SER: Blood – ABAcard HemaTrace® Immunoassay Test

Principle

The ABAcard HemaTrace® Immunoassay Test is a one-step test for the detection of human hemoglobin (Hb). The test devices use a conjugated dye labeled antibody which forms a complex with the human hemoglobin antigen.

A positive result for this test confirms the presence of blood when a positive result is also obtained for a presumptive test for blood (e.g. Kastle-Meyer). A presumptive test for blood (e.g. Kastle-Meyer) should be performed prior to the HemaTrace® test.

The identification of possible human hemoglobin in a stain using this method indicates but does not confirm the presence of *human* blood since other species have been shown to produce a positive result for this test.

Equipment

The following equipment is used for this procedure:

- microcentrifuge tubes
- pipettes and barrier pipette tips

Reagents

The following prepared reagents are used in this procedure:

- ABAcard one-step HemaTrace® immunoassay test device
- ABAcard extraction buffer

Controls

The following controls are used when checking a new lot of test devices:

- positive control (human bloodstain)
- negative control (ABAcard extraction buffer)

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Quality control

Each new lot of ABAcard HemaTrace® 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.

Positive control preparation

Prepare a positive control using the following procedure:

Step	Action
1	Add known liquid human blood to a sterile cotton swab or cloth and let air dry.
2	Once dry, divide the stained cloth into approximately 3-5mm^2 cuttings or the stained swab into 2-4 pieces.
3	The prepared cuttings may be stored in the refrigerator or freezer for later use.

Negative control preparation

Prepare a negative control using the following procedure:

Step	Action
1	Use 4-5 drops with the supplied dropper or 150 µL of extraction buffer from one of the provided tubes.

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Sample preparation

Prepare samples for analysis using the following procedure:

Step	Action
1	Allow all samples to warm up to room temperature.
2	Prepare cuttings to be tested using the following guidelines: • For stains on cloth or fabric, use an approximately 1 mm thread to a 5 mm^2 cutting. • For swabs, use ¼ to ½ of the swab.
3	Soak the cutting in the entire volume of extraction buffer in the provided tube for 1-5 minutes. Older less soluble bloodstains (e.g. older than 5 years) can be soaked for up to 30 minutes.
4	Gently mix the contents of the tube for approximately 10 seconds making sure the sample does not foam up. The supernatant is now ready for immediate testing.

Sample preparation for concentrated stains

Due to the *High Dose Hook Effect* (a false negative reaction when very high levels are present in the sample), samples that appear highly concentrated (e.g. dark red-brown sample extract) or initially gave a negative result can be prepared as follows:

Step	Action
1	Dilute the extract 1:10 with distilled water.
2	If the extract still appears too dark or is still negative, dilute the extract 1:100 with distilled water.

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Procedure

The ABAcard HemaTrace® test is performed using the following procedure.

Note: Each sample will require a separate test device.

Step	Action
1	Remove each test device from its sealed pouch and label appropriately. Test devices must be used the same day they are removed from the sealed pouch. 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	For each sample to be tested, add 4-5 drops of sample with the supplied dropper or 150 µL to the sample window of the test device.
3	Record the results at ten minutes.

Interpretation

A visible pink band at both the Control (C) and the Test (T) positions is a positive (+) result for the presence of human hemoglobin. Hemoglobin in bloodstains from other animals, specifically the pigeon and domestic ferret, has produced positive results with this test. Bloodstains from the domesticated ferret cannot be differentiated from the human hemoglobin because domestic ferret Hb and human Hb share a common amino acid sequence utilized by this test. Several primates also share this sequence. For this reason, a positive result is indicative of the presence of human hemoglobin. Because of the sensitivity of this test, hemoglobin may be detected in samples other than blood such as urine, semen, feces, saliva, vaginal fluid and perspiration.

A visible pink band at the Control (C) position only is a negative (-) result for human hemoglobin. Because of the possibility of the *High Dose Hook Effect*, samples that yield negative results where blood is strongly suspected may be diluted and re-tested, particularly when the extract appears dark red in color.

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

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Reporting guidelines

If the following combination of results is obtained, the report may read:

- **KM positive, HemaTrace® negative, and Takayama positive:**
  "Blood was detected but may not be of human origin."

- **KM and HemaTrace® positive – OR-**
  KM positive, HemaTrace® positive, and Takayama negative:
  "Human blood was detected. However, primate and ferret blood has also been shown to produce a positive result for the HemaTrace® test for human blood."

- **KM positive and HemaTrace® negative (Takayama not performed):**
  "Possible blood was detected but could not be confirmed. I did not perform a Takayama test (confirmatory test for blood)."

- **KM positive, HemaTrace® and Takayama negative**:
  "Possible blood was detected but could not be confirmed."