Laboratory Orientation and Testing of Body Fluids and Tissues for Forensic Analysts

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Online Training: Laboratory Orientation and Testing of Body Fluids and Tissues for Forensic Analysts

This course provides information in the two lessons:

Laboratory Orientation. Addresses basic procedures, safety requirements, and the laboratory's quality assurance program(s).

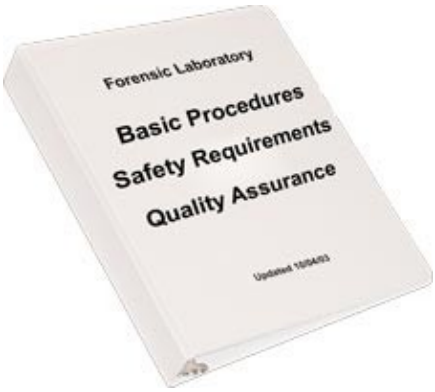
Testing of Body Fluids and Tissues. An overview of historical and contemporary techniques used to characterize body tissues. Some techniques are still used in the examination of body fluids, while knowledge of others, such as ABO and multi-enzyme systems, is of value if old cases are opened for review.

Laboratory Orientation: Introduction

Training to become a reporting forensic scientist is rigorous and time consuming. The first steps are to understand basic procedures, safety requirements, and the laboratory's quality assurance program(s).

The forensic scientist's job requires accurate record keeping, chain-of-custody documentation, stringent quality control, and data management. Forensic scientists must make sure that

- the examination is comprehensive,
- appropriate tests are selected,
- tests are performed correctly,
- interpretation of the data is accurate and thorough,
- the written report is correct and easily understood by a non-scientist,
- testimony is complete and truthful.



Laboratory safety programs are designed to prevent employees or visitors from being subjected to any unusual health or safety risks. Additionally, many safety programs strive to meet the Quality Assurance Standards (QAS) and relevant OSHA or regulatory requirements.

Laboratory quality assurance programs are designed to establish and monitor requirements for all work performed by the staff. New analysts must have a basic understanding of the laboratory's quality control requirements for clean techniques, facilities, equipment, and reagents prior to beginning training in specific laboratory methods.

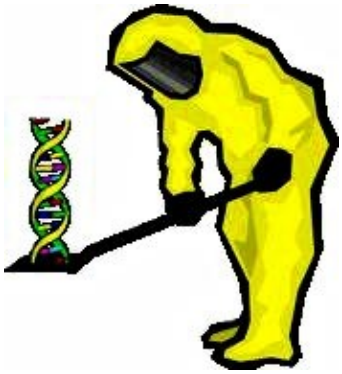
Objectives

Upon successful completion of this unit of instruction, the student shall have the following:

- A basic awareness of safe laboratory practices
- A basic competency in general forensic laboratory quality control measures and QA standards related to the following:
    - ♦ laboratory clean techniques

- ♦facility
- ♦equipment
- ♦reagents

Safety



Numerous hazards exist in forensic laboratories. The risks associated with these are reduced or eliminated if proper procedures, practices, and precautions are followed. The documentation of, and adherence to, practices and procedures in a laboratory safety manual is an essential requirement of an effective laboratory safety program.

Laboratory safety programs generally include an overall safety manual, chemical hygiene, and bloodborne pathogen plans.

Laboratory safety can be addressed through policy, practice, and use of safety equipment. Laboratory safety needs should be evaluated on an ongoing basis to assess the need for policy changes and appropriate safety equipment. Laboratory safety equipment may include, but is not limited to:

- Safety Hoods
- Respirators
- Personal protective equipment (PPE)
- Safety showers and eye washes

Safety Hoods

Laboratory safety hoods are designed to prevent or minimize the escape of air contaminants into the laboratory. Chemical fume hoods provide a safe environment for working with chemicals and can be equipped with various filters that handle different categories of chemicals. Biological safety cabinets are designed to work exclusively with biological materials; they are equipped with HEPA filters (of specific pore size) that prevent the release of biological agents into the surrounding laboratory areas.

Respirators

Respirators have two primary functions:

- to remove contaminants from the air
- to supply respirable air from another source

Respirators responsible for contamination removal include gas masks that filter out chemicals and particulate respirators that filter out airborne particles.

Those respirators that supply respirable air include:

- compressed air respirators that provide air from a remote source
- self-contained breathing apparatuses (SCBA) that include their own air supply

Personal Protective Equipment

Personal protective equipment (PPE) includes: gloves, gowns, lab coats, face shields, masks, and eye protection. Laboratories should define the tasks that require the use of PPE(s).

After identifying the tasks that require these types of precautions, the laboratory should provide all of the following:

- annual training
- necessary PPE(s)
- safety program that encourages the usage of PPEs to prevent and/or limit unnecessary biological or chemical exposures.

Safety Showers and Eyewash Stations

Safety showers and eye wash stations provide on the spot decontamination. It is essential to look beyond the use of goggles, face shields, and other personal protective equipment since accidental chemical exposures can occur even with a well-established safety program. Emergency showers and eyewash stations are a necessary backup to minimize the effects of accidental exposures.

The Safety Course is available as a PDF file.

Clean Technique

Clean techniques refer to laboratory practices employed to reduce the risk of contamination. Clean techniques are employed in the forensic DNA laboratory to prevent the transfer of DNA from analyst to sample, environment to sample, and cross-contamination between samples. Contamination can adversely affect the outcome of a case; therefore, it is essential that the laboratory have procedures in place to limit, recognize, and address contamination.

Effective clean techniques procedures assist the laboratory in meeting the QAS 6.1.4, which requires that laboratories have and follow a written procedure for monitoring, cleaning, and decontaminating facilities and equipment. Specific techniques are not delineated by the standards. It is the responsibility of laboratory management to design and implement appropriate clean techniques protocols. Some recommended practices are provided.

Read more about the QAS on the FBI Site.

Recommended Practices for Clean Technique

Work Surfaces and Equipment

- Work surfaces should be cleaned before contact with evidence, between evidence items, and after evidence processing is complete.
- It is common practice for glassine weigh paper, Kimwipes®, butcher paper, or Benchkote® paper to be placed on the bench top while processing evidence to act as a barrier. The paper should be changed and the bench top cleaned between items.
- Centrifuges, thermal cycler, tube racks, pipettes, and any other equipment deemed appropriate should be cleaned before and after each use.
- Instruments such as forceps, scissors, scalpels, and tube openers should be cleaned just prior to use. Some laboratories purchase sterile disposable instruments. These should be opened just prior to

sample processing and discarded after one use.
- Cleaning should be done with a 10% bleach solution or a commercially available sterilization reagent such as Cidex® Plus.
- After an item or surface is cleaned with bleach it must be rinsed with purified water or alcohol to prevent the build up of sodium hypochlorite crystals. Instruments or equipment cleaned with bleach should be rinsed to avoid corrosion.

Reagents and Sample Processing

- When appropriate, reagents should be prepared in bulk. Each analyst is then provided with an aliquot for his/her individual use.
- Reagents should be kept closed when not in use.
- Samples should be processed individually. Only one sample should be open at a time.
- Unknown samples should be processed separately from reference samples. Processing may be separated by time and/or space. When possible, small/dilute samples should be worked prior to large/concentrated samples.
- Autoclave sample tubes.
- Only one microcentrifuge tube should be open at a time. Close each tube immediately after labeling and after the addition of sample or reagents to prevent cross-contamination.
- Use a tube opener, clean Kimwipe®, or other suitable barrier, rather than gloved fingers, to open microcentrifuge tubes.
- Aerosol-resistant pipette tips should be used. Place the sterile tip on the pipette immediately prior to use. If the pipette is set down with the tip on, discard the tip. A new pipette tip should be used for the addition of each reagent to a sample tube.
- Centrifuge microcentrifuge tubes prior to opening to remove any liquid clinging to the lid.
- Use basket tubes, such as Spin-X® tubes, to centrifuge stain extraction buffers from sample matrices.

Good Lab Practices

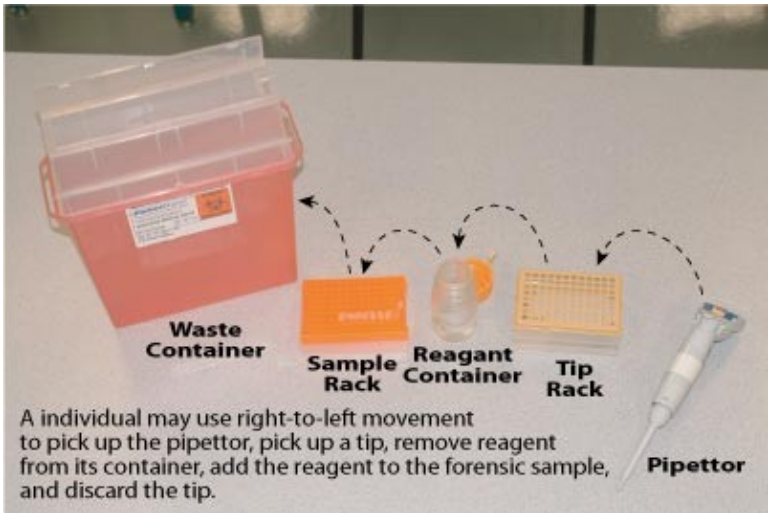
- Gloves should be worn throughout sample processing. At a minimum, gloves should be changed at the completion of each step of the process. If gloves become contaminated, discard them and replace with new ones.
- Lab coats should be worn at all times while processing evidence. It is essential for a lab coat and gloves to be worn at all times in the post-amplification room.
- The post-amplification room should contain lab coats, gloves, and equipment that do not leave that area without decontamination. Adherence to this practice prevents contamination of pre-amplification areas with amplified product.
- The movement of paperwork from post-amplification into pre-amplification areas should be limited. The most common solution is to send data to printers outside the laboratory.

Facilities

The QAS outlines facilities requirements with the intent to minimize contamination risks. The application of the PCR process to forensic DNA analysis has dramatically improved the technological abilities in the field. The use of PCR does, however, require that laboratories have appropriate facility design and procedures in place to minimize the risk of contamination. QAS standard 6.1.4 requires the following:

- The laboratory must be designed to provide adequate security and minimize contamination. Note 1
- Access to the laboratory must be controlled and limited. Note 2
- Evidence examinations, liquid sample examinations (for offender laboratories), DNA extractions, and PCR setup must be conducted at separate times or in separate spaces. Note 3
- Amplified DNA product must be generated, processed, and maintained in a room(s) separate from the evidence examination, DNA extractions, and PCR setup areas. Note 4

- For offender laboratories, if a robotic workstation is used to carry out DNA extraction and amplification in a single room, the laboratory must demonstrate that contamination is minimized and equivalent to that when performed manually in separate rooms. Note 5
- The laboratory follow written procedures for monitoring, cleaning, and decontaminating facilities and equipment. Note 6



[1] The laboratory must have documented and well-understood procedures for security, and use procedures that minimize contamination.

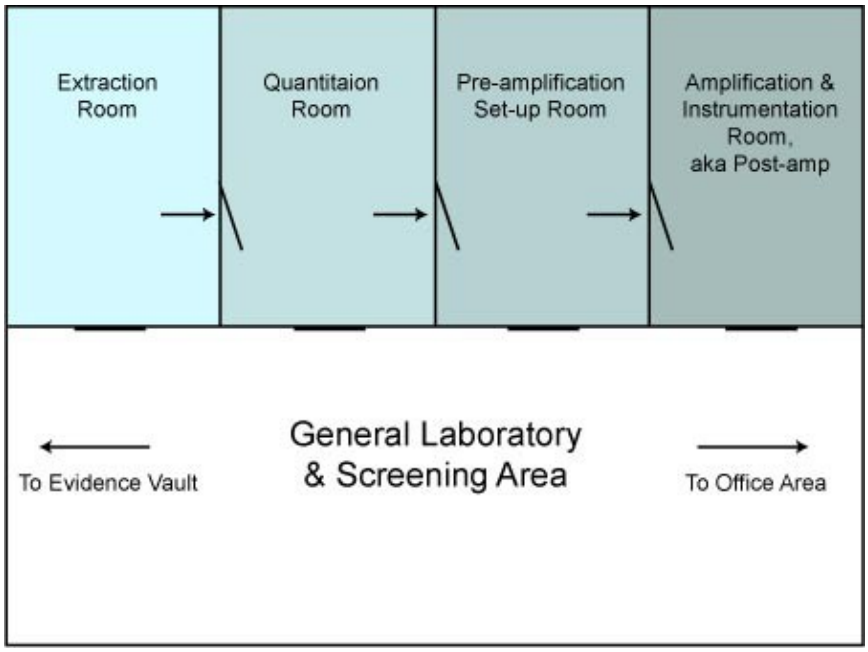
[2] Additionally, the laboratory must be secured and controlled in a manner to prevent access by unauthorized personnel and limit access to authorized personnel.

[3] The laboratory's approach to sample processing for PCR-based procedures (extraction and amplification) must demonstrate a separation in time or physical space for each activity. This can be accomplished through a combination of clearly written technical procedures, casework notes, and/or personal observation. The laboratory's design must demonstrate that evidence flow, through the various steps of DNA processing, does not compromise the integrity of the sample. The amplification room must be enclosed with walls from the floor to the ceiling and door(s) for passage, and the amplification room(s) must physically separate amplified DNA from all other areas of the laboratory by maintaining doors in the closed position.

[4] When robotic workstations are used to carry out DNA extractions through PCR setup on casework samples, a single room may be used. Internal validation must show that if contamination occurs, it is minimized, addressed, and less than or equivalent to that observed when these procedures are performed manually in separate rooms.

[5] Robotic workstations may be used to carry out DNA extraction through amplification in a single room provided that they are separated from the sample extraction and sample amplification areas and that it can be demonstrated through internal validation that if contamination occurs, it is minimized, addressed, and less than or equivalent to that observed when these procedures are performed manually in separate rooms.

[6] A laboratory may employ a variety of methods to monitor its facilities, such as the use of appropriate controls in the analysis process. Whichever approach(es) the laboratory selects to use, the method(s) must be documented. This may be accomplished through a variety of ways at the discretion of the laboratory.



A very generic schematic of a laboratory layout that would comply with standards to prevent contamination. Note the one-way flow of the samples. Additional rooms may be present for reagent preparation, film development, computer use, etc. Each area should have dedicated equipment, reagents, and PPE.

Reagents

The QAS require the following:

- Analytical procedures must describe reagents, sample preparation, extraction, equipment, and controls that are standard for DNA analysis and data interpretation.
- The laboratory must use reagents that are suitable for the methods employed.

Note:
Laboratories must have written procedures detailing the quality control measures in place for evaluating reagents and materials, the acceptable range of results, procedures for acting upon data that are unacceptable, and the mechanisms used for documentation and the subsequent approval/rejection of quality control data.

- The laboratory must have written procedures for documenting commercial supplies and for formulating reagents.
- Reagents must be labeled with the identity of the reagent, the date of preparation or expiration, and the identity of the individual preparing the reagent.

Note:
Laboratories must follow their written procedures for documenting commercial supplies and formulating reagents. Reagents must be labeled with the identity of the reagent and a tracking mechanism identifying preparation or expiration date and component sources. Records must be maintained that identify the preparer of the reagent and the quality control measures (if any) used to check the reliability of the reagent .

- The laboratory must identify and evaluate the reagents critical to the analysis process prior to use in casework.

- The laboratory must identify and evaluate the following critical reagents (for PCR based systems: commercial kits for performing genetic typing, primer sets, and thermostable DNA polymerase).

Note:

The laboratory must identify the reagents critical to the analytical processes used and evaluate each, prior to their use on evidence and convicted offender samples. This list must include, at a minimum, those critical reagents listed in Standard 9.2.3(b).

Reagent Preparation

Given the number of reagents prepared in the laboratory and the importance of effective quality control procedures, laboratories may wish to include some instruction on preparation, documentation, and quality control testing of reagents. Additionally, it is important to document specific storage conditions and expiration dates, as appropriate.

Methods employed by laboratories may differ with respect to the exact concentrations and/or volumes of reagents used, reagent container labeling procedures, and documentation. These should be addressed in the laboratory's Quality Manual and procedures. Trainees should be familiar with their laboratory's requirements for documentation, preparation, quality control, and use of both purchased and prepared reagents.

Examples of Written Formulations

Written formulations may be presented in different ways. For example, the instructions for preparation of the four reagents below use different styles to present the correct required information.

5% Chelex (w/v)
(Bio-Rad 143-2832 or equivalent):
Add 5 grams of Chelex 100 Resin to 100 ml of sterile deionized water. Properly label and date the container. Store at room temperature. Expires 1 year after preparation.

Proteinase K, 10 mg/ml (Sigma P-2308 or equivalent):
Dissolve 250 mg proteinase K in 25 ml deionized water. Aliquot and freeze. Properly label, date, and initial the container. If possible, individual aliquots may be labeled. Expires in approximately 3 years per manufacturer's recommendation.

20% (w/v) SDS:
1L bottle
800mL DI H_2O
200g SDS (must be ultra pure – electrophoresis grade)
warm if necessary to dissolve
200ml DI H_2O
store at RT (no expiration)

Citrate Buffer:
(0.1M Sodium citrate, pH 5.0)

1L bottle
800mL DI H_2O
18.4g trisodium citrate dihydrate ($Na_3C_6H_5O_5 \cdot 2H_2O$)
adjust pH to 5.0 (takes about 6g citric acid monohydrate, $C_6H_8O_7 \cdot H_2O$)
store at RT (no expiration)

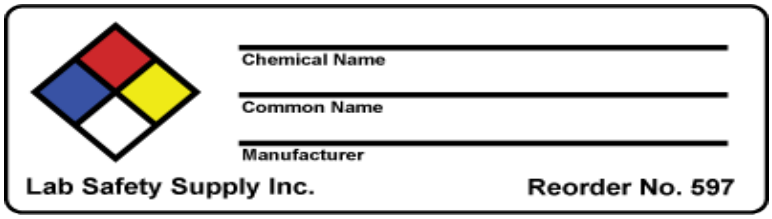
Sample Excerpt from Log Book

The following generic format represents one method of documentation for recording information on specific reagents. For those reagents requiring quality control, this form may be modified to record the date of QC of the reagent and by whom.

Reagent	Batch #	Initials / Date	Chemical / Lot#; Reagent / Batch#

Sample Label for Reagent Containers

Alternatively, laboratory tape may be used to mark containers/aliquots with the appropriate information.



The QAS requires reagents to be labeled with the identity of the reagent, the date of preparation or expiration, and the identity of the individual preparing the reagent. Quality assurance and quality control will both be discussed at length in a subsequent module.

Decontamination Practices

Surfaces

Household bleach is 5.25% sodium hypochlorite (52,500ppm), so a 1% bleach solution is 525ppm and sufficient for inactivating most viruses. Per the Centers for Disease Control and Prevention (CDC), chlorine solutions gradually lose strength, so diluted solutions should be replaced daily.01 Some laboratories have changed from the traditional 10% mix in a bottle to a type of container that mixes neat bleach and water at the time it is expressed from the bottle. Biocidal activity is affected by the presence of organic material, which consumes available chlorine. Some laboratories may employ a detergent based germicidal disinfectant, e.g. Coverage Plus®, or a decontaminant such as DNA Away™ per manufacturer's directions. Cleaning with bleach or detergents may be followed by water and/or EtOH to eliminate the possibility of introducing bleach or detergent into the sample or build up. EtOH alone, even 70% as employed in microbiology settings to prevent transfer of pathogens, is not sufficient to rid a surface of exogenous DNA.

Another means of decontaminating hoods, reagents, pipettes, tubes, etc., is exposure to UV light. Most biological safety cabinets are equipped with a UV light source. It is generally accepted that UV exposure at 254nm for a minimum of 5 minutes is sufficient for disinfection to include the deactivation of nucleases and destruction of extraneous DNA on surfaces. Laboratory SOPs often include UV exposure steps as long as 30 minutes before and after use of hoods for PCR work. Wiping with bleach and/or detergents is still warranted as the penetrating power of UV light is minimal.

Reagents, Consumables, and Tools

Applicable consumables and tools such as scissors, microcentrifuge tubes, spin baskets, racks, glassware, containers, and other items may be autoclaved. Many laboratories, as a part of their quality control program, autoclave some reagents prior to use, and may prepare some reagents from autoclaved solutions. It is a good practice to apply autoclave tape to items being sterilized to test the autoclave's efficacy and differentiate between items that have and have not been autoclaved. Laboratories should pay careful attention to the plastics used in the laboratory if they are to be autoclaved, as some can break down, becoming more porous and prone to harboring contaminants. When using tubes or supplies that have been autoclaved in a container, it is good laboratory practice to pour them onto a clean surface rather than reaching into the container to retrieve them.

Use of Basic Equipment

The QAS require the following:

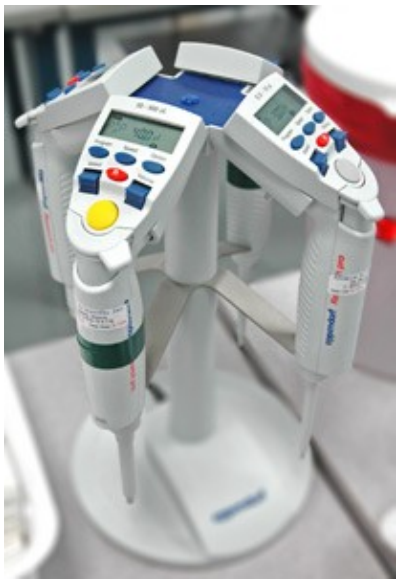
- The laboratory must use equipment that is suitable for the methods employed.
- The laboratory must have a documented program for calibration of equipment and instruments.
- When available and appropriate, standards traceable to national or international standards must be used in the calibration of equipment.
- Where traceability to a national standard of measurement is not applicable, the laboratory must provide satisfactory evidence of correlation of results.
- Each instrument requiring calibration must have the frequency of calibration documented and have such documentation retained in accordance with applicable federal or state law.
- The laboratory must have a documented program to ensure that instruments and equipment are properly maintained.
- New instruments and equipment, or instruments and equipment that have undergone repair or maintenance, must be calibrated before being used in casework analysis.
- Written records or logs must be maintained for maintenance service performed on instruments and equipment and such documentation must be retained in accordance with applicable federal or state law.

*Note: The laboratory's documentation must include the identification of all critical equipment and instruments that require calibration. Critical equipment or instruments are those requiring calibration prior to use and periodically thereafter when the accurate calibration of that instrument directly affects the results of the analysis. Calibration is the set of operations that establish, under specified conditions, the relationship between values indicated by a measuring instrument or measuring system or values represented by a material and the corresponding known values of a measurement. The laboratory's documentation must include the schedules for and records of all calibrations for the critical equipment and instruments. Standard 10.3.1 does not apply to instruments and equipment that cannot be calibrated by laboratory personnel (e.g., fluorescence-based detection instruments). Traceability is the property of a result of a measurement whereby

it can be related to appropriate standards, generally international or national standards, through an unbroken chain of comparisons.

Most newly hired DNA analysts have a general familiarity with the principles and operation of basic laboratory equipment. However, it is recommended that an overview, practical sessions, and trainer oversight be provided before trainees use the equipment.

Pipettes



Pipettes are used as a tool for transferring fluids. Many new analysts are familiar with glass and/or plastic volumetric and Mohr pipette that are commonly used in college laboratories. Many new analysts are not as familiar with the micropipettes used in forensic laboratories. Generally, forensic laboratories do not use glass and/or plastic pipettes in forensic DNA analysis, so these will not be covered. It should be noted that proper pipette calibration is essential. Pipette calibration can be done by laboratory staff or through an outside vendor. It is important to ensure the reference standards used be traceable to the National Institute of Standards and Technology (NIST).

Single Channel Pipettes (fixed or adjustable)

Single Channel Pipettes are those which dispense fluids via one channel. These can come in a fixed volume (only one volume can be dispersed from the pipette) or an adjustable volume (more than one volume can be dispersed based on the pipette settings). Most single channel pipettes have built in tip ejectors so that the analyst need not remove the tips by hand, and utilize an air-displacement mechanism for volume measurement.

Multi-Channel Pipettes (fixed or adjustable)

Multi-channel pipettes are those which dispense fluid via more than one channel utilizing an air-displacement mechanism. Most multi-channel pipettes are adjustable volume and have built in tip ejectors. Multi-channel pipettes can be obtained in 8 channel varieties, which make them quite useful in setting up multi-welled plates used in thermal cyclers and genetic analyzers.

Motorized/Electronic Pipettes (fixed or adjustable)

Motorized/electronic single and multi-channel pipettes are available. These pipettes have electronic operation from aspiration to tip ejection. Pipette use can contribute to Repetitive Strain Injuries (RSI), and many users find that tip ejection and pipette operation are significant contributing factors. Motorized/electronic pipettes negate the issues by allowing these processes to be accomplished by touching a button. Volume adjustments are generally made with a stepping motor to control piston action, which are usually controlled by a microprocessor.

Positive Displacement Pipettes

Positive displacement pipettes are generally used for difficult liquids. Air displacement pipettes reach their limits with high density, viscosity, and vapor pressure liquids. Positive displacement pipettes work by having a piston-integrated tip. The piston makes contact with the liquid, and a positive wiping action of the piston

against the capillary walls of the tip assures dispensing without residual droplets. Positive displacement pipettes can come in fixed and adjustable, single or multi-channel varieties.

Repeat Pipettes (fixed or adjustable)

Repeat pipettes are those which allow for multiple dispensings from a single aspiration. These are quite useful when the same volume of a reagent must be place into multiple tubes or wells. These can come in fixed and adjustable volumes, single and multi-channel, and air-dispersing and positive displacement varieties.

Rocker/Shaker

The online version of this course contains a multimedia [or downloadable] file. Visit this URL to view the file:
http://beta.forensicbiology.dna.devis.com/Lab_Orientation/Basic_Equipment/Rocker_Shaker/default_page
Rockers provide a repeated side-to-side motion and are generally variable in speed. They come in a variety of sizes, and many have adjustable table angle.

Shakers are designed to provide a uniform motion that is both mixing and vortexing in nature. They come in a variety of sizes and have adjustable speed settings.

Rockers and shakers can be used for the mixing of reagents, gel staining, and hybridization steps.

The online version of this course contains a multimedia [or downloadable] file. Visit this URL to view the file:
http://beta.forensicbiology.dna.devis.com/Lab_Orientation/Basic_Equipment/Rocker_Shaker/default_page

Balances

Measurements

The term "weight" is commonly used synonymously for mass. Mass is the amount of matter in an object, and the International System of Units (SI) unit for mass is the kilogram (kg).02

A discussion and demonstration of the use of all types of balances in the laboratory, such as top-loaders and analytical balances, should be provided. If necessary, a review of basic math and standard measurements can be presented. Traceable standards, such as weight sets, are easily obtained for this purpose, or a laboratory may employ an outside entity to conduct the calibration. It should be noted that the outside entity must use traceable standards to conduct the calibration. A balance should be calibrated whenever it is moved.

Top-loading Balances

Top-loading balances are generally portable and ideal for easy weighing tasks. They have a larger weighing pan and may offer ranges such as 210g x 0.01g and 8100g x 1g, and are thus applicable for weighing chemicals to make stock reagents, etc. It is imperative that anyone using a balance know how to properly use features such as the external calibration and taring functions.

Analytical Balances

Analytical balances offer higher resolution and measuring quality, in ranges typically to 2.1g x 0.001mg and 510g x 0.1mg. Such tools are necessary in the preparation of some reagents in the forensic biology laboratory.

pH Meters

Basic Acid – Base Chemistry

Acid Definitions:

- Brønsted-Lowry – a compound capable of donating hydrogen ions
- Lewis – a compound that can accept an electron pair from a base
- Arrhenius – a compound that releases hydrogen ions in solution

Base Definitions:

- Brønsted-Lowry – a molecule or ion that accepts hydrogen ions from solution
- Lewis – a molecule or ion that donates an electron pair to an acid
- Arrhenius – a compound that releases hydroxide ions in solution

Acid – Base Reactions

- Neutralization
    - The neutralization reaction of acid and base results in water and salt.
    - H^+ (aq) + OH^- (aq) H_2O
- Examples of acid – base reactions:
    - HBr + KOH H_2O + KBr
    - HCl + NaOH H_2O + NaCl

In keeping with the Brønsted-Lowry definitions of acids and bases, the criterion is the concentration of hydrogen ions present. Acids are proton donors and bases proton acceptors. The pH scale denotes the acidity or basicity of something through measurement of hydrogen ion concentration.

Given pH = -log [H^+], a solution where [H^+] = 1 x 10^{-7} moles/liter is of pH 7. Thus, solutions with lower pH designations are more acidic, and those with higher pH are more basic. Ranging from pH 0 (e.g., HCl) to pH 14 (e.g., NaOH), pH 7 is considered neutral. Acidic and/or basic solutions may require titration to a desired pH through the addition of acid and/or base. Thus, the pH meter is a tool in determining the pH of the solution

in question.

pH Meters

pH meters utilize a probe bearing a thin glass bulb at the tip. Two electrodes inside the probe measure voltage. One electrode is contained within a liquid of fixed pH, while the other is free to respond to the [H+] of the sample. The voltage difference is measured by a voltmeter and translated into pH. Trainees must be able to properly care for, calibrate, and use the pH meters available in the laboratory before making reagents. Most pH meters are readily calibrated using standard pH solutions available from the manufacturer or a variety of suppliers; however these are not traceable to a national standard of measurement so the laboratory must provide satisfactory evidence of correlation of results.

Centrifuges

Many techniques in forensic biology and DNA analysis call for centrifugation. Analysts should understand proper use of specific centrifuges in the laboratory, as they may be designated for specific uses and/or be set to specific speeds for certain steps in a procedure. Given the relatively small volumes handled, the maximum throughput of a centrifuge for efficient use is generally not calculated, but rather analysts depend upon empirically determined and validated or manufacturer recommended volumes and speeds.

The centrifugal force created by spinning separates mixtures based on particle size and the difference in density between liquid and solid phases in the tube. Equations considering many variables such as particle size, solution density, and angular velocity may be used to determine a rate of centrifugation. Commercial products generally indicate appropriate settings in a bulletin or user's manual. This is particularly important when dealing with smaller and thin-walled tubes as well as spin baskets or other inserts with filters, as excessive force may cause them to fail. Overall, centrifuges play an important role in the forensic DNA laboratory in procedures such as extraction and preparation for quantitation and analysis. It should be noted that relative centrifugal force (RCF) and/or rotations per minute (RPM) can be verified by outside vendors.

Other

Autoclaves

Because autoclaves must be used properly to effectively decontaminate materials, it is important that trainees be instructed on their care and use. According to Rutty, autoclaving alone may not rid instruments or glassware of DNA, but proper autoclave sterilization would prevent contamination by enzymes with nuclease activity.03

Heat Blocks

Laboratories may use a heat block or dry bath for heated incubation. For example, steps such as heat denaturation of amplified DNA product in a microcentrifuge tube may be conducted in a heat block in the amplification room or in a thermal cycler with appropriate settings. A heat block is often preferred for heating samples during extraction, as there is less risk associated with water seepage and contamination. When

necessary, the temperature can be checked using a NIST traceable thermometer.

Hotplates & Magnetic Stirrers

Hotplates and magnetic stir plates are often used in reagent preparation. Analysts should be well versed in their care and use and to only use them when a reagent recipe specifically warrants. Laboratories may also use hotplates for heat fixing slides, so it is important that they be clean and in good working order.

Incubators

Laboratories utilizing certain types of biological screening tests, such as precipitin or Ouchterlony plates and/or radial gel diffusion assays for -amylase, may maintain a laboratory incubator. These can be used to create an environment of optimum temperature and humidity for incubating assays or for storing warm reagents, such as those used in slot blot quantitation. Temperatures can be sustained at room ambient or higher, and trainees should be instructed in the care and use of incubators. Cleanliness is imperative in these environments given the propensity for microbial growth. When necessary, the temperature can be checked using a NIST traceable thermometer.

Thermometers

The majority of thermometers used in a forensic science laboratory are liquid in glass thermometers. Thermometers deemed critical by the laboratory should be calibrated using standards traceable to national or international standards. Oftentimes laboratories purchase thermometers that have been verified and calibrated against a NIST traceable source.04 Specialized thermometers using shaped probes are also used for monitoring and calibrating of equipment such as heat blocks and thermal cyclers. Trainees should understand the appropriate application of thermometers, how to read them, and the importance of calibration proper use and safety.

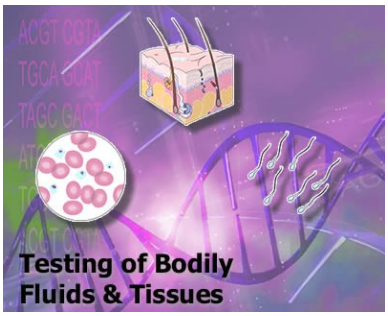
Water Baths

Water baths are used for a variety of laboratory applications and include general-purpose, shaking, immersion circulating, boiling, high temperature, and low temperature water baths. Trainees should be made familiar with the types of baths available in the lab and their respective uses. Emphasis should also be placed on proper filling and maintenance. When necessary, the temperature can be checked using a NIST traceable thermometer.

Water Purification

It is important that a DNA analyst understand the effects of resistivity and total organic carbon level in monitoring water quality and when ultrapure, sterile, and/or deionized water is used. Using sterilized water in various stages of the forensic DNA analysis process is very important. Most laboratories employ a commercial water purification system, and these have outputs that alert the laboratory when filters and/or UV lights need to be changed.

Testing of Body Fluids and Tissues: Introduction



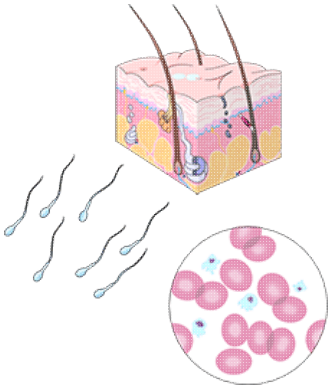
This module provides an overview of historical and contemporary techniques used to characterize body tissues. DNA typing has become such an effective tool in forensic biology that almost all of the traditional techniques have fallen into disuse. Some techniques are still used in the examination of body fluids, while knowledge of others, such as ABO and multi-enzyme systems, is of value if old cases are opened for review.

Objectives

Upon successful completion of this unit of instruction, the student shall be able to do the following:

- Select and apply methods to screen evidence for body tissues and proceed to confirmatory tests where appropriate
- Have a basic understanding of the principles of ABO typing and other red cell surface antigen polymorphisms used in forensic biology
- Have a basic understanding of the principles of enzyme and other protein polymorphisms used in forensic biology

Overview



Serology is the detection, identification, and typing of body tissues, either in native form or as stains or residues left at a crime scene. Most often, the tissue of interest is a body fluid such as blood or semen; however, other tissues such as hair or bone are encountered. The detection and identification process begins with a physical examination, followed by a screening test, and may include confirmatory identification and sample typing. Typing is the detection of genetically determined characters in the sample. A difference in type between the stain and reference sample from the postulated source may provide a positive elimination. If there is no elimination, the results can be evaluated against population frequency data to give a measure of the significance of the failure to eliminate.

The features of a good typing system are that it

- shows variability from person to person but is constant within one individual,
- is stable in shed form,
- can be detected reliably at the concentrations found in forensic samples,
- has a known and stable frequency of occurrence within the population.

Note:

It is not required that a typing system be based on an inherited characteristic, although in practice they all are. The very presence of biological fluids, specifically blood and semen, may be an indication of a serious crime and of evidential value. The physical distribution of blood stains at the scene or on clothing may produce valuable information about the crime.

Characterization of biological fluids is often used to associate forensic evidence with an individual. Testing allows conclusions to be drawn as to the person from whom the fluid originated.

Presumptive v. Confirmatory Tests

Most tissues possess characteristics that are typical of the specific material but not unique to it. For example, semen has a high concentration of the enzyme acid phosphatase, but the enzyme is found at lower levels in other body fluids, including vaginal secretions. Screening or presumptive tests make use of a target chemical to establish the possibility that a specific body tissue or fluid is present. Confirmatory tests are then used to identify the specific biological material, which can then be typed.

The line between screening and identification is not always clear. For example, while examining the clothing of a suspect, a forensic biologist might visually locate a brown stain that presumptively tested positive for blood and was then DNA typed. The DNA type is found to match the victim. Knowing that the loci tested are higher primate specific, what conclusions can be drawn?

The only unqualified conclusion that can be offered is that the stain contains DNA that matches the victim. It has not been proven to be blood.

If asked "Could the results have arisen because the material tested was the blood of the victim?" then an answer of "Yes" is justified. However, it would be wrong to report that the material was human blood with a DNA type that matched the victim. The material was not subjected to confirmatory testing for blood or proven to be human in origin.

Read more examples of how to report tests in the Communicating Results PDF file.

Immunological Tests

Many traditional tests used in forensic biology are immunological in nature. A brief account of basic immunological principles is given before discussing the tests.

Antibodies are proteins, called immunoglobulins (Ig), which are produced by white blood cells (WBC) in response to stimulation by foreign materials (antigens). They are found in serum in the gamma globulin fraction. All immunoglobulins have the same basic structure consisting of two pairs of peptide chains linked to form a Y-shaped molecule. The chains in the longer pair are designated as "heavy" or H and those in the shorter pair as "light" or L chains. The chains within each pair are identical. However, these pairs (H and L) differ from each other. There are five classes of immunoglobulins, each of which are differentiated by their chemical structure.

Immunoglobulin Classes	
Immunoglobulin	H-subtype
IgG (serological interest)	gamma ()
IgM (serological interest)	mu (μ)
IgA	alpha ( )
IgD	delta ()
IgE	epsilon ()

* Note: There are two L chain sub-types,
namely kappa () and lambda .
The antibodies involved in precipitin reactions are mainly IgG, whereas IgM molecules are the class responsible for agglutination reactions.

Antigens (also referred to as immunogens) are variously described as compounds that stimulate production of antibodies in an immune response or as substances that combine with an antibody. Immunogens are usually large molecules, such as proteins. A chemical complex is formed when an antibody binds to the epitope region on an antigen. It is possible to have an antibody-antigen reaction where the antigen would not produce an immune response except in combination with a carrier molecule; such an antigen is correctly termed a hapten.

The immune response to an antigen challenge is the production of antibodies. Each cell line produces identical antibodies; cultures of these cells will generate highly pure and specific monoclonal antibodies. Monoclonal antibodies interact with a specific epitope region, whereas polyclonal antibodies interact with numerous sites on the antigen. Each epitope on the antigen produces specific antibodies. Serum containing antibodies is called antiserum.

The antibody-antigen reaction is specific. This can be visualized as a lock and key; the binding site on the antibody fits exactly with the epitope region on the antigen. For example, each IgG is a bivalent antibody having two identical receptor sites specific to an epitope on the antigen. This enables cross-linking to occur.

This is the basis of the precipitin and agglutination reactions. In the former, divalent IgG molecules cross link with binding sites on proteins to form a high-molecular weight, insoluble precipitate. In the latter, the IgM molecules exist as pentamers that are able to cross link with binding sites on the cell surface, resulting in clumping, or agglutination, of the cells. In both cases (precipitin and agglutination), the aggregates can be seen with a low power microscope or the naked eye.

Learn more about precipitin and agglutination reactions.

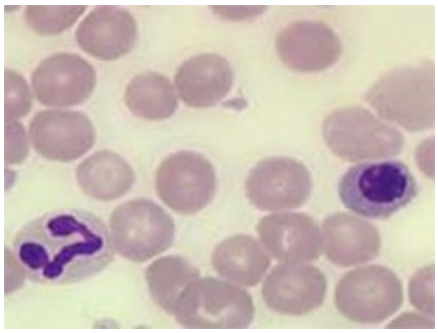
Blood

Blood is a suspension of cells in an aqueous solution, consisting of three types of materials:

- salts (sodium, potassium, and chloride ions)
- organic chemicals (glucose, hormones, and vitamins)
- proteins

There are three cellular components to blood:

- red blood cells (RBCs) or erythrocytes
- platelets or thrombocytes
- white blood cells (WBCs) or leukocytes



Red and white blood cells.

The white blood cells are the larger

ones with dark, irregular nuclei.

The small particles are platelets.

(Photo: U.S. National Institutes of Health)

The cellular component of blood is mainly comprised of red blood cells, which account for about 45% of the total volume and is referred to as the hematocrit. RBCs are unique because the mature circulating cells contain no DNA. Their function is to transport oxygen to tissues as a hemoglobin complex.

White blood cells, which possess a nucleus (and therefore DNA), are involved in the body's responses to infection. Lymphocytes, one type of WBCs, are responsible for antibody production.

The fluid portion of unclotted blood is called plasma. Blood clots through the conversion of a dissolved protein, fibrinogen, to a precipitated polymer, fibrin. Fibrin traps platelets to form the clot. The liquid fraction obtained from clotted blood is called serum. Serum can be further separated into fractions by electrophoresis. The simple and not very discriminating forms of electrophoresis that were first used, such as those employing cellulose acetate membranes, typically produced only four fractions. These are, in order of electrophoretic mobility, albumin, followed by three globulin fractions designated as alpha, beta, and gamma. These designations have become accepted terms used to describe serum proteins.

About half of the serum consists of albumin, which is one of the factors that preserves blood volume by regulating osmotic pressure. In contrast, each globulin fraction consists of many different proteins. This is particularly true of the gamma globulin fraction, which contains antibodies.

Screening Tests

Most screening tests for blood depend on the peroxidase activity of hemoglobin. The most common tests depend on the oxidation of colorless reduced indicators, many of which are conjugated systems and are known or suspected carcinogens.

Screening Tests

Test	Indicator	Sensitivity	Comment
Benzidine	Blue color	++++	Carcinogen
Tetra methyl benzidine	Blue color	+++	Probable carcinogen
o-Tolidine	Dark green	+++	Probable carcinogen
	Pink color	++	Relatively safe

Phenolphthalein or Kastle Meyer test			
Leucomalachite green	Green color	++	Relatively safe
Luminol (5-amino-2,3-dihydro-1,4 phthalazinedione)	Fluorescence	+++++	Probable carcinogen

Note: The product "Hemastix", manufactured by Bayer and widely used in clinical screening tests for detection of blood in urine, provides a safe and simple alternative to the above reagents. It contains o-Tolidine immobilized behind a membrane. The membrane acts as a barrier to contact by the user, but permits aqueous extracts to pass and react with the reagent.

These tests are not specific for blood. Other biological materials, such as fruits, possess peroxidase activity, and oxidizing agents may be present on surfaces. Some laboratories have used two or more of the tests listed in the table and reported the second as confirming the first. However, since they are all presumptive tests, carrying out two or more tests does not substitute for or constitute a confirmatory test.

The online version of this course contains a multimedia [or downloadable] file. Visit this URL to view the file: http://beta.forensicbiology.dna.devis.com/Testing/Blood/Screening_Tests/default_page

Confirmatory Tests

Many different tests have been used to confirm that a stain contains blood. The oldest is chemical confirmation of the presence of hemoglobin or its derivatives by the formation of specific crystals. For example, the Takayama or hemochromogen test, in which ferrous iron from hemoglobin reacts with pyridine to produce red feathery crystals of pyridine ferroprotoporphyrin. Another confirmatory test uses the Teichman reagent, consisting of a solution of potassium bromide, potassium chloride, and potassium iodide in glacial acetic acid, and is heated to react with hemoglobin. The reaction first converts the hemoglobin to hemin, and then the halides react with the hemin to form characteristic brownish-yellow rhomboid crystals.

Blood can be identified as being of human origin by precipitin reactions with antisera specific for components of human blood. Usually this is an antihuman serum serum - that is, an antiserum to human serum. Strictly speaking, this is a test for human origin not for human blood, as serum constituents such as albumin and some globulins are found in the extra-vascular space.

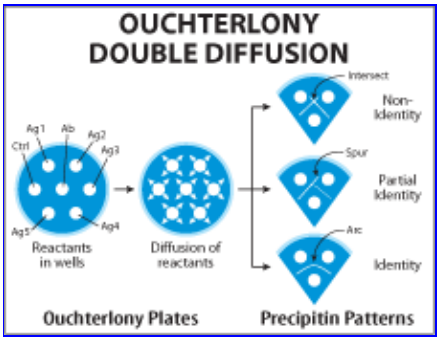
The original precipitin reaction was carried out by layering a solution of antibody on top of a solution of stain extract in a tube, and left for a period of time to allow the development of a precipitin band at the interface. This is referred to as the tube method, and is still used in a few laboratories today.

Ouchterlony

However, most species identification uses radial diffusion of antigen and antibody through agar gel. This is the Ouchterlony test. A variant of the Ouchterlony test, called cross-over electrophoresis, uses an electric field rather than diffusion to move the extract and antibody through the gel. Ouchterlony plates can be purchased or made in the laboratory.

Extracts are made from stained areas of interest, and from nearby unstained areas (substrate controls). *Note that the use of unstained controls is a fundamental principle in forensic immunologic testing.*

Stain and controls samples are loaded in the outer wells and a drop of anti-human antiserum is loaded into the center well. The process is repeated for antisera to other species, such as dog, cat, and cow; this may include the species from which the antiserum was obtained (e.g., rabbit).



The plates are left at 4°C for a suitable period (which can range from a few hours to overnight) and the serum proteins and antibody molecules diffuse outward from the wells. A precipitin band is formed when the diffusing stain contains proteins that are recognized by IgG molecules in the diffusing antiserum. The precipitin band is sometimes clearly visible to the naked eye, but it is normal to stain the plates with amido black or other general protein stain to enhance sensitivity and clarity.

Cross-over Electrophoresis

Cross-over electrophoresis for species identification is conducted using agar at a pH of 8.6. Stain extracts are loaded into wells arranged in a line at the cathode end of the plate and the antiserum is loaded into wells at the anode end. During electrophoresis, the electric field drives the serum proteins towards the anode, but the IgG molecules, which are essentially neutral at this pH, are driven to the cathode by the process of electroendosmosis. The antigen-antibody precipitation occurs at the interface between the two rows of wells. Electroendosmosis occurs because the supporting medium acquires a net negative charge. If free, the negatively charged molecules would migrate to the anode, but this is not possible because the agar is immobilized on the plate. Instead, the effect is countered by positively charged water molecules migrating to the cathode. The migrating water molecules carry any dissolved neutral molecules (such as IgG) with them.

ABAcard®

The method of choice today is the ABAcard® HemaTrace test strips manufactured by Abacus Diagnostics, Inc. Stain extract is applied to the bottom of the test strip, where any human hemoglobin present in the extract will combine with a monoclonal antihuman hemoglobin antibody. The antibody is labeled with a dye. Any antibody-antigen formed then migrates through an absorbent membrane to the test area of the strip. The test area has an immobilized polyclonal antihuman hemoglobin that will capture the Ag-Ab complex to form an Ab-Ag-Ab sandwich. The pink dye becomes visible as a band in the test region at concentrations of human hemoglobin above about 0.05 µg/ml. An internal control consisting of human hemoglobin antibody–dye conjugate cannot bind to the antibody in the test area but is captured by an antibody in the control area. A correctly functioning positive test will therefore show two pink bands, one in the test area and one in the control area. A correctly functioning negative test will show only one pink band, in the control area. If there is any problem with the test, there will be no visible bands.

The ABAcard® test has been extensively validated and shown to be sensitive, specific, and rapid.

ABO Groups

"Blood grouping" conventionally means assignment of its ABO blood type. The concept originated with the work of Landsteiner in 1901. While investigating the properties of blood, he showed that serum separated from the blood of some individuals would cause clumping, or agglutination, of the RBCs isolated from some other individuals, but not RBCs from themselves. Not all serum and not all RBCs would react.

He identified the response as being due to interaction of antibody in serum with antigens on red cell surfaces, and was able to describe four blood types and measure their frequency of occurrence in the population:

Blood Types

Type	Cells	Serum	Population frequency*
A	A antigen	anti-B	42.3%
B	B antigen	anti-A	9.4%
AB	A and B antigen	no antibody	3.5%
O	no antigen	anti-A and anti-B	44.8%

*These are approximate figures for Caucasians in the U.S. There are differences depending on race and geographical location. For example, group B blood is more common in persons of negroid race (around 20%).

The online version of this course contains a multimedia [or downloadable] file. Visit this URL to view the file: http://beta.forensicbiology.dna.devis.com/Testing/Blood_Grouping/ABO_Groups/default_page

It took about 60 years for Landsteiner's work to be developed into a usable test for classifying blood type in stains. The first partially successful attempt was introduced by Lattes in 1915. The basic problem is that the RBCs are destroyed when a stain is formed, and so there is nothing to subject to an agglutination reaction. Lattes realized that antibodies were less susceptible to degradation in stains and might be detectable. He developed a method for extraction of antibody and identification with indicator A and B cells. However, two problems remained. The first is that the low levels of antibody extractable from stains made the test somewhat unreliable. The second is that identification of AB blood depends on making a call from a negative observation (no agglutination with A and no agglutination with B indicator cells), which is not a scientifically acceptable thing to do.

Various attempts to type stains by identifying the antigen were tried for the next 45 years, and a sufficiently reliable method, absorption–elution, was eventually introduced in the early 1960s by Kind and by Outterridge. 01, 02

Absorption-elution depends on detecting antibody that has been bound by A or B antigen on immobilized fragments of the lysed cell surface. The antibody is then eluted and detected with A or B indicator cells. Since group O blood has no antigen, there will be no bound antibody to elute and detect. That means that almost half of the samples tested would produce no detectable result. However, group O cells do in fact possess antigen on their surface. It is a precursor of the A and B antigens and is designated as H substance. Certain botanical extracts called lectins can act like antibodies, and the extract from *Ulex europaeus* reacts with H substance to agglutinate group O cells. The extract is called H-lectin. The chemistry of A, B and H is dealt with in the section on Semen.

The procedure works because the IgM antibody molecules have an optimum reaction temperature of around 4°C. The first step, specific binding, is conducted at 4°C and then the temperature raised to 56°C which disrupts the non-covalent Ag-Ab interactions and elutes antibody from its bound state. H-lectin behaves

sufficiently similarly to permit identification of group O stains. Note that since H is a precursor of A and B, and since absorption-elution is very sensitive, A and B stains will usually also display H activity.

Immobilization of stain can be achieved in various ways. The two most widely used are fixing a single thread from the stain (or from a swab of the stain if it is not on fabric) onto a glass or plastic plate with glue (nail polish) or heat, or making an extract of the stain in 5% ammonia and heat fixing it to the plate.

Learn more about absorption-elution reactions.

Characterizing body fluid stains by absorption-elution typing for ABO group was one of the most significant advances in forensic biology. However the technique has its problems. The major difficulty is the presence of adventitious blood group substance. That is, stains can sometimes produce false positive results, especially for group B. Running appropriate negative controls is a vital step and results from any sample that shows a false positive has to be discarded. Common evidential substrates such as denim fabric and soiled shoes often give false positives.

The end-point — agglutination of test cells by eluted antibody — is subject to some variability also. Different observers may read weak results differently, and many samples will come up positive if left long enough. Confirmation of test readings by a qualified second reader is a key quality control measure.

Other Red Cell Blood Groups

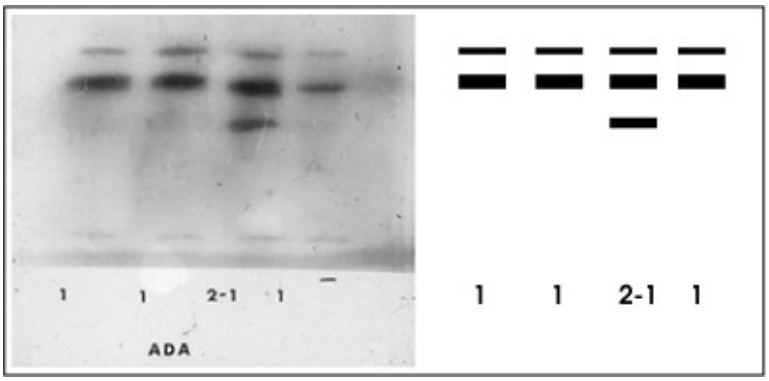
Hundreds of blood groups characterized on the basis of red cell antigens have been documented. They include clinically important groups such as Rhesus, a range of variants of A and B, and a whole suite of groups, such as Kell, Kidd, and Duffy, that were used in tissue typing before DNA became the method of choice. None of them proved to be of sufficient value for typing stains to become much used in forensic work.

Enzyme Groups

Because of these limitations in blood groups, forensic serologists had to look to different kinds of inherited biochemical markers to extend the discriminating power of typing. Fortunately, many of the systems of forensic interest turned out to be enzymes found on the red cell membrane. The main example is the enzyme phosphoglucomutase (PGM). PGM catalyzes the reversible conversion of glucose-1-phosphate and glucose-6-phosphate, with glucose-1.6-diphosphate as a co-factor. PGM is an important metabolic enzyme and is found throughout the body. It is expressed at many loci, and the form found in red cells is designated as the PGM 1 locus, usually written as PGM_1. The PGM_1 locus is also expressed in semen, which increased its value in forensic serology. There are two alleles, designated "1" and "2", giving the phenotypes PGM-1. PGM-2, and PGM 2-1. Note that the locus is assumed, and the subscripted identifier has been omitted. The population frequencies for the three phenotypes are approximately 59%, 36%, and 5%, respectively. The actual frequencies vary by race and ethnicity. Rare variants of the 1 and 2 alleles have been found.

Other red cell enzymes used in forensic biology include the following:

- erythrocyte acid phosphatase (EAP)
- esterase D (EsD)
- adenylate kinase (AK)
- adenosine deaminase (ADA)
- glyoxalase (GLO)



A typical ADA plate, with a schematic alongside to identify the isoenzyme bands.

The enzymes vary in their stability in stains, the reliability of typing, the sensitivity of tests, and in their discriminating power. Although discriminating power can be increased by testing for more than one enzyme, each individual test consumes sample, typically about six one-centimeter threads from a stain on cotton cloth. One partial solution is to run more than one system at a time, and Multi Enzyme Systems (or MES) became popular for a time. Typical combinations included PGM, EsD, and GLO, and PGM, ADA, and AK.

Identification of the polymorphisms in all the above systems depends on the same basic principles:

- The changes in structure affect the net charge of the isoenzymes.
- The isoenzymes can be separated by simple electrophoresis.
- The locations of the separated isoenzymes can be visualized by reactions that depend on the specific enzyme activity.

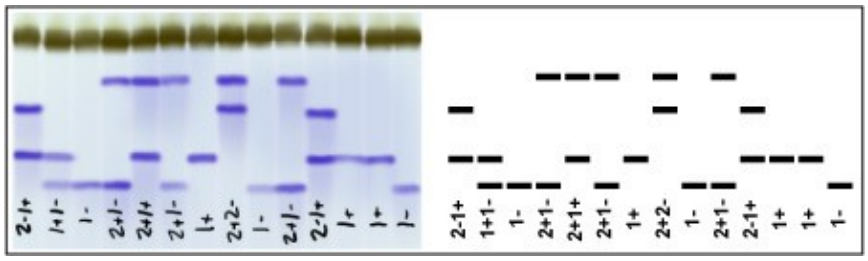
Starch gel was the usual separation medium, but cellulose acetate, polyacrylamide, and agarose were also used. Most of the detection systems used a biochemical chain reaction in which the enzyme of interest reduced nicotinamide adenine dinucleotide phosphate (NADP) to NADPH with the concomitant conversion of MTT tetrazolium (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazoliumbromide) to the purple colored formazan in the presence of phenazine methosulfate (PMS).

Some of the enzymes – EsD and EAP for example – can hydrolyze esters to produce a fluorescent compound that can be visualized under UV light.

A variant of electrophoresis is isoelectro focusing (IEF) where a pH gradient is formed during the electrophoresis and molecules move until the point in the gel at which they carry no charge. IEF produces much sharper bands than slab gel electrophoresis. Attempts to improve PGM separation by using IEF gave a surprising result, namely the discovery of a further two alleles, the expression of which was not detected by starch gel separations. Each of the alleles detectable by starch gel electrophoresis had two alternate forms, designated as the "+" and "-" alleles. Thus, the 10 phenotypes were comprised of the four homzygous forms PGM-1+, PGM-1-, PGM-2+, PGM-2-, and their heterozygous expressions.

View an animation on isoelectric focusing.

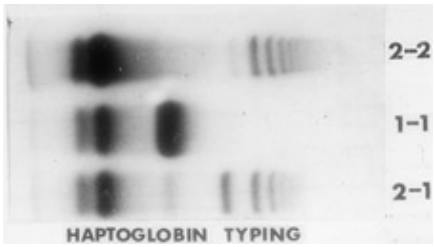
Sometimes referred to as "PGM sub-typing," IEF was probably one of the best techniques available before the advent of DNA typing. The technique could type very low concentrations of enzyme and the enzyme itself was stable in blood and semen stains. A diagram showing the PGM sub-types is shown below.



Serum Protein Polymorphisms

Some of the proteins circulating in serum display detectable polymorphisms, with alleles that have sufficient frequency differences to be of value in blood typing. Transferrin (Tf) and Group Specific Component (Gc) were two that offered considerable promise and were becoming routinely used just before the advent of DNA typing. However, haptoglobin (Hp) was the most widely used of the polymorphic serum proteins in forensic biology.

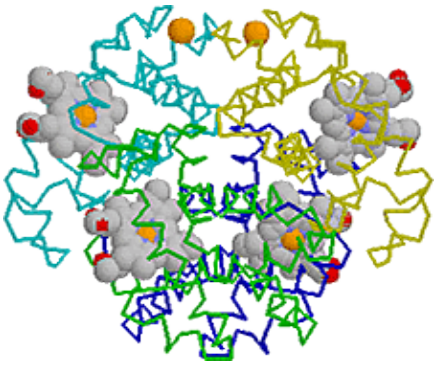
Haptoglobin is a hemoglobin-binding protein found in the -globulin fraction of serum. There are two alleles, designated Hp 1 and Hp 2, with several rare variants at each allele. The alleles are separated by electrophoresis on a gradient polyacrylamide gel (that is, one in which the concentration of polyacrylamide varies from 5% at the top to 30% at the bottom, so giving enhanced separation by molecular sieving).



Haptoglobin 1 is a monomer consisting of two pairs of peptide chains (and) joined by disulfide bridges. Electrophoresis of serum from someone who is homozygous for Hp 1 shows only one band. In contrast, samples from someone who is homozygous for Hp 2 display multiple bands on electrophoresis. Curiously, electrophoresis of a sample from a heterozygous Hp 2-1 shows a band matching the Hp 1 band along with multiple other bands, but these do not align exactly with those from a haptoglobin 2 homozygous person. The Hp 2 proteins are similar to Hp 1 in that they are composed of and β peptide chains cross-linked by disulfide bridges. However, the α peptides (α^{2}) are not the same as those in Hp 1. Furthermore, the proteins are found as polymers of the structure α$^{2}_{n}$β$_{n}$ where n is between 3 and 8. In heterozygotes, some of the polymers incorporate α^{1} chains as well as α^{2} ones.03, 04,

Haptoglobin is a reasonably good system for use in forensic serology. It is stable in stains and the assay is quite sensitive, using one of the hemoglobin screening procedures, such as leucomalachite green, to visualize the bands by reacting with the bound hemoglobin.

Hemoglobin



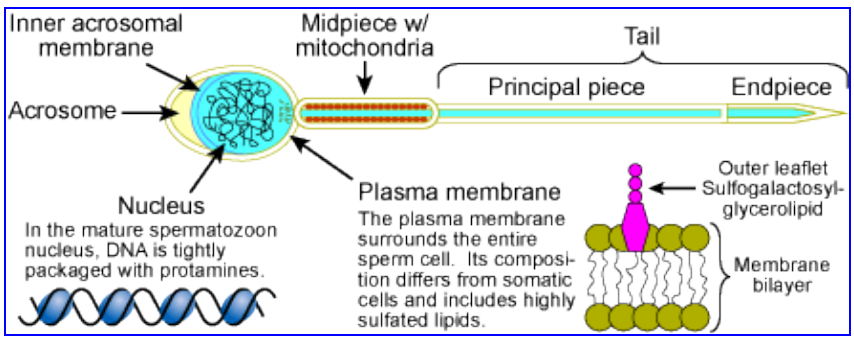
The crystal structure of human deoxy-haemoglobin at 1.74 Angstrom resolution. Gene Gateway, U.S. Department of Energy Genome Programs.

Mention has been made of the role of hemoglobin in screening and confirmatory tests for blood. Hemoglobin is yet another protein formed from two pairs of polypeptide chains. There are several variants of hemoglobin. All have the same structure for one of the pairs of polypeptide chains – designated as α. The dominant form found in adult humans is termed Hemoglobin A (Hb A) and is composed of two α and two β chains. About 2 to 3 per cent of human adult Hb consists of a variant called HbA_2 in which the β chains are replaced by two δ chains. A more significant variant is HbF, which makes up about 70% of the hemoglobin in fetal blood. HbF has a pair of γ chains instead of β. HbF is rapidly replaced by HbA after birth, and only a trace remains by age 1 year.

Detection of HbF in a blood stain is an indicator of fetal blood. The usual test is a combination of electrophoresis and the resistance of HbF to alkali denaturation.

Semen

Semen is a fluid of complex composition, produced by the male sex organs. There is a cellular component, spermatozoa, and a fluid component, seminal plasma. An average ejaculate is 3 to 4 ml containing 70 to 150 million sperm. Sperm are the male reproductive cells. Each consists of a head, tail and mid-piece. In humans, the head is a tiny disc, about 4.5 µm long and 2.5 µm wide. The tail is about 40 µm long, and is rapidly lost in ejaculates. The head is where the DNA is preserved. Ape sperm are similar in size and shape. Dogs have similarly shaped sperm but about one third the size of human sperm. Other animals have differently shaped sperm.



Seminal plasma contains proteins, salts, organics (including flavins which are the source of its UV fluorescence, and choline), and some cellular material. The components originate from several sources, including seminal vesicles and the prostate gland. The prostate is the source of the enzyme acid phosphatase and the protein prostate specific antigen, or p30 protein.

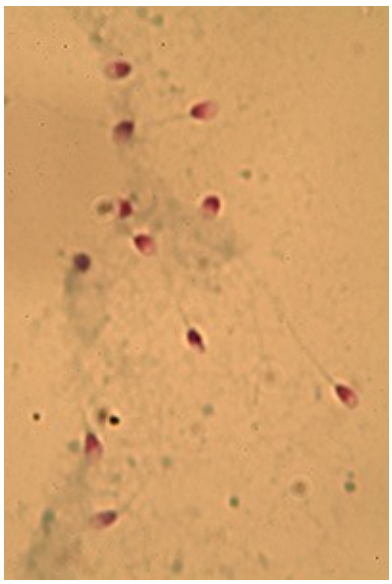
Vasectomy severs or ligates the ducts carrying sperm to the penis. Thus vasectomized men will have no sperm but will have the plasma components present in their ejaculate.

After ejaculation during intercourse, semen is lost by drainage and by biochemical change. Microscopical examination of vaginal swab samples shows a sequence of changes with time, since there is some biochemical evidence for the persistance of tails as long as heads. Tails are lost first - the damage begins immediately and about 25% will have no tails by 6 hours. By 12 hours, there will be few sperm with intact tails and by 24 hours there will be mainly heads left. These proportions and times are highly variable. Sperm survival in stains outside the body depends on environmental conditions, but a small stain that has dried quickly may have intact sperm preserved for months or even years.

Screening and Identification Tests

Human semen contains unusually high concentrations of acid phosphatase, which can therefore be the basis of a screening test. The reaction is based on the hydrolysis of phosphate esters and detection of the liberated organic moiety by production of a color complex. For example, the reaction of acid phosphatase with sodium alphanapthylphosphate and fast blue B to produce a purple-blue coloration. As with the screening test for blood, a positive result is the rapid formation of the intensely colored product – less than 20 seconds or so, or 30 seconds at most.

The best identification of semen is from its microscopy. The morphology and dimensions of the human spermatozoon are unique. The small sperm, particularly if they have lost their tails, can be difficult to locate microscopically especially in samples which have bacterial or yeast infection. Detection is simplified by histopathological staining. The most usual stain is popularly known as Christmas tree stain because of the bright colors. It utilizes nuclear fast red that differentially stains the DNA-containing head bright crimson, and a counter-stain of picric acid - indigocarmine (PIC) that stains the tails green-blue-gray. The traditional histological staining of hematoxylin and eosin (H&E) is also used, as is Giemsa stain.



Problems may be encountered if the seminal fluid is from a man who has a low sperm count (oligospermia) or who has no spermatozoa present in his seminal fluid (aspermia). In situations where the presumptive alkaline phosphate test indicates the presence of semen, but the microscopical analysis yields no detectable spermatozoa, tests are carried out to determine the presence of a protein, P30, or prostate specific antigen (PSA), which is only found in high concentration in human semen. Some laboratories even use P30 testing in place of microscopical examination for semen identification. It can be detected by precipitin reaction with a specific antiserum using the Ouchterlony process. There is also a quantitative immunological test utilizing an enzyme-linked reaction (ELISA).

However, the currently accepted method of choice for identification of semen in all circumstances is detection of p30 using the ABAcard® test strips manufactured by Abacus Diagnostics, Inc. The strips work in the same way as described above for confirmation of blood, except that they use anti-p30 monoclonal and polyclonal antisera, and a pink dye.

Typing

The ability to draw inferences as to the origin of semen in a sexual assault case is obvious. The power to do this in traditional serology was limited, and depended mainly on ABO and PGM typing. The PGM_1 locus is expressed in semen and vaginal secretions, and the methodology and interpretation used in its typing are

exactly the same as for blood.

ABO typing is somewhat different. Almost everyone has at least trace levels of antigen in their body secretions that correspond to their ABO blood type. However, about 80% of the population has very high levels of these antigens in body secretions. These persons are described as secretors. The remaining 20% of the population are described as non-secretors and have concentrations of the antigens that are too low for normal detection. The quantitative difference is genetically determined. The gene responsible has two alleles, a dominant form, Se, and a recessive one, se. Thus SeSe and Sese persons are secretors and sese persons are non-secretors.

The A, B, and H antigens are polysaccharides. They are found on RBC surfaces as lipo-polysaccharides and in secretions as glycoproteins. The biochemical genetics involves four genes as shown in the table below.

Biochemical Genetics

Gene	Expression	RBC genotype	Secretions genotype
Le, H and secretor	Dominant H allele in presence of dominant Se allele converts Le^a substance to mixture of H, Lea and Le^b substances	Depends on A and B alleles	$Le^a$and Le^b substances present together with A and/or B depending on A and B alleles
Le, ABO A allele together with H and Se	A blood group, Lewis a and b	A, H, Le(a-b+)	Le^a, Le^b , A, H
Le, ABO B allele together with H and Se	B blood group, Lewis a and b	B, H, Le(a-b+)	Le^a, Le^b , B, H
Le, ABO O (silent) allele together with H and Se	O blood group (No A or B blood group), Lewis a and b	H, Le(a-b+)	Le^a, Le^b , H
Le, H and sese	A, B or H depending on ABO gene	A. B. H depending on ABO gene, Le(a+b-)	Le^a only
Le, hh and Se	no ABH or Lewis	No ABO activity (Bombay phenotype)	Le^a only
lele, H and Se	ABO depending on ABO gene alleles, no Lewis	A, B, H (depending on ABO alleles), Le(a-b-)	A, B, H (depending on ABO alleles), no Lewis
lele, H and sese	A, B, or H depending on ABO gene	A, B, H depending on ABO gene, Le(a-b-)	No ABH, no Lewis
lele, hh and Se	No ABH or Lewis	No ABO activity (Bombay phenotype) Le (a-b-)	No ABH, no Lewis

Note: Secretors all have an Le allele, an H allele and an Se allele. For simplicity, AB heterozygotes are not listed in the Table – they will have A and B.

ABO Typing Techniques

ABO typing of semen in secretors is conventionally conducted using the technique of absorption-inhibition. The principle behind the technique is simple, and can be illustrated with reference to an A secretor. If anti-A is added to a sample (semen, saliva, or a semen stain extract), the antibody will complex with the antigen in the sample. If a suspension of A cells is now added, there will be no agglutination since there is no free

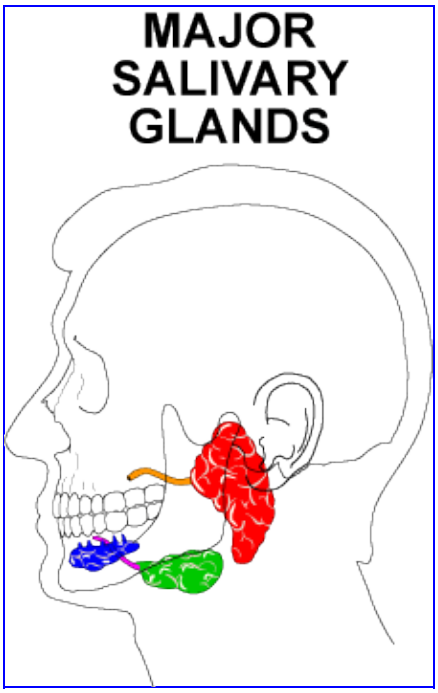
antibody in solution. A matrix can be set up to cover A, B, and H. There are some important factors to remember when conducting the test:

- The indicator cells are best prepared as a weak suspension in a saline-albumin solution.
- The antisera should be titrated against the indicator cells and used at the weakest dilution that will give a reliable result.
- The titration is conducted using serial dilutions.
- Negative results in inhibitions may be due to small sample size or a weak expression of the Se gene.
- Negative results should be reported as "No ABH activity detected."
- Absolute definition of secretor status requires Lewis typing of RBCs and confirmation of the presence of Lewis b substance.

Some laboratories do not perform inhibitions but go straight to absorption-elution. The principle behind this approach is that it will detect ABH activity in all cases, not just secretors. However, certain issues must be noted:

- Invoking conclusions about secretor status from absorbtion-elution results is not reliable since the difference is quantitative.
- High levels of antigen can result in false negatives in absorption-elution as the Ab-Ag complex dissolves in the excess Ag.
- It is always best to prepare at least a 1:10 dilution of extract in absorption-elution to try to overcome these problems.

Saliva



Saliva is the fluid that moistens the mouth. It is secreted from three sets of glands – the sublingual, submandibular, and parotid. The saliva from the parotid glands contains amylases, which aid in the digestion of carbohydrates.

Saliva can be the source of evidence in sexual offenses where oral contact is alleged, bite marks, or on cigarette butts discarded at a scene.

Screening for saliva is based on detection of high levels of amylase in the sample. It is not a confirmatory test as amylase is found in other body fluids.05

Saliva contains ABH substances, especially in secretors. Saliva samples (spit or buccal swabs) are often taken as reference materials for determination of secretor status. Stains can be typed using absorption-elution or absorption-inhibition.

Saliva is also a rich source of DNA, and buccal swabs are routinely collected for reference DNA typing.

Vaginal Secretions

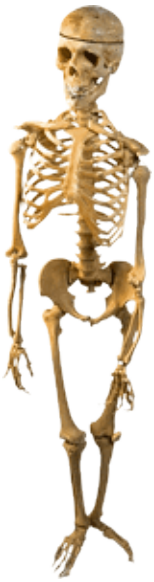
Vaginal secretions are a complex mixture of cells and secretions. There is no absolutely reliable test to identify material as being from the vagina. Several screening tests based on microscopy have been proposed. Vaginal epithelial cells are large, and many contain glycogen which can be demonstrated by staining with iodine in the form of a solution or exposing to iodine vapor. However, the presence of glycogenated cells is variable depending on the stage of the estrous cycle.

The most important aspect of vaginal secretions in traditional serology is the presence of markers that are also used to type semen, specifically ABH and PGM1. It is not possible to distinguish grouping results by physiologic origin with an acceptable degree of reliability. This includes situations where the woman is a non-secretor and the man is a secretor. For example, consider a situation where a rape victim is A, sese, and the suspect is A, Se. If absorption-elution testing on the swab shows the presence of group A substance, then the results should be reported with a qualified interpretation since it cannot be discounted that the results came from the victim.

Feces

Feces are food residues passed after completion of travel through the digestive system. Feces have a characteristic odor mainly due to skatole. Laboratories may be requested to test stains or other samples for the presence of feces. This occurs in the investigation of anal intercourse or where perpetrators have fouled a crime scene. The screening of samples depends on the detection of urobilinogen, a bile pigment excreted in feces, which may be detected using its fluorescent reaction to Edelman's reagent.

Bone



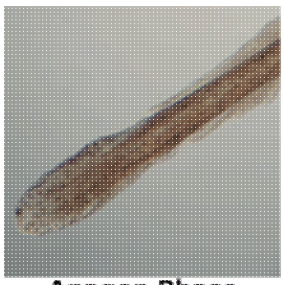
Bone is the tissue that makes up the skeleton. Bone is composed mainly of minerals and collagen, with the minerals (mainly calcium carbonate) making up about 65% of the total mass. The structure makes bones resistant to decay, and skeletal remains may be submitted to the laboratory for identification. Physical attributes such as size and shape, and the presence of injury sites, are probably the most valuable aspects of skeletal remains to examine. However, on occasions serological typing is required. This was not a fruitful area in traditional serology, but does yield excellent evidence in DNA typing.

Teeth

Teeth are similar to bone in that they incorporate a stable, mineralized component in their structure. Dental evidence is one of the most powerful tools for identification of remains by comparing dentition with known dental records. Where no records are available, or when there is only a tooth fragment, typing can be attempted. The dental pulp in the center of the tooth can be extracted and subjected to ABO and enzyme typing.

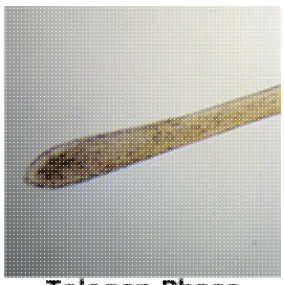
Hair

Hair is an appendage of mammalian skin. It grows outwards from its root, which lies below the surface of the skin and is housed within a hair follicle. The root is surrounded by an inner root sheath and an outer root sheath. The hair shaft that protrudes above the skin surface is made of a strong structural protein called keratin; the same protein that makes up the nails and the outer layer of skin.



Anagen Phase

Catagen Phase



Telogen Phase

Hair follicles grow in repeated cycles, termed the anagen, catagen, and telogen phases. Most hairs are in the anagen or growth phase, during which they have a full-sized and active follicle.

At the end of the anagen phase, hairs enter into the transition, or catagen, phase. The hair follicle shrinks, and metabolic activity and hair growth begins to slow down. The hair then enters the resting, or telogen phase. Metabolism and growth cease. The follicle eventually reenters the anagen phase, and a new hair grows, pushing out the old, dead, one.

Although there is some evidence that the hair shaft can contain ABO substances, identification of origin of a hair by the techniques available to traditional serology depends on the shed hair having root material attached. In these cases, ABO and enzyme typing can produce good results.

Today, nuclear and mitochondrial DNA typing allow excellent assignment of origin of hairs even when there is no root material. There is some controversy about nuclear DNA typing of shafts. It may be that results depend on the presence of adsorbed material from sweat or other body fluids, therefore thorough washing is critical. Mitochondrial DNA typing is generally reliable but is, at present, a lengthy and costly procedure. It is best conducted on hairs that have been screened using conventional microscopy and found not to be distinguishable from the target source.

Read more about mitochondrial DNA in the "Other DNA Markers & Technologies PDF file.

Urine

Urine contains a large amount of urea, a chemical byproduct of normal metabolic processes in the body. Identification of high levels of urea can therefore serve as a screening test for urine in fluids or stains. The presence of creatinine is also used for screening purposes. Creatinine forms a red compound with picric acid (known as the Jaffe Test). Urine also has a characteristic odor, which can help in locating its presence. Gentle heating of urine-stained materials gives rise to a distinctive odor.

Urine from secretors will contain ABH substances. This is a source of contamination in testing underclothing.

Author: Leigh Clark

Leigh Clark is a DNA Analyst with the Florida Department of Law Enforcement. Previously, Leigh was Academic Program Specialist at the National Forensic Science Technology Center, where she authored this module.

Author: Bill Tilstone

Bill Tilstone has a B.Sc. and Ph.D. from the University of Glasgow, Scotland. He was on the faculty of the Forensic Science program at the University of Strathclyde for 12 years before moving to Adelaide, Australia, to be Director of the State Forensic Science facility. He was appointed Executive Director of the newly created National Forensic Science Technology Center in 1996, and now serves NFSTC as Director of Instructional Technology and Education.

Thank you!

We appreciate you taking the time to contact us. If a response is necessary, we will attempt to do so within 48 hours.

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