

# DNA Analyst Training Laboratory Training Manual

## Subject 2: Forensic Biology

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PRESIDENT'S  
**DNA**  
INITIATIVE



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## Purpose

To instruct the trainee in basic laboratory techniques, including safety, clean technique, laboratory equipment, and reagent preparation.

To instruct the trainee in biological evidence screening methods, including blood, semen, saliva, urine, fecal material, vaginal secretions, and overall case processing.

The trainee will also be introduced to the laboratory's procedures for quality control, to include procedures for limiting the risk of contamination.

## Objectives

Upon successful completion of these exercises, the trainee will be able to:

- Describe the theories and procedures used for screening biological evidence, to include blood, semen, saliva, urine, fecal material, vaginal secretions, and hair (optional).
- Describe the laboratory's safety program (upon completion of safety training), to include Material Safety Data Sheets (MSDS).
- Explain the laboratory's clean technique practices.
- Identify and operate laboratory equipment.
- Demonstrate the ability to properly prepare reagents and complete related documentation
- Describe the controls used for biological evidence screening.
- Describe the reagents used for biological evidence screening.
- Perform all of the laboratory's procedures for biological evidence screening, per laboratory SOPs.
- Describe the sensitivity and limitations of each procedure used for biological evidence screening.
- Describe the laboratory's quality system procedures employed to avoid, detect, and document contamination.

## Preparation for Exercises

### Trainer Responsibilities

1. Provide documented safety practices specific to chemicals used in the screening process, to include the pertinent MSDSs.
2. Provide the necessary clean technique training.
3. Provide all pertinent manufacturers' user manuals.
4. Demonstrate operation, calibration (if applicable), and cleaning of equipment, as outlined in the Individual Training Plan.
5. Provide the necessary reagent preparation training.
6. Provide documented safety practices specific to chemicals used in biological evidence screening.
7. Assign required samples to be analyzed, as outlined in the Individual Training Plan.
8. Demonstrate each method used for biological evidence screening.
9. Observe the trainee performing each method used for biological evidence screening.
10. Determine the assessment criteria.

11. Review, verify, and document exercise completion.

### **Trainee Responsibilities**

1. Review laboratory safety program and all relevant MSDSs.
2. Review all clean technique procedures.
3. Review all pertinent manufacturers' user manuals.
4. Operate, calibrate (if applicable), and clean equipment, as outlined in the Individual Training Plan.
5. Prepare reagents and complete related documentation, as outlined in the Individual Training Plan.
6. Review safety practices specific to the chemicals used in biological evidence screening.
7. Perform each exercise, as outlined in the Individual Training Plan.
8. Document and submit exercise completion, as required by the Trainer.

## **Literature**

[Return to Laboratory Training Manual User Guide](#)

## **Exercise 1: Safety**

### ***Purpose***

To acquire the necessary safety training and to review the pertinent Material Safety Data Sheets (MSDSs).

### ***Tasks***

- Complete the laboratory safety training program(s), which may include:
  - Chemical hygiene plan
  - Hazard communication
  - Bloodborne pathogens
  - Laser/ALS safety
  - Radiation safety (if necessary)
  - Emergency action plan
  - Fire extinguisher training
  - Unit-specific training (e.g. Firearms, special handling procedures based on the laboratory's physical plant)
  - Personal protective equipment (PPE)
  
- Read the relevant Material Safety Data Sheets (MSDSs)

## **Exercise 2: Clean Technique**

### ***Purpose***

To acquire an understanding of the laboratory's SOPs for clean techniques.

### ***Tasks***

Complete the training on the laboratory's clean techniques procedures, as outlined in the Individual Training Plan

### ***Resources***

Sample Protocols: [2.02](#)

## Exercise 3: Laboratory Equipment

### **Purpose**

To acquire an understanding of the operation, maintenance, calibration (when applicable), and cleaning of laboratory equipment.

### **Tasks**

Become acquainted with laboratory equipment:

- Read equipment user's manuals.
- Read laboratory's SOPs regarding maintenance, calibration (if applicable), and cleaning of laboratory equipment.
- Observe operation, calibration (if applicable), and cleaning of equipment as outlined in the Individual Training Plan.

Suggested equipment exercises, (if applicable), as outlined in the Individual Training Plan.

- **Centrifuge**
  - Calculate the acceleration for one of the laboratory's centrifuges (14,000 x g)
- **Balances**
  - Perform calibration check following the laboratory's SOPs, using NIST traceable weights
- **Microscopes**
  - Clean and focus a microscope
- **Pipettes**
  - Perform calibration check on several pipettes, following the laboratory's SOPs
  - Practice pipetting on each type of pipette used in the laboratory
- **Thermometers**
  - Perform calibration check on several thermometers, following the laboratory's SOPs
- **Thermal cycler**
  - Perform a calibration check on a thermal cycler, following the laboratory's SOPs
- **pH meter**
  - Perform a calibration check on the pH meter, following the laboratory's SOPs
- **Incubators**
  - As outlined by the Individual Training Plan
- **Water baths**
  - As outlined by the Individual Training Plan
- **Heat blocks**
  - As outlined by the Individual Training Plan
- **Hotplates and magnetic stirrers**
  - As outlined by the Individual Training Plan
- **Autoclave**
  - As outlined by the Individual Training Plan

- **UV light sources**
  - As outlined by the Individual Training Plan
- **Water Purification System**
  - As outlined by the Individual Training Plan
- **Hoods**
  - As outlined by the Individual Training Plan
- **Vortex**
  - As outlined by the Individual Training Plan
- **Refrigerator/freezers**
  - As outlined by the Individual Training Plan
- **Power supply**
  - As outlined by the Individual Training Plan
- **Rockers**
  - As outlined by the Individual Training Plan
- **Rotators**
  - As outlined by the Individual Training Plan
- **Genetic analyzers**
  - As outlined by the Individual Training Plan

## **Exercise 4: Reagent Preparation**

### ***Purpose***

To prepare reagents and complete any related documentation, as per laboratory SOPs.

### ***Tasks***

Following the Individual Training Plan:

- Prepare reagents according to laboratory SOPs.
- Label and document each reagent preparation as outlined in the laboratory SOPs.

## Exercise 5: Blood

### ***Purpose***

To perform presumptive and confirmatory tests on body fluid stains to determine the presence of blood following the laboratory's SOPs, including controls and blanks.

To perform tests to determine human antigen activity in body fluid stains following the laboratory's SOPs, including controls and blanks.

### ***Tasks***

1. Locate stains visually using the methods outlined in the laboratory's SOPs, by:
  - Flashlight or other light source
  - Illuminated magnifier

#### Suggested Samples:

- Neat blood
- Blood/semen mix (1:10)
- Blood/saliva mix (1:10)
- Grape
- Bleach
- Ketchup
- Potassium permanganate
- Bloodstain on denim
- Bloodstain on leather
- Washed bloodstain (with or without bleach)
- Bloodstain soaked in water
- Bloodstain exposed to sunlight or UV light for one week or longer
- Bloodstain covered in dirt
- Bloodstain exposed to high temperature
- Bloodstain exposed to amido black and/or coomassie blue

2. Examine stains for characteristics of dried blood, such as color and texture. Use low power magnification, per laboratory's SOPs.

#### Suggested Samples:

- Neat blood
- Blood/semen mix (1:10)
- Blood/saliva mix (1:10)
- Grape
- Ketchup
- Potassium permanganate
- Bloodstain on denim
- Bloodstain on leather
- Washed bloodstain (with or without bleach)
- Bloodstain soaked in water
- Bloodstain exposed to sunlight or UV light for one week or longer

- Bloodstain covered in dirt
  - Bloodstain exposed to high temperature
  - Bloodstain exposed to amido black and/or coomassie blue
3. Perform sensitivity tests using the methods outlined in the laboratory's SOPs, such as:
- Kastle-Meyer (KM)
  - Leucomalachite green (LMG)
  - Luminol
  - Hemastix
  - ABACard® HemaTrace
  - Ouchterlony

Suggested Samples:

- Neat blood
  - 1:1 blood dilution
  - 1:10 blood dilution
  - 1:100 blood dilution
  - 1:1000 blood dilution
  - 1:10,000 blood dilution
4. Perform specificity tests using methods outlined in the laboratory's SOPs, such as:
- Kastle-Meyer (KM)
  - Leucomalachite green (LMG)
  - Luminol
  - Hemastix
  - ABACard® HemaTrace
  - Ouchterlony

Suggested Samples:

- Neat saliva
  - Neat urine
  - Blood/semen mix (1:10)
  - Blood/saliva mix (1:10)
  - Grape
  - Bleach
  - Horseradish
  - Ketchup
  - Potassium permanganate
  - Animal blood (dog, ferret)
5. Perform presumptive and confirmatory tests on stains on various substrates and environmentally challenged samples using methods outlined in the laboratory's SOPs such as:
- Kastle-Meyer (KM)
  - Leucomalachite green (LMG)
  - Luminol
  - Hemastix
  - ABACard® HemaTrace
  - Ouchterlony

Suggested Samples:

- Bloodstain on denim
  - Bloodstain on leather
  - Washed bloodstain (with or without bleach)
  - Bloodstain soaked in water
  - Bloodstain exposed to sunlight or UV light for one week or longer
  - Bloodstain covered in dirt
  - Bloodstain exposed to high temperature
  - Bloodstain exposed to amido black and/or coomassie blue
6. Perform presumptive and confirmatory tests on a minimum of 10 – 20 training samples (to include non-probative and/or mock case samples), using methods outlined in the laboratory's SOPs.

## ***Resources***

**Sample Protocols:** [2.14](#), [2.15](#), [2.16](#), [2.17](#), [2.18](#), [2.19](#), [2.20](#)

## Exercise 6: Semen

### ***Purpose***

To perform presumptive and confirmatory tests on body fluid stains to determine the presence of semen following the laboratory's SOPs, including controls and blanks.

### ***Tasks***

1. Locate stains visually using the methods outlined in the laboratory's SOPs, by:
  - Flashlight or other light source
  - Illuminated magnifier
  - Alternate light source

Suggested Samples:

- Neat semen
- Blood/semen mix (1:10)
- Semen/saliva mix (1:10)
- Bloodstain on denim
- Semen stain on dark fabric
- Semen stain on light fabric
- Washed semen stain (with or without bleach)

2. Perform sensitivity tests using the methods outlined in the laboratory's SOPs, such as:
  - Acid phosphatase (AP)
  - P30 analysis by ABACard® PSA
  - Microscopic exam using the Christmas tree stain

Suggested Samples:

- Neat semen
- 1:1 semen dilution
- 1:10 semen dilution
- 1:100 semen dilution
- 1:1000 semen dilution

3. Perform specificity tests using the methods outlined in the laboratory's SOPs, such as:
  - Acid phosphatase (AP)
  - P30 analysis by ABACard® PSA

Suggested Samples:

- Neat semen
- Neat saliva
- Neat urine
- Neat blood
- Neat breast milk
- Semen/blood mix (1:1)
- Semen/saliva mix (1:1)
- Grape stain

- Time lapsed sex kit – samples collected at:
    - 0 hour
    - 6 hour
    - 12 hour
    - 24 hour
    - 48 hour
    - 72 hour
4. Perform microscopic evaluation of spermatozoa from various species using the methods outlined in the laboratory's SOPs.

Suggested Samples:

- Dog spermatozoa
  - Primate spermatozoa
  - Yeast
  - Bacteria
5. Perform microscopic evaluation of samples containing yeast and bacteria using the methods outlined in the laboratory's SOPs.
6. Perform mapping of body fluid stains using the methods outlined in the laboratory's SOPs.
7. Perform presumptive and confirmatory tests on a minimum of 10 – 20 training samples (to include non-probative and/or mock case samples), using methods outlined in the laboratory's SOPs.

## ***Resources***

**Sample Protocols:** [2.03](#), [2.04](#), [2.05](#), [2.06](#)

## Exercise 7: Saliva

### ***Purpose***

To perform presumptive tests on body fluid stains to determine the possible presence of saliva following the laboratory's SOPs, including controls and blanks.

### ***Tasks***

1. Locate stains visually using the methods outlined in the laboratory's SOPs, by means of:
  - Flashlight or other light source
  - Illuminated magnifier
  - Alternate light source

#### Suggested Samples:

- Neat saliva
- Blood/saliva mix (1:10)
- Semen/saliva mix (1:10)
- Saliva stain on dark fabric
- Saliva stain on light fabric

2. Perform sensitivity tests using the methods outlined in the laboratory's SOPs, such as:

- Radial gel diffusion test
- Phadebas® Amalyse Test
- SALIGaE® Test for Presence of Saliva

#### Suggested Samples:

- Neat saliva
- 1:1 saliva dilution
- 1:10 saliva dilution
- 1:100 saliva dilution
- 1:1000 saliva dilution
- 1:5000 saliva dilution
- 1:10,000 saliva dilution

3. Perform specificity tests using the methods outlined in the laboratory's SOPs, such as:

- Radial gel diffusion test
- Phadebas® Amalyse Test
- SALIGaE® Test for Presence of Saliva

#### Suggested Samples:

- Neat saliva
- Neat urine
- Neat semen
- Blood/saliva mix (1:1)
- Semen-saliva mix

4. Perform mapping of body fluid stains using the methods outlined in the laboratory's SOPs.
5. Perform presumptive tests on a minimum of 10 – 20 training samples (to include non-probative and/or mock case samples), using methods outlined in the laboratory's SOPs.

## ***Resources***

**Sample Protocols:** [2.08](#), [2.09](#), [2.10](#), [2.11](#)

## Exercise 8: Urine

### ***Purpose***

To perform presumptive tests on body fluid stains to determine the possible presence of urine following the laboratory's SOPs, including controls and blanks.

### ***Tasks***

1. Locate stains visually using the methods outlined in the laboratory's SOPs, by means of:
  - Flashlight or other light source
  - Illuminated magnifier
  - Alternate light source

Suggested Samples:

- Neat urine
- Urine/saliva mix (1:10)
- Urine/Blood mix (1:10)
- Urine stain on dark fabric
- Urine stain on light fabric

2. Perform sensitivity tests using the methods outlined in the laboratory's SOPs, such as:
  - Creatinine test

Suggested Samples:

- Neat urine
- 1:1 urine dilution
- 1:10 urine dilution
- 1:100 urine dilution
- 1:1000 urine dilution
- 1:5000 urine dilution
- 1:10,000 urine dilution

3. Perform specificity tests using the methods outlined in the laboratory's SOPs, such as:
  - Creatinine test

Suggested Samples:

- Saliva / urine mix (1:1)
- Blood / urine mix (1:1)
- Semen/ urine mix (1:1)

4. Perform presumptive tests on a minimum of 10 – 20 training samples (to include non-probative and/or mock case samples), using methods outlined in the laboratory's SOPs.

## ***Resources***

**Sample Protocols:** [2.12](#)

## Exercise 9: Fecal Material

### *Purpose*

To perform presumptive tests on body fluid stains to determine the possible presence of fecal material following the laboratory's SOPs, including controls and blanks.

### *Tasks*

1. Locate stains visually using the methods outlined in the laboratory's SOPs, by:
  - Flashlight or other light source
  - Illuminated magnifier
  - Alternate light source

Suggested Samples:

- Fecal material
- Fecal material/urine mix (1:10)
- Fecal material/Blood mix (1:10)
- Fecal material on dark fabric
- Fecal material on light fabric

2. Perform specificity tests using the methods outlined in the laboratory's SOPs, such as:
  - Urobilinogen

Suggested Samples:

- Fecal material / urine mix (1:1)
- Fecal material / blood mix (1:1)
- Fecal material / semen mix (1:1)

3. Perform presumptive tests on a minimum of 10 training samples (to include non-probative and/or mock case samples), using methods outlined in the laboratory's SOPs.

### *Resources*

Sample Protocols: [2.13](#)

## Exercise 10: Vaginal Secretions

### ***Purpose***

To perform presumptive tests on body fluid stains to determine the possible presence of vaginal secretions following the laboratory's SOPs, including controls and blanks.

### ***Tasks***

1. Locate stains visually using the methods outlined in the laboratory's SOPs, by:
  - Flashlight or other light source
  - Illuminated magnifier
  - Alternate light source

Suggested Samples:

- Neat vaginal secretions
- Vaginal secretions /semen mix (1:10)
- Vaginal secretions /saliva mix (1:10)
- Vaginal secretions stain on dark fabric
- Vaginal secretions stain on light fabric

2. Perform sensitivity tests using the methods outlined in the laboratory's SOPs, such as:
  - Lugols

Suggested Samples:

- Neat vaginal secretions
- 1:1 vaginal secretions dilution
- 1:10 vaginal secretions dilution
- 1:100 vaginal secretions dilution
- 1:1000 vaginal secretions dilution

3. Perform specificity tests using the methods outlined in the laboratory's SOPs, such as:
  - Lugols

Suggested Samples:

- Neat vaginal secretions
- Neat saliva
- Neat urine
- Neat semen
- Vaginal secretions /semen mix (1:1)
- Vaginal secretions /saliva mix (1:1)

4. Perform presumptive tests on a minimum of 10 training samples (to include non-probative and/or mock case samples), using methods outlined in the laboratory's SOPs.

## ***Resources***

**Sample Protocols: [2.07](#)**

## Exercise 11: Case Processing

### ***Purpose***

To obtain case processing skills by performing analysis on mock casework, following the laboratory's SOPs.

### ***Tasks***

- Observe a qualified analyst processing casework.
  - Take notes during observation
  - Compare notes with analyst's notes
  
- Practice case processing skills on mock case evidence
  1. Describe the evidence
  2. Locate stains visually, using laboratory methods
    - Flashlight or other light source
    - Illuminated magnifier
    - Alternate light source
  3. Mark stains
  4. Perform testing on stains
  5. Note results
  6. Interpret results
  7. Write a report

### ***Resources***

**Sample Protocols:** [2.01](#), [2.02](#), [2.03](#), [2.04](#), [2.05](#), [2.06](#), [2.07](#), [2.08](#), [2.09](#),  
[2.10](#), [2.11](#), [2.12](#), [2.13](#), [2.14](#), [2.15](#), [2.16](#), [2.17](#), [2.18](#),  
[2.19](#), [2.20](#), [2.21](#), [2.22](#)

## Subject Review

After completion of the laboratory manual exercises and having previously completed the corresponding theory modules, the trainee should be able to answer the following questions:

### Safety

- Where are Material Safety Data Sheet (MSDS) located?
- What is the purpose of an MSDS?
- What is the proper disposal method for:
  - Sharps
  - Organic chemicals
  - Biohazard waste
- What personal protective equipment (PPE) is required by the laboratory during each step of the analysis process?
- Where are the laboratory chemical spill kits located?
- What is the laboratory's emergency evacuation plan?

### Clean Technique

- What are the laboratory's procedures for clean technique?

### Laboratory Equipment

- What is the purpose of each type of instrumentation or piece of equipment?
- Which laboratory equipment requires calibration? Why?
- What is the laboratory's procedure for handling equipment that is not functioning properly? How is this documented?
- What are the laboratory's procedures for cleaning equipment?

### Reagent Preparation

- Which reagents are prepared in the laboratory?
- Which reagents are purchased outside the laboratory?
- What are the quality control measures taken for both in-house prepared and externally purchased reagents?
- What documentation is required for each reagent used in the laboratory?

### Blood

- What are the different components of blood?
- What are the presumptive tests for blood? What chemical reactions occur?
- What are the confirmatory tests for blood? What chemical reactions occur?
- What are the limitations of each test? (sensitivity, specificity, etc?)
- What is the purpose of positive controls? Negative controls?

### Semen

- What are the different components of semen?
- What is the approximate longevity of the different constituents of semen on different substrates?
- What are the presumptive tests for semen? What chemical reactions occur?
- What are the confirmatory tests for semen? What chemical reactions occur?
- What are the limitations of each test? (sensitivity, specificity, etc?)
- What is the purpose of positive controls? Negative controls?

#### Saliva

- What are the different components of saliva?
- What are the presumptive tests for saliva? What chemical reactions occur?
- What are the limitations of each test? (sensitivity, specificity, etc?)
- What is the purpose of positive controls? Negative controls?

#### Urine

- What are the different components of urine?
- What are the presumptive tests for urine? What chemical reactions occur?
- What are the limitations of each test? (sensitivity, specificity, etc?)
- What is the purpose of positive controls? Negative controls?

#### Fecal Material

- What are the different components of fecal material?
- What are the presumptive tests for fecal material? What chemical reactions occur?
- What are the limitations of each test? (sensitivity, specificity, etc?)
- What is the purpose of positive controls? Negative controls?

#### Vaginal Secretions

- What are the different components of vaginal secretions?
- What are the presumptive tests for vaginal secretions? What chemical reactions occur?
- What are the limitations of each test? (sensitivity, specificity, etc?)
- What is the purpose of positive controls? Negative controls?

#### Case Processing

- What wavelength(s), when using an alternate light source, is best for locating a stain?
- What are the laboratory's procedures for collecting stains for further analysis?
- What are the laboratory's procedures for trace evidence collection?
- How are evidence and testing procedures documented?

# Laboratory Training Manual

## *Subject 2: Forensic Biology*

### Optional Exercise 12: Hair

#### ***Purpose***

To perform macroscopic / microscopic evaluation of hair following the laboratory's SOPs, including controls and blanks.

#### ***Tasks***

1. Evaluate hair from multiple species (including human hair) for characteristics such as length, color, shaft profile, presence/absence of the medulla, type of scales, etc.
2. Evaluate training samples from multiple species to determine human or non-human origin.
3. Evaluate human hair from multiple individuals for presence/absence of root materials.
4. Perform microscopic evaluation on a minimum of 50 training samples (to include non-probative and/or mock case samples).
5. Remove mounted hair from multiple slides, in preparation for possible DNA analysis.

#### ***Resources***

Sample Protocols: [2.21](#)

#### ***Exercise Review Questions***

Upon completing this exercise, the trainee should be able to answer these questions:

- What are some macroscopic features of hair?
- What are some microscopic features of hair?
- What are differentiating characteristics of animal hair versus human hair?
- What are the different stages of hair growth?
- What type of DNA analysis can be performed on hair?
- Can sex, race, or species be determined from a hair comparison?

[Return to Laboratory Training Manual User Guide](#)