB Best Practice Recommendations 171, *Recommendations for the Management and Use of Quality Assurance DNA Elimination Databases in Forensic DNA Analysis*, First Edition, 2023). Similarly, some laboratories compare the data obtained within certain test batches to screen for possible contamination events that may have occurred between DNA extracts processed concomitantly. During these evaluations, the source of the contaminating DNA may be identified. In this situation, it may be possible to evaluate the DNA test results even in the presence of contaminating DNA from a known individual, similar to the interpretation steps used to evaluate mixed DNA test results when a known contributor to a DNA mixture is assumed. The use of an assumed contributor in the interpretation and comparison of the data should be reported according to the laboratory's protocol and best practice recommendations for reporting evaluations performed using assumed contributors.

Additional standards and best practice recommendations are referenced in the Bibliography that may be used in conjunction with this standard and provide additional useful information

Annex B
(informative)

Supplemental Information – Examples

The following examples describe different scenarios where samples are associated with a failed control or contamination event with some possible outcomes responsive to the requirements of this standard. When reporting interpretations and comparisons associated with a failed control or contamination event, the report identifies the associated DNA test results and describes the nature of the event. Every situation must be evaluated on a case-by-case basis in conjunction with the laboratory's documented interpretation and comparison protocol(s) and quality assurance program, including evaluations, root cause analyses, risk assessments, and applicable corrective actions. As with many situations of compromised or complex profiles, there may be scenarios where the data are sufficient for excluding individuals as a possible contributor but insufficient for reporting a possible association and providing a statistical value.

1) No results were obtained for the amplification positive control. The associated forensic samples provided partial or full profiles that corresponded logically to their respective quantitation results and, where predictable, the expected results (e.g., single source DNA profile from a presumed blood stain or "sperm" fraction; "non-sperm/epithelial" fraction results consistent with complainant; duplicate amplifications of a DNA extract). Amplification results consistent with expectations confirm the PCR amplification was successful and that the allele calling by the software was appropriate. Based on the laboratory's root cause investigation, it was determined that the analyst most likely did not add the known DNA to the amplification positive control sample, and the associated profiles were interpreted and used for comparison purposes. It was expected that re-testing would generate a similar DNA profile. The issue and resolution were documented in the case record and the results were reported per the laboratory protocol since the results were not directly impacted by the failed control.

2) The DNA profile from the forensic sample associated with a failed positive control demonstrated the presence of a mixture of at least six individuals. The assessment of the impact of the failed positive control determined that the interpretation of the forensic sample profile was not affected since the laboratory's protocol does not permit the interpretation of mixtures of greater than four individuals. No retesting was performed; the forensic sample profile was reported as not suitable for comparison purposes due to the high number of contributors.

3) The DNA profile of a member of the laboratory was detected as a minor component of a two-person mixture profile detected from a forensic sample. The laboratory staff member was the individual who performed the latent print examination on the sample prior to the DNA testing. A similar profile was expected upon re-testing due to the nature of the contamination event. The DNA profile was interpreted and used for comparison under the assumption that the laboratory staff member was one of the contributors to the mixture.

4) A low-level DNA profile was detected in the extraction reagent blank that was consistent with the low-level DNA profile detected from the forensic sample. The forensic sample and DNA extract were consumed during testing. Investigation could not determine the cause of the contamination event (e.g., whether cross contamination occurred or whether the reagents themselves were contaminated). The results for the forensic sample were reported as not suitable for comparison purposes.

5) The DNA profile of the analyst was detected in the "epithelial cell fraction" of a sexual assault kit sample and there was no indication of contamination of the "sperm fraction" profile. Because the remaining contributor profile in the epithelial cell fraction was consistent with the complainant, retesting was not performed. Results from both the "epithelial cell fraction" and "sperm fraction" were interpreted, used for comparison, and reported.

6) The DNA profile of the analyst who performed an amplification set up was detected in the negative amplification control. A review of the associated sample profiles showed that the profiles were not impacted by the contamination and no retesting was performed. The issue and resolution were documented in the case record and the results were reported per the laboratory protocol.

7) The DNA profiles from an amplification plate showed a low-level contaminant throughout, indicating that there may have been contamination of the amplification master mix. Because of the way the contaminant presents, the associated forensic sample profiles were determined to be unsuitable for comparison. The DNA amplified included the consumed extract of a single swab (also consumed) from the neckline of a shirt. The neckline of the shirt was resampled by taking and consuming a second swab, and an interpretable and comparable profile was obtained. The laboratory report addressed both the first and second sampling of the neckline of the shirt.

8) The DNA profile of an individual in the chain of custody prior to the receipt of the evidence into the laboratory was detected as the major component of a mixed DNA profile likely from two individuals. No re-testing was performed since it was not expected that the contaminating DNA would be eliminated upon re-testing, even if any remaining forensic sample or DNA extract existed. The issue and resolution were documented in the case record and reported according to the laboratory protocol. The exclusion of individuals from the low level minor component profile was also reported according to the laboratory protocol.

Annex C
(informative)

Bibliography

The following bibliography is not intended to be an all-inclusive list, review, or endorsement of literature on this topic. The goal of the bibliography is to provide examples of publications addressed in the standard.

1] ANSI/ASB Standard 018, *Validation Standards for Probabilistic Genotyping Systems,* First Edition, 2020.[a]

2] ANSI/ASB Standard 020, *Standard for Validation Studies of DNA Mixtures, and Development and Verification of a Laboratory's Mixture Interpretation Protocol*, First Edition, 2018.[a]

3] ANSI/ASB Standard 040, *Standard for Forensic DNA Interpretation and Comparison Protocols*, First Edition, 2019.[a]

4] ANSI/ASB Standard 139, *Reporting DNA Conclusions*, First Edition, 2024.[a]

5] ANSI/ASB Best Practice Recommendations 171, *Recommendations for the Management and Use of Quality Assurance DNA Elimination Databases in Forensic DNA Analysis*, First Edition, 2024.[a]

6] Forensic Science Regulator. *Forensic Science Providers: Codes of Practice and Conduc*t. 2014.[b]

7] Federal Bureau of Investigation. *Quality Assurance Standards for DNA Databasing Laboratories.*[c]

8] Federal Bureau of Investigation. *Quality Assurance Standards for Forensic DNA Testing Laboratories*.[c]

9] ISO/IEC 17025 *Testing and Calibration Laboratories*.[d]

10] SWGDAM. Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories.[e]

[a] Available from: https://www.aafs.org/academy-standards-board
[b] Available from: https://www.gov.uk/government/collections/forensic-science-providers-codes-of-practice-and-conduct
[c] Available from: https://www.swgdam.org/publications
[d] Available from: https://www.iso.org/ISO-IEC-17025-testing-and-calibration-laboratories.html
[e] Available from: https://www.swgdam.org/_files/ugd/4344b0_c4d4dbba84f1400a98eaa2e48f2bf291.pdf

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