# Scientific Working Group on DNA Analysis Methods Guidelines for the Collection and Serological Examination of Biological Evidence

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## SWGDAM Guidelines for the Collection and Serological Examination of Biological Evidence

The Scientific Working Group on DNA Analysis Methods, better known by its acronym of SWGDAM, is a group of approximately 50 scientists representing federal, state, and local forensic DNA laboratories in the United States and Canada. During meetings, which are held twice a year, subcommittees discuss topics of interest to the forensic DNA community and often develop documents to provide direction and guidance for the community. This document was approved by the SWGDAM membership and posted for public comment in December 2014. The SWGDAM Executive Board reviewed and approved minor revisions to address comments on January 15, 2014.

This document provides guidance for collecting and conducting serological examinations on biological evidence. Forensic serology is the identification and characterization of biological fluids on evidentiary

items. Serological testing is often a precursor to DNA examinations on items of evidence where biological material is potentially present. Therefore, the quality and integrity of the serological testing process is critical for the success of subsequent DNA analysis. These guidelines are intended for agencies and organizations that collect items of evidence and/or conduct serological examinations to conclude that the evidence contains or may contain a specified biological fluid or material. The results of these serological examinations may be used by a laboratory conducting forensic DNA testing. For the purposes of this document, an agency or organization that conducts and reports results of serological examinations, whether in a laboratory setting or at a crime scene location, shall be referred to as a laboratory.

Laboratories conducting serological examinations are encouraged to review their standard operating procedures in light of these guidelines and to update their procedures as needed. This document does not invalidate any biological fluid identification testing previously performed and the intent is that the guidance be applied prospectively and not retroactively. With the underlying assumption that work (validation, training, analysis, interpretation) performed prior to the issuance of this document was appropriate and scientifically valid, these guidelines are not intended to invalidate or call into question the previous work. It is anticipated that these guidelines will evolve further as future technologies emerge (e.g., RNA).

## 1. Accreditation

**1.1** The laboratory should seek accreditation in Body Fluid Identification (or an equivalent category of testing) and follow the accrediting body's assessment scheme.

## 2. Facilities

**2.1** The laboratory should have a facility that is designed to ensure the integrity of all evidence and testing.

**2.2** Access to the laboratory should be controlled and limited to prevent access by unauthorized personnel. All exterior entrance/exit points should be security controlled. The distribution of all keys, combinations, etc., should be documented and limited to personnel as designated by laboratory management.

## 3. Personnel

**3.1** The laboratory should have written job descriptions for personnel that may be augmented by additional documentation, to define responsibilities, duties and skills.

**3.2** Personnel should have the education, training and experience commensurate with the responsibilities, duties and skills of the assigned position.

## 4. Training

**4.1** The laboratory should have a documented training program for qualifying all personnel that will collect potential biological evidence and/or all personnel that will conduct serological examinations.

**4.2** Any personnel collecting or processing potential biological evidence should have documented training that includes safety, handling, packaging, storing, and tracking biological evidence. For more guidance on these topics, a laboratory may refer to the *Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers* by the Technical Working Group on Biological Evidence Preservation.

**4.3** In addition, the training program for personnel conducting and reporting serology examinations (serologists or equivalent role, position, or title as designated by the laboratory), should define the technical skills and knowledge required to perform serological analysis and the level of detail should be applicable to the individual job responsibilities[1].

**4.4** The training program for personnel conducting and reporting serology examinations (serologists or equivalent role, position, or title as designated by the laboratory) should include:

**4.4.1** Policies and procedures pertaining to

- The preferred order in which evidence is to be tested
- What information to take into account when establishing which assay to use

[1] Because the identification of body fluids on items of evidence submitted to forensic laboratories is often a precursor to further characterization using DNA testing for human identification, the SWGDAM Training Guidelines are recommended for additional information.

- Preservation of biological material (e.g., for additional characterization, Bloodstain Pattern Analysis (BPA) considerations, DNA testing, or in accordance with applicable law)

**4.4.2** Fundamental scientific knowledge

- History of serological testing
- Composition of body fluids
- Mechanism of serological tests
    - Visual (A visual examination is conducted to locate possible areas of staining and may include using alternate light sources (ALS).)
    - Chemical (A chemical test is an analytical procedure that exploits the properties of chemicals as they interact with biological molecules under specific conditions to yield a detectable change, such as a color change, luminescence or fluorescence, or formation of crystals.)
    - Histological (A histological test is one that uses microscopic study to identify cell types often using differential staining to visualize different cellular structures.)
    - Immunological (An immunological test is an analytical procedure employing antibodies to detect antigens.)
- Test specificity and limitations
    - Presumptive tests (A screening test which may be positive in the presence of a biological material of interest. Presumptive tests are sensitive, but not specific. A positive result indicates that further testing could be informative.)
    - Confirmatory tests (A test that is specific for the presence of a particular biological material. Confirmatory tests are specific for the biological material of interest and reduce or eliminate false positive results.)

- Limit of Detection (The point at which the sensitivity of the test is such that the biological material present is insufficient to produce a positive test result. The result may be categorized as a false negative.)

**4.4.3** Procedures for the serological tests utilized

**4.4.4** Practical exercises to include the examination of a range of samples routinely encountered in casework

**4.4.5** Interpretation of the serological examination results

**4.4.6** Reporting the serological examination results according to policy

**4.4.7** Preparation for testimony and the legal system of the applicable jurisdiction

**4.5** When hiring experienced serologists, their previous training should be assessed and documented. Modification to the training program may be appropriate and will be dependent upon the extent of any previous, documented training.

**4.6** Prior to completion of the training program, a competency/qualification test should be successfully completed. The test should encompass the range of serological examinations personnel will be performing.

**4.7** Successful completion of the training program should be documented.

**4.8** The training program should be updated to incorporate any new methodologies that will be conducted by the laboratory.

**4.9** The laboratory should have a documented program to ensure serologists' technical qualifications are maintained through documented continuing education and review of scientific literature. This should include subject areas relevant to the collection, processing, or testing of forensic biological evidence.

## 5. Proficiency Testing

**5.1** The laboratory should have and follow a program for annual proficiency testing.

**5.2** Proficiency testing should be external (if available) and the test results should be submitted to the proficiency test provider.

**5.3** When possible, relevant tests routinely performed should be conducted on the items contained in the proficiency test.

**5.4** The laboratory should maintain records for proficiency tests. These records should include:

**5.4.1** Test-set identifier

**5.4.2** Identity of the individual completing the test

**5.4.3** Date of analysis and completion

**5.4.4** Copies of all data and notes supporting the conclusions

**5.4.5** Proficiency test results

**5.4.6** Any discrepancies noted

**5.4.7** Corrective actions taken

**5.5** Proficiency test results should be evaluated and the participant should be notified.

**5.5.1** Proficiency test results should be graded as satisfactory or unsatisfactory. A satisfactory grade is attained when the target/consensus result is obtained. Results reported as "no result" or "inconclusive" should be consistent with the written laboratory guidelines. When the target/consensus result is not obtained corrective action should be initiated.

**5.5.2** The laboratory should document that the participant received notification of the graded results.

## 6. Evidence Control

**6.1** The laboratory should have and follow a documented evidence control system to ensure the integrity and security of biological evidence.

**6.1.1** At a minimum, evidence should be marked with a unique identifier on the evidence package.

**6.1.2** The laboratory should define what constitutes evidence and what constitutes work product.

**6.1.3** The laboratory should have and follow written procedures for documenting, collecting, handling and preserving biological evidence.

**6.2** Laboratories that collect evidence from crime scenes should have and follow written policies and procedures for the documentation, collection and preservation of evidence.

**6.3** The laboratory should have and follow written policies and procedures for acceptance of evidence, including categories and conditions of evidence that are and/or are not acceptable.

**6.4** The laboratory should have and follow written policies and procedures for the receipt of evidence addressing at a minimum:

**6.4.1** Condition of the package and seal upon receipt

**6.4.2** Improperly packaged or sealed evidence

**6.4.3** Evidence damaged during storage and transfer

**6.5** Chain-of-custody for all evidence should be documented and maintained in hard copy or electronic format.

**6.5.1** Chain-of-custody should include:

**6.5.1.1** Signature, initials, or electronic equivalent of each individual receiving or transferring the evidence

**6.5.1.2** Corresponding date for each transfer

**6.5.1.3** Evidentiary items(s) transferred

**6.6** The laboratory should have secure storage space with controlled access for evidence and work product while testing is in progress.

**6.7** The laboratory should have and follow documented procedures designed to minimize loss, contamination, and/or deleterious change of evidence and work product while testing is in progress.

**6.8** When possible, the laboratory should retain or return a portion of any evidence stain.

**6.9** The laboratory should have and follow a documented policy for the disposition of evidence.

## 7. Contamination Prevention

**7.1** The laboratory should have and follow cleaning and sample handling procedures to prevent the potential indirect transfer of cellular material onto items of evidence. Additional information on personal protective equipment (PPE), sample handling and packaging guidance may be found in the *Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers* by the Technical Working Group on Biological Evidence Preservation.

**7.2** Disposable gloves should be used at all times when handling evidence.

**7.2.1** When wearing gloves, contact with person or personal items should be avoided to prevent the possibility of secondary transfer.

**7.2.2** Gloves should be replaced if they become visibly or potentially soiled or are defective.

**7.2.3** Gloves should be replaced when moving between items of evidence, dissimilar areas of collection or testing, and/or distinct areas of a crime scene/evidence.

**7.3** Laboratory coats, face masks, and/or other suitable personal protective equipment should be worn. Personal protective equipment should be laundered or properly disposed of upon becoming visibly or potentially soiled, when deemed defective or according to written laboratory procedure.

**7.4** Tools and work surfaces should be thoroughly decontaminated in an effort to remove possible adventitious sources of biological material. For example, using bleach or other commercial products suitable for eliminating biological material, with subsequent steps (e.g.,

water rinses) to ensure residual bleach or cleanser does not remain on the tools and work surfaces.

**7.4.1** Tools should be decontaminated before use, between items or areas of examination, as they become visibly or potentially soiled, and after their final use on a given workday.

**7.4.2** Work surfaces should be decontaminated before use, as they become visibly or potentially soiled, and after their final use on a given workday.

**7.4.3** Disposable paper and tools may be used and should be changed between items or areas of examination or when visibly or potentially soiled.

**7.5** Examinations should be conducted on one item or set of similar items (e.g. a set of vaginal swabs from the same individual) at a time to prevent potential indirect transfer between items.

**7.6** Materials suitable for use in forensic biology (e.g. swabs, filter paper, tubes, water) should be used for collection and testing procedures.

**7.7** Elimination samples of personnel collecting potential biological evidence or performing serological exams should be made available to the forensic DNA testing laboratory to assist in identifying potential sources of contamination.

**7.8** The laboratory should have methods (e.g. controls) in place to monitor for contamination.

## 8. Validation/Method Introduction

**8.1** The laboratory should validate new serological methodology that will be used to conduct examinations. A validation should include, where applicable, studies to evaluate a method's reliability, reproducibility, specificity, sensitivity, and stability. Validation studies should be reviewed and approved prior to use.

**8.1.1** The laboratory should identify scientific literature describing the test and/or the scientific principles that serve as its foundation.

**8.1.2** The laboratory should conduct studies of the method on known and non-probative/mock evidence samples that represent the types of samples expected to be commonly encountered in casework.

**8.1.3** The laboratory should conduct studies to establish the necessary controls for each procedure, the frequency with which the controls should be performed (e.g., concurrently, daily, before use, etc.) and the performance expectations for each control.

**8.2** Before the introduction of a methodology into the laboratory, serologists should successfully complete a competency/qualifying test.

**8.3** Modifications made to validated procedures should be documented. The performance of a modified procedure should be evaluated prior to use on evidence. The evaluation may be accomplished by comparison to the original procedure using similar samples.

**8.4** For laboratory systems that consist of more than one laboratory, validation studies may be shared as long as each serologist receives the necessary training and successfully completes a competency/qualifying test.

## 9. Equipment Calibration and Maintenance

**9.1** The laboratory should use equipment suitable for the methods employed.

**9.2** The laboratory should have and follow a documented program for conducting performance checks and calibrating equipment.

**9.3** The laboratory should have a schedule and follow a documented program to ensure that instruments and equipment are properly maintained. The laboratory should retain documentation of maintenance, service, or calibration.

**9.4** Instruments and equipment that would affect the outcome of a test (e.g. automated sperm search) that have undergone repair, service or calibration, should be checked before use in casework. This check may be accomplished by evaluating a positive and negative control.

## 10. Analytical Procedures

**10.1** The laboratory should have and follow written analytical procedures for each test used.

**10.1.1** The laboratory's standard operating procedures should be reviewed annually by Technical Management and the review should be documented.

**10.2** Procedures should specify safety information, reagents, equipment, controls, sample preservation and processing used in the testing and its interpretation.

**10.3** Reagents and equipment used should be appropriate to the testing being performed.

**10.3.1** The laboratory should have written procedures specifying acceptable commercial reagents and for the formulation of in-house reagents, as appropriate.

**10.3.2** Reagents should be labeled with the identity of the reagent and the expiration date as provided by the manufacturer or as determined by the laboratory.

**10.3.3** Reagents should be stored separately from evidentiary items.

**10.3.4** The laboratory should define and document quality assurance procedures for the evaluation of reagents (e.g., sensitivity or specificity requirements).

**10.4** The laboratory should have written procedures for monitoring the performance of its reagents and/or analytical procedures using positive and negative controls.

**10.4.1** The laboratory should define the frequency with which such controls should be performed (e.g., concurrently, daily, before use, etc.).

**10.4.2** The controls should yield the appropriate results as defined by the analytical procedures prior to the use of a reagent on evidentiary items or the interpretation of the results of a concurrently tested item of evidence. The results or successful performance of the controls should be recorded.

**10.5** The laboratory should have and follow documented procedures designed to minimize loss, contamination, and/or deleterious change of evidence and work product while testing is in progress (e.g. avoid adding reagent directly to an item, avoid indirect transfer to and between items of evidence).

**10.5.1** The laboratory should have a policy for the preservation of biological material for DNA testing. If a limited amount of staining is observed on an item, the serologist should evaluate whether testing should be conducted on the stain(s). For a stain that has tested presumptively positive and which may be limited in size or quality, the laboratory should have a policy that addresses best practices for confirmatory testing and potential future DNA testing aimed at maximizing reliable and informative test results. For example, multiple attempts at presumptive and confirmatory tests should be avoided.

**10.6** The laboratory should utilize written procedures for performing analytical testing.

**10.6.1** Serological procedures should be based upon validation studies, scientific literature, and experience.

**10.6.2** Written procedures should include the order in which serology tests should be performed. Conducting testing in a specific order may assist the serologist in efficiently testing large numbers of potential stains while preserving biological material for DNA testing.

**10.6.3** Written procedures should address:

**10.6.3.1** Preferred order in which evidence from the same case is to be tested

**10.6.3.2** Information (e.g. limitations of the tests) to take into account when determining which test to perform

**10.6.3.3** Recording examination notes

**10.6.3.4** Requirements for deviating from written procedures

**10.6.4** The laboratory should have written procedures for conveying the serological results and location of any potential biological material identified.

**10.6.5** The laboratory should document all the serological tests performed and the results.

**10.6.5.1** The laboratory should have and follow written guidelines for the interpretation of test results. The guidelines should include the acceptance criteria

for all controls, the conclusions that can be drawn based on the results of a test or combination of tests, and the limitations of the testing procedures.

## 11. Reporting and Reviews

**11.1** The laboratory should have and follow written procedures for taking and maintaining case notes to support the conclusions drawn in reports. The laboratory should maintain all analytical documentation generated by serologists related to the testing. The laboratory should retain, in hard copy or electronic format, sufficient documentation for each technical analysis to support the reported conclusions such that another qualified individual could evaluate and interpret the test results.

**11.2** Casework reports should include the following elements:

**11.2.1** Case identifier

**11.2.2** Description of evidence examined

**11.2.3** Results and/or conclusions

**11.2.4** Date issued

**11.2.5** Disposition of evidence

**11.2.6** A signature and title, or equivalent identification, of the person accepting responsibility for the content of the report

**11.3** The reported conclusions should clearly convey and accurately represent the result of the test(s). As an example, a positive presumptive blood test provides an indication that blood may be present on an item of evidence but it does not constitute an identification of blood. In contrast, while a negative presumptive test indicates that no blood was detected in a stain, the failure to detect blood in biological material is not the basis for an absolute determination that blood was not present.

**11.4** The laboratory should have written procedures for the release of reports and case files.

**11.5** The laboratory should conduct and document technical and administrative reviews of all case files and reports to ensure conclusions and supporting test results are reasonable and within the constraints of scientific knowledge.

**11.5.1** An individual conducting technical reviews should be or have been a serologist qualified in the methodology being reviewed.

**11.6** The laboratory should have and follow a documented procedure to address unresolved discrepant conclusions between serologists and reviewers.

**11.7** The laboratory should have and follow a program that documents the annual monitoring of the testimony of each serologist, as applicable.

## 12. Corrective Action

**12.1** The laboratory should establish and follow a corrective action plan to address when discrepancies are detected in proficiency tests and casework analysis. A laboratory corrective action plan should define the different types/levels of possible discrepancies and should describe the different elements required for a specific action plan. These elements should document the cause of the discrepancy that necessitated the corrective action, the potential consequences of the discrepancy, and the preventive measures to be taken to minimize the recurrence of the discrepancy.

**12.2** Corrective actions should not be implemented without the documented prior approval of technical management.

**12.3** Technical management should be responsible for directing the implementation of a corrective action and document the closure or completion of all of its required actions.

## 13. Document Retention

**13.1** The laboratory should maintain and follow a procedure regarding document retention that specifically addresses proficiency tests, corrective action, accreditation records/annual review, training records, continuing education, case files, and court testimony monitoring.

## 14. Safety

**14.1** The laboratory should have and follow a documented environmental health and safety program. This program should include documented training in blood borne pathogen and chemical hygiene plans.

**14.2** The laboratory should review its environmental health and safety program once each calendar year. This review should be documented.

**14.3** Safety information should include the use of personal protective equipment. Additional information on personal protective equipment, sample handling and packaging guidance may be found in the *Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers* by the Technical Working Group on Biological Evidence Preservation.

> **14.3.1** Any item that comes into contact with biological material should be placed into appropriate containers for disposal. Follow applicable local, state, and federal regulations for proper disposal requirements.

## 15. Definitions

**Accredited laboratory** is a laboratory that has received formal recognition that it meets or exceeds a list of standards by a nonprofit professional association of persons actively involved in forensic science that is nationally recognized within the forensic community and in accordance with relevant laws within the laboratory's jurisdiction.

**Confirmatory test** is a test that is specific for a biological material or substance of interest and is necessary for the conclusive identification of a biological fluid.

**Contamination** is the unintentional introduction of exogenous biological material into a sample.

**False negative** is a negative test result despite the presence of the specified biological material. This can be attributed to limitations of the tests or limitations of detection.

**False positive** is a positive test result despite the absence of the specified biological material. This can be attributed to a non-specific reaction occurring between the evidence and the test reagents.

**Methodology** is used to describe the analytical processes or procedures used to support a serological conclusion.

**Negative control** consists of the reagents used in testing without the introduction of sample. It is an analytical control used to detect contamination of reagents used in the test.

**Performance check** is a quality assurance measure to assess the functionality of laboratory instruments and equipment that affect the accuracy and/or validity of forensic sample analysis.

**Positive control** consists of the test reagents plus a known sample that will provide a positive response in the test. It is an analytical control used to determine if a test performed properly.

**Presumptive test** is a screening test that indicates that a biological fluid of interest may be present on an item of evidence but the result does not constitute the identification of that biological fluid. A negative presumptive test indicates that a biological fluid of interest was not detected.

**Procedure** (protocol, SOP or other equivalent*)* is an established practice to be followed in performing a specified task or under specific circumstances.

**Proficiency test** is a quality assurance measure used to monitor performance and identify areas in which improvement may be needed. Proficiency tests may be classified as:

1. An internal proficiency test, which is produced by the laboratory undergoing the test.

2. An external proficiency test, which may be open or blind, is a test obtained from an approved proficiency test provider that is not part of the laboratory undergoing the test.

**Serologist** (or equivalent role, position, or title as designated by the laboratory) conducts and/or directs the identification and characterization of biological fluids on forensic samples, reaches conclusions, and prepares final reports.

**Technical management** are personnel that have responsibility over the technical operations of the laboratory. Technical management should be defined by the laboratory and may include quality assurance personnel.

**Validation** is a process by which a procedure is evaluated to determine its efficacy and reliability for forensic casework analysis and includes the following:

1. Developmental validation is the acquisition of test data and determination of conditions and limitations of a new or novel methodology for use on forensic samples.

2. Internal validation is an accumulation of test data within the laboratory to demonstrate that established methods and procedures perform as expected in the laboratory.

## 16. Reference Materials

The following resources may be helpful to the laboratory in developing protocols for collecting and conducting serological examinations on biological evidence. This list is not meant to be all inclusive. The laboratory should develop a list appropriate to its specific needs. Updated references should be added to the laboratory's list when new methodologies or technologies are incorporated into the laboratory protocols.

Technical Working Group on Biological Evidence Preservation: National Institute of Standards and Technology, *NISTIR 7928 The Biological Evidence Preservation Handbook: Best Practices for Evidence Handlers* (2013), available at http://nvlpubs.nist.gov/nistpubs/ir/2013/NIST.IR.7928.pdf

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**Blood**

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**Feces**

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Metropolitan Police Forensic Science Laboratory, *Biology Methods Manual*. (1978) 4-1 through 4-9.

Natelson, S., *Microtechniques of Clinical Chemistry*, Charles C. Thomas Publishers, Springfield, IL (1957).

**Semen**

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Bedrosian, J.L. *Development of a radial gel diffusion technique for the identification of urea in urine stains*, Journal of Forensic Sciences (1984) 29:601-606.

## Saliva

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