2020-S-0004
Standard for Interpreting, Comparing and Reporting DNA Test Results Associated with Failed Controls and Contamination Events

Human Forensic Biology Subcommittee
Biology Scientific Area Committee
Organization of Scientific Area Committees (OSAC) for Forensic Science

OSAC Proposed Standard

2020-S-0004
Standard for Interpreting, Comparing and Reporting DNA Test Results Associated with Failed Controls and Contamination Events

Prepared by
Human Forensic Biology Subcommittee
Version: 2.0
May 2021

Disclaimer:

This OSAC Proposed Standard was written by the Human Forensic Biology Subcommittee/Biology Scientific Area Committee of the Organization of Scientific Area Committees (OSAC) for Forensic Science following a process that includes an open comment period. This Proposed Standard will be submitted to a standards developing organization and is subject to change.

There may be references in an OSAC Proposed Standard to other publications under development by OSAC. The information in the Proposed Standard, and underlying concepts and methodologies, may be used by the forensic-science community before the completion of such companion publications.

Any identification of commercial equipment, instruments, or materials in the Proposed Standard is not a recommendation or endorsement by the U.S. Government and does not imply that the equipment, instruments, or materials are necessarily the best available for the purpose.

To be placed on the OSAC Registry, certain types of standards first must be reviewed by a Scientific and Technical Review Panel (STRP). The STRP process is vital to OSAC's mission of generating and recognizing scientifically sound standards for producing and interpreting forensic science results. The STRP shall provide critical and knowledgeable reviews of draft standards or of proposed revisions of standards previously published by standards developing organizations

(SDOs) to ensure that the published methods that practitioners employ are scientifically valid, and the resulting claims are trustworthy.

The STRP panel will consist of an independent and diverse panel, including subject matter experts, human factors scientists, quality assurance personnel, and legal experts, which will be tasked with evaluating the proposed standard based on a comprehensive list of science-based criteria.

For more information about this important process, please visit our website at: https://www.nist.gov/topics/organization-scientific-area-committees-forensic-science/scientific-technical-review-panels

Foreword

Controls are routinely incorporated during DNA testing of forensic and reference samples in forensic DNA testing laboratories. If all controls generate the expected results, there is a high level of confidence in the profile data from the associated sample set. A control may fail for various reasons. In addition, the profile data may indicate a handling error or the presence of contaminating DNA. Retesting 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, additional testing would exhaust the remaining portion of the sample or DNA extract eliminating the possibility of future testing, or the associated profile(s) would not be suitable for comparison even if the controls produced the expected results.

There are scenarios where it may be possible to interpret, compare, and report data with some level of confidence, even if the data are associated with the failure of a control or a contamination event (of a sample or control). Evaluation and reporting of the possibly compromised data may provide critical and valid information to support the investigation of a criminal case, for example excluding a person of interest. To the extent determination of contamination may be influenced by judgmental bias, persons making that determination should be shielded from irrelevant information.

It is intended that this standard be used in conjunction with the laboratory's documented quality assurance program. This would ensure that proper evaluations, root cause analyses, risk assessments, and corrective actions, when necessary, have been performed and appropriately documented for each instance of a failed control or contamination event that occurs in the laboratory. It is also intended that the laboratory perform the requirements in this standard using documented protocols for data interpretation, comparison and reporting with appropriate accompanying validation and protocol verification studies along with the strong reliance on other available standards for forensic DNA testing (e.g., FBI Quality Assurance Standards for DNA Testing Laboratories, ANSI/ASB Standards 18, 20, 40, 136 and 139 and OSAC 2020-N-0007, Best Practices Recommendations for the Management and Use of Quality Assurance DNA Elimination Databases in Forensic DNA Analysis; see Bibliography). This document is not intended to support the reporting of data associated with failed controls or contamination events without the associated prerequisite for thorough evaluation of the possible cause and impact of the events on the data obtained.

The draft of this standard was developed by the Biological Data Interpretation and Reporting Subcommittee of the Organization of Scientific Area Committees for Forensic Science.

Keywords: *contamination, failed control, reporting DNA results, DNA interpretation*

Table of Contents

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. DNA data associated with a failed control or a contamination event may still be scientifically valid and may be relevant to an investigation.

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 or other biological material in a DNA sample, PCR reaction, or item of evidence; the exogenous DNA or biological material could be present before the sample is collected or introduced during collection or testing of the sample.

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

3.4
forensic sample
A biological sample originating from and associated with evidence from a crime scene. A sample associated with evidence from a crime scene may include a sample that has been carried away from the crime scene.

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

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

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

3.10
unsuitable for comparison
Data that cannot be used for comparisons for reasons including, but not limited to, poor or limited data quality, mixture complexity, or a failure to meet quality assurance requirements. This decision is 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:

4.1.1 Contamination in a negative control

4.1.2 Contamination in a positive control

4.1.3 Contamination in forensic or reference sample DNA test results

4.1.4 A failed positive control

4.1.5 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. The assessment shall be based on scientifically valid principles in DNA analysis and 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.

4.2.1 If the DNA test results are determined to be suitable for interpretation 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.

4.2.2 If the DNA test results are determined to be compromised to the extent of being unsuitable for interpretation and retesting is not conducted, the results shall be reported as not suitable for interpretation according to laboratory policy.

NOTE If the DNA test results are determined to be compromised to the extent of being unsuitable for interpretation and retesting is conducted, it may be necessary to report results, interpretations, and comparisons from both the original and second tests.

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

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 include documentation of 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 The source 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.

Annex A

(informative)

Supplemental Information – Historical 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.

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 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 [3]. 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., pipette 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 are 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 Best Practice Recommendations for the Management and Use of Quality Assurance DNA Elimination Databases in Forensic DNA Analysis). 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:

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. 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 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. 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. Since the interpretation was directly impacted, the contamination event was described in the report.
3. 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. Since the contamination event directly impacted the interpretation of the profile from the forensic sample, the contamination event was described in the report.
4. 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.
5. The DNA profile of the working DNA 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. 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 cell fraction were interpreted, used for comparison and reported. Since the contamination event directly impacted the interpretation of the forensic sample profile, the contamination event was described in the report

6. The DNA profile of the technician who performed amplification set up was detected in the negative amplification control. A review of the associated samples shows that they 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 since the results were not directly impacted by the contamination.
7. The DNA profiles from an amplification plate show 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 includes the consumed extract of a single swab (also consumed) from the neckline of a shirt. The neckline of the shirt is resampled by taking and consuming a second swab, and an interpretable profile is obtained. The laboratory report should address both the first and second sampling of the neckline of the shirt.

Annex C
(informative)

Bibliography

1] Forensic Science Regulator. Forensic Science Providers: Codes of Practice and Conduct. 2014. Available at https://www.gov.uk/government/collections/forensic-science-providers-codes-of-practice-and-conduct

2] SWGDAM Contamination Prevention and Detection Guidelines for Forensic DNA Laboratories. Available at www.swgdam.org

3] Federal Bureau of Investigation. Quality Assurance Standards for Forensic DNA Testing Laboratories. Current version available at www.swgdam.org

4] ISO/IEC 17025 Testing and Calibration Laboratories. Current version available at https://www.iso.org/ISO-IEC-17025-testing-and-calibration-laboratories.html

ANSI/ASB and/or OSAC Standards and Best Practices Applicable to this Standard

5] OSAC 2020-N-0007, Best Practice Recommendations for the Management and Use of Quality Assurance DNA Elimination Databases in Forensic DNA Analysis; Current version available at https://www.nist.gov/osac/osac-registry *(accessed May 2021)*

6] ANSI/ASB Standard 136, Forensic Laboratory Standards for Prevention, Monitoring, and Mitigation of DNA Contamination, Ed. #1 *(currently in review at ASB; has been out for public comments)*

7] ANSI/ASB Standard 018, Validation Standards for Probabilistic Genotyping Systems First Edition, 2020 http://www.asbstandardsboard.org/wp-content/uploads/2020/07/018_Std_e1.pdf (accessed October 2020)

8] 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 https://asb.aafs.org/wp-content/uploads/2018/09/020_Std_e1.pdf (accessed December 2020)

9] ANSI/ASB Standard 040, Standard for Forensic DNA Interpretation and Comparison Protocols, First Edition, 2019 http://www.asbstandardsboard.org/wp-content/uploads/2019/10/Std_040_e1.pdf (accessed December 2020)

10] ANSI/ASB Standard 139, Reporting DNA Conclusions *(currently in review at ASB; has been out for public comments)*