SER: Seminal Fluid – ABAcard p30 Immunoassay Test

Principle

The ABAcard p30 Immunoassay Test is a one-step test for the detection of p30. The test devices use a conjugated dye labeled antibody which forms a complex with the p30 antigen.

The identification of p30 in a stain using this method indicates the presence of seminal fluid.

Equipment

The following equipment is used for this procedure:

- microcentrifuge tubes
- pipettes and barrier pipette filters

Reagents

The following prepared reagents are used in this procedure:

- ABAcard one-step p30 immunoassay test device
- TE Buffer

Quality control

Each new lot of ABAcard p30 test devices must be tested with a positive and negative quality control to evaluate the "test area" band. If problems are found, that lot will not be used for casework.

A record of the quality check for new lot numbers including a photograph documenting the results is kept in the *Quality Control Log Book* located in the Biology Laboratory.

NOTE: Refer to the log book for previous band intensities observed for the positive control to determine whether a new lot of test devices is acceptable for casework.

Continued on next page

SER: Seminal Fluid – ABAcard p30 Immunoassay Test,
Continued

Positive control preparation

Prepare a positive control using the following procedure:

Step	Action
1	Add 50 µl of known semen or seminal fluid to a sterile cotton swab and let air dry.
2	Extract the entire swab in 1 mL of TE Buffer for 1-2 hours.
3	Prepare for analysis by adding 1 µl of extract to 199 µl of TE Buffer.

Prepare frozen pre-aliquoted positive controls using the following procedure:

Step	Action
1	Follow steps 1 and 2, above.
2	Place 1 µl of the extract and 19 µl of deionized water or TE Buffer into small tubes and freeze. Prior to freezing, test one of the tubes to ensure the batch is working properly.

Prepare a previously frozen aliquoted control for analysis using the following procedure:

Step	Action
1	Add 180 µl of TE Buffer to a thawed 20 µl aliquot.

Negative control preparation

Prepare a negative control for analysis using the following procedure:

Step	Action
1	Pipet 200 µl of TE Buffer into a labeled tube.

Continued on next page

SER: Seminal Fluid – ABAcard p30 Immunoassay Test,
Continued

Sample preparation

Prepare samples for analysis using the following procedure:

Step	Action
1	Allow all samples to warm up to room temperature.
2	Extract the stain cutting or the entire swab in 300 µl of TE Buffer for approximately 30-60 minutes on the ThermoMixer.
3	Centrifuge the sample for 5 minutes to form a cellular pellet.
4	Transfer 270 µl of the supernatant to a labeled microcentrifuge tube.

NOTE: The p30 test devices are only suitable for use on dried stains. Liquid samples should not be tested directly.

Continued on next page

SER: Seminal Fluid – ABAcard p30 Immunoassay Test,
Continued

Procedure

The ABAcard p30 test is performed using the following procedure.

NOTE: Each sample will require a separate test device.

Step	Action
1	Add 200 µl of prepared supernatant or control to the sample well of the test device. NOTE: Positive and negative controls are run for the lot of test devices when the quality check is performed and may also be run again with the set of samples. The lot number must be recorded in the examination records.
2	After ten minutes, evaluate the test devices. Record the results.

NOTE: Due to *High Dose Hook Effect* (a false negative reaction when very high levels are present in the sample), negative results for samples that are AP positive with rapid color development must be re-tested as follows:

Step	Action
1	Add 2 µl of the original sample supernatant to 198 µl of TE Buffer.
2	Add the entire 200 µl sample to the sample well of the test device and record the result at 10 minutes.

Continued on next page

SER: Seminal Fluid – ABAcard p30 Immunoassay Test,
Continued

Interpretation

A visible pink band at the Control (C) position only is a negative (-) result for p30.

A visible pink band at both the Control (C) and the Test (T) positions is a positive (+) result for p30 which indicates the presence of seminal fluid, a component of semen.

If no Control(C) band appears, the test is invalid and should be repeated.