Decoding DNA:
The Expert Explanation

Old, but not forgotten...

Forensic Serology in the Modern Laboratory

by: Suzanna R. Ryan, MS

If you read the headlines, you may have gotten the impression that DNA analysis is the only thing going on in the crime lab's biology section. However, forensic serology, or, in it's more recent incarnation, “biological evidence screening”, still plays a major role in forensic casework. The majority of the evidence items that go on to DNA testing are first examined for the presence of biological materials. The typical examinations that are performed by the forensic laboratory include screening for the presence of blood, semen, and saliva. In addition to these biological fluids, some labs screen for the presence of urine and/or feces and some will examine “touch” DNA evidence for the presence of nucleated, or DNA-bearing, cells.

Testing that is not routinely performed in forensic laboratories includes screening for the presence of vaginal fluid, sweat, and menstrual blood (as opposed to circulatory blood). In fact, for the most part, the tests simply do not exist in forensic laboratories at this time.

The following is a discussion of the common methodologies used in order to screen biological evidence and a brief glimpse into the future of forensic serology. In addition, false positives and improper interpretation of the test results will be covered.

Blood

It is important to understand that many of the tests performed by the lab are merely presumptive tests. These tests only indicate that a particular substance might be present. However, there are other known substances which can also yield a positive result. For example, the blood presumptive tests used by most forensic laboratories are quick color change tests that have multiple known false positives. Orthotolidine and Leuchomalachite Green for instance, will react in the presence of many plant peroxidases. Therefore it is important to read the results of a positive carefully. For example, a reddish stain located in the same area as a possible grass-stain on a pair of jeans could potentially yield a positive result even if the reddish stain is not actually blood.

Later testing may yield a DNA profile, but it is possible that the DNA profile is actually from skin cells and the presumptive positive blood test was reacting with plant material, not blood. In fact, unless a blood confirmatory test is performed, it is improper to state on a report that blood was identified even if a DNA profile is obtained from the stained area.

Confirmatory tests for blood are routinely available at most forensic laboratories. However, these tests do have cross-reactions with other higher primates and they have been shown to react with ferret blood as well. Also, the majority of forensic labs no longer perform species of origin testing. The older blood confirmation test (called the Ouchterlony test) was more difficult and time-consuming to prepare, use, and interpret and has been superseded in most, if not all, forensic labs by the much quicker and easier HemDirect or HemaTrace card assays. However, with the transition to the “easier” confirmatory tests, labs have lost the ability to determine if the blood detected is from an animal such as a dog, pig, or cow.

Saliva

The saliva tests commonly used in forensic laboratories are actually presumptive tests for the presence of amylase, which is merely a component of saliva. Amylase is also a component of a multitude of other body fluids at lower levels than generally found with saliva. The amylase tests (usually either the phadebas test or the starch-iodine radial diffusion test) will indicate if amylase is present and will also give a general indication for the quantity of amylase present. Typically a strongly positive amylase test is a good indicator for the presence of saliva. However, it is important to be aware of what sort of sample is yielding the positive result. For example, a positive amylase result originating from a stain in the crotch area of a pair of women’s underwear has the potential to be from sources other than saliva. It should be taken into consideration that vaginal secretions, urine, feces, and sweat (all possible contributors to the crotch area of
underwear) also contain amylase at varying levels and the positive presumptive amylase test might actually be detecting a combination of these body fluids and not saliva.

**Semen**

Forensic labs generally perform both presumptive and confirmatory tests on semen stains. The presumptive test, usually an AP, or Acid Phosphatase test, is a quick color change test that indicates that semen might be present. However, it is well known that false positives do exist with this test. In fact, one of the most common false positives is vaginal acid phosphatase (VAP). VAP is present in varying concentrations in the vaginal fluid and pregnant women are known to exhibit higher levels of VAP. In addition, if an AP test is not read at the appropriate time (typically within 2 to 3 minutes), a false positive may be observed simply due to the reaction of the chemicals used in the test with exposure to air.

It is important to note that neither an Alternate Light Source, nor a Woods lamp, is considered to be a presumptive test. These are simply tools that can be used to aid the investigator in locating possible semen-stained areas for further testing. Therefore it might be quite common for a forensic nurse examiner to locate and swab a “Woods lamp positive” area on the body of a rape victim, and yet the laboratory be unable to detect the presence of semen from this swab as it simply does not exist.

Confirmatory tests for the presence of semen include a microscopic examination and identification of spermatozoa as well as the P30 assay. The P30 assay is typically used only when a sample is AP positive, but no sperm can be identified under the microscope. P30 is a component of semen found in very high concentrations and it’s level in seminal fluid is unrelated to the amount of sperm present. Therefore, a P30 positive result can be obtained from an aspermic individual.

It is generally best to have visual confirmation of the presence of sperm cells via a microscopic exam. Additional information, such as the approximate number of sperm cells can also be gleaned at this time. Importantly, the number of sperm cells observed is often only noted in the analyst’s notes, and not in the actual serological report. Instead it is often reported that a sample is “positive for the presence of semen” even if only one or two sperm cells could be located. This can be somewhat misleading as the presence of a single sperm cell may mean something far different than the presence of a multitude of sperm cells.

Low levels of sperm can mean that an extended length of time between alleged assault and collection of the sample has taken place (especially in the case of body cavity samples), or it could mean that the sperm located are present due to transfer from an object. For example, a few sperm cells located on a pair of underwear may be present simply because the underwear was placed in the same laundry hamper with a semen-stained item or the underwear was washed in the same washer as a semen-stained item. Kafarowski, et al illustrated in their 1996 paper that sperm could be retained on an item of clothing even after washing and that sperm could transfer in a washing machine to previously unstained items. Additionally, Allard and Rankin report on a presentation given by Gerry Davidson of the Forensic Science Service which discussed the possibility of transfer of spermatozoa due to contact and/or laundering. Davidson reports that while he did not observe the transfer of sperm cells onto clean children’s underwear through storage in a laundry basket with semen-stained items alone, he did observe the presence of trace amounts of sperm cells on both the inside and the outside of each pair of underwear stored and washed with semen-stained items. In addition, trace amounts of spermatozoa were able to be collected from the door, drum and seal of the washer.

The possibility of transfer of sperm cells should be carefully considered, especially if the area where the sperm cells are collected is:

1. AP negative (AP is an enzyme that loses its reactivity when exposed to water. If an item has been washed, sperm cells may still be present, but it is unlikely that the AP test would yield a positive result.)
2. on the exterior, or some area other than the crotch, of underwear
3. exhibiting very low levels of sperm

**Future methods**

The future of forensic serology may lie with nucleic acids - but this time it is RNA, not DNA that plays the starring role. Researchers have been working on using bacteria for the identification of vaginal secretions as well as menstrual blood. A 2010 article by Fleming and Harbison discussed their use of rRNA for the identification of vaginal specific bacteria. They have created an 11-plex assay that can identify circulatory blood, menstrual blood, saliva, semen (in the presence and absence of spermatozoa) and vaginal secretions.
Other researchers (Sakurada, et al) are using real-time RT-PCR and ELISA assays to detect a component of sweat called Dermicidin (DCD). They found that the Real Time RT-PCR test could detect DCD in 7-day-old stains containing as little as 10ul of sweat and the test showed high specificity when testing stains containing 30ul of other body fluids. Their ELISA assay was able to detect sweat diluted up to 10,000-fold and could specifically detect DCD in 10ul of body-fluid stains.

A benefit of using Real-Time Reverse- Transcriptase PCR is that it allows for the identification of body fluids, and the co-extraction of both RNA and DNA. This ultimately means that little to no sample is wasted on identifying the body fluids present in the sample.

Unfortunately, these RNA testing methods are still in the research stages and have not yet made their way to the forensic laboratory.

In conclusion:

Biological screening results must be performed and interpreted correctly in order to be useful and have the maximum impact in a case. If presumptive testing is not performed on an item, it is not appropriate to “assume” that a substance is present. For example, if a sample from a drinking glass yields a DNA profile, it cannot be assumed that saliva was present as the DNA could just as easily come from skin cells transferred through the handling of the glass.

Additionally, it should be remembered that while confirmatory tests do exist for blood, semen, and saliva, they are not always used by the lab. It is important to carefully read the report to determine if a substance has been identified via a confirmatory test, or if it merely tested positive with a presumptive test.

Serological testing can be very important in corroborating or negating the victim’s version of events. Likewise, untested items can be useful in helping to substantiate an alternate version of events and should not be overlooked. In addition, it is important to be aware of the possibility of false positives and incorrect inferences regarding serological results.
DNA case? Consider hiring a Expert DNA Consultant

Ryan Forensic DNA Consulting

DNA cases are becoming more and more complex thanks in part to the multitude of “touch” DNA samples being collected and submitted to the laboratory. If you have a DNA case, you may want to consider hiring an expert to help explain exactly what the results do (and do not) mean as well as to confirm that the results obtained by the DNA laboratory are accurate and complete and that the conclusions drawn in the report are supported by the data.

Ryan Forensic DNA Consulting is willing and able to assist you in all aspects of DNA case review, attorney preparation, testimony review, and observation of DNA testing (which can be especially important if the lab will consume the entire item of evidence to complete it's testing). In addition, we can provide attorney training, either formal for MCLE credit, or informal. Topics for training range from the basic (“What is DNA?”) to much more advanced and can be tailored to meet your exact needs. Contact us today for more information!

ph. 949-973-7588
www.ryanforensicdna.com