Experimental Design for the Validation of the p30 ABAcard for the Identification of Human Semen

1. The development of a written protocol for the p30 Test Card derived from the product insert provided with the test cards produced by Abacus Diagnostics.

2. Dilution series of SERI semen standard.

   SERIS semen standard will be serial diluted in a tenfold dilution series. The dilution series will start at 1/10 and will extend to 1/1,000,000 (this dilution series does not take into account that the standard is shipped as a 1/50 dilution). Once a negative test is encountered at a particular dilution the testing of further dilutions will not be necessary. The dilution series will also be tested by our current procedure involving crossover electrophoresis.

   Purpose: To determine how sensitive the p30 Test Card is.

3. Dilution series of liquid human semen (two semen donors will be used).

   Human semen will be serial diluted in a tenfold dilution series. The series will start with a 1/10 dilution and will extend to 1/1,000,000. Once a negative test is encountered at a particular dilution the testing of further dilutions will not be necessary. The dilution series will also be tested by our current procedure involving crossover electrophoresis.

   Purpose: To determine how sensitive the p30 Test Card is.

4. Testing of the p30 Test Card with other human body fluids (three donors will be used).

   Urine, blood, feces (stain), and saliva will be diluted in a tenfold dilution series. The series will start as undiluted and will extend to 1/1,000.

   Purpose: To determine if other human body fluids besides semen can yield a positive result with the p30Test Card.

5. Testing of the p30 Test Card with semen stains.

   A tenfold dilution series of human semen stains will be prepared as described in step #2, spotted onto various clothing substrates, and the stains allowed to dry. The stains will then be dehydrated and tested with the p30 Test Card.

   Purpose: To determine if human semen stains can be effectively tested using the p30 Test Card.

6. If needed, minor refinement of the protocol developed in step #1.

7. Implementation of the p30 Test Card if the validation experiments prove that it is an effective and reliable tool for the identification of human semen.

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Summary of the Abacus *OneStep* p30 ABAcard Validation Studies Performed by the San Diego Police Department

Intended Use

The San Diego Police Department currently utilizes the crossover gel electrophoresis technique for detection of the p30 protein found in human semen. The p30 crossover technique requires approximately twenty-four hours to obtain results that yield a relatively low degree of sensitivity (1:8 fold dilution for semen stains, 1:16 fold dilution for SERI p30 standard). In efforts to increase the efficiency and sensitivity of semen detection, the San Diego Police Department has performed a validation study of the Abacus OneStep p30 ABAcard. The OneStep ABAcard is designed to detect semen in samples containing p30 concentrations exceeding 4ng/ml, including samples collected from vasectomized or azoospermic individuals. The results of the ABAcard test appear within ten minutes. Serial dilutions of liquid semen, liquid body fluids, semen stains, and body fluid stains were used in this experiment to determine if the OneStep ABAcard would provide reliable, specific results that exceed the efficiency and sensitivity of the current p30 crossover technique.

Principle Behind p30 Crossover Electrophoresis

The San Diego Police Department currently utilizes the p30 gel electrophoresis technique to detect the presence of semen in a given sample. In crossover electrophoresis, antigen is placed in a well of an agarose gel that is parallel to a well that holds an antibody (SERI Anti-p30). The antigen is placed in the well on the cathodic side of the gel, while the antibody is placed in the anodic well. An electrical current is applied to the gel medium, causing the antigen and antibody to migrate towards each other. The formation of a visible line midway between the two wells signifies the presence of p30 in the sample. The p30 crossover method requires approximately twenty-four hours to obtain results.

Principle Behind OneStep p30 ABAcard

Each test card includes a test area (S) labeled with the monoclonal p30 antibody, a test area (T) labeled with a polyclonal antihuman p30 antibody, and a positive control area (C) labeled with an immobilized anti immunoglobulin antibody. If p30 is present in a semen sample, it will react with the monoclonal p30 antibody to form a mobile antigen-antibody complex. The antigen-antibody complex migrates from the test area (S) towards the polyclonal antihuman p30 antibody in the test area (T). An antibody-antigen-antibody sandwich is formed upon contact with the test area, producing a conjugated pink dye band when the amount of p30 present exceeds 4ng/ml.
An internal positive control in area (C) consists of an immobilized anti immunoglobulin antibody that binds all p30 antibody-dye conjugates that are unable to bind to the antibody in the test area (T). The presence of a colored line in both the test area (T), and the control area (C) indicates a positive reaction. A negative result is indicated by a single colored line that appears in the control area (C).

Reagents and Materials Required

1. Test Device card
2. Nanopure water
3. Swatch stain cards
4. Cotton swabs
5. 1.5 ml microcetrifuge tubes
6. Centrifuge
7. Clock or timer

Results

The validation study performed on the Abacus OneStep p30 ABAcard proved the test cards successful in detecting the p30 protein at a considerably higher sensitivity than the current p30 gel electrophoresis method. The ten-minute test yielded a positive result at a 1:3125 fold dilution for the semen swatch stains, while the liquid semen samples produced a positive reaction at a 1:10,000 fold dilution. The human and SERI standard liquid semen samples produced slightly weaker positive reactions in the more heavily concentrated samples. This result is explained by the "high dose hook effect" that occurs when there is too much p30 present in the sample. Consequently, the excess p30 that is unable to bind to the antibody migrates towards the test area (T), blocking the antibody from binding to the antibody-antigen-pink dye complex. A false negative result is produced even though there is a large amount of p30 present in the sample.

Blood, saliva, and feces were eliminated as detectable human body fluids, yielding negative results at a 1:5 fold dilution. Male urine was detectable at a 1:75 fold dilution in the liquid urine samples. This was predicted as a potential result, given that p30 has been detected in male urine at 260ng/ml. However, the male urine stain samples did not yield a positive result at any dilution. The male urine stain sample results are more informative because they closely mimic the casework samples received by the department.

Finally, four p30 crossover gels were run to provide a reference to measure the sensitivity of the Abacus test cards. Each gel produced a reaction at a 1:8 fold dilution of the semen stain samples. The SERI standard was visible at a 1:8 fold dilution but has demonstrated visible detection at a 1:16 fold dilution in previous work performed by analyst Zach Gaskin. This is considerably less sensitive than the results given by the ABAcards.

Conclusion

The goal of this validation study was to determine the efficiency and sensitivity of the Abacus Onestep p30 ABAcard when applied to samples that mimic casework samples

received by the forensic laboratory at the San Diego Police Department. When compared to the p30 crossover electrophoresis method currently utilized by the department, the ABAcard successfully demonstrated a higher degree of sensitivity and efficiency. Reliable results were obtained within ten minutes, significantly decreasing the amount of time currently necessary for semen detection by crossover electrophoresis.

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