Procedures for the Confirmatory Identification of Blood

1 Scope

These procedures describe the method by which blood is conclusively identified on evidentiary items submitted to the FBI Laboratory for examination by the DNA Casework unit (DCU) or Biometrics Analysis Unit (BAU) using the Takayama hemochromogen (aka Taky) test.

2 Equipment/Materials/Reagents

- Takayama hemochromogen reagent
- Known positive blood control sample
- Glass microscope slides, 1" x 3" (Fisher Scientific, 12-544-1, or equivalent)
- Glass microscope slide coverslips (Fisher Scientific, 12-542A, or equivalent)
- Fume Hood (Fisher Hamilton, SAFEAIRE®., 54L0335, or equivalent)
- Stirrer/Hot Plate (Corning Model PC-220, or equivalent)
- Microscope with lenses for 100x magnification (Leitz, Laborlux 11, or equivalent)
- General laboratory equipment and supplies (e.g., scalpel, forceps)

Refer to the appropriate DNA QA procedure for reagent and control preparation information.

3 Standards and Controls

The known positive (KP) blood control is a dried human blood sample. The known negative (KN) blood control is a portion of unstained cotton sheeting or swab. The Takayama hemochromogen reagent must be tested on a KP and KN prior to first daily use on evidentiary items to verify the continued detection efficacy. A bottle of Takayama hemochromogen reagent that does not yield a positive reaction with a KP blood control, or that yields a positive reaction with a KN blood control, must not be used for casework.

4 Sample Selection

4.1 Items with an indication that blood may be present (e.g., phenolphthalein positive red/brown staining, scenario information) may be tested using the Takayama hemochromogen test. Generally, at least one stain on an item that tested positive using the phenolphthalein test will be tested using the Takayama hemochromogen test.

4.2 If a limited amount of biological material is observed on an item that is being tested for the presence of blood, Takayama hemochromogen testing may not be conducted on a stain(s) that has tested presumptively positive for blood. Foregoing confirmatory testing for blood on such stains ensures that as much stain material as possible is available for potential DNA testing in the Laboratory, as well as for any potential future testing that may be necessary.

4.2.1 If the presumptive blood test is positive, but the Takayama hemochromogen confirmatory test could potentially consume the majority of a stain, the Takayama hemochromogen confirmatory test must not be conducted. This circumstance is recorded in the case notes as quantity not sufficient (or QNS).

4.2.2 If the presumptive blood test is positive, but circumstances other than the physical characteristics of a stain (i.e., non-probative item, multiple blood stains, the potential for latent print examinations, etc.) limit the potential value of any additional consumptive testing; the Takayama hemochromogen confirmatory test may not be conducted. This circumstance is recorded in the case notes as not further characterized (or NFC).

5 Procedures

Refer to DNA Procedures Introduction (DNA QA 600) for applicable laboratory quality assurance and cleaning instructions.

Ensure the appropriate fields (i.e., reagents, KP) in STACS are completed from any network computer, as necessary.

5.1	Place a small portion (e.g., an approximately 2 mm length of textile fiber, a visible amount of scraping, an approximately 1 mm^2 fabric cutting, etc.) of the questioned stain on a glass microscope slide.	

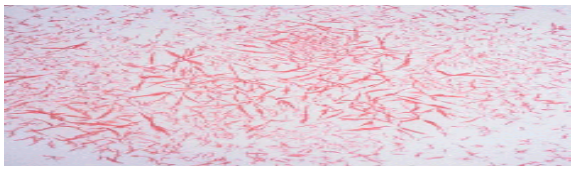
5.2	Cover the sample with a piece of coverslip that closely matches the size of the sample.	

5.3	Place the slide on a warm hot plate (generally at a heat setting of 3) in a fume hood. Place ~1-2 drops of Takayama Hemochromogen reagent sufficiently close to the coverslip piece so that some of the solution is drawn under the coverslip and into contact with the sample. Warm the solution on the slide (generally for 30 to 60 seconds).	

If necessary, additional Takayama Hemochromogen reagent may be added to the slide to prevent the sample from evaporating to dryness.

The slide may be chilled to facilitate crystal formation. Generally less than 10 minutes in a refrigerator or less than 60 seconds in a freezer is sufficient. Slides may be left in a refrigerator overnight prior to viewing.

5.4	View the slide under a microscope (generally at 100x magnification or higher if necessary).	

5.4.1	The observation of characteristic red or salmon-pink, feathery and branched, rhomboid crystals (See Figure 1) is recorded in the case notes as a positive result (or POS).  **Figure 1: Pyridineferroprotoporphyrin Crystals** The identification of crystals must be confirmed by a serology qualified examiner. The examiner will be recorded in the case notes.	

5.4.2	The observation of no characteristic crystals is recorded in the case notes as a negative result (or NEG).	

If the Takayama hemochromogen test yields a negative result, the test may be attempted additional times provided that these attempts do not consume the stain material. When the test is repeated, a notation of the number of times the test was performed must be made in the examination notes. Generally, the test will be performed either one or two times depending on the stain.

5.4.3	The observation of crystals of any other color and/or morphology is recorded in the case notes as an inconclusive result (or INC).	

The formation/presence of non-Hemochromogen crystals may consume the Takayama Hemochromogen reagent (making it unavailable for reaction with any heme groups present) or potentially mask or alter any potential Hemochromogen crystals that may be present. If such an observation is made, an Examiner should be consulted prior to conducting any additional testing.

The language an Examiner should use to report the test results from this testing and others is contained within the appropriate procedure (i.e., Sero 100) in the *DNA Procedures Manual.*

6 Quality Control Procedures

6.1 Each new batch of Takayama hemochromogen reagent will be tested for efficacy at the time of its preparation using the analytical procedures above on a KP blood control and against a KN blood control.

6.1.1 A positive test result (i.e., characteristic crystals) for the KP blood control establishes that the new batch of Takayama hemochromogen reagent is yielding the expected positive result for dried human blood. A new batch of Takayama hemochromogen reagent that does not yield a positive reaction with a KP blood control is not assigned a unique identifier (i.e., batch number or barcode) and must not be used for casework.

6.1.2 A negative test result (i.e., no characteristic crystals) for the KN blood control establishes that the new batch of Takayama hemochromogen reagent is not itself yielding a positive result. A new batch of Takayama hemochromogen reagent that yields a positive reaction with a KN blood control is not assigned a unique identifier (i.e., batch number or barcode) and must not be used for casework.

6.2 If the expected results for both the KP and KN blood controls are obtained using the new batch of Takayama hemochromogen reagent, that preparation of Takayama hemochromogen reagent may be assigned a unique identifier (i.e., batch number or barcode) and may be used for casework.

7 Calculations

Not applicable.

8 Measurement Uncertainty

Not applicable.

9 Limitations

9.1 While a negative Takayama hemochromogen test indicates that no blood was detected in a stain, the failure to detect blood in biological material is not the basis for a conclusive determination that blood is not present. False-negative test results (i.e., no characteristic crystals when blood is present) may be obtained in the presence of blood when it is present in a quantity below the detection limit of the Takayama hemochromogen test.

9.1.1 A false-negative test result may be obtained from a stain that fails to efficiently solubilize into the Takayama hemochromogen reagent (e.g., generally older stains that have dried to the point of having lost their layer of molecular hydration). The temperatures and incubation times used in these procedures were optimized to facilitate pyridineferroprotoporphyrin crystal formation in the types of bloodstains encountered in forensic casework.

9.1.2 The sensitivity (i.e., detection limit) of the Takayama hemochromogen test procedure described here has not been empirically determined in the Laboratory; however, based on the nature of the test, this procedure is expected to be less sensitive than the phenolphthalein test.

10 Safety

10.1 All evidence containing or contaminated with blood or other potentially infectious materials will be considered infectious regardless of the perceived status of the source individual or the age of the material. All DNA personnel who work with such material will follow the

"Bloodborne Pathogen (BBP) Exposure Control Plan (ECP)" found in the most current version of the *FBI Laboratory Safety Manual*.

10.2 Refer to "Safe Work Practices and Procedures," "Bloodborne Pathogen (BBP) Exposure Control Plan (ECP)," "Personal Protective Equipment Policy," and "Chemical Hygiene Plan" sections of the *FBI Laboratory Safety Manual* for important personal safety information prior to conducting these procedures.

10.3 Refer to the "Hazardous Waste Disposal" section of the *FBI Laboratory Safety Manual* for important information concerning proper disposal of the chemicals used in these procedures as well as the biohazardous wastes generated. Unused Takayama hemochromogen reagent, as well as slides that contain the reagent, must be handled and disposed of as a regulated hazardous material.

10.4 Procedural Specific Chemical Hazard: Sodium Hydroxide can be hazardous. Wear appropriate protective clothing and eyewear; be careful not to expose face or hands to splashes. A rapid release of heat can be produced when dissolving sodium hydroxide pellets.

11 References

FBI Laboratory Quality Assurance Manual (QAM)

FBI Laboratory Safety Manual

DNA Procedures Manual

Camps, F.E., editor. *Gradwohl's Legal Medicine*. Baltimore: Williams and Wilkins, 1968.

Gaensslen, R.E. *Sourcebook in Forensic Serology, Immunology, and Biochemistry.* U.S. Department of Justice, National Institute of Justice, Washington, D.C., 1983.

Hatch, A.L., A Modified Reagent for the Confirmation of Blood, *Journal of Forensic Sciences,* (1993) 38(6):1502-1506.

Lee, H. C. Identification and grouping of bloodstains. Saferstein, R., ed., In: *Forensic Science Handbook.*, Prentice-Hall, 1982; 267-337.

Rev. #	Issue Date	History
5	04/24/13	Changed “pink, feathery and branched, rhomboid crystals” to “characteristic crystals” and “filter purified” water to “reagent grade” water throughout document. 3: Removed equipment: autoclave, filter purified water, bleach. Changed “Hotplate/stirrer” to “Stirrer/Hot Plate”. Added purchased water throughout. 4.1: Changed “lot” to “batch” throughout. 4.1.1: Removed directions to make KP. User is directed to SOP 106. 7.1: Updated reference to SOP 100. 7.2.2: Revised for clarification and added a footnote #1 to remind the user that the KP used to evaluate the Takayama reagent may be different from the one used to evaluate the Phenophthalin reagent. 7.3: Footnotes regarding glass containers, expiration information, and autoclaving were deleted and renumbered remaining footnotes. Added that volumes can be adjusted and that purchased reagents may be substituted. 7.3.1: Removed directions to make 10% bleach solution. Renumbered remaining sections. 7.3.2: Revised for consistency with SOP 200. 7.3.4: Removed directions to make cotton swatches and directed the user to SOP 117. 7.4.3: Removed “slide warmer” since a hot plate is usually used. 7.4.6: Added “characteristic red or salmon-pink”. 7.4.8: Added that an FE should be consulted for an INC result. 7.4.9: Added “generally twice”. 7.5: All reporting procedures were removed from this SOP. The user is directed to SOP 100 for reporting language. Footnote #8 of 10.1: Updated reference in footnote to SOP 106. 10.2.1: Changed “consume a stain in its entirety” to “consume the majority of a stain”. 11.3: Added that slides with Takayama reagent also disposed with hazardous material. 12: Deleted Miscellaneous Procedures Manual reference.
6	05/25/16	Removed nDNAU throughout. Changed to DCU. Added BAU. Updated references to DNA SOPs. Simplified entire procedure. Added sample selection information. Relocated 4.2 through 4.2.2 from limitations. Relocated reagent QC to end of procedure. 9.1.1: Moved from footnote.

Approval

Redacted - Signatures on File