

DNA Analyst Training Laboratory Training Manual

Protocol 2.02 Clean Technique



This laboratory protocol (or part thereof) has been provided as an example of a laboratory SOP, courtesy of the Illinois State Police. It has been included for training and example purposes only.

**PRESIDENT'S
DNA
INITIATIVE**



INTRODUCTION

All Forensic Biologists and DNA analysts must follow clean technique. The purpose of clean technique is to prevent unwanted DNA from entering a sample. The first line supervisor through the laboratory director is responsible for ensuring this policy is followed.

There are many sources of contamination such as: aerosols, liquids or dry flakes/dust, unclean tools, unclean gloves, and contaminating materials on lab coats. There are also laboratory areas that are more at risk for the introduction of unwanted DNA such as evidence examination areas and extraction areas. Proper precautions must be taken to reduce the risk of contamination.

SAFETY CONSIDERATION

Observe Standard Laboratory Practices.

Warning: Treat all reagents/samples as potential biohazards.

Refer to safety considerations under the DNA Isolation/Methods section.

Personal Protective Equipment (PPE)

Gloves must be worn whenever an individual is handling equipment or instruments that are used for casework within the Forensic Biology/DNA laboratory.

Forensic Biologist/DNA Analysts must wear a mask, gloves and disposable lab coat or reusable lab coat in conjunction with disposable sleeve covers while examining all items of evidence. The gloves must either be sterile or after putting the gloves on, they must be bleached with 10% bleach and dried. Gloves must be changed or bleached between exhibits. Gloves must also be changed after handling non-evidence items prior to returning to casework. These non-evidence items may include but are not limited to, refrigerators/freezers, biohazard waste bins, equipment, computers, and telephones. Gloves should be changed often.

Personal Protective Equipment (PPE) in Post-PCR

Disposable or designated lab coats must be worn in the post-PCR rooms and these lab coats must be worn only in the post-PCR rooms. Non-disposable lab coats must be removed from the laboratory for cleaning in a closed container.

PREPARATION

10% Bleach Solution

INSTRUMENTATION

Standard Laboratory Instrumentation

MINIMUM STANDARDS AND CONTROLS

Not applicable.

PROCEDURE OR ANALYSIS

General Information

1. Analysts should avoid taking phone calls when working in the laboratory. Conversations between laboratory personnel should be kept at a minimum when an analyst is working with evidence samples.
2. Tube openers should be used to uncap tubes (microcentrifuge tubes, Microcons, etc.). The tubes must be centrifuged prior to opening. Open only one tube at a time. Tube openers should be cleaned in a 10% bleach solution after each use.
3. Each analyst must use individual mini-stocks of each reagent. These mini-stocks may not be shared between analysts. Mini-stocks may be replenished from the large stock solution.
4. Do not pipette from a stock reagent bottle. Reagents must be poured from the stock reagent bottle into a disposable beaker. When finished, discard the beaker and its contents. All stock reagents must be closed when processing stains for extraction.
5. Exhibits will be processed one at a time. Only one exhibit will be open at a time.
6. The analyst will attempt to process unknown samples in an order from small or dilute samples to large or concentrated samples.
7. Unknowns must be processed first and separately from standards throughout the screening and DNA processes.

Decontamination:

1. Decontaminate the surface on which samples are to be processed with a 10% bleach solution. Ensure the surface is dry before examining evidence. Make a new bleach solution daily.
2. All instruments which will be used to process forensic samples (e.g., forceps, scissors, centrifuge rotors, bone cutting equipment, pipettors and metal probes) must be decontaminated by autoclaving or rinsing with a 10% bleach solution. In addition, these items may be placed under an ultraviolet (UV) light source for at least 15 minutes.
3. Use a 10% bleach solution to rinse or wipe tools between samples. Tools may be rinsed with distilled water. After rinsing with a 10% bleach solution, use a disposable cloth to dry. Use a new disposable cloth each time.
4. Fresh paper must be used for each exhibit throughout the screening process. The work area must be bleached with a 10% bleach solution between each exhibit. The only exception to this procedure is during the examination of exhibits from a Criminal Sexual Assault (CSA) kit.

Sample Processing - DNA Extraction:

1. The process of examining DNA evidence, cutting samples for DNA extraction, and adding reagents will take place in a biohood, if available. The biohood provides a clean environment for DNA extraction and must have a hepa filter and a UV light. It must be cleaned thoroughly with a 10% bleach solution. All tubes that will be used in the extraction process must also be exposed to UV light for 30 minutes prior to their use. When finished, the biohood must be bleached with a 10% bleach solution.

If a biohood is not available, the analyst may process samples on a freshly bleached bench top. Tubes that will be used in the extraction process must still be exposed to 30 minutes of UV light prior to their use.

2. A separate extraction process can be accomplished as follows:
 - A. Extraction of standards and unknown samples can be conducted on different days or at different times.
 - B. Extraction of standards and unknowns samples can be conducted in different biohoods.
 - C. If samples are to be processed on the same day using the same hood, the analyst must keep the unknown samples and their manipulation blank(s) in their own rack and process them first. The standard samples and their manipulation blank(s) will be kept in their own rack and processed after the unknowns throughout the analysis, when applicable.
3. The order the samples are extracted and processed must be clearly documented in the case notes. This documentation must show the separate handling of the unknowns and their manipulation blank(s) from the standards and their manipulation blank(s). This can be accomplished by listing the samples in the order of extraction in the case notes along with the date the extraction is started.
4. For each extraction protocol followed, a sterile swab or piece of cotton material must be processed as a manipulation blank. A manipulation blank must be processed with each set of unknown samples and each set of standard samples (separate manipulation blanks). Process the manipulation blank identically to all of the other samples. Unknowns from different cases may be batched together with one manipulation blank for each extraction protocol used. Standards from different cases may also be batched together with one manipulation blank.
5. Exhibits will be processed one at a time. Only one exhibit will be opened at a time. Unknowns must be processed first and separately from standards throughout the DNA analysis. Unknowns will be cut, placed into tubes and placed into their own samples rack. A manipulation blank will then be cut for the unknown samples and added to the unknown samples rack. After the unknowns have been processed, the hood will be thoroughly bleached. The standards can then be cut, placed into tubes and placed into their own samples rack. A manipulation blank will then be cut for the known samples and added to the standard samples rack.

Amplification Set-up:

1. All amplifications must be set up in designated biohoods. If the face shield on the hood does not shield the analyst's face, the analyst must wear a face mask during amplification set up.
2. Pipettors that are dedicated for amplification set-up must be used.
3. The set-up biohood must be cleaned thoroughly with a 10% bleach solution. Any tubes that will be used in amplification set-up must be exposed to 30 minutes of UV light prior to their use. The biohood must be on during amplification set-up.
4. One master mix of amplification chemicals can be used for both unknown and standard samples. The amplification chemicals can be added to tubes in the rack of unknown samples and the tubes in the rack of standard samples. Close all tubes. Unknown samples and their manipulation blank(s) will continue to be contained within their own rack and processed prior to the rack of standard samples and their manipulation blank(s). The positive and negative amplification controls will be setup last.
5. All samples can be amplified at the same time using the same thermal cycler. Do not set the tube rack down in the post-amplification room while transferring samples to the thermal cycler. If the rack is set down, it must be bleached with a 10% bleach solution before being used in the main laboratory again.

Post-Amplification:

1. After working with amplified DNA, an analyst must not work with any other biology or non-amplified evidence.
2. The door to the post-amp room must remain closed.
3. The sample preparation hood and pipettors must be cleaned with a 10% bleach solution before and after the samples are prepared.
4. The unknown samples and their manipulation blank(s) are prepared for injection first and separately from the standards and their manipulation blank(s), when applicable.

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