One of the most important developments in the field of forensic science in the past few decades has been the implementation of DNA testing. With this testing, crime labs can match stains to individuals and test extremely small amounts of biological material to assist forensic investigations. Wisconsin DNA laboratories work to adhere to the quality standards put forth by the FBI in their Quality Assurance Standards for Forensic DNA Testing Laboratories that influence guidance provided below.

I. Introduction

DNA, which stands for deoxyribonucleic acid, is the genetic material (or “blueprint”) that determines the characteristics of all living organisms. It is what provides uniqueness among human beings. While the vast majority of DNA is the same in humans, enough variations exist to allow discrimination between individuals with the exception of identical twins. DNA is found in all nucleated cells (except red blood cells) and is the same throughout an individual’s body.

The DNA of a person does not change over the course of his life so that person’s DNA profile always remains the same. (The only exception would be a bone marrow transplant recipient.) Half of each person’s DNA is inherited from their mother and half from their father. This is why DNA can be used in paternity testing. DNA is also a very robust molecule and can yield results years after a stain is deposited.

There are many applications of DNA testing beyond the field of forensics. It has been used in paternity testing, plant research, identification of missing persons and unknown remains, tissue matching for transplants, and identification of remains after mass disasters. DNA testing was used extensively after 9/11 to identify victims.
There are three types of forensically interesting DNA: nuclear, Y chromosome, and mitochondrial.

**Nuclear DNA**
Nuclear DNA is found in the nucleus of cells and is the type of DNA testing generally utilized in crime laboratories. Nuclear DNA is unique to an individual with the exception of identical twins. The areas of DNA that are forensically significant provide no significant information about that individual other than sex.

**Y Chromosome DNA**
Y chromosome DNA is found only on the male sex (Y) chromosome and is therefore present only in males. This type of DNA is passed on from father to son through the *paternal* bloodline, so a man, his brothers, his father and his male sons will all have identical Y DNA (barring a mutation). Y DNA testing is very useful for samples where a high level of female DNA is mixed with a much lower amount of male DNA. If nuclear testing was used on such a sample, the female DNA may mask any other profile present; however with Y chromosome testing, the female DNA is in effect ignored, revealing only the male DNA. While not as discriminating as nuclear DNA, Y chromosome DNA may provide valuable information in a case.

**Mitochondrial DNA**
Mitochondrial DNA is not found in the nucleus of cells but rather in the mitochondria, the part of the cell where energy is produced. Like Y DNA, mitochondrial DNA is not unique to an individual and is passed through the *maternal* bloodline. Barring a mutation, all maternally-related family members will have identical mitochondrial DNA. As a result, forensic comparisons can be made using a reference sample from any maternal relative. This type of DNA testing is used primarily on hairs and bones, very old remains, and remains that are severely degraded such as after a mass disaster.

Mitochondrial DNA testing is not available at the Wisconsin State Crime Laboratory Bureau but is available at no charge.
from the FBI. A number of private laboratories also conduct mitochondrial DNA testing for a fee.

**STR Analysis (STRs)**
The type of nuclear and Y DNA testing currently in use in Crime Laboratory Bureau is called Short Tandem Repeat analysis (STRs). STR analysis looks at short pieces of DNA which are repeated a specific number of times at a particular location on the DNA molecule. Think of it as analogous to railroad cars: each person has two tracks, one from each parent. On one track an individual might have five repeats (or boxcars); on the other, six repeats. At that particular location, that person has a type of 5, 6. Other people in the population may be the same type at that location, but as more and more locations are typed, differences between individuals will be obvious. The Laboratory currently analyzes 23 different areas of DNA.

The advantages of STR DNA testing are many. Due to the sensitivity of this method, results can be obtained from extremely small and/or degraded samples. This method is extremely sensitive and uses very small amount of material. It is also highly discriminating among individuals.

There are also disadvantages to this type of testing as well. Because of its sensitivity, care must be taken to prevent the introduction of extraneous DNA at a crime scene or during collections. Mixtures of DNA from different people are also common, especially on touched items, which can make interpretation of the results difficult or impossible. In instances when an adequate amount of material cannot be obtained, a minimum amount of DNA is still necessary for testing.

Despite the revolutionary ways that DNA testing has changed forensic analysis, there are still some limitations to be aware of:
• casual contact does not generally transfer enough DNA for analysis,
• DNA can be removed by washing,
• DNA analysis cannot be performed in a few hours or
overnight,
• analysts need to be able to identify a stain on an item or know where DNA might be deposited on an item,
• DNA analysis cannot determine when a stain was deposited on an item, and
• environmental factors such as mold, heat, humidity, bacteria and sunlight can destroy DNA very quickly.

II. Collection of DNA Evidence

Commonly Collected
• Bloodstains – Can be on anything including clothing, bedding, weapons, objects, vehicles, and fingernail scrapings.
• Semen Stains – Can be found on clothing, bedding, swabs from victims or suspects (sexual assault kits), condoms, carpeting, upholstery, objects, and vehicles.
• Saliva Stains – Commonly found on cigarette butts, swabs from victims, bite marks, stamps, envelopes, bottles, cans, and clothing.
• Vaginal Secretions – Clothing from suspects, external penile swabbings, condoms, objects, and fingers.
• Tissue (Skin cells) – Fingernail scrapings, visible material from a vehicle or a weapon, tape or ligatures, intimate objects, eyeglasses, earrings, clothing (for wearer), guns or knives (grips/ handles).
• Hair Roots – Head hairs from intimate objects, pubic hairs from clothing/intimate objects/ pubic hair combings. NOTE: Must have cellular material attached for STR analysis.
• Perspiration – Hats, jackets, ski masks, bandannas, gloves, handled objects, weapons, eyeglasses, etc.

May Be Collected
• Urine – Large dried stains are generally not useful due to lack of cellular material present. May collect very small dried stains such as a droplet or two of urine by swabbing the stains.
• Feces – Lack of results in many cases due to large amount
of bacteria present in fecal material. Fecal material must be collected by swabbing the exterior surface. (Must be swabbed, see collection technique below.)

- Vomit – Contains acids and bacteria as well. Possible to get results or partial results. (Must be swabbed, see collection technique below.)
- Food – With a bite or bites out of it.
- Drug baggies – The Laboratory chooses a representative sample from submitted baggies for analysis.
- Fetal tissue – For criminal paternity only. Have tissue screened by a doctor or pathologist to obtain fetal (not maternal) tissue and cut a small piece for analysis.
- Cars – Can be considered a crime scene. Determine what question(s) need to be answered and transport to the laboratory in a manner that will preserve any evidence. Consider the length of time between an incident and the seizure of the car as well as environmental conditions.
- Touched items – Frequently result in uninterpretable mixtures. DNA works best on continually touched items (steering wheel of stolen car, hammer/slide of gun). Do NOT collect the following swabs from public places unless there is body fluid present or direct evidence that the object was touched or handled by the individual in question:
  - Floor swabs
  - Countertop swabs
  - Door knob/handle swabs

**Contamination Prevention During Collection**

The prevention of contamination is of vital importance in DNA testing at all stages of an investigation. The most important steps to always take are as follows:

1. Wear proper protective clothing:
   - Gloves
   - Mask/face shield
   - Glasses

2. **Do not handle any items without gloves. Change gloves after handling each item.**

3. Double glove if desired. May change only top gloves after
each item collected.
4. Avoid handling any item where the DNA may be deposited – you will wipe it off (even with gloves).

5. **Do NOT talk, cough or sneeze on or near DNA evidence.**

6. Put each item of evidence in new paper bags or envelopes – one item per bag/envelope. This includes swab boxes (except for multiple swabs from the same item or area of an item if multiple areas were swabbed, which may be packaged together).

7. **Do NOT** put your gloves in with the item of evidence. Dispose of them properly.

**Basic Collection Techniques**

There are three basic collection techniques for both stains and touched items:

1. Collect the entire item and package in paper or a cardboard box. This is not recommended for extremely large items, such as furniture or large pieces of rugs/carpets.

2. Cut out a piece of the item with the stain for items that can be cut, such as cloth, carpet and bedding.

3. Swab the stain(s) or area(s) of interest on item. Whenever able, the “two swab” method of collection is preferred.

   a. Proper swabbing technique for the “two swab” method is as follows:
      - Use a sterile or DNA-Free swab lightly moistened with DI water. Shake off any excess water away from the evidence.
      - Use the moistened swab to swab the targeted area gently but with enough pressure to collect the cellular material.
      - Follow the moistened swab with a sterile or DNA-Free dry swab to collect any cells remaining. This is especially important on items with hard surfaces (such as weapons, wood, etc.)
      - Thoroughly air dry both swabs and package together.

   b. For body fluid stains, two well-coated swabs are sufficient for analysis purposes.
      i. If the stain is dry collect at least two well-coated swabs
using the “two swab” method above.
ii. If the stain is still wet use at least two sterile or DNA-Free swabs and collect sufficient stain to coat the swabs well.

c. **Control swabs are not needed.**
d. For food, vomit, fecal material, gum and anything biodegradable:
   • Freeze item before swabbing
   • Swab bitten portion of food
   • Swab exterior of vomit and fecal material to collect only the surface cells

When a sample is collected from an object, whether cut or swabbed, document where the sample was obtained. This documentation may consist of notes, a diagram and/or a photograph detailing where the sample was taken.

![Diagram](image)

**Fig. 6-1** Above is an example of a diagram that documents where stains were collected from a door.
IV. Submission of DNA Evidence

Packaging
All submitted items should be **thoroughly** dry, always packaged in **paper** not plastic. Acceptable paper containers include clean bags, envelopes, and boxes. Each item should be packaged separately – one item per package (this includes swabs with the exception that all the swabs collected from the same stain or touched area should be packaged together [wet and dry together].) Each package should be properly sealed and marked. It is also vital that the item be sealed in appropriately sized packages. For example, a comforter should be placed in a large paper leaf bag. Once at the laboratory, the packages will be opened and resealed, requiring room to remove the item of evidence, return it to the package after analysis and reseal the package. If enough room is not provided in the original packaging for this process, the item will be repackaged at the laboratory. All layers of packaging should be labeled (one envelope inside another, swab box inside a bag, etc.).

Knives and other sharp items (such as broken glass, syringes, etc.) **should be protected so as not to penetrate the packaging.** Place these items in a sharps container or tie them down in a heavy cardboard box. Any holes or gaps in the box or other packaging must be covered with tape to prevent loss of or contaminating the evidence. For submitted weapons, wrap handles/touched areas separately from any body fluid stains on the weapon if DNA analysis of those areas is desired. This prevents cross-contamination.

Submission
Proper submission of evidence, including all relevant paperwork, is important at the Laboratory to process evidence in a timely fashion. The submitting agency should collect everything that may be of value in the investigation. If a large number of items are collected, do not submit everything to the laboratory at once – pick the most probative items to submit initially, keeping the remaining evidence available.
to be submitted later if necessary. Please see the Crime Laboratory DNA Unit evidence submission guidelines for further specifications on acceptable item submission. This document can be found on WILEnet (https://wilenet.org/).

All evidence packaging should be clearly marked with the agency number and exhibit number, the officer’s initials, and the date of collection. Documentation of evidence on the Laboratory transmittal forms should accurately reflect the contents of the package. The submitter should explain on the Laboratory transmittal how DNA may be of use for each item submitted. A detailed summary of the incident, including ALL types of contact in a sexual assault should accompany each case, as well as a list of all items collected whether or not they were all submitted to the laboratory. If you have any questions, please contact the DNA Analysis Unit at either laboratory:

(414) 382-7500 – Milwaukee | (608) 266-2031 – Madison

III. Storage of Evidence

Proper storage of evidence is vital in preserving any DNA present on the items submitted before submission to the Crime Laboratory. For short term storage, room temperature is fine for most items. Liquid samples (blood, urine, etc.) should be refrigerated; food, fecal material, fetal tissue and vomit should be frozen to prevent growth of bacteria and mold. The storage area should be a dry, cool environment (use a dehumidifier if necessary). DO NOT store these items packaged in plastic as it promotes growth of mold and bacteria which destroys DNA rapidly. Transport biological evidence to the Laboratory as soon as possible.

For long term storage (after analysis at the laboratory), the agency will need to properly store and preserve the evidence per state law. All DNA return packets need to be frozen in a FROST-FREE FREEZER sealed in plastic. Liquid samples (blood, urine, etc.) should be refrigerated or frozen. Food, fecal
material, fetal tissue and vomit should also be frozen to prevent growth of bacteria and mold.

VI. Standard Samples for DNA Analysis

The submission of standard and particularly elimination samples allow for comparison of DNA from known individuals to DNA from items of evidence. The comparison provides opportunity for potential individual inclusion or exclusion as contributor to a DNA profile.

**Standard Samples**

Standard samples are presumed to have been present at the scene in question and are free from contamination. Standard samples are recognized during investigation and subsequently collected as known standards. Primarily, the only acceptable types of known standards received for DNA analysis at the Laboratory are:

- Buccal swabs (preferred) – taken from the inner cheek
- Dried blood stains spotted on blood preservation cards from drawn liquid blood.
- Liquid blood samples drawn into an EDTA (purple-top) blood collection tube.

All layers of packaging for known standards need to be labeled at a minimum with the individual’s name and date of birth. This includes labeling each swab box and tube of blood. When using buccal swabs for suspect standard collection, a fingerprint from the individual may be placed on the swab label as well for additional identification.

**Elimination Samples**

An elimination sample is a particular form of a standard sample and is important in all types of DNA cases, especially with the dramatic increase in submission of touched items. In many cases, elimination samples are now **REQUIRED** for the Combined DNA Index System (CODIS) upload purposes (See Chapter 8 DNA Databank for more on CODIS).
Elimination samples are needed for the following individuals:

- prior consensual partners in sexual assault cases (contact of up to 120 hours prior to assault)
- owners of vehicles/homes/businesses and individuals with legitimate access to swabbed items (i.e., the regular driver of a stolen car or owner of a burgled home)

The reason to collect elimination samples is similar to the reason for collecting elimination fingerprints. The samples are used by the Laboratory for elimination purposes – to intentionally exclude samples due to legitimate access to the scene in question. These samples are not eligible for entry into the State DNA Database and are returned to the submitting agency after analysis is completed.

Prior to return of standard samples, they are entered into a quality assurance index at each respective DNA laboratory for the investigation of any possible contamination event. The quality assurance index represents all local DNA lab contact where standard and elimination sample profiles are maintained within the respective laboratory’s larger local index system for a limited time and are deleted in approximately 90 days.

**VII. Local DNA Index System (LDIS)**

The Local DNA Index System (LDIS) is a compilation of different database indices established by the local laboratory. As earlier mentioned, the quality assurance index is a part of LDIS where casework DNA profiles are also stored as forensic profiles to include mixture profiles, partial profiles, and single source unknown profiles. LDIS is maintained locally and administered locally for compliance and connectivity to larger database indices outside of the laboratory while providing the most comprehensive local storage of DNA profile data to assist forensic investigations. There is an LDIS laboratory in Madison and one in Milwaukee.