



Abacus Diagnostics, Inc.

ABAcard[®] p30 Test For The Forensic Identification of Semen

For Forensic Use

Immunoassay for the qualitative detection of p30 for the forensic identification of semen.

Catalog # 308332 (25 Test/kit)

Technical Information sheet

Intended Use

ABAcard[®] p30 test is designed to qualitatively detect p30 for the forensic identification of semen. p30 is an accepted marker for detecting semen in criminal cases including vasectomized or azoospermic individuals.

Summary

In 1971 Hara et al. first described a protein in the seminal fluid, named gamma-seminoprotein. In 1978, Sensabaugh et al. characterized the protein in detail, found that its molecular weight corresponds to 30,000 Dalton and named it p30. In 1980 first immunometric assays were developed and Graves and Sensabaugh demonstrated that p30 is a reliable forensic marker for the identification of semen. The range of p30 is 200,000 to 5.5 million nanogram per ml of semen. The sensitivity of ABAcard[®] p30 test is 4 ng/ml and therefore seminal fluid diluted up to 1 in a million should also be detectable. Various methods of detection of p30 include Ouchterlony double diffusion, crossover electrophoresis, rocket immunoelectrophoresis, radial immunodiffusion, and ELISA. A disadvantage of all the above conventional methods is that they are either not sensitive enough or cumbersome and time consuming to perform in forensic laboratories. Abacus Diagnostics's ABAcard[®] p30 is, however, very sensitive with results only within 10 minutes.

Principle Behind This Test

In this test procedure, 200 µl of sample is added to the sample well 'S' and allowed to soak in. If p30 is present in the semen specimen, it will react with the mobile monoclonal anti-human p30 antibody and a mobile antigen-antibody complex is thus formed. This mobile antibody-antigen complex migrates through the absorbent device towards the test area 'T'. In the test area 'T', a monoclonal anti-human p30 antibody is immobilized. This immobilized antibody captures the above complex so that an antibody-antigen-antibody sandwich is formed. The conjugated pink dye particles concentrate in a narrow zone on the membrane. When the p30 concentration in the sample exceeds 4 ng/ml the pink dye particles will form a pink colored band in the test area 'T' indicating a positive test result. As an internal positive control, p30 antibody-dye conjugates cannot bind to the antibody in the test area 'T', but are captured by an immobilized anti-immunoglobulin antibody present in the control area 'C' forming a complex. The captured pink dye particles will thus form a band in the control area 'C', indicating that the test has worked properly and proper procedures have been followed. Thus, presence of two colored lines, one in the test area 'T' and other in the control area 'C', indicates a positive result, while a line only in the control area 'C' would indicate a negative result (provided no "high dose hook effect").

Reagents And Materials Provided

1. Test Device (25 pcs, each sealed individually in a test pouch)
2. A Dropper and a desiccant sealed inside each of the test pouch.
3. Test Instructions

Materials Required But Not Included

1. Clock or timer.
2. Centrifuge

Stability, Storage and Shelf Life

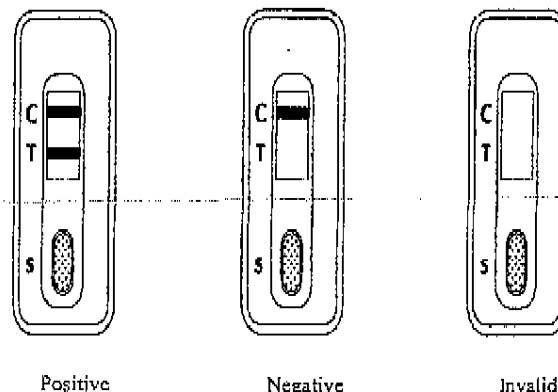
ABAcard[®] p30 Detection Test should be stored below 82°F (28°C). The test can be stored in the sealed pouch below 82°F (28°C) until the expiration date as printed on the sealed test pouch. Do not Freeze. Do not use the test after the expiration date.

Sample Collection, Preparation and Storage

- The frozen specimens/swabs/stains must be thawed completely and brought to 2-8 °C.
- Extraction of specimens from swab or stain may be performed in 750 µL of HEPES buffered saline for 2 hours at 2-8 °C. Distilled water or other buffers suitable for further DNA extraction may be used as well. This procedure recovers approximately 99% of the extractable p30 on the swab.
- Centrifuge the above sample for 3 minutes after the above extraction step. Remove 300 µl of supernatant for testing purposes. This aliquot may be stored between 2-8 °C if not used immediately. Immediately before use with ABAcard[®] p30 test, the sample should be brought back to room temperature. Remaining sample may be used for further DNA analysis without affecting the DNA yield.

Test Protocol

1. Allow the sample to warm to room temperature if it has been refrigerated.
2. Remove the device and the dropper from the sealed pouch.
3. Label the device with the case number.
4. Add 200 µL (or 8 drops with the dropper) of sample to the sample well 'S' of the test device.
5. Read result at 10 minutes. Positive results can be seen as early as 1 minute depending upon the p30 concentration. For negative results, one must wait for full 10 minutes.



1. **Positive.** If there are two pink lines, one each in the test area 'T' and in the control area 'C', the test result is positive and indicates that the p30 level is at or above 4 ng/ml.
2. **Negative.** If there is only one pink line (in the control area 'C'), the test result is Negative. This may indicate that (a) No p30 is present above 4 ng/ml or (b) Presence of "High Dose Hook Effect". Presence of "High Dose Hook Effect" may give false negative result due to the presence of high concentration of p30 in the sample, as for example in undiluted seminal fluid. In such cases the sample may be retested using a 10 to 10,000 fold dilution.
3. **Invalid.** If there is no pink line visible in the control area 'C', the test is inconclusive. Repeat the test and reexamine the test procedure carefully.

PRECAUTIONS

- For the in vitro qualitative detection of p30 for the forensic identification of semen only.
- Do not use beyond the expiration date which appears on the sealed pouch.
- Disposable gloves should be worn while handling kit reagents or specimens. Wash your hands after the test.
- A fresh transfer pipette for each specimen should be used.
- Do not smoke, eat or drink in areas in which specimens or kit reagents are being handled.

- Handle all forensic samples as if they were capable of transmitting disease. Follow standard procedures for proper disposal of specimens.
- Kit reagents contain sodium azide as a preservative which may react with lead or copper in plumbing to form potentially explosive metal azides. Upon disposal, always flush with large volumes of water to prevent build up in drains.

Quality Control

The control line in the control area 'C' can be considered an internal procedural control. A distinct pinkish line will always appear if the test has been performed correctly. If the control line 'C' does not appear, the test is invalid and a new test should be performed following the correct test procedure. A quality control test using positive and negative control standards may also be performed.

Limitations

1. ABACard_® p30 Test is only for in vitro detection of p30 for the forensic identification of semen.
2. The test must be performed in strict accordance with these instructions to obtain accurate and reproducible results.
3. Even if the test result is positive, careful forensic judgment should be made in conjunction with other information and information from other test results.
4. If elevated p30 levels is suspected but a negative result is obtained, the test should be repeated and with fresh specimen.
5. Positive results may be obtained with male urine, which has a reported mean value of 260 ng/ml. Seminal vesicle specific antigen should not be present when tested with urine. Use of another appropriate test is recommended when male urine is in question.
6. Appropriate specimen should be used since p30 is detectable in the vaginal tract only up to a maximum of 2 days.

Performance Characteristics

Sensitivity

The minimum detection limit of ABACard_® p30 Test is 4 ng/ml in 10 minutes (using Stanford's seminal plasma derived Standard, Catalog # L F500, Phone # (650)725-5542 with PBS/BSA Buffer, pH 7.4, Sigma Catalog # P3688). Results with specimens having high levels of p30 may be obtained as early as 1 minute. For negative results, one must wait for full 10 minutes. The range of p30 is 200,000 to 5.5 million nanograms/ml of semen. Therefore, depending on p30 concentration, seminal fluid diluted up to 1 in a million may also be detectable.

Specificity

Hemoglobin (10 g/L), bilirubin (100 mg/L) and lipemic samples, as indicated by triglyceride (5 g/L), do not interfere with the test results. High protein concentration such as prostatic acid phosphatase (1000 ng/ml), albumin (20 g/L), chorionic gonadotropin (900 IU/ml), transferrin (5g/L) and prolactin (1mg/L) did not interfere with test results. Besides semen from both normal and vasectomized men, positive results were obtained from post-ejaculate urine and male urine from adult men, when the urine samples were directly added to the test. However, it is well established that p30 does occur in these urine samples with a reported mean value of 260 ng/ml. Seminal vesicle specific antigen should not be present when this test is used with urine.

Intra Assay and Inter Assay Studies

Intra-assay

An Intra Assay variability study was performed. Ten replicates of known positive and negative p30 samples were tested. The results demonstrated a 100% agreement with the expected results.

Inter-assay

Independent assays were performed on the above samples with three lots of ABACard_® p30 Test over a three month period. The assay results were 100% in agreement with the expected results.

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Some Frequently Asked Questions

Q1. What is "High Dose Hook Effect"?

A1. "High Dose Hook Effect" occurs when the p30 concentration is too high since ABACard_® p30 test is very sensitive. The mechanism behind this effect is that huge amounts of human p30 bind both to the antibody to form an antigen-antibody complex but also free p30 migrates towards the test area "T". The antibody in the test area "T" is blocked by this free p30. Therefore the mobile antigen-antibody complex with the pink color cannot bind to the antibody. As a result no pink line will form in the test area "T" although a lot of p30 is present in the sample giving a false negative result.

Q2. Is there any minimum and maximum times for reading the results.

A2. Yes there is a maximum time of 10 minutes. The minimum time in a positive result is the time at which both lines appear. The time of the reaction depends upon p30 concentration and other characteristics of the specimen. However if the test line did not appear before ten minutes, one should wait for full 10 minutes to allow the reaction to occur. Specimen with lowest concentration of p30 should take longest time to react. It is to be noted that the results should not be read after 10 minutes since non specific reactions may occur and may result in false positives.

Q3. What does control band 'C' represent?

A3. The built-in procedural positive control is provided by the appearance of a pink line next to the letter 'C', validating the integrity of the test, assuring that the correct test procedure was followed and indicating that proper volume of the fluid entered the test cassette and capillary flow occurred. If the line in the control area 'C' does not develop within 10 minutes, the test result is invalid. Repeat the test using proper procedures.

Q4. Does the intensity of the test band 'T' and control band 'C' matter?

A4. The intensity of either the control band or the test band should not be compared between tests or to each other for ABACard_® p30 Test and no quantitative interpretation should be made based upon differences in the intensity. The mere appearance of both lines proves the presence of p30.

Q5. Where can we order the standard from?

A5. You may order L-F500 standard from Stanford by calling (650) 725-5542. You may order PBS/BSA buffer from Sigma (Cat # P3688) by calling (800)-325-3010

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