# DNA
# Procedures for the Presumptive Testing of Blood

## 1 Scope

These procedures apply to DNA personnel that screen evidentiary items in the DNA Casework Unit (DCU) for the possible presence of blood.

## 2 Equipment/Materials/Reagents

- Swabs, Puritan or equivalent
- Water, Reagent Grade or equivalent
- Known Positive (KP) blood control, dilute blood
- Phenolphthalin solution
- Hydrogen peroxide ($H_2O_2$), 3% solution

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

## 3 Standards and Controls

The phenolphthalin solution and hydrogen peroxide must be tested prior to first daily use on evidentiary items to verify the continued detection efficacy. A known positive (KP) and known negative (KN) must be tested by the biologist, each day, prior to using the phenolphthalin test reagents for casework.

A KP blood control is a sample of dried human blood. A KP prepared with diluted blood (generally diluted to 1:250 with water) will be tested following the procedures in this document. The diluted KP is used to reestablish the necessary swabbing pressure and the expected test result for less concentrated stains. Internal studies indicate that blood diluted to 1:250 was consistently detected using these procedures. A neat blood sample may be tested to demonstrate the expected color changes that occur with a concentrated blood stain but is not required to be tested each day. A clean swab will be tested as the KN blood control. Aliquot(s) of the phenolphthalin reagents that do not yield a positive reaction (i.e., a distinct pink color change) with a KP blood control or that yield a positive reaction with a KN blood control must not be used for casework.

## 4 Sampling

Items with an indication that blood may be present (e.g., red-brown staining, scenario information), may be tested using the phenolphthalin test. Any area of potential staining will be spot tested and, at minimum, areas that test positive will be described in the case notes.

## 5 Procedures

Refer to DNA Procedures Introduction (i.e., 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.

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| **5.1** | Using a new, clean swab moistened to dampness with reagent grade water, rub the stained area until a visible amount of stain has been transferred to the swab, or the swab appears matted. | |
| **5.2** | Add ~1-3 drops of phenolphthalin solution to the swab. | |
| **5.3** | Observe the swab tip for any color change for ~3 seconds. | |
| **5.3.1** | If no color change is observed, continue with the procedure. | |
| **5.3.2** | If a pink color is observed, the procedure should be stopped at this step.<br>Record this result as inconclusive (INC).<br>Consult an Examiner prior to conducting any additional testing. | |
| **5.3.3** | If an unexpected non-pink color is observed, the procedure should be stopped at this step.<br>Record this result as INC.<br>Consult an Examiner prior to conducting any additional testing. | |

**Note:** Heavily saturated blood stains are known to turn greenish gray after addition of the phenolphthalin solution.

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| **5.4** | Add ~1-3 drops of 3% hydrogen peroxide solution to the swab | |
| **5.5** | Observe the swab for a color change within 10 seconds.<br>Record the test results as listed below:<br>The observation of a pink color… Positive (POS)<br>The observation of no color… Negative (NEG)<br>The observation of a non-pink color… Inconclusive (INC) | |

Generally, the color change will occur instantly. Due to oxidation, the swabs used for negative stains may turn pink after ~2 minutes. It is generally expected that most swabs inoculated with both phenolphthalin solution and 3% hydrogen peroxide solution may display a pink color within approximately 30 minutes of their exposure to air.

The presence of a non-pink color could mask any potential pink color that would result from the presence of blood. If such an observation is made, an Examiner should be consulted prior to conducting any additional testing.

The reporting language used for the results from this testing and others is contained within the appropriate procedure (i.e., Sero 100) in the *DNA Procedures Manual.*

## 6 Reagent Quality Control

**6.1** Each new batch of phenolphthalin solution and 3% hydrogen peroxide solution will be tested for efficacy at the time of its preparation using the analytical procedure. The phenolphthalin solution and 3% hydrogen peroxide may tested concurrently or independently with an in use lot of the counterpart reagent.

**6.1.1** A positive test result (i.e., a pink color) for the KP blood control establishes that the new batch of phenolphthalin solution and/or 3% hydrogen peroxide solution is yielding the expected positive result. A new batch of phenolphthalin solution and/or 3% hydrogen peroxide solution that does not yield a positive reaction with a KP blood control must not be used for casework.

**6.1.2** A negative test result (i.e., no color) for the KN blood control establishes that the new batch of phenolphthalin solution and/or 3% hydrogen peroxide solution is not itself yielding a positive result (i.e., a pink color) in the absence of blood. A new batch of phenolphthalin solution and/or 3% hydrogen peroxide solution that yields a positive reaction (i.e., a pink color) with a KN blood control 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 phenolphthalin solution and/or 3% hydrogen peroxide solution, that preparation of phenolphthalin solution and/or 3% hydrogen peroxide solution may be used for casework.

## 7 Calculations

Not applicable.

## 8 Measurement Uncertainty

Not applicable.

## 9 Limitations

**9.1** A positive reaction with the phenolphthalin test provides a presumptive indication that blood may be present on an item but does not constitute an identification of blood. A confirmatory testing procedure (i.e., Takayama) is required to identify the presence of blood in a questioned stain.

**9.2** A positive phenolphthalin test is not required for the identification of blood. The utility of the phenolphthalin test is to determine the stain(s) that may be blood, so that further testing (e.g., confirmatory test, DNA testing) can be focused on those stains most likely to yield additional information.

**9.3** For the phenolphthalin test to be considered positive, a pink color must be observed within 10 seconds of the addition of the 3% hydrogen peroxide solution. Due to the nature of this chemical reaction (i.e., reduction-oxidation), any pink color observed after this period may be mediated by other oxidizing agents (e.g., oxygen).

**9.4** Should the color of an item preclude the interpretation of the phenolphthalin test, or should the test give an inconclusive result, a confirmatory testing procedure may be used to identify the presence of blood in a questioned stain.

**9.5** While a negative phenolphthalin test indicates that no blood was detected in a stain, the failure to detect blood is not the basis for an absolute determination that blood was not present. Negative test results may be obtained in the presence of blood when it is present in a quantity below the detection limit of the phenolphthalin test.

**9.6** Presumptive blood testing should not be conducted on areas of items with potential value for latent print examination.

## 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 the "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 this procedure.

**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 this procedure as well as the biohazardous wastes generated.

**10.4** Procedural Specific Chemical Hazards. The phenolphthalin solution contains the following:

- Ethyl alcohol can be hazardous. Wear appropriate protective clothing and eyewear when handling ethyl alcohol. Be careful not to expose face or hands to splashes.
- 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.
-Zinc is flammable. Avoid exposure to open flames or sparks.

## 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).

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

| Rev. # | Issue Date | History |
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| 7 | 05/25/16 | Changed nDNAU to DCU or BAU and updated references to DNA procedures.<br>Simplified entire procedure.<br>2 and 3: KP will be a dilute blood sample. Testing by biologists has demonstrated up to 1:250 consistently yielded positive results.<br>Removed reagent preparation info now contained in QA SOP.<br>Moved reagent QC to Section 6.<br>4: Added information to sample selection.<br>5.5: Removed FT POS result in 10-15 second option.<br>9: Revised limitations section.<br>10.4: Added zinc hazards |
| 8 | 02/03/20 | 1 Updated scope<br>2 Updated swab supplier<br>3 Added option to test neat blood<br>5.3.3 Revised to unexpected and added note for expected color change with heavily saturated blood stains<br>Changed phenolphthalein to phenolphthalin throughout. |

**Approval** Redacted - Signatures on File

DNA Technical Leader Date: 01/31/2020

DCU Chief Date: 01/31/2020