

Body fluid identification



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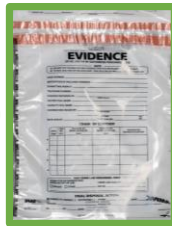
Division of
Forensic Medicine
& Toxicology



When an unknown stain is found at a crime scene or swabbed from a victim of a crime or an assailant, presumptive and confirmatory tests can be performed to confirm the presence of biological material. If the sample is biological, it may contain DNA that can be used to establish a DNA profile, or it may contain other useful information about the alleged crime.

Forensic serology involves the examination and analysis of a variety of body fluids which includes blood, saliva, semen and urine. How are body fluids identified? What is a presumptive and confirmatory test? Are there different tests for different fluids?

Identification of body fluids



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If a stain is found at a crime scene on a piece of evidence, and it is possible that is biological in nature, the first step is to swab this item to indicate the presence of biological material. If the stain is found on a piece of evidence that can be removed from the scene like a hammer, knife, blanket or t-shirt, the evidence is packed and sent to the laboratory. When a stain is found on a wall, floor or any other item that cannot be removed from the scene, the use of a sterile swab is sufficient for recovery of biological evidence.

While television shows often show biological fluid identification at a crime scene, this is very rare in real forensic cases. In most cases, the evidence, or a swab of the evidence is sent to the laboratory, where body fluid identification takes place.

Identification of body fluids

Presumptive testing

- Indicates **presence** of a biological substance

Confirmatory testing

- **Confirms type** of biological fluid present.



Semen?
Blood?

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There are two categories of tests used to identify body fluids: Presumptive tests and confirmatory tests. Presumptive tests are used to test for the presence of a biological substance. These answer the question, could this stain or piece of evidence contain any biological material. Presumptive tests are used to screen samples and have limited specificity, which means that they indicate the presence of certain substances that are not only present in biological fluids. It is for this reason that presumptive tests are only the first step in body fluid identification. The results from this test will indicate the potential for DNA, histological and biochemical analyses.

Confirmatory tests are more specific. These tests confirm the presence of specific biological material such as saliva, blood or semen. Confirmatory tests are not routinely used in all countries as these tests are expensive and time consuming. It is important to note that presumptive tests can only establish the possibility that a particular substance is present. Determining how the substance got there, who it came from need to be determined by other tests.

Visual examination

Red/Brown stain:

-Presumptive test for blood



Translucent/white/yellow stain:

-Presumptive test for semen



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The first step in body fluid identification involves visual examination of the evidence. If the stain or mark appears to be a red-brown colour, A presumptive test for blood will be performed. If a translucent stain or greyish white stain is observed on a piece of evidence, a presumptive test for semen may be performed. A fresh semen stain on non-absorbent material may be difficult to see as you can see in the images included here. Semen appears greyish-white, or yellowish-grey as it dries, and it is sometimes easier to see when it dried. So if a stain appears white, translucent, grey or yellow, a test for semen is likely to be used.

Presumptive tests for semen

The presumptive tests for semen detect specific proteins and enzymes present in the seminal fluid, contributed by the prostate gland

- Semen = Sperm + Seminal Fluid
- Seminal fluid: contains acid phosphatase, amino acids, fructose, prostate specific antigen (PSA)



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The presumptive tests for semen detect specific proteins or enzymes present in the seminal fluid, which is contributed by the prostate gland. To understand these tests, we need to understand that semen is made up of sperm and seminal fluid. The seminal fluid contains important proteins and enzymes like acid phosphatase, amino acids and prostate specific antigens or PSA.

Presumptive tests for semen

Acid Phosphatase Test



Short demonstration of
Acid Phosphatase test

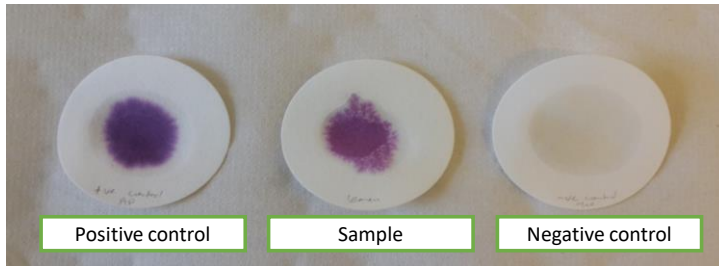


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But how do the presumptive tests for Semen work? The first test we will describe and the most commonly used is the acid phosphatase test. As mentioned before, seminal fluid contains an enzyme called acid phosphatase. When a molecule called alpha-naphthyl is added to semen, it reacts with the acid phosphatase present in the semen and undergoes a coupled reaction with a special dye called ortho-dianisidine. This reaction produces a purple colour. And if there is no acid phosphatase then there will be no colour change. If a colour change to purple is observed acid phosphatase is present. If the stain contains lots of acid phosphatase, then a positive result may be seen quickly. The weaker the stain, the longer the colour change will take.

Presumptive test for semen: Controls

Acid Phosphatase Test



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The acid phosphatase test is performed by pressing a piece of filter paper onto a section of the evidence containing the stain or a swab of evidence. Filter paper is first moistened with water and pressed onto the item. The previously mentioned chemical reagents and special dyes are added onto the filter paper and used to detect acid phosphatase. When a presumptive test for semen is performed, it is performed alongside a negative control and a positive control.

A positive control is done by performing the same presumptive test on a sample contain known to contain acid phosphatase. A negative control is performed on a substance known to have no acid phosphatase, like water for example.. If the negative control yields no colour change and the positive control yields a colour change to purple, then the result on the presumptive test on the evidence are valid. This means that a positive result is reliable in indicating the presence of acid phosphatase and a negative result is reliable in indicating its absence.

Presumptive test for semen: False positives

Acid Phosphatase Test

- AP present in most body fluids
- AP also present in vaginal secretions
- Tea, albumin, corn and onions



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A positive result for this presumptive test on semen can still occur when the suspect has no sperm in their semen. Why is this? Well, a presumptive test tests the presence of acid phosphatase, and this is present in ejaculations, even those that have low sperm counts. While semen contains 500-1000 times more acid-phosphatase than any other body fluid, if a presumptive test for semen yields a colour change to purple, this simply means that there is acid phosphatase present, but doesn't tell us if the source is Semen. This is because various things contain acid phosphatase for example vaginal fluid, fruit and vegetable, tea and mushrooms. It is for this reason that this test is considered presumptive for Semen and requires confirmation through microscopy or DNA analysis

Presumptive test for semen

Prostate Specific Antigen (PSA) Test

- Targets p30 which is present in semen ONLY.
 - p30: protein produced in prostate gland.
- p30 PSA Test:
 - Immunological and uses antibodies to detect P30
 - More specific than the AP test.

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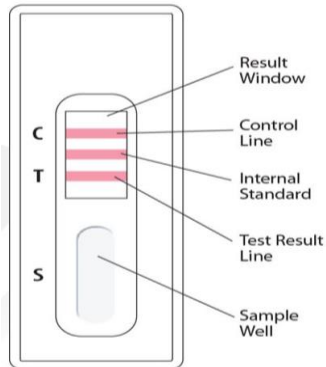


The PSA test targets a protein produced in the prostate gland called P30. P30 is specific to semen and no other body fluid. It is for this reason that the PSA test is more specific than the AP test. The PSA test is an immunological test and it uses antibodies to detect the P30 protein.

Presumptive test for semen

Prostate Specific Antigen (PSA) Test

Test Device



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This is what a PSA test looks like. The test is done using a device that forms a bands in the presence of P30 which is only found in semen. The presence of p30 in the sample reliably identifies semen regardless of whether acid phosphatase is elevated or sperm are detected. A positive test result would be indicated by three bands. Two under the "C" which indicate that the test is working as it should, and one band under the T. A band will only appear under the T if there is semen present in the sample.

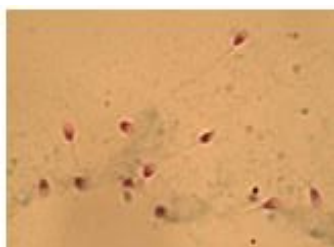
Confirmatory test for semen

Microscopy

H & E Stain



Christmas Tree Stain



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But how is the presence of semen confirmed? Confirmatory tests for semen are performed using microscopy. A section of the evidence thought to contain semen or a sample of semen is prepared on a glass slide and fixed with staining reagents. The Hemotoxylin and Eosin stain, more commonly known as the H and E stain, seen in the image on the left, is where the head of the sperm is stained pink and the tail is stained purple. For the Christmas tree stain seen in the image on the right, the head of the sperm cell is stained red and pink, while the tail is stained bright green, allowing for the visualisation of the sperm cell. If a sperm cell with a tail is observed, then it is confirmed that semen is present. Some laboratories conduct microscopic examinations on items that have negative acid phosphatase test results, based on the circumstances of the case and evaluation of the item of evidence.

Presumptive tests for blood

Luminol

Sodium Hydroxide (**Base**) + Hydrogen Peroxide (**Oxidizing agent**) + Luminol

Pros

- Visualise latent stains
- More sensitive than colour change tests



Cons

- Inhibits DNA analysis
- Short reaction
- Bleach: False positive

Short video demonstrating the Luminol test



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Now, I'm sure you have seen a similar image on a crime scene in an episode of CSI. This blue light seen in the image is due to a solution called Luminol. Luminol is used to visualise latent blood stains. These are stains that are not visible to the naked eye. The luminol reagent, hydrogen peroxide and sodium hydroxide are mixed and sprayed onto the area that is suspected to contain blood stains. The hydrogen peroxide reacts with a molecule called heme that is found in red blood cells. This reaction emits a light that is visible under a UV light. This reaction is short lived and is photographed immediately with reaction time noted. There are various materials which produce the same reaction when exposed to peroxides, this includes bleach, pus, saliva, mucus, milk, formalin and plant juices. It is important to know that if bleach is used on a crime scene, then the luminol solution could result in a false positive.

Presumptive tests for blood

Hemastix®



Short demonstration
of the Hemastix® test

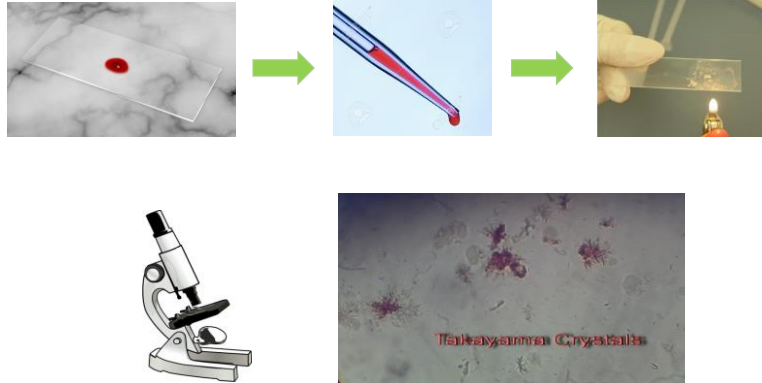


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Another common presumptive test is Hemastix strips. These strips are lined with reagents that react with the haemoglobin in blood. The colour change is from yellow to green and will darken as the concentration of the blood increases. A colour change should be read within 60 seconds and the colour change should be compared to the chart on the bottle as seen in the image. False positives for this test can include: oxidizing agents, vegetable peroxidases found in tomatoes and potatoes and certain cosmetics.

Confirmatory tests for blood

Takayama Test

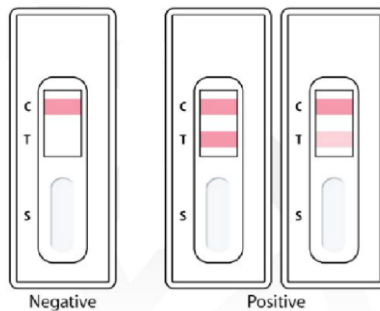


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The Takayama Test is a microscopy based confirmatory test for blood. The method works like this: a drop of the fluid that you think is blood is spotted onto a microscope slide. A chemical solution is then added to this drop which induces the formation of crystals. The slide is then heated so that the cells are fixed onto the slide. Mounting media are then applied to facilitate viewing under the microscope. If the characteristic crystals of blood are observed under microscopy then it is confirmed that blood is indeed present. This technique is not routinely used in many labs around the world.

Confirmatory tests for blood

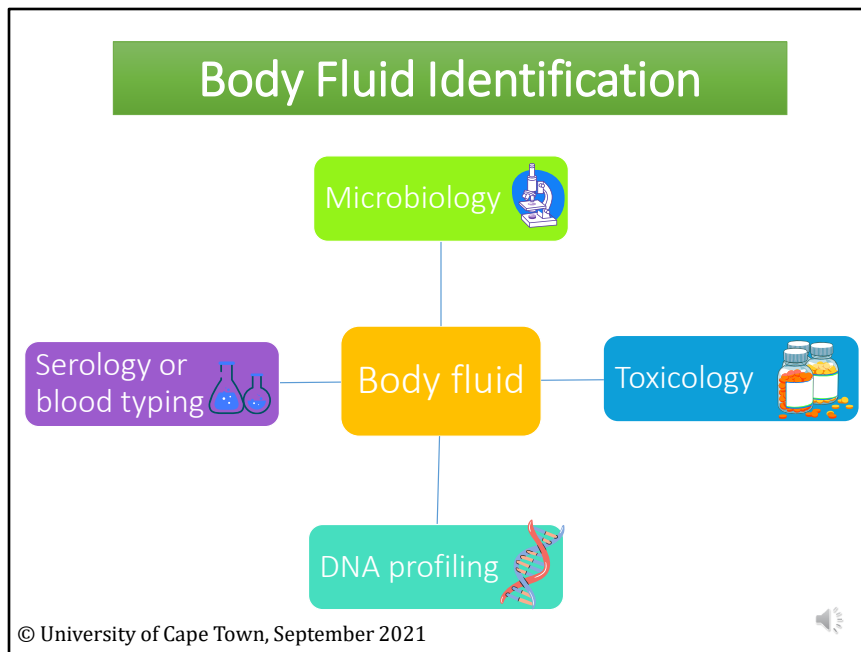
ABAcad® Hematrace® test



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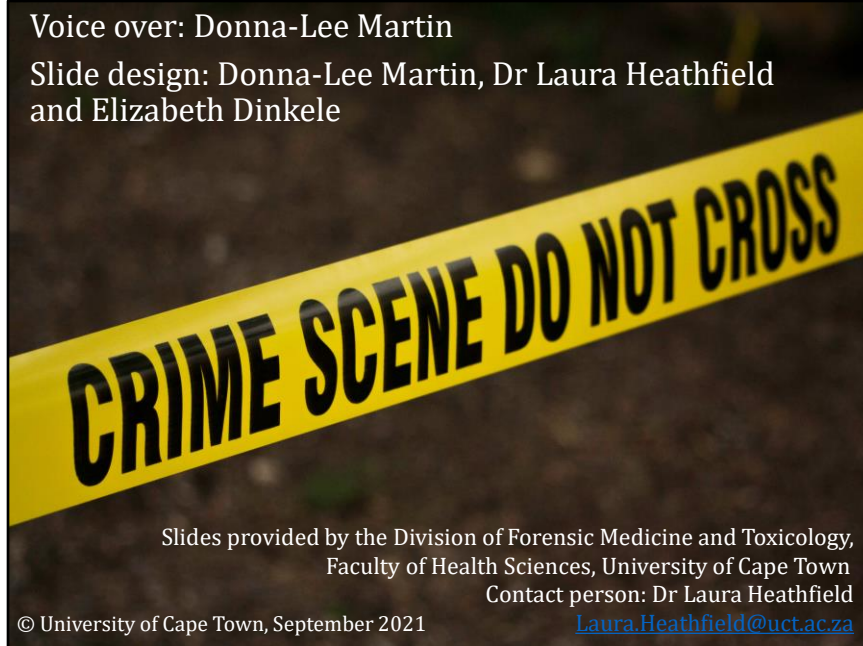
Rather the ABAcad Hematrace test is more commonly used. The image shows a positive and negative result. This immunological test works in a very similar way to the PSA test that has been described earlier in this lecture. It targets human hemoglobin and is therefore more specific than the presumptive tests for blood. However, false-positives are still possible, as the HemaTrace ABA card has been shown to yield a positive result from ferret blood.



Visual observation of an untested stain, coupled with positive chemical presumptive tests are considered sufficient for further confirmatory tests or chemical tests. There include: DNA profiling, biochemical analyses, blood typing or serology, and even toxicological analyses. More information on these downstream tests can be found in other lectures within the series. Thank you.

Voice over: Donna-Lee Martin

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