

**This document sets forth background materials on the scientific research supporting examinations as conducted by the forensic laboratories at the Department of Justice. It also includes a discussion of significant policy matters. This document is provided to assist a public review and comment process of the related Proposed Uniform Language for Testimony and Reports (posted separately). It is not intended to, does not, and may not be relied upon to create any rights, substantive or procedural, enforceable by law by any party in any matter, civil or criminal, nor does it place any limitation on otherwise lawful investigative and litigative prerogatives of the Department.**

**SUPPORTING DOCUMENTATION FOR DEPARTMENT OF JUSTICE  
PROPOSED UNIFORM LANGUAGE FOR TESTIMONY AND REPORTS  
FOR THE FORENSIC EXAMINATION OF SEROLOGY**

**Background**

Forensic serology describes testing procedures employed to detect and/or confirm the presence of body fluids on items of evidence. The Department currently performs serological tests for blood and semen only.

**A. Blood**

Blood is a connective tissue composed of both a liquid portion (plasma) and a cellular portion. Blood circulates through the heart, arteries, capillaries and veins. Blood is 6-8% of the total body weight of an individual and comprises about one third of the fifteen liters of extracellular body fluid in an adult man.<sup>1</sup> Blood has many functions to include maintaining fluid balance, maintaining body temperature, transporting oxygen, carbon dioxide, nutrients, waste, metabolites, and hormones, and providing defense against blood loss and invading pathogenic material such as viruses and bacteria.<sup>2</sup>

Plasma represents approximately 55% of the total blood volume.<sup>3</sup> Blood plasma differs from blood serum in that serum is the clinical term that refers to the liquid portion of blood, minus specific clotting factors, that remains in a liquid blood tube containing a blood clot.<sup>4</sup>

The cellular component of blood, which is suspended in the plasma, consists of erythrocytes (red blood cells), leukocytes (white blood cells), and thrombocytes (platelets) which collectively constitute approximately 45% of the blood volume in humans<sup>5</sup>. Red blood cells are the most

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<sup>1</sup> Rhoades & Pflanzner, *Human Physiology*, 578 (2d ed 1992).

<sup>2</sup> *Id.* at 578.

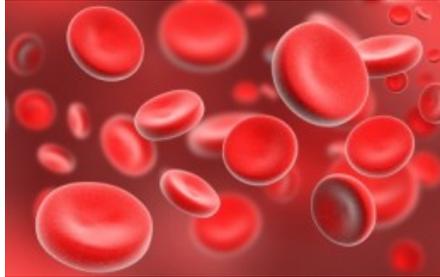
<sup>3</sup> Alberts et al., *Molecular Biology of the Cell*, 1164 (3d ed 1994).

<sup>4</sup> Rhoades & Pflanzner, *supra* note 1, at 580.

<sup>5</sup> Blood cellular components are also referred to as formed elements. *Dorland's Illustrated Medical Dictionary* (27<sup>th</sup> ed 1988).

abundant formed element in blood and number approximately 5 – 6 million per microliter<sup>6</sup> of whole blood. They lack a nucleus and any cellular organelles (see Figure 1).<sup>7</sup> The function of red blood cells is to transport oxygen and carbon dioxide using the protein hemoglobin. There are approximately 250 million molecules of hemoglobin per red blood cell.<sup>8</sup> It is the presence of hemoglobin, specifically the heme group, which most forensic chemical tests for blood are detecting.

Figure 1: Red Blood Cell  
*freedigitalphotos.net*



White blood cells are nucleated cells<sup>9</sup> that are present in blood and their primary function is immunological. Whole blood has approximately 5,000 – 10,000 white blood cells per microliter and they are larger than red blood cells.<sup>10</sup> Platelets are small detached cell fragments that adhere specifically to the cell lining of damaged blood vessels, where they help repair damage and initiate blood clotting.

## **B. Semen**

Semen is the male reproductive fluid which consists of both liquid (referred to as seminal plasma) and cellular (sperm cells, white blood cells and epithelial cells<sup>11</sup>) components.<sup>12</sup> The cellular component of semen is comprised largely of spermatozoa (sperm cells) that are present in concentrations ranging from 50 million to 150 million sperm cells per milliliter of semen, with an average value of approximately 100 million per milliliter.<sup>13</sup> However, sperm cell count varies

<sup>6</sup> Dean, L. *Blood Group and Red Cell Antigens*, (2005). See also A microliter is 1/1,000,000 of a Liter. *Dorland's Illustrated Medical Dictionary* (27<sup>th</sup> ed 1988).

<sup>7</sup> Image courtesy of dream designs at freedigitalphotos.net (last visited August 22, 2013)

<sup>8</sup> Rhoades & Pflanzner, *supra* note 1, at 587.

<sup>9</sup> A nucleated cell is a cell that contains a nucleus. Red blood cells are anucleated, meaning mature red blood cells do not contain a nucleus.

<sup>10</sup> Rhoades & Pflanzner, *supra* note 1, at 594.

<sup>11</sup> Epithelial cells are cells that line the bodies' cavities or flat surfaces and are the most abundant cell type in the body. *Dorland's Illustrated Medical Dictionary* (27<sup>th</sup> ed 1988).

<sup>12</sup> Saferstein, *Forensic Science Handbook volume 2*, 338-340 (2d ed 2005).

<sup>13</sup> Mann and Lutwak-Mann, *Male Reproductive Function and Semen*, 335 (1981). See also A milliliter is 1/1,000 of a liter. *Dorland's Illustrated Medical Dictionary* (27<sup>th</sup> ed 1988).

between men. Oligospermic men produce semen in which the sperm cell count is 20 million per milliliter or less (referred to as low sperm cell count), azoospermic men are unable to produce semen with sperm cells and aspermia refers to a condition where men are unable to produce semen.<sup>14</sup> Other conditions which result in low or no sperm cell count in semen can be transient (due to illness, nutrition, drug usage or other environmental factors) or permanent (such as a vasectomy,<sup>15</sup> an elective surgery which should result in permanent azoospermia).<sup>16</sup>

Human spermatozoa are approximately 50 micrometers<sup>17</sup> in length and consist of a head, midpiece, and tail. The sperm head is generally oval and flattened with dimensions of approximately 4.6 micrometers x 2.6 micrometers x 1.5 micrometers. Other mammalian species have sperm cells of comparable size, but they possess head shapes distinct from those of human sperm cells.<sup>18</sup> The anterior half of the head of the sperm is covered by a small, cap-like structure called the acrosome, which contains enzymes that aid the sperm in penetrating the female egg during fertilization. The midpiece of the sperm contains a spiral arrangement of mitochondria that generate the energy needed for the sperm cell to be mobile. The tail of the sperm cell moves back and forth to propel it forward.<sup>19</sup> The physical structure of sperm cells (in which nuclear DNA is located in the head of the sperm cell protected by an acrosomal cap) enables the forensic scientist to perform a specialized DNA extraction procedure that can separate sperm cell DNA from non-sperm cell DNA (such as DNA from epithelial cells) that may be present from female vaginal fluids.

Spermatozoa are produced by the testes during a process called spermatogenesis (Figure 2)<sup>20</sup>.

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<sup>14</sup> *Dorland's Illustrated Medical Dictionary* (27<sup>th</sup> ed 1988).

<sup>15</sup> Vasectomy should result in permanent azoospermia; however, approximately 1-2% of these procedures fail to prevent the passage of sperm cells through the reproductive tract. [Kaplan, K.A. and Huether, C.A., *A Clinical Study of Vasectomy Failure and Recanalization*, 113 *Journal of Urology*, 71 (1975) ] Successfully vasectomized men should have no motile sperm in their semen 3 months after the procedure, although immotile cells have been seen for up to 1 year [Mann, *supra* note 13, at 335] depending on the frequency of post-vasectomy ejaculation. Sperm cells may transiently reappear in the semen of successfully vasectomized men within several months post-surgery. [Marshall, S. and Lyons, R.P., *Transient Reappearance of Sperm after Vasectomy*, 219 *Journal of the American Medical Association*, 1753 (1972)].

<sup>16</sup> [www.mayoclinic.com/health/low-sperm-count/DS01049/DSECTION=causes](http://www.mayoclinic.com/health/low-sperm-count/DS01049/DSECTION=causes) (last visited May 24, 2016)

<sup>17</sup> A micrometer is 1/1,000,000 of a meter.

<sup>18</sup> Mann, *supra* note 13, at 63.

<sup>19</sup> Rhoades & Pflanzler, *supra* note 1, at 980.

<sup>20</sup> Image from Encyclopedia Britannica- spermatogenesis  
[www.britannica.com/EBchecked/topic/559418/spermatogenesis](http://www.britannica.com/EBchecked/topic/559418/spermatogenesis) (last visited May 24, 2016).

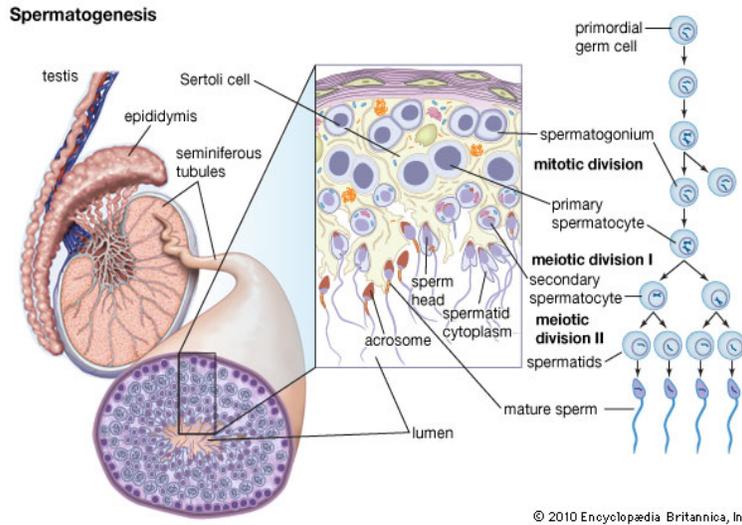


Figure 2:  
Spermatogenesis  
Occurring in the  
Seminiferous Tubules of  
the Testis

The glands of the male reproductive system (i.e., seminal vesicles, prostate, and Cowper's glands) contribute the components of seminal plasma to the ejaculate. The seminal vesicles secrete a viscous material rich in prostaglandins, fructose, flavins,<sup>21</sup> phosphorylcholine, MHS-5 protein,<sup>22</sup> and other substances. The prostate gland contributes approximately 30% of the total volume of semen.<sup>23</sup> The prostatic contributions to semen include, among others, acid phosphatase (AP) and prostate specific antigen (PSA, also known as p30). Several substances from the prostate gland can serve as biochemical markers to detect the presence of semen on forensic evidence. The Cowper's glands provide approximately 10% of the volume to seminal plasma and, during sexual arousal, a small amount of fluid from the Cowper's glands can appear at the tip of the penis before ejaculation. This fluid is termed the pre-ejaculate, which can also contain a small number of sperm cells.<sup>24</sup> The volume of ejaculate can range from approximately 2 milliliters to 6 milliliters with an average value of 3.5 milliliters.<sup>25</sup>

### Theory of Serological Examination

The use of chemical and/or biological tests as a means to identify body fluids rests on the premises that specific molecules biochemically react with specific compounds and that specific cells are only present in certain body fluids. The chemical and biological tests utilized in forensic serology have been used by scientists for decades and, in some instances, over a

<sup>21</sup> <http://www.ncbi.nlm.nih.gov/pubmed/16196041> (last visited May 24, 2016).

<sup>22</sup> Herr, J.C. et al., *Characterization of a Monoclonal Antibody to a Conserved Epitope on Human Seminal Vesicle-Specific Peptides: a Novel Probe/Marker System for Semen Identification*, 35 *Biology of Reproduction*, no. 3, 773-784 (1986).

<sup>23</sup> Huggins, C. et al., *Chemical Composition of Human Semen and the Secretion of the Prostate and Seminal Vesicles*, 136 *American Journal of Physiology*, 467-473 (1942).

<sup>24</sup> Rhoades & Pflanzler, *supra* note 1, at 983.

<sup>25</sup> Saferstein, *supra* note 12, at 335.

century<sup>26</sup>. The specific molecules and cells examined in serology are stable as dried stains, which are often encountered in forensic casework. The FBI Laboratory currently conducts serology tests for the presence of blood and semen only on items of evidence.

## **Serological Examination Process**

There are different methodologies and processes for conducting a serological examination. The Department shares information regarding some appropriate processes below. The Department does not suggest that the processes outlined here are the only valid or appropriate processes.

Detecting stains for serological testing and subsequent DNA testing on items of evidence is essential to the forensic examination process. Serological examinations may include a visual examination of the evidence which can be enhanced using techniques such as chemicals or alternate light source.<sup>27</sup> Two categories of testing can be performed to determine if a body fluid is present on forensic evidence: tests used for screening, “presumptive” tests, and tests used to identify a body fluid (blood or semen), “confirmatory” tests. Both types play an important role in the identification of body fluids.

### **A. Presumptive tests used to screen for the presence of body fluids**

A screening test is used to locate and preliminarily characterize biological stains. Screening tests are fast, use very little of a stain, and generally detect very small amounts of the body fluid of interest. Therefore, if a negative result is obtained using a screening test, then the biological fluid being tested is either not present or is below the test’s limit of detection. A known limitation of these tests is that other substances, besides the body fluid of interest, can also give positive test results. Therefore, it is important to conduct an appropriate identifying test, sample permitting, to confirm that any stains are the specific body fluid of interest.

### **B. Confirmatory tests used to identify body fluids**

The ability to identify a particular body fluid on an item of evidence allows an examiner to state that a specific body fluid (blood or semen) has been detected. These tests are used to achieve the level of specificity needed to identify blood or semen to the exclusion of all other substances. However, these tests generally are not as sensitive as the screening tests. This

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<sup>26</sup> M. Takayama, *A method for identifying blood by hemochromogen crystallization*, 306 Kokka Igakkai Zasshi, 463 (1912). See also J. H. Kastle and O.M. Shedd, *Phenolphthalin as a Reagent for the Oxidizing Ferments*, 26 American Chemical Journal, no. 6, 526-539 (1901); F. S. Baechtel, J. Brown and L. Terrell, *Presumptive Screening of Suspected Semen Stains In Situ using Cotton Swabs and Bromochloroindolyl Phosphate Acid Phosphatase activity*, 32 J. Forensic Sciences, no. 4, 880-887 (1987); M. N. Hochmeister et al., *Evaluation of Prostate Specific Antigen (PSA) Membrane Test Assays for the Identification of Seminal Fluid*, 44 J. Forensic Sciences, 1057-1060 (1999).

<sup>27</sup> Saferstein, *Forensic Science Handbook volume 1*, 527 (2d ed 2005).

limitation is widely recognized<sup>28</sup> by the scientific community and is taken into consideration when the forensic examiner decides if further testing, or characterization, is warranted.<sup>29</sup>

The process of confirming that a body fluid is present on evidence can either be done using a single identifying test or by coupling multiple screening tests. It is a scientifically acceptable practice to use two screening techniques that are based on different chemical principles to confirm the presence of a body fluid if the limitations of one test are not subject to the same limitations as the other.<sup>30</sup>

### C. Methods of Detecting Blood

- **The Kastle Meyer Test**

The most common techniques utilized to detect blood involve biochemical tests that detect the presence of the iron containing heme group of hemoglobin. This category of test is referred to as a catalytic test.<sup>31</sup> The basis for this test is that hemoglobin exhibits peroxidase-like activity. This means that it can catalyze the oxidation of a number of organic compounds by peroxide to yield either colored or chemiluminescent products.<sup>32</sup>

The Kastle Meyer test, which uses phenolphthalin, is the screening test that is used by the FBI Laboratory.<sup>33</sup> This test is generally represented by equation 1<sup>34</sup>



The  $[AH_2]$  represents the reduced chemical reagent (e.g., reduced form of phenolphthalin),  $[ROOH]$  represents the oxidizing chemical reagent (e.g., typically hydrogen peroxide). When these two reactive chemicals are applied to a stain containing blood, the heme component of blood catalyzes (accelerates) the reaction to oxidize phenolphthalin (detected by a color change) and create a molecule of water.

To perform a phenolphthalein test, a small portion of the evidence stain is collected by touching a moistened cotton-tipped swab to the stain. A few drops of the phenolphthalin reagent

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<sup>28</sup> Kirk, P., *Blood: General Testing*, 189 (2d ed 1953). See also Thomas, J.C., *The Examination of Blood and Seminal Stains*, J. Police, 490-503 (1937).

<sup>29</sup> Because of the potential for a false negative result with serological confirmatory tests, a Forensic examiner may select a negative stain to go on for DNA testing.

<sup>30</sup> *SOFT/AAFS Forensic Toxicology Laboratory Guidelines*, 2006 version, §8.2.

<sup>31</sup> Gaensslen, R., *NIJ Sourcebook in Forensic Serology, Immunology, and Biochemistry*, 113 (1983).

<sup>32</sup> *Id.* at 101.

<sup>33</sup> DNA Casework Unit Serology Manual, SOP 106, Rev. 6 (04/24/13), p.1.

<sup>34</sup> Gaensslen, *supra* note 31, at 101.

(a mixture of reduced phenolphthalin and alcohol, at an alkaline pH<sup>35</sup>) are added to the swab. After the addition of the reagent to the swab, the swab is observed to determine if a color change occurs. If no color change occurs, then the second reagent (a diluted hydrogen peroxide solution) is added. If blood is present, a pink color change will occur. This color change must be observed within a prescribed time period to be considered a positive result. If the color change occurs after the prescribed time period, either no blood is present, or the quantity is limited and the color change could be the result of the phenolphthalin being oxidized without the aid of a catalyst (i.e., heme).<sup>36</sup>

A two step phenolphthalein test is utilized. Using a two step approach, an examiner is able to determine if a chemical oxidant is present in the sample if a color change occurs before the second reagent (typically hydrogen peroxide) is added.<sup>37</sup> The presence of a color change before all of the chemicals are added is a false-positive reaction and such a result is reported as inconclusive.

It has been noted that the use of the common catalytic tests (i.e., phenolphthalin) provide considerable proof that blood is present and "...that few materials other than blood will react to [these] reagents and positive test [results] given by the [common] reagents, particularly the alkaline reagents, leave little doubt that hemoglobin is present."<sup>38</sup> That said, phenolphthalein has been shown to give false positive results with fresh green bean, potato, horseradish and legume root nodules.<sup>39</sup>

- **Hemochromogen Crystal Test**

Crystal tests for blood are conducted by removing a small portion of a suspected bloodstain, placing it on a microscope slide, adding certain chemical reagents and applying heat. Characteristic crystals that are visible microscopically will form if blood is present in the sample. The presence of these crystals identifies the stain as blood.<sup>40</sup>

The Hemochromogen Crystal test, also known as the Takayama test, has been used to identify blood since its development in 1912 and is the method currently used by the

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<sup>35</sup> "Phenolphthalin oxidation occurs more rapidly in an alkaline solution"; Kastle, J.H. and Shedd, O.M., *supra* note 26.

<sup>36</sup> Ponce and Pascual, *Critical Revision of Presumptive Tests for Bloodstains*, 1 Forensic Science Communications, no. 2 (July 1999). See also FBI, DNA Casework Unit Serology Manual, SOP 106, Rev. 6 (04/24/13).

<sup>37</sup> Blake, E.T. and Dillon, D.J., *Microorganisms and the Presumptive Tests for Blood*, 1 Journal of Police Science and Administration, 395-400 (1973).

<sup>38</sup> Kirk, *supra* note 28, at 184.

<sup>39</sup> Cox, Milton, *A Study of the Sensitivity and Specificity of Four Presumptive Tests for Blood*, 36 J. Forensic Sciences, no. 5, 1503-1511 (Sept. 1991). See also Petersen, Daniel and Kovacs, Frank, *Phenolphthalein False-Positive Reactions from Legume Root Nodules*, 59 J. Forensic Sciences, no. 2 (March 2014).

<sup>40</sup> Saferstein, *supra* note 27, at 535. See also Gaensslen, *supra* note 31, at 79; Beam, W. and Freak, G.A., *A greatly improved haemin test for blood, with notes on some recently proposed methods*, 9 Biochemical Journal, 161-170 (1915); DNA Casework Unit Serology Manual, SOP 107, Rev. 5 (04/23/13).

Department.<sup>41</sup> The Takayama reagent includes sodium hydroxide which denatures the hemoglobin protein and separates the heme group from the rest of the protein. A reducing sugar (glucose) present in the reagent reduces the iron in heme so that it is free to bind to pyridine (also present in the reagent). When the heme binds to pyridine, it forms characteristic pink, rhomboid shaped crystals that are visible microscopically (see Figure 3).<sup>42</sup> Bloodstains that are aged or have been exposed to harsh environmental conditions are more difficult to resolubilize, hindering the ability of heme to form crystals.<sup>43</sup>



Figure 3: Takayama Crystals

The Takayama test and other crystal tests<sup>44</sup> are similar in sensitivity and specificity for blood detection; however, the Takayama test is used within the Department because it is easier to perform and is not inhibited by wood or leather materials.<sup>45</sup> Crystal tests can conclusively identify the presence of blood- “when crystals are formed under the proper testing conditions, there is no doubt of the nature of the stain tested” and “specificity...was checked by reacting it with a variety of materials, including chemicals, plant extracts...we have found no false positives.”<sup>46</sup> Because crystal tests are far less sensitive than blood screening tests it is well established that the failure to obtain a positive result from a confirmatory test does not necessarily indicate the absence of blood.<sup>47</sup> The discrimination power of the Takayama test is such that if it is positive, blood is conclusively present. However, a negative result can mean either that blood is not present or that blood is present but is of a quality or quantity below the threshold necessary for the test to function.

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<sup>41</sup> Saferstein, *supra* note 27, at 534. See also Lee, H.C., *Identification and Grouping of Bloodstains*, Forensic Science Handbook (edited by R. Saferstein 1982) 271-283; DNA Casework Unit Serology Manual, SOP 107, Rev.5 (04/24/13), p.1.

<sup>42</sup> Image generated by the FBI and from FBI DNA Casework Unit Serology Manual, SOP 107, Rev. 5 (04/24/13).

<sup>43</sup> Gaensslen, *supra* note 31, at 87.

<sup>44</sup> Such as the Teichmann Crystal test.

<sup>45</sup> Kirk, *supra* note 28, at 189.

<sup>46</sup> Kirk, *supra* note 28, at 189. See also Hatch, Alan, *A Modified Reagent for the Confirmation of Blood*, 38 J. Forensic Sciences, no. 6, 1502-1506 (November 1993).

<sup>47</sup> Gaensslen, *supra* note 31, at 87.

## D. Methods of Detecting Semen

A visual examination of the evidence is the first step in the serological testing process for the presence of semen. Semen stains have a white to yellow cast in normal light and can be heavy and crusted.<sup>48</sup> However, if a low quantity of semen is present, or if the material is patterned or dark, semen stains may be difficult to visualize in normal light. In these instances, an alternate light source (ALS) may be employed to screen the evidence for the presence of semen. Semen stains fluoresce<sup>49</sup> at wavelengths in the 450 to 530 nanometers range.<sup>50</sup> Other body fluids (such as saliva, urine or sweat) will also fluoresce, but generally not as brightly.<sup>51</sup> The contrast afforded by the alternate light source allows the forensic scientist to focus on stained areas that can be further tested for the presence of semen.

After visually examining the evidence, there are three tests utilized by the Department to aid in the identification of semen: biochemical tests, antigenic tests and biological tests.

- **Biochemical Tests**

Seminal acid phosphatase (AP) is present in extremely high quantities in semen and is secreted by the prostate during ejaculation.<sup>52</sup> Seminal AP concentration is low in prepubescent males but maximum levels are obtained by sexual maturity. The presence or concentration of seminal AP has no relation to the presence or absence of spermatozoa.<sup>53</sup> AP is a glycoprotein of approximately 100 kDa<sup>54</sup> that is composed of two approximately equal subunits.<sup>55</sup> AP is an enzyme, which means that it catalyzes, or accelerates, a chemical reaction. In semen, AP helps break down the phosphorylcholine secreted by the seminal vesicles to provide energy for sperm cell motility. This catalytic functionality is used by the presumptive biochemical tests used to detect semen.

The AP test currently used in the Department is the One-Step Acid Phosphatase Test manufactured by Serological Research Institute (SERI). The test is performed by collecting a portion of the evidence stain on a moistened swab, and then applying the reagent to the swab. This liquid reagent contains alpha naphthyl acid phosphate, which is broken down in the presence of AP. The resulting free naphthyl group reacts with o-dianisidine, also contained in the reagent, and forms a pink to purple colored compound. The swab is observed for a color change.

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<sup>48</sup> Saferstein, *supra* note 12, at 330.

<sup>49</sup> Flavins contained in semen result in fluorescence under alternate wavelengths of light. <http://www.ncbi.nlm.nih.gov/pubmed/16196041> (last visited 9/11/13).

<sup>50</sup> A nanometer is 1/1,000,000,000 of a meter. *See also* Saferstein, *supra* note 12, at 331.

<sup>51</sup> Saferstein, *supra* note 27, at 538.

<sup>52</sup> Saferstein, *supra* note 12, at 331.

<sup>53</sup> Saferstein, *supra* note 27, at 536.

<sup>54</sup> A kiloDalton (kDa) is a measurement of protein weight.

<sup>55</sup> Saferstein, *supra* note 12, at 332.

If a color change occurs within a prescribed time period, the test is positive, however, if not the test is negative and indicates that AP was not detected.<sup>56</sup>

The AP test is considered to be a screening test because AP is present in other body fluids.<sup>57</sup> Though the concentration of AP in semen is 20-400<sup>58</sup> times the concentration in other body fluids, there is still the possibility for other substances to give a positive result (i.e. false positive). Validation studies conducted within the Department have shown that the One-Step AP Test is specific to seminal AP and will not react with erythrocytic AP found in blood or vaginal AP.

- **Antigenic Tests**

Antigenic tests to detect the presence of semen have been in use since the 1970s.<sup>59</sup> The seminal protein Prostate Specific Antigen (PSA), also referred to as p30, has been widely used in the forensic community to detect the presence of semen on evidence.<sup>60</sup> Prostate Specific Antigen is a glycoprotein of approximately 30kDa consisting of a single subunit.<sup>61</sup> The concentration of p30 in semen is high (average of 1300 micrograms/milliliter<sup>62</sup> with a range of levels between 390 to 3000 micrograms/milliliter);<sup>63</sup> however, other body fluids such as male urine, peripheral male blood, rectal secretions, and breast milk have been reported to have low levels of p30.<sup>64</sup> The levels of p30 present in semen are approximately 1000 times more concentrated than the levels of p30 in male urine.<sup>65</sup>

The p30 test used in the Department is a commercially available rapid antigen-antibody immunoassay called the Seratec® p30 Semiquant card. This immunoassay card includes antibodies to human p30 embedded in the membrane. Liquid extract from an evidence stain is added to the card, and, if p30 is present in the extract, it will bind the mobile anti-human-anti-p30 antibody. This complex will then bind to a different epitope of the p30 protein. When this binding occurs, the antigen-antibody complex is captured and will accumulate in a specific area

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<sup>56</sup> DNA Casework Unit Serology Manual, SOP 117, Rev. 0 (10/02/12).

<sup>57</sup> Saferstein, *supra* note 27, at 537.

<sup>58</sup> Stefanidou, M. et al., *Fundamental Issues in Forensic Semen Detection*, 59 West Indian Medical Journal, no. 3, 280-283 (2010).

<sup>59</sup> Sensabaugh, G.F. et al., *Isolation and Characterization of a Semen-Specific Protein from Human Seminal Plasma: A Potential New Marker for Semen Identification*, 23 J. Forensic Sciences, 106-115 (1978).

<sup>60</sup> Saferstein, *supra* note 27, at 539.

<sup>61</sup> Sensabaugh, *supra* note 59, at 110.

<sup>62</sup> Graves, H.C.B, G.F. Sensabaugh, et al., *Postcoital Detection of a Male-Specific Semen Protein: Application to the Investigation of Rape*, 312 The New England Journal of Medicine, 338-343 (1985).

<sup>63</sup> Sensabaugh, *supra* note 59, at 110. *See also* Wang, T. et al., *PSA Concentrations in Seminal Plasma*, 44 Clinical Chemistry, no. 4, 894a-896 (1998).

<sup>64</sup> Saferstein, *supra* note 27, at 539. *See also* P30 concentration in male urine is reported to be average of 260 nanograms/milliliter of urine. Graves, H.C.B, *supra* note 62, at 338.

<sup>65</sup> Average p30 concentration in semen is 1300 micrograms/milliliter compared to 0.260 micrograms/milliliter in urine; therefore approximately 1000 times higher concentration in semen.

on the card. This accumulation of p30 complex creates a pink line on the card. The Seratec® p30 Semiquant card has an additional control area that can provide a level of quantitative information regarding the amount of p30 protein detected.<sup>66</sup>

The sensitivity of the commercial p30 immunoassay cards used by the Department is approximately 0.25 nanograms/milliliter.<sup>67</sup> The procedure used within the Department utilizes a specific size cutting from the evidence and a specific volume of buffer to extract the sample. The extract is then further diluted. This deliberately desensitizes the p30 immunoassay to the level where a non-specific reaction with male urine is unlikely.

Because the AP test and p30 tests detect different seminal plasma components and are based on different chemical tests (with different limitations), a positive AP test together with a positive p30 test is used to identify the presence of semen.<sup>68</sup>

- **Biological Tests**

Scientists recognized in the 19<sup>th</sup> century that sperm cells are only present in semen.<sup>69</sup> Therefore, the identification of a sperm cell confirms the presence of semen. Identification of sperm cells is possible using microscopic visualization. Sperm cell visualization can be enhanced by using a differential cell staining technique, such as Kernechtrot-picroindigocarmine (also referred to as Christmas Tree stain).<sup>70</sup>

The procedure used to differentially stain sperm cells in the Department uses the Christmas Tree stain. Liquid extract from an evidence stain is deposited onto a microscope slide. A nuclear fast red stain is added to the slide. After a water wash to remove the excess red stain, the green stain (picroindigocarmine) is added to the microscope slide. After an ethanol wash to remove the excess green stain, the microscope slide can be viewed under a microscope at the appropriate magnification. Sperm cells will be differentially stained, with the sperm heads being stained red and the tails being stained green. Epithelial cells typically appear blue/green with large red/purple nuclei.<sup>71</sup>

Observation of sperm on an item of evidence confirms that semen is present, even in the absence of a positive screening test result.

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<sup>66</sup> DNA Casework Unit Serology Manual, SOP 105, Rev. 5 (10/02/12). *See also* Kearsey, J. et al., *Validation Study of the OneStep ABACard® PSA Test Kit for RCMP Casework*, 34, Canadian Society Forensic Science Journal, 63-72 (2001).

<sup>67</sup> DNA Casework Unit Serology Manual, SOP 105, Rev. 5, §7.2.1 (10/02/12).

<sup>68</sup> *SOFT/AAFS Forensic Toxicology Laboratory Guidelines*, 2006 version, §8.2.

<sup>69</sup> Gaensslen, *supra* note 31, at 149.

<sup>70</sup> Saferstein, *supra* note 27, at 540.

<sup>71</sup> DNA Casework Unit Serology Manual, SOP 112, Rev. 1 (09/10/14). *See also* Leubitz, S. et al., *Sensitivity of Picroindigocarmine/Nuclear Fast Red (PIC/NF) Stain for Detection of Spermatozoa: A Serial Dilution Study of Human Ejaculate*, 81 American Journal of Clinical Pathology, 90-93 (1984).

## E. Persistence of Semen

Frequently Forensic examiners are asked how long semen can persist on an item. This is dependent upon the particular seminal fluid constituent (AP, P30, or sperm cells) and the environmental conditions to which it has been exposed. Studies have shown that AP activity can persist for at least 3 years in dried semen stains under certain conditions.<sup>72</sup> P30 can be easily detected in semen stains for up to 1 year,<sup>73</sup> and has routinely been detected from dried stains decades after semen was deposited.<sup>74</sup>

The survival time of the various components of semen in the vagina and other body orifices is often important in the context of forensic casework. Detectable levels of AP have been reported in postcoital vaginal samples collected several hours to several days after the encounter, with most estimates ranging from 16 – 72 hours.<sup>75</sup> Studies have been performed that report p30 can be detected in postcoital vaginal samples for up to 1-2 days.<sup>76</sup> The times are generally longer for samples collected post mortem. AP has been detected post-mortem in vaginal samples for up to 7 days, in the oral cavity for up to 36 hours, and in the rectum up to 24 hours.<sup>77</sup>

There are many factors which can affect sperm cell survival time. Shorter survival times have been reported if the concentration of sperm cells in the ejaculate is low.<sup>78</sup> Factors such as the timing of the female's menstrual cycle and pregnancy can also affect sperm cell survival.<sup>79</sup> There have been reports that sperm cells may last longer in the vaginal cavity of a pre-pubescent female than a menstruating female.<sup>80</sup> It is known that sperm cells last longer in the cervix than in the vagina and there is information regarding the survival times in other bodily orifices (such as mouth and rectum). A summary of this information is provided in Table 1.

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<sup>72</sup> Kaye, S., *The acid phosphatase test for seminal stains. A study of the reliability of aged stains*, 41 *Journal of Criminal Law and Criminology*, 834-835 (1951).

<sup>73</sup> Sensabaugh, *supra* note 59, at 113; It was noted that the anti-p30 antisera was tested with negative results to stomach contents, bile, cow's milk, cat semen, chimpanzee semen, egg yolk, egg white, along with other house hold items.

<sup>74</sup> Spear, T. and Khoskebari, N., *Analysis of Old Biological Samples: A Study on the Feasibility of Obtaining Body Fluid Identification and DNA Typing Results*, presented at the Spring 2002 meeting of the California Association of Criminalists

<sup>75</sup> Saferstein, *supra* note 27, at 542. See also McCloskey, K.L. et al, *Prostatic Acid Phosphatase Activity in the Postcoital Vagina*, 20 *J. Forensic Sciences*, 630-638 (1975).

<sup>76</sup> Saferstein, *supra* note 27, at 542

<sup>77</sup> Standeffer, J.C. and Street, E.W., *Postmortem stability of prostatic acid phosphatase* 22 *J. Forensic Sciences*, 165-172 (1977). See also Schiff, A.F., *Reliability of Acid Phosphatase Test for Identification of Seminal Fluid*, 23 *J. Forensic Sciences*, 833-844 (1978).

<sup>78</sup> Gaensslen, *supra* note 31, at 153 .

<sup>79</sup> Morrison, A.I., *Persistence of Spermatozoa in the Vagina and Cervix*, 48 *British Journal of Venereal Diseases*, 141-143 (1972).

<sup>80</sup> Gaensslen, *supra* note 31, at 153.

Table 1: Sperm Cell Survival Times<sup>81</sup>

Body Orifice	Length of Time
Vagina: motile	Up to 6-8 hours
Vagina: non-motile	Up to 3-4 days
Cervix: motile	Up to 6 days
Cervix: non-motile	Up to 17 days
Mouth	Up to 8–12 hours
Rectum	Up to 24 hours

Generally seminal fluid survivability is less than that of sperm cells<sup>82</sup> due to their protein nature (i.e., proteins will be broken down more quickly than sperm cells). It has also been reported that sperm cell survival time is longer in body orifices postmortem.<sup>83</sup> The environmental conditions of the body will have a major impact in determining survival times.<sup>84</sup> Differences in sensitivity of AP, p30, and sperm cell detection assays will result in variations on reported survivability of the constituents of seminal fluid. It is also known that seminal fluid drainage can occur, resulting in the transfer of semen from one location to another (e.g., drainage from the vaginal area to the rectal area or drainage from vagina to the panties). This seminal fluid drainage can result in the loss of detection.<sup>85</sup> Because of this, as well as the limits of detection of the tests used, a negative result means either semen is not present or semen is present but is of a quality or quantity below the threshold necessary for the test to function.

## Policy Considerations

Over time, a number of factors have contributed to policy considerations regarding serological testing within the Department. These factors include, but are not limited to, the discovery of DNA, accreditation of the Laboratory and reports generated by the National Academy of Sciences (NAS)/ National Research Council (NRC)<sup>86</sup>.

<sup>81</sup> Gaensslen, *supra* note 31, at 153. See also Enos, W.F and Beyer J.C, *Spermatozoa in the Anal Canal and Rectum and in the Oral Cavity of Female Rape Victims*, 23 J. Forensic Sciences, 231-233 (1978); Menger, R., *Microscopic Investigation of Supposed Seminal Spot on a Child's Clothing*, 4 Texas Cour.-Rec. Med., no. 6, 250-255 (1887); Sharpe, N., *The Significance of Spermatozoa in Victims of Sexual Offense*, 89 Canadian Medical Association Journal, 513-514 (1963).

<sup>82</sup> University of North Texas Health Science Center website.

<sup>83</sup> Wilson, E.F., *Sperm's Morphological Survival After 16 days in the Vagina of a Dead Body*, 19 J. Forensic Sciences, 561-564 (1974).

<sup>84</sup> Willott, J.R., *The Role of the Forensic Biologist in Cases of Sexual Assault*, 15 J. Forensic Sciences, 269-276 (1975).

<sup>85</sup> Davies, A. and Wilson, E., *The Persistence of Seminal Constituents in the Human Vagina*, 3 Forensic Science, 45-55 (1974).

<sup>86</sup> National Research Council, Committee on DNA Technology in Forensic Science, *DNA Technology in Forensic Science*, National Academy of Sciences Press, (1992), available at [www.nap.edu/catalog/1866.html](http://www.nap.edu/catalog/1866.html). See also National Research Council, Committee on DNA Forensic Science: An Update, *The Evaluation of Forensic DNA Evidence*, National Academy of Sciences Press, (1996), available at <http://www.nap.edu/catalog/5141.html> and National Research Council, Committee on Identifying the Needs of the

## **A. Discovery of DNA and the Effect on Serological Testing**

The advent of DNA testing, with its superior ability to distinguish between individuals, eliminated the need to differentiate between individuals using serological methods in investigative analysis. Historically, blood group testing, such as ABO blood group typing, was utilized to differentiate bloodstains. While ABO blood group typing could not differentiate a bloodstain to a specific person (the rarest blood group is type AB and is found in approximately 4% of the human population in the United States<sup>87</sup>), it was useful for excluding individuals. Because of the discriminatory power of DNA testing, forensic laboratories no longer perform historic ABO or other blood grouping tests. However, serology – now used to describe the identification of biological fluids (e.g. blood and semen) -- remains a vital sub-discipline in forensic biology. Serology testing today allows for the identification of specific stains which have the best chance of yielding a DNA profile and also allows the forensic scientist to select an optimal DNA extraction method. Serology and DNA are used in tandem to obtain the most information from items of evidence.

## **B. A Framework for Safeguarding Against Error in Serological Testing**

Errors can occur in any laboratory setting. One way to safeguard against errors is by the use of reagent controls. For each serological examination performed, there is a positive control and a negative control.<sup>88</sup> These controls are performed in forensic casework, in parallel with the serological examination being performed, or prior to that specific lot of chemical reagents' use in casework. These precautions are taken to ensure that the reagents give accurate, reproducible and reliable results. If the controls do not produce the expected results, then the reagent is not approved for use on casework and another lot must undergo the same quality assurance testing procedures before being approved for use.

The Scientific Working Group on DNA Analysis Methods (SWGDM) is composed of approximately 50 scientists from federal, state, and local forensic DNA laboratories in the United States and Canada. The mission of SWGDAM includes discussing and evaluating forensic biology methods and research to further forensic biological services. SWGDAM published guidelines for serological testing<sup>89</sup> in January 2015. This set of guidelines is specific to serological testing and addresses validation, training, proficiency testing and analytical procedures. The Department follows the guidelines issued by SWGDAM.

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Forensic Sciences Community, *Strengthening Forensic Science in the United States: A Path Forward*, (2009), available at <http://www.nap.edu/catalog/12589.html>.

<sup>87</sup> [www.redcrossblood.org/learn-about-blood/blood-types](http://www.redcrossblood.org/learn-about-blood/blood-types) (last visited May 24, 2016).

<sup>88</sup> DNA Casework Unit Serology Procedures Manual, DNA Casework Unit Quality Manual 609, Rev. 5 (12/15/11).

<sup>89</sup> Scientific Working Group on DNA Analysis Methods, *SWGDM guidelines for the Collection and Serological Examination of Biological Evidence* (2014), available at [www.swgdam.org](http://www.swgdam.org).

### **C. ASCLD/LAB**

While there are a number of entities that provide laboratory accreditation, the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB), Forensic Quality Services (FQS) and American Association for Laboratory Accreditation (A2LA) are accrediting bodies for forensic science laboratories employing management and technical requirements following ISO/IEC 17025 standards. These standards pertain to document control, corrective action, audits, personnel, validation, equipment, assuring the quality of test results, reporting of results, reports, proficiency tests, technical records, education of personnel, competency testing, security of facilities and evidence, evidence handling, training, and technical and administrative review.<sup>90</sup> These ISO/IEC 17025 standards may be employed by laboratories conducting a variety of forensic analysis, for example, fingerprint analysis or firearms analysis. Laboratories within the Department are accredited by the American Society of Crime Laboratory Directors/Laboratory Accreditation Board (ASCLD/LAB). In order to maintain accreditation, laboratories undergo a full re-assessment every five years.

### **D. National Research Council (NRC) / National Academy of Sciences (NAS) Reports**

In 2009, the National Academy of Sciences published *Strengthening Forensic Science in the United States: A Path Forward*, which assessed important issues facing the forensic science and medical examiner communities. The report addressed biological evidence which encompasses serology and DNA testing. While DNA testing received the greater focus in this report, other biological testing (i.e. serology) was also discussed. The report states that serological testing has well-established scientific bases and reliability.<sup>91</sup>

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<sup>90</sup> ISO/IEC 17025 - *General Requirements for the Competence of Testing and Calibration Laboratories*, International Organization for Standardization, Geneva, Switzerland (2005). See also American Society of Crime Laboratory Directors/Laboratory Accreditation Board, *ASCLD/LAB-International Supplemental Requirements for the Accreditation of Forensic Science Testing Laboratories*, Garner, N.C. (2011).

<sup>91</sup> National Research Council, Committee on Identifying the Needs of the Forensic Sciences Community, *Strengthening Forensic Science in the United States: A Path Forward*, National Academy of Sciences Press, p. 129-133 (2009), available at <http://www.nap.edu/catalog/12589.html>.