FORENSIC USE OF ABACUS ABACARD[®] TEST FOR THE IDENTIFICATION OF THE p30 ANTIGEN

<u>Kristin Koch</u>, Lewis O. Maddox, Ph.D., Bryan Suit, Jacki Higgins, Charlotte J. Word, Ph.D., and Robin W. Cotton, Ph.D.

Cellmark Diagnostics, Germantown, MD

The detection of the p30 antigen on forensic samples is often helpful because it confirms the presence of semen even in samples that involve vasectomized or azospermic individuals. The p30 antigen is a glycoprotein produced by the prostate gland and secreted in seminal fluid at concentrations ranging from 2.0x105 to 5.5x106 ng/ml. Unlike the acid phosphatase presumptive test, detection of the p30 antigen requires the presence of the protein without the need of the target to perform an enzymatic function. This may aid in the identification of semen in aged evidence samples in which the acid phosphatase enzyme is functionally inactive.

Methods used to detect the p30 antigen include Ouchterlony double diffusion, crossover electrophoresis, rocket immunoelectrophoresis, radial immumodiffusion, and ELISA testing. These techniques are labor intensive and require specialized training. The Abacus ABAcard[®] is a product that can be used for rapid and qualitative identification of the p30 antigen. The Abacus ABAcard[®] p30 test confirms the presence of semen by utilization of a dye-labeled mobile monoclonal antibody that binds to the p30 antigen. The p30 antigen-antibody complex diffuses horizontally on a membrane with two regions of fixed antibodies. Immobilized polyclonal p30 antibodies are located in a test region and antihuman immunoglobulin antibodies are present in a control region on the Abacus ABAcard[®] test membrane. In these regions, the mobile p30 antigen-antibody complex and/or free dye labeled p30 antibody interact with the immobilized antibody regions. Following the application of 200 µl of sample extract and a 10 minute diffusion time, the accumulation of the dye labeled antibodies results in the formation of pink bands at the test and control regions on the Abacus ABAcard[®] membrane. A pink band at the control region signifies that the test was run appropriately. Immobilization of the ap30 antigen antibody complex at both the test and control region positively identifies the presence of the p30 antigen.

Validation studies were performed to evaluate the Abacus ABAcard[®] p30 test for use in our laboratory as a confirmatory test for the presence of semen. The sensitivity of the Abacus ABAcard[®] membrane for the p30 antigen was determined using liquid and dried serial dilutions of a SERI semen sample. The specificity of the Abacus ABAcard[®] was evaluated by analysis of various body fluids (both neat and mixed stains) on different substrates. These included male and female urine, blood, saliva, fecal material, and vaginal secretions on swabs, wood, cotton, metal, carpet, and leather.

Our validation studies have demonstrated that the Abacus ABAcard[®] p30 test is a rapid and simple test for confirmation of semen in forensic evidence samples. Using the Abacus ABAcard[®], p30 antigen was detected at a concentration as low as 100 ng/ml. Although p30 antigen could be detected in neat male urine, analysis of a 1:10 dilution of male urine showed no detectable amount of p30 antigen. This test detects the p30 antigen found in seminal fluid even in the mixtures with other body fluids. A summary of the studies performed will be presented.