B48 Masking of Blue Color of Positive Seminal Acid Phosphatase by Red Color of Red Blood Cells

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After attending this presentation, attendees will gain a better understanding of the Seminal Acid Phosphatase test and how red blood cells can mask the blue color of the Seminal Acid Phosphatase test.

This presentation will impact the forensic community by bringing better understanding on how the color of red blood cells can affect the ability of the criminalist to observe the Seminal Acid Phosphatase test.

Sexual assault evidence is submitted to forensic laboratories for biological screening and DNA analysis. A screening method for detection of seminal fluid utilizes a chemical test for seminal acid phosphatase (SAP). This is a two-step test that uses 1 drop of thymolphthalein monophosphate (SAP #1) and after one minute, 2-3 drops of Na_2CO_3/NaOH solution (SAP #2). The SAP test rapidly produces a blue color in the presence of seminal fluid and remains colorless when seminal fluid is not present. When an SAP test is performed on a mixture of semen and red blood cells (RBC), the red color of the cells can often mask the characteristic blue color of a positive SAP test, leading to an inconclusive result. This experiment examines the use of deionized water as a means of diluting the red color of the blood to better observe the characteristic blue color of a positive reaction.

In this experiment, five samples were used (neat blood, neat semen, and 1:10, 1:50, and 1:100 semen/blood ratios). Neat semen, neat blood, and samples of each of the three ratios were spotted onto swabs (a total of five swabs), and allowed to dry in a fume hood over night. Cuttings (2-3mm by 1mm) were taken from each swab, placed into Fresh Plate® spot wells in row #1, and re-hydrated using one drop of deionized water (~ 20 micro liters). After waiting one minute, 20 micro liters of the solution were transferred down into a well in row #2 and 1 drop of deionized water was added. After another minute elapsed, 20 micro liters of the solution from the well in row #2 of the Fresh Plate® was then placed in a well in row #3 of the Fresh Plate®. This dilution procedure was performed on all samples. The 20 micro liters of water taken from wells in rows #1 and #2 had an observable red color. The SAP test was performed on all wells in rows #1-3 for all the samples following the SAP procedure using 1 drop of SAP #1 and 2 drops of SAP #2. The SAP test produced the expected blue color on the samples containing seminal fluid and the blue color was observable.

When water was used as a diluent, the characteristic blue color could be observed in each of the semen/blood dilutions and the neat semen sample on the cutting in the wells of row #1. The neat semen and the 1:10 semen/blood dilution produced a positive SAP result on the cutting and the water dilutions in all rows. The SAP test did not produce positive results in the 1:50 and 1:100 semen/blood dilutions in the lower wells in rows 2 and 3. This could have occurred because of a lowering in concentration of seminal acid phosphatase.

When testing for the presence of seminal fluid in samples with blood present, adding one drop of water to the Fresh Plate® well containing the dry cutting appeared to reduce the concentration of red blood cells in the well enough so that the analyst could observe the blue color, a positive SAP test.

Seminal Acid Phosphatase, Blood and Semen Mixture, Rape Evidence

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