DNA, Semen, and Saliva

DNA

The following module discusses the properties of DNA, Semen, and saliva so that we can better understand their use in forensic science.

Historically, the Federal Bureau of Investigation is often considered the 'go-to' resource for forensic science information and knowledge. In their 1975 issue of "Handbook for Forensic Science," they briefly discuss serology and the fact that, "it is not possible to identify human blood as coming from a particular person. The race and sex of the person from whom blood came cannot be ascertained.

Of course, since 1975, the advances in DNA have been extraordinary. Following is an excerpt from the 2007 FBI Handbook, which is significantly different from a short 32 years prior.

"Deoxyribonucleic acid (DNA) is analyzed in body-fluid stains and other biological tissues recovered from items of evidence. The results of DNA testing on evidence samples are compared with the results of DNA analysis of reference samples collected from known individuals. Such analyses can associate victims and suspects with each other, with evidence items, or with a crime scene.

"There are two types of DNA used in forensic analyses. Nuclear DNA (nDNA) is the more discriminating of the two types and is typically analyzed in evidence containing blood, semen, saliva, body tissue, and hairs that have tissue at their root ends. The power of nDNA testing done by the DNA Analysis Unit I (DNAUI) lies in its ability to potentially identify an individual as being the source of the DNA obtained from an evidence item to a reasonable degree of scientific certainty, as well as the definitive power of exclusion. Additionally, where appropriate, the DNA-typing results from evidence items (including items related to missing persons) examined in the DNAUI may be uploaded into the Combined DNA Index System (CODIS) database."

FBI Handbook of Forensic Services, 2007

IN PLAIN LANGUAGE

What is DNA, what are the analysts doing with it, and how can we use it in our cases?

Intro to Forensic Science

What is DNA?

Dexoxyribonucleic acid (DNA) is molecular-level information. Other types of evidence can convey meaning based on their morphology or form, but this is unseen evidence that is the genetic fingerprint for every living creature. This molecule is present in the nucleus of cell and encodes the whole set of information needed for the development and the cycle of life for organisms. It's the instruction book for the cell's life.

DNA is found in many forensically important sources – bodily fluids and tissues, tooth pulp, residue from fingerprints, and so on. Anywhere nucleated cells are, we might find DNA.

To begin, please watch the following overview video of the forensic DNA process - https://www.youtube.com/watch?v=ZxWXCT9wVoI

Don't worry about knowing all the terminology yet, but this is just to give you an easy introduction into the topic.

Human DNA

Our DNA is a double helix of about 6 billion paired nucleotides, one set from each parent, forming that familiar twisted ladder. Made of a phosphate associated with a sugar, they are linked with one of four organic bases:

G/C		T/A	
Guanine (G)	Cytosine (C)	Thymine (T)	Adenine (A)

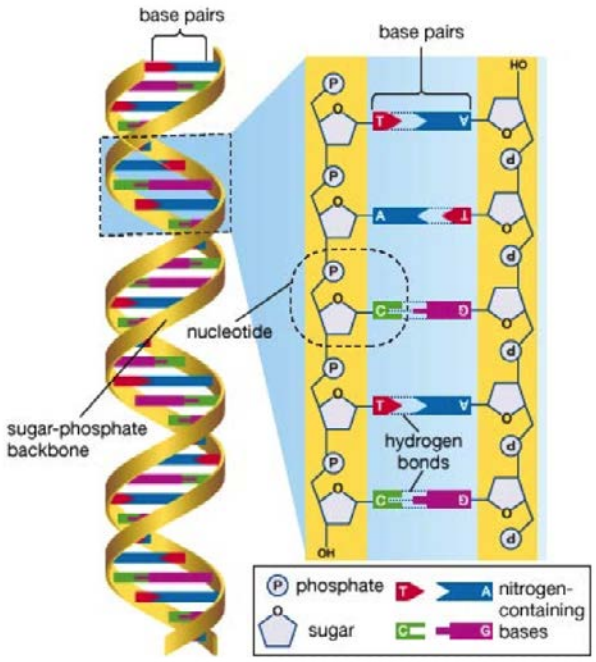
The G/C or T/A bases constitute the links between the two sides of the helix chain, or, the rungs on the ladder.

The material in the cells of biological evidence is so tiny, that it must be enhanced in order to essentially 'see' it well enough for analysis. Real-time Polymerase Chain Reaction (PCR) is used to amplify the DNA strands so that they can be used.

This was mentioned in the introductory video, and also please visits the National Human Genome Research Institute page at http://www.genome.gov/10000207 for a thorough, but very easily understood explanation of the PCR process.

Afterwards, watch this short video on PCR denaturing and annealing. https://www.youtube.com/watch?v=iQsu3Kz9NYo

The end results of the PCR are exact copies of the targeted areas, called "loci."

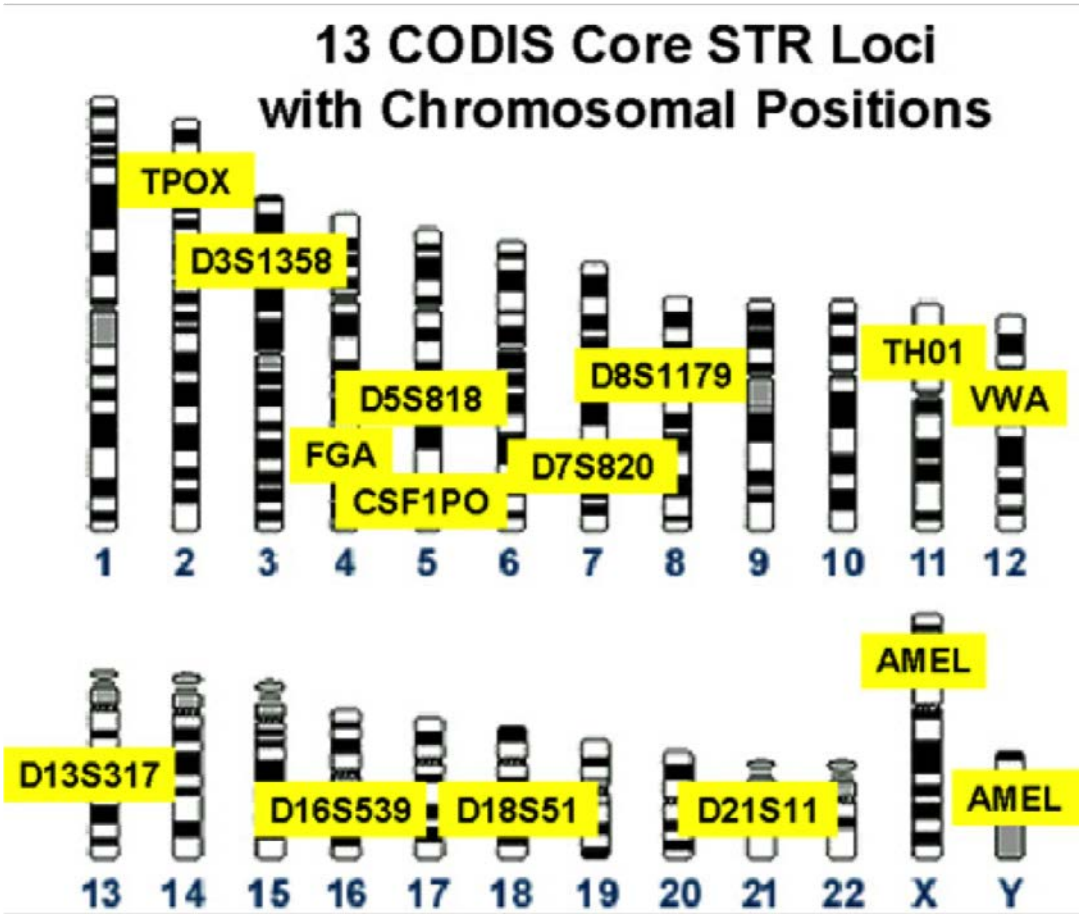


Intro to Forensic Science

What are loci?

These are noncoding locations along our genome that do not produce proteins. Chosen for ethical and privacy reasons, they create enough of a statistical difference that we can be mathematically excluded/included as the contributor of that genetic material.

Beginning in 1996, the FBI Laboratory launched a nationwide forensic science effort to establish core STR loci for inclusion within the national database known as CODIS (Combined DNA Index System). The 13 CODIS loci are CSF1PO, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 and D21S11. These loci are nationally and internationally recognized as the standard for human identification.



http://www.cstl.nist.gov/strbase/images/codis.jpg

Read this open letter from the Scientific Working Group on DNA on the subject of loci. http://swgdam.org/SWGDAM%20Abernathy%20Open%20Letter%20APPROVED%2001172013.pdf

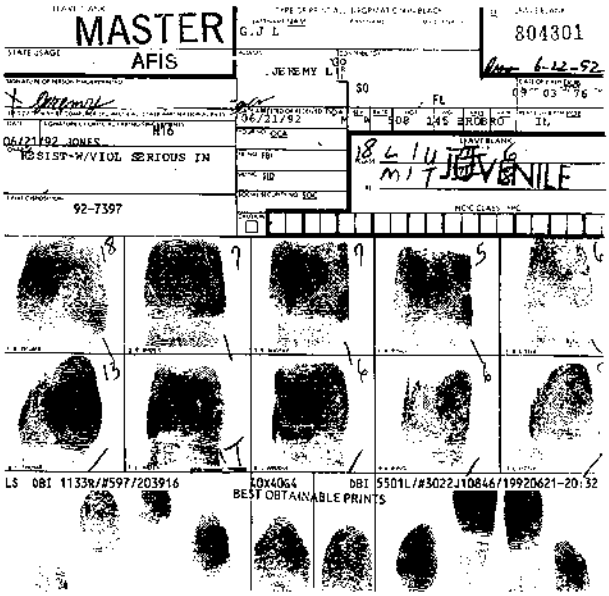
Do you think the SWGDAM successfully defended the use of the 13 loci?

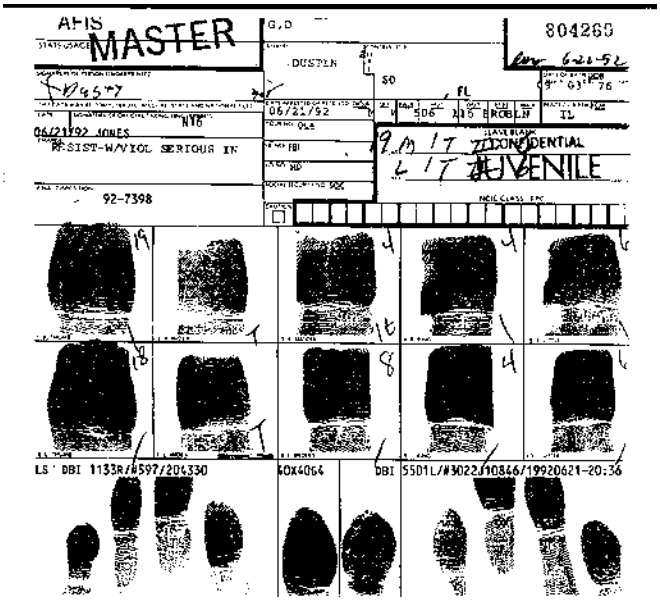
Intro to Forensic Science

What about identical twins?

The genetic profile of identical twins will be the same on the surface level, however researchers have been able to go deep into the genome to find differences – read more about it here. http://www.telegraph.co.uk/news/science/science-news/10511087/Identical-twins-need-never-be-tried-for-same-crime-after-DNA-breakthrough.html

Identical twins might even have nearly identical fingerprints





Gel Electrophoresis

Now that we have an understanding of the structure of DNA, DNA loci, and PCR we can better understand how it can be used for Identification purposes. Central to DNA identification is Gel Electrophoresis, which is a technique that separates

DNA segments based on size and produces a particular pattern unique to an individual.
The scientists at Simon Fraser University Museum of Archaeology and Ethnology in BC, Canada explain the process further:
"Another technique called gel electrophoresis separates the different DNA parts based on size. The sample is searched for special areas of DNA that repeat themselves. Although humans share over 99% of their DNA, these particular segments, called Short Tandem Repeats (STR), vary between individuals. Because a person inherits different genes from each parent, every individual has a particular set of STR markers and the chance of two unrelated people having the same pattern is very low. As a result, DNA 'profiles' can be used to assist in the identification process. For example, sex identification is an important part of generating a DNA profile. To determine sex from nDNA, analysts use the fact that females have two X chromosomes and males have one X and one Y chromosome to target genes that differ between males and females. Three common techniques in forensics focus on the SRY gene, the amelogenin gene and repetitive sequences on the Y-chromosome (Y-STR). The SRY gene is responsible for the development of a fetus into a male. As a result, its presence suggests a male individual, while its absence suggests a female. The amelogenin technique targets a

gene that is found on both the X and Y-chromosomes. However, the gene sequence is longer in males than females and once visualized, the length can be used to determine the sex of the individual. The Y-STR technique targets DNA on the nuclear genome specifically. This technique looks for short repeats of the Y-chromosome that are only present in males. Since males inherit this portion of their Y-chromosome from their fathers, this technique can be used to test paternal relationships."

Watch the complete process video at https://www.youtube.com/watch?v=ZxWXCT9wVoI

Potential Problems

At least 0.6 ng of DNA is necessary to produce a DNA profile of good quality, which means that around 100 cells are required. If such a small quantity of DNA is collected and later profiled, what does it mean to have found so low an amount of material?

CONSIDER THIS

Is the DNA found relevant to the case at hand?

Doesn't it pose a risk that someone unrelated to the case could have touched the item innocently?

Touch DNA

http://www.forensicmag.com/sites/forensicmag.com/files/legacy/u763/art81210_image1.jpg

Read the following and watch the accompanying video for the basic explanation of touch DNA and its uses in forensic investigations.

http://www.cleveland.com/metro/index.ssf/2014/10/touch_dna_police_harness_advan_1.html

http://videos.cleveland.com/plain-dealer/2014/10/60-second_know-it-all_touch_dn.html

Also read the following for excellent explanations of "touch" DNA: Minor 2013. Touch DNA:
http://www.forensicmag.com/articles/2013/04/touch-dna-crime-scene-crime-laboratory

Williamson 2011. Touh DNA:
http://www.acsr.org/wp-content/uploads/2012/01/Williamson.pdf

In the absence of nuclear DNA our last resort is Mitochondrial DNA (mtDNA). Found in the subcellular structure of human cells, the mitochondria are small energy factories found in each cell and have their own genetic material. The mtDNA is a hardy molecule that is sometimes the last biological vestige for molecular analysis. Unfortunately, it has a far weaker ability for individualization than with nuclear DNA for several reasons:

1. Inherited only from the mother during reproduction
2. Some nonrelated sequences repeat

"TOUCH" DNA AND THE INNOCENT

Think about a crime where an innocent person's DNA could end up at the scene.

Nuclear and mitochondrial DNA differ in important ways. While each cell contains only one copy of nuclear DNA, it may contain up to 10,000 copies of mitochondrial DNA. In addition, nuclear DNA is a combination both parents' genes, while mitochondrial DNA is inherited solely from the mother.

The FBI explains the use of mtDNA further, and how they use it – Mitochondrial DNA (mtDNA) is typically analyzed in evidence containing naturally shed hairs, hair fragments, bones, and teeth. Typically, these items contain low concentrations of degraded DNA, making them unsuitable for nDNA examinations. The high sensitivity of mtDNA analysis allows scientists to obtain information from old items of evidence associated with cold cases, samples from mass disasters, and small pieces of evidence containing little biological material.

Additionally, the maternal inheritance of mtDNA allows scientists to compare a mtDNA profile to reference samples from that person's mother, brother(s), sister(s), or any other maternally related individuals. All of these individuals have the same mtDNA profiles because all maternal relatives inherit their mtDNA from their mother. Because multiple individuals can have the same mtDNA type, unique identifications are not possible using mtDNA analysis. However, mtDNA performed by the DNA Analysis Unit II is an excellent technique to use for obtaining information when nDNA analysis is not feasible. Additionally, the mtDNA-typing results related to missing-person cases may be uploaded into the CODIS database."

Degradation of DNA

Degradation refers to the breakdown or destruction of cellular structures after death. Because DNA is contained in the cell, exposure of tissues to the environment, fire, water or chemicals will eventually lead to the physical breakdown of the DNA strands and the alteration of its chemical structure. These changes can lead to incorrect assignments of base pairs and the incorrect identification of a species or individual.

If a sample is very degraded, DNA analysts must be careful to ensure they are testing the right material. Importantly, samples must be collected and extracted without contaminating them. Because everyone has it, if a sample is mishandled, DNA that does not belong to the target sample (for example from a police officer) may be detected instead. This could result in a false profile.

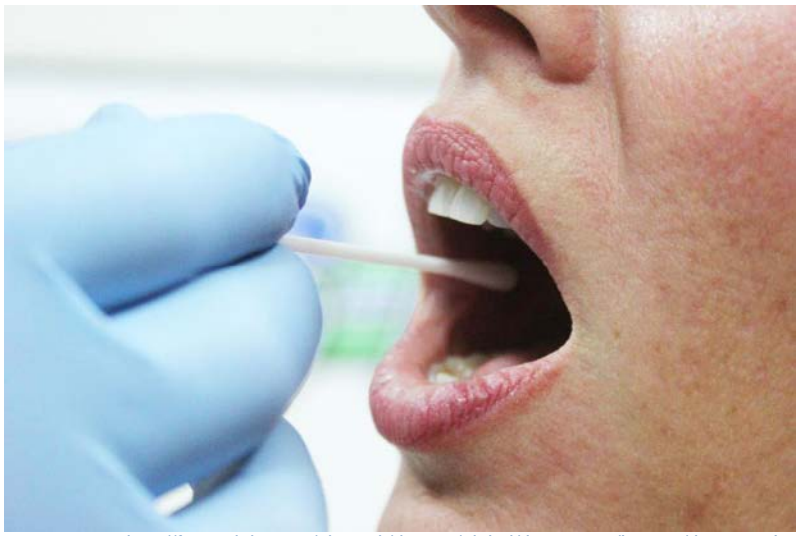
In addition, every analysis must be repeated more than once to control for random chemical changes. Overall, DNA testing facilities must be extremely clean and follow strict protocols to ensure that the results obtained are accurate.

Simon Fraser Universtiy Museum of Archaeology and Ethnology in BC, Canada

Intro to Forensic Science

Reference, or "Known" Samples

Just collecting and analyzing DNA from a crime scene is not enough, as we must have a source to link the information back to. Buccal (cheek cell) swabs from suspects, witnesses, victims, or other involved persons are an easy way to obtain a known sample. Blood draws are another option.



http://fastestlabs.com/php-oak/themes/global/documents/images/dna-tests.jpg

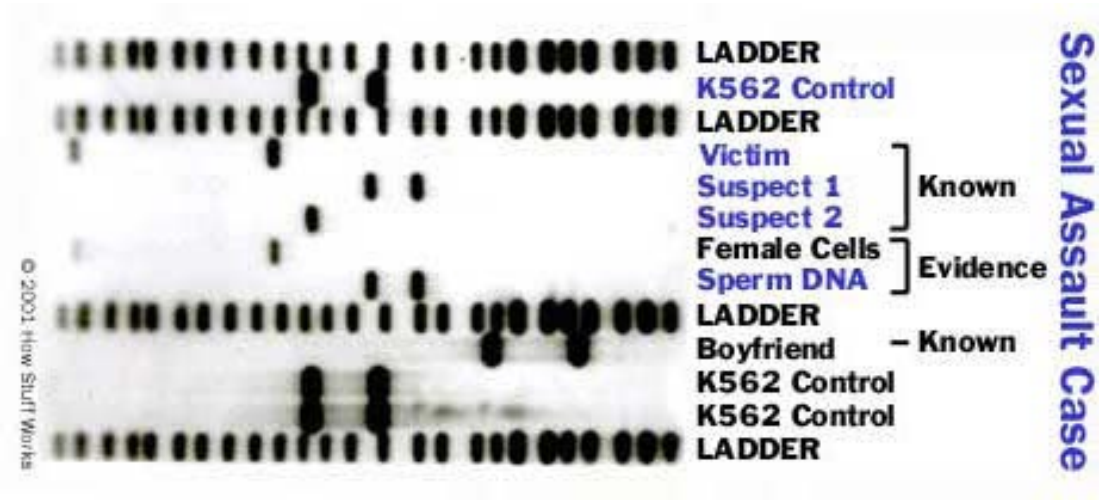
In missing person cases, what might you look for in order to get DNA from your victim? Examples may include: rooted hair from a brush, a toothbrush, bedding or clothing known to be worn by the victim, or swabs from any item linked closely to the victim, such as a cell phone/computer, vehicle, wallet or purse, a desk phone at their workplace, or tools in the garage. Think outside the box!

In the end we hope to find a link between the crime scene DNA evidence collected and the known samples in the case.

The DNA analysts generate detailed reports and statistical probabilities as to the linkages between the evidence. The information is added to the appropriate CODIS databases for reference as well.

Read more about CODIS (FAQs) here: http://www.fbi.gov/about-us/lab/biometric-analysis/codis/codis-and-ndis-fact-sheet

Intro to Forensic Science



http://s.hswstatic.com/gif/dna-evidence2.jpg

CODIS Indices:

"In its original form, CODIS consisted of two indices: the Forensic Index and the Convicted Offender Index. The Forensic Index contains evidentiary profiles developed from biological material such as semen, saliva, or blood found at crime scenes. The Convicted Offender Index contains profiles of individuals convicted of crimes specified by State laws. All 50 states have passed DNA legislation authorizing the collection of DNA profiles from certain convicted offenders for submission to CODIS. In recent years, CODIS has added new indices: the Arrestee Index, the Missing or Unidentified Persons Index, and the Missing Persons Reference Index. CODIS automatically searches across these indices for a potential match to aid criminal investigations of crimes from which unknown biological evidence has been recovered. It is important to note that if a hit is obtained from a convicted offender or arrestee sample, the hit is typically used as probable cause to obtain an additional DNA sample from that suspect so that the match can be confirmed by the crime laboratory before an arrest is made."

http://www.goccp.maryland.gov/dna/database.php

An extraordinarily unusual case in California is an example of the fears that some in law enforcement have concerning giving up their DNA - if their profile is found in a case, they may be considered as a suspect, even if contamination is a more likely culprit. In this particular murder, there may be evidence that the lab analyst was in fact the perpetrator, but the initial suspicions of DNA cross-contamination remain.

Please read the following news stories regarding the case, or check the Internet for more recent information.

http://www.nbcsandiego.com/news/local/Incriminating-DNA-Found-Inside-Cold-Case-Vicitm-Claire-Hough-Warrant-Kevin-Brown-Ronald-Tatro-281162272.html

http://www.utsandiego.com/news/2014/oct/31/warrant-brown-hough-murder-dna-photo/

http://www.kusi.com/story/27665318/dna-evidence-could-have-been-contaminated-in-cold-case

Looking Further

DNA lab personnel are required to give their own biological profiles in order to eliminate them from any instances of possible accidental contaminations.

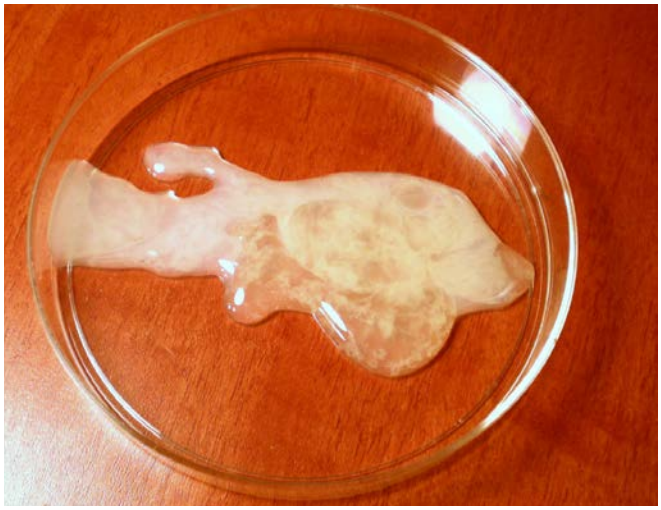
THINK ABOUT IT

Currently, most law enforcement agencies, even though they are asking their officers to collect DNA samples at crime scenes, are NOT asking them to be part of an elimination bank. Under what circumstances could this cause a problem when evidence is analyzed?

SEMEN

What is Semen?

Semen is a mixture of various fluids that carry live spermatozoa to the female ovule for fertilization. A fertile semen sample holds tens of millions of spermatozoa per milliliter and can provide useful DNA evidence. The volume of ejaculate is anywhere between 2-6 milliliters, with roughly 100-150 million spermatozoa in each mL. Because of illness, drug use, or injury, the sperm count may be lower.



The main constituents of semen:

- Solid components
  - Sperm
  - Miscellaneous solids, like skin cells
- Fluid component
  - Seminal plasma

The solid fraction is primarily the spermatozoa themselves, plus any fragments thereof and miscellaneous skin cells shed along the way. 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 (AP) and the protein prostate specific antigen, or p30 protein.

Exploitable semen for analysis can be detected up to:

- 31 hours in the mouth
- 44 hours in the anus
- 110 hours in the rectum
- 10 days in the vagina
- 19 days in the cervix

Read the Davies and Wilson article on "Persistence of Seminal Constituents in the Human Vagina".

Confirming Semen?

This is done through microscopy and stained identification of sperm cells. An article on three staining methods of semen is attached here for further reading. "Cytological Detection of Spermatozoa: Comparison of Three Staining Methods"

Vasectomized males require other tests targeting known components of semen. Obtaining DNA from aspermatic samples is possible, but less successful. On aspermatic men, a prostate specific protein, or p30, test may be used to confirm semen.

Common Semen Tests

The easiest and least intrusive to the evidence is the Alternate Light Source. UV light and the ALS detect seminal stains by phosphorescence and luminescence of the various materials in the semen.

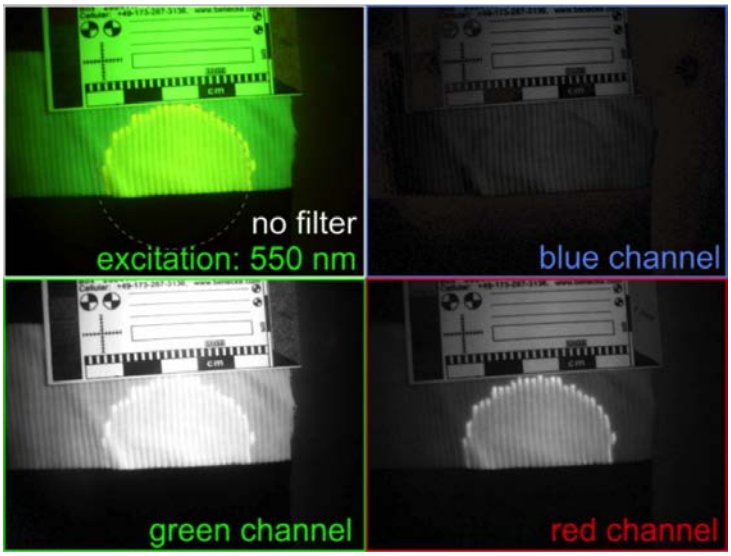
Also very easy is the presumptive test for the enzyme, acid phosphatase (AP). Many animal and vegetable tissues have AP, but it is a very high concentration in semen.
Both of these are extremely easy to use in the field or at the lab, and both are looking at fluid portion, not solids.

JUST IN CASE YOU ARE WONDERING

The AP tests are significantly less expensive than the p30 tests (roughly $4 each vs. $16 each). They are both good for screening, so if your state lab is going to confirm anyway, it may be more cost effective to do a simple AP test, even though there's some cross reactivity.

Alternate Light Source

As we mentioned in a previous module, the Alternate Light Source is our first step. It works on the principle that most items will have luminescent qualities at certain wavelengths of light, and a band pass filter allows only that wavelength to reach our eyes/the camera.



Using ALS

The alternate light source is the least destructive technique we have (other than plain white light). Use it to save yourself time and effort locating stains. Be sure you understand how it works and why it works before going to court. Remember, while we examine the items with ALS, we might mark it lightly with a sharpie, since once were in regular light, we may not be able to see the stain any longer.

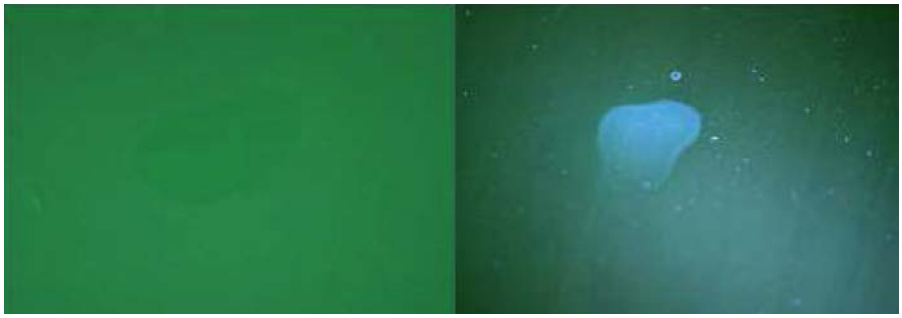
When using ALS, the flavins and bacteria in seminal fluid cause the glow. Drops and stains can have a distinctive ring at the outer perimeter, caused by the migration of the fluid out through the material or on the surface. Smears are still bright, but without the ring. Best in UV light or range just above.

Testing for Semen using Acid Phosphatase

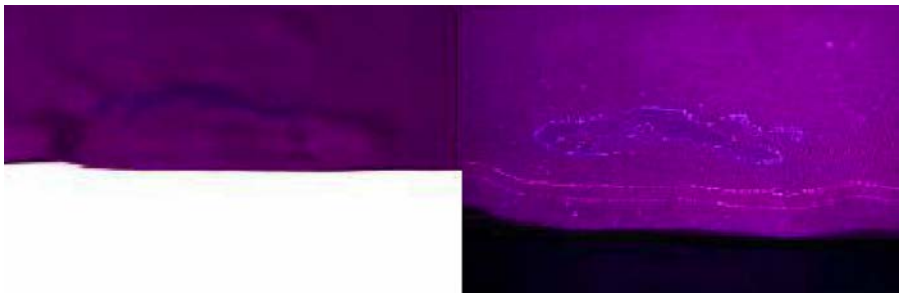
ALS examination is obviously the first step. However, all stains that fluoresce are not necessarily semen and not all semen stains will fluoresce. Acid Phosphatase testing is easy, fast, and inexpensive. If you have lab capabilities, you can actually mix your own from reagent powder. But for field-testing, there are several kits.

WHITE LIGHT UV LIGHT

BELT



PLASTIC



T-SHIRT

Intro to Forensic Science

The Chemistry Behind Acid Phosphatase
The AP spot test contains sodium-α-naphthyl phosphate and o-dianisidine (Brentamine Fast Blue). If acid phosphatase is present in a sample and a drop of the reagent is added, the enzyme catalyzes the sodium-α-naphthyl phosphate producing free naphthyl. That reacts with o-dianisidine producing a purple colored compound.

Please read the Laux article "Forensic Detection of Semen I. The Acid Phosphatase Test"

Are There False Positives?
Of course there are false positive. Feminine products, fecal stains, plants, pregnant women and prepubescent girls are all potential intereferences with the test. But remember, semen is a heterogeneous fluid and a single stain will contain various levels of acid phosphatase, P30, and sperm. Since AP can be found in lower levels in other bodily fluids, it's important to view the test immediately and report it accurately.

Time Frame for Testing
How long would you expect to find the following in the vagina after intercourse?

Acid Phosphatase is detectable for approximately 72 hours after intercourse and sperm can be found approximately 3 to 5 days depending on activity after the assault.

Testing Other Items
How long would you expect to find AP in a dried stain, such as clothing or bedding?

AP may survive for many years in a dried state; however, environmental conditions (heat, moisture, bacterial growth) could affect the survival rate.

Intro to Forensic Science

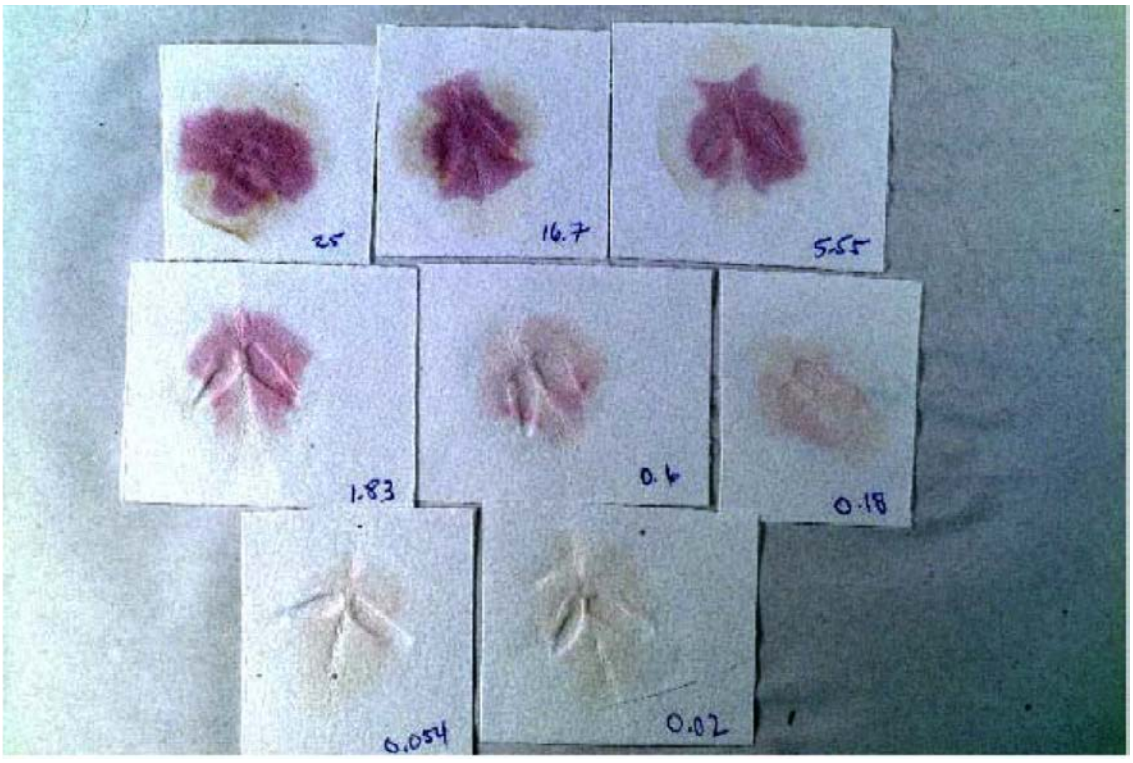
Reporting Your Result

"A swab of the unknown substance was tested with the AP spot test and gave a positive presumptive result, indicating the substance may be seminal fluid."	Or	"Analysis of _____ gave presumptive chemical indications for the presence of acid phosphatase, a component of semen."

In court, explain in a similar manner to the KM test – it's a color change based on the chemicals reacting to a substance in seminal fluid.

Watch the video demonstration of the AP field test in the module course page.

Perform your own tests using the wheat germ AP and the test kit.



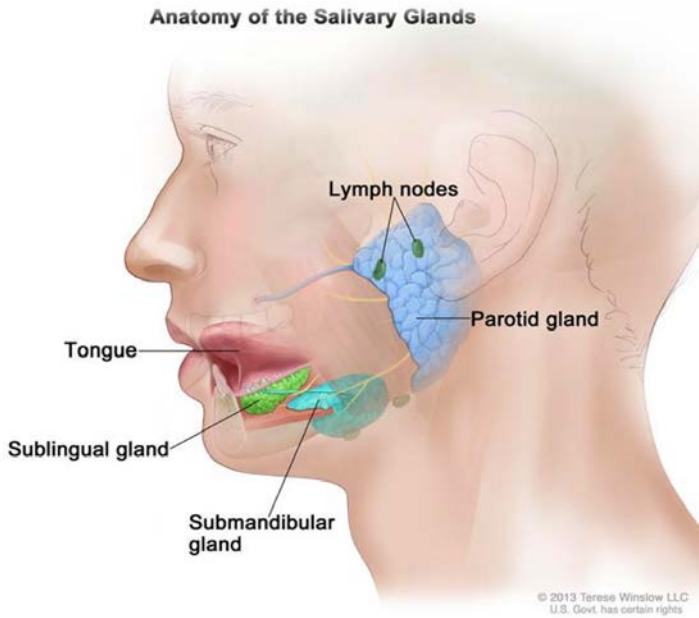
Several 2012 studies explored the possibility of letting the test reaction be read after the standard two-minute mark.

Please read the Redhead and Brown 2012 article "The acid phosphatase test two minute cut-off: An insufficient time to detect some semen stains."

SALIVA

What is saliva?

Saliva is a biological secretion inside the mouth that is primarily a digestive aid as the salivary amylases break down the starches in our food. It contains urea, glucose, progesterone, various traces of acids, amino acids, creatinine, and more than 1,000 different proteins. No specific test exists for its detection, although the presence of alpha-amylase strongly supports the identification of saliva.



Additionally, saliva is a carrier of cells for genotyping. Cells might be found on stamps, food, drinking glasses, cosmetics, pillows, bite marks on skin, and so forth.

Saliva is secreted from three sets of glands; the sublingual, submandibular, and parotid.

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.

What's in Your Spit?

Your spit contains mostly water, but bacteria, skin cells from the inside of the mouth (buccal cells), and the substance we test for – α-amylase – is also present. This enzyme helps break down carbohydrates, but can vary widely between people.

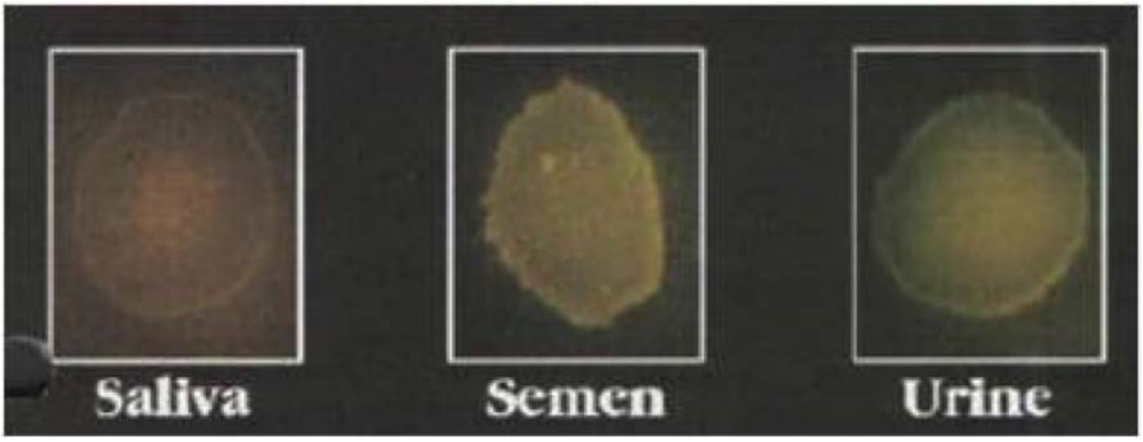
Studies have also suggested that vegetarians and people from cultures that consume a high carbohydrate diet may have higher levels of amylase than others. In a completely non-scientific local experiment, two CSIs who came from Central American and East Indian heritages and ate often of their cultures' foods (corn, beans, breads, rices, etc) had stronger reactions in testing than a Caucasian CSI who was avoiding starchy carbohydrates and eating primarily meats, nuts, and vegetables.

The Breslin 2013 article on "An Evolutionary Perspective on Food Review and Human Taste" begins a discussion of the possible evolutionary aspects of this on page 6.

Also found in other bodily fluids, so again, not a confirmatory test!

Saliva and the ALS

Saliva visually represents with soft edged white spots, sometimes less intense than other stains because of fewer solids.



Saliva under UV

WHITE LIGHT UV LIGHT

ROBBER MASK

PAPER

Testing for Saliva

We're going to look at a lab technique for the RSID-Saliva kit. Be careful when deciding to do this test though, since case information may indicate sending an item off for DNA analysis might be better.

The RSID Saliva test is specific for human salivary α-amylase.
No cross-reaction has been observed with blood, semen, urine, vaginal secretions, or menstrual blood.

RSID test is kind of like a pregnancy test. You're going to get two stripes for positive, or one for negative.

Saliva

50 µl of saliva extracted in 1 ml PBS

Extract 0µl 1µl 5µl 10µl 20µl

Lateral Flow Immunochromatographic Strip Tests for the Bdentification of body Fluids Using the RSID-Saliva Test

First specific, non-enzymatic tests for salivary amylase. No observed cross reaction with blood, urine, sweat, semen, domestic animals, exotic species, body fluid mixtures. Minor, but consistent signals seen with breast milk and fecal samples. Fast: Test results in 10 minutes post extraction. Sensitive: Detects as little as 50 ml of saliva – stated detection limit: 1 ml. No High Dose Hook effect observed: little or no dilution required, less possibility of false negatives. Efficient: Assay procedure integrated in DNA-STR analysis

RSID-Saliva should be evaluated exactly 10 minutes after the addition of sample.

A visible red line at the Control (C) position only, indicates a negative result. No α-amylase detected.

Visible red lines at both the Control (C) and Test (T) positions, indicate a positive result. A-amylase detected.

A visible red line at the Test (T) position only, indicates a failed test. Test failure, no conclusion possible.

No cross reactivity has been observed with saliva from the following animals and pets: dog, opossum, guinea pig, woodchuck, cow, domestic cat, domestic rabbit, tokay gecko, cuckoo, mongoose, chameleon, domestic pig, llama, sheep, horse, goat, grey gull, ferret, hedgehog, skunk, lion, tiger, rhinoceros, marsh snake, Sykes monkey, Capuchin monkey, tamarin, and marmoset.

A positive signal was obtained from the saliva of gorilla.

Read more about RSID here. Old et al. 2009. Developmental Validation od RSID-Saliva: A Lateral Flow Immunochromatographic Strip Test for the Forensic Detection of Saliva.

So next, we'll do a little demo. There is no hands-on or written component for this particular lab. Please just watch the associated video in the course page.

Fun with the RSID-Saliva kit
The lab version does require a pipetter with a low setting, however there is also a 'field friendly' kit.

So why show it here, because it can be done with just a little training and understanding of the process. And, since it's a bit more advanced than average, it can be intimidating. We're here to see it's actually not that scary.

CONCLUSION

This module has covered the properties of biological evidence including DNA, Semen, and Saliva and the detection of these substances.