MICROCRYSTAL TEST FOR BLOOD (TAKAYAMA)

9.1 PURPOSE

9.1.1: To determine the presence of blood in Forensic samples.

A. Theory

1. The Takayama (Hemochromogen) test is a microcrystal test which employs a series of reactions that are specific for the detection of the hemoglobin portion of blood.

2. Sodium hydroxide releases the heme groups from the globin through alkaline hydrolysis. Glucose reduces the heme iron and pyridine combines with it to form the product, pyridine ferroprotoporphyrin, which is visualized as insoluble, red, needle-shaped crystals, also known as hemochromogen crystals.

B. Limitations

1. This test does not determine the origin of species.

2. The stain's substrate may interfere with the formation of crystals.

3. Crusty reddish-brown stains are best for the formation of crystals.

9.1.2: To prepare the reagent for the microcrystal test for blood and to perform quality control on the prepared reagent.

9.2 RESPONSIBILITY

9.2.1: Forensic Science Examiners (however titled) from the Division of Scientific Services who have been trained in the discipline of the Takayama test procedure according to FB SOP-26 (Training Manual and Checklist).

9.2.2: Forensic Science Examiner in the Forensic Biology Unit. Ordering information is maintained in a log book in the Forensic Biology Unit. New chemicals are purchased according to GL-6 (Purchasing). For additional information, refer to the Biological Inventory (Appendix 3) in the FB folder on the shared drive.

9.3 SAFETY

Use appropriate measures for the proper handling of biohazardous materials, pyridine, sodium hydroxide and the Takayama Reagent according to GL-2 (Safety Manual) and the Safety Data Sheets.

A. The Takayama test is typically conducted in cases when animal blood is suspected and the Rapid Immunoassay Test(s) for human blood are negative.

B. A sample is considered limited when it appears to be of low quantity and compromised when it appears to be in poor condition. The conditions the evidence may have been exposed to prior to submission shall be considered when assessing the sample tested and/or collected.

9.4 DEFINITIONS

QRW(s): Quality Record Worksheet(s) (Appendix 1)

9.5 TEST PROCEDURE

This test will be performed at the discretion of the examiner, with input from the Unit Lead(s), based on the submitting agency requests, case information and the condition of the evidence.

9.5.1: Materials

A. Takayama Reagent
B. Controls: positive (known bloodstain) and negative (blank substrate)
C. Microscope slides
D. Cover slips
E. Alcohol burner or oven

9.5.2: Procedure

A. Record the reagent lot number used on the General Reagent Sheet (FBQR-09).

B. Test a positive and negative control with the following procedure (steps 9.5.2.C - 9.5.2.H).
   1. The controls may be run concurrently with the questioned samples as an intermediate check. Reagent QC is always conducted prior to use on case samples.

   2. If the questioned sample is limited/compromised, run the controls prior to testing the questioned sample. If the controls yield the appropriate results then immediately test the questioned sample.

   3. If the controls do not yield the appropriate results, review the procedure and retest the controls prior to the questioned sample. If the controls still do not yield the appropriate results, then inform the Unit Lead to try to determine the root cause.

C. Place a portion of the questioned sample on a microscope slide and cover with a cover slip.

D. Let 1-2 drops of reagent flow slowly under the cover slip and come in full contact with the sample.

E. Under a hood, heat the slide gently over an alcohol burner until small bubbles begin to appear under the cover slip. Alternately, the slide may be placed in a 37°C oven for 5-10 minutes.

F. Allow slide to cool under a hood.

G. Examine under the microscope at 100-400x, as soon as the slide cools.

H. If no crystals are observed, re-examine the sample periodically for 2-3 hours, as weak samples may need a longer time for crystals to develop.

I. Record the results of the controls and samples on the appropriate QRW(s).

J. A second qualified examiner will observe and confirm the results and initial the appropriate QRW(s).

9.5.3: Results and Conclusions

A. Positive

1. The formation of bright red crystals indicates a positive test and the presence of blood. Crystals may form on the surface of the substrate in older samples.

2. Suggested Report Wording:

a.

Testing Performed	Result	Conclusion
Microscopic - Crystal - Blood	Positive	Blood identified

b. *[ ] tested gave a positive result(s) with a microcrystal test for the presence of blood.*

B. Negative

1. The absence of bright red crystals indicates a negative test and blood is not detected.

2. Suggested Report Wording:

a.

Testing Performed	Result	Conclusion
Microscopic - Crystal - Blood	Negative	Blood not detected

b. *A microcrystal test for the presence of blood was performed on [ ]. Blood was not detected with this test.*

9.6 Preparation/QC PROCEDURE

A. Takayama Reagent will be prepared as needed.

B. Manufacturer's expiration dates with only month and year indicated (i.e. 04/2014) expire the last day of the month noted.

9.6.1: 10% Sodium Hydroxide Solution

Materials

A. Sodium Hydroxide 1g
B. dH_2O ~10ml

Procedure

A. Make a 10% weight/volume solution: Mix 1g of sodium hydroxide in less than 10 ml of dH_2O until dissolved. Bring to a total volume of 10ml with dH_2O.

B. Discard excess sodium hydroxide solution after preparing Takayama reagent.

9.6.2: Glucose solution

Materials

A. Glucose 5g
B. dH_2O 5ml

Procedure

A. Mix together and heat until dissolved.

B. Discard excess glucose solution after preparing Takayama reagent.

9.6.3 Takayama Reagent

Materials

A. 10% Sodium hydroxide solution 5ml
B. Pyridine 5ml
C. Glucose solution 5ml
D. dH_2O 10ml

The control date will be one (1) year from the date of preparation.

Procedure

A. Mix all materials together and place in a brown dropper bottle.

B. Test each new batch of reagent before use according to the test procedure and the Takayama Reagent Log Sheet. Record the required information.

C. If the appropriate results are not obtained, discard the reagent, review the procedure, make new reagent and retest. If the reagent still does not yield the appropriate results, then inform the Unit Lead to try to determine the root cause.

D. If the reagent is acceptable for use, record the reagent, lot # (date of preparation), control date and examiner's initials on the bottle and store in the refrigerator.

1. The reagent is acceptable for use when a positive result is obtained with the blood control and a negative result is obtained with the blank/negative control.

2. A second qualified examiner will observe and confirm the results and initial the Takayama Reagent Log Sheet.

E. Discard on the control date.

9.7 REFERENCES

A. Takayama, M. " A Method for Identifying Blood by Hemachromogen Crystalization" Kokka Igakkai Zasshi 306: 15-33 (issue); 463-481 (cumulative), (1912) 15.
B. Gaensslen, R.E., Sourcebook In Forensic Serology, Immunology, and Biochemistry, U.S. Government Printing Office, Washington D.C., 1983, pp. 85-87.
C. GL-2 (Safety Manual)
D. GL-6 (Purchasing)
E. Safety Data Sheets