



Forensic Chemistry Section

Identification of Blood Method

1. Scope

This document outlines the methods for examining various items for the presence of blood.

2. Safety

- 2.1 Disposable lab coats and disposable gloves will be worn during reagent preparation and handling and during the examination of evidence.
- 2.2 Reagents will be prepared in a laboratory total exhaust hood.
- 2.3 Safety glasses and disposable face masks will be available for use when heavily blood stained items are being processed.
- 2.4 Ortho-Tolidine is a carcinogen and must be handled accordingly.

3. Presumptive Chemical Testing

- 3.1 Prior to evidence examinations, the bench top will be cleaned with a minimum of 10% bleach solution, followed by alcohol and lined with clean brown paper.
- 3.2 Potential bloodstains will be tested with ortho-Tolidine / Sodium Perborate. The reagents will be prepared as follows:
 - 3.2.1 Glacial acetic acid is mixed 1:1 with reagent water (50% solution). The total volume of the solution is 360ml.
 - 3.2.2 180ml of the 50% solution is added to each of two beakers.
 - 3.2.3 Approximately 4g of ortho-Tolidine dihydrochloride is added to one beaker. The solution is allowed to stir for several hours. **Do not heat.**
 - 3.2.4 Approximately 10g of sodium perborate is added to the second beaker. The solution is allowed to stir for several hours. **Do not heat.**
 - 3.2.5 The above constituent amounts may be varied as long as the final concentrations remain the same.
 - 3.2.6 The stock solution is tested with both positive and negative controls.
 - 3.2.7 The solutions are distributed in individual amber dropper bottles. Each bottle is labeled with the name of the chemical, lot number, and expiration date.



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- 3.2.8 The dropper bottles should be retained in the hood for several hours prior to placing on lab benches. The lid of the sodium perborate should be left loosely closed.
- 3.2.9 These reagents expire two months from the date of preparation.
- 3.3 Positive and negative controls are conducted on a daily basis prior to performing casework.
- 3.4 If inappropriate results are obtained from the control, the examiner will make changes to ensure that, before beginning casework, positive and negative controls react appropriately.
- 3.5 The examiner will screen an item of evidence for the presence of blood using the following method:
 - 3.5.1 A visual examination is conducted first to identify any potential bloodstains, flakes, crusts, etc.
 - 3.5.2 Any potential bloodstains, flakes, etc. will be documented through notes, sketches, and/or photographs.
 - 3.5.3 If the examiner is unable to visually see a stain or sample, a stereomicroscopic examination or an ortho-tolidine general sweep may be conducted.
 - 3.5.4 The examiner will use an unused piece of filter paper or a cotton tip swab to rub the sample to transfer the suspected blood to the filter paper or the swab. Ortho-Tolidine, then sodium perborate, are added to the filter paper in a 1:1 ratio. If the suspected blood sample was transferred to a swab, the swab will be tested indirectly using filter paper as described.
 - 3.5.5 A positive reaction results in an instant blue green color. A negative reaction results in no color change or a color change other than the instant blue green.
4. **Confirmatory Testing**
 - 4.1 If a sample reacts positively with the presumptive test, a cutting, extract, or crust may be tested for confirmation of human blood by using Abacus Diagnostics HemaTrace™. If the examiner believes that confirmation testing will consume the sample, no confirmation will be conducted. The examiner may submit the sample to the Forensic Biology Section for confirmation and/or DNA analysis.



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- 4.2 HemaTrace™ may also be performed using the supernatant from PSA / sperm cell extractions, if necessary. If samples are to be extracted in TE buffer for HemaTrace™ testing, positive and negative controls will be conducted with each combination of HemaTrace™ and TE buffer lots to be used. This check must be completed and documented prior to being used on casework.
- 4.3 Reagent water will be used whenever water is needed.
- 4.4 Confirmation with HemaTrace™ is performed as follows:
 - 4.4.1 A small amount of stain is placed in the extraction buffer provided with the kit. Sample size will depend on the concentration of the suspect stain. However, approximately 1mm² or one red brown stained thread is generally appropriate.
 - 4.4.2 Extraction time may be varied depending on stain concentration. However, a negative result will only be reported after a stain has been allowed to extract for a minimum of 30 minutes.
 - 4.4.3 Two (2) drops or a volume of 80uL of the extract are added to the sample well, marked 'S', of the test card.
 - 4.4.4 Two lines, at the 'C' and 'T' areas, indicate a positive result. This indicates that higher primate hemoglobin is present in the stain at the level of 0.05 µg/ml or above.
 - 4.4.5 Positive results may be read as early as 2 minutes. A card that appears to be negative must be allowed to react for 10 minutes. The test card cannot be read after 10 minutes.
 - 4.4.6 A negative result is indicated by one line, at the 'C' area. A negative result indicates that no higher primate blood is present or that the blood is below 0.05µg/ml.
 - 4.4.7 Extremely concentrated samples have been known to cause false negatives known as 'High Dose Hook Effect'. Any extract which exhibits heavily stained buffer and produces negative results should be diluted 1:100 and retested.
 - 4.4.8 If no pink line develops in the 'C' area of the test device, the test is invalid and must be repeated. Also, if the results from the human positive control or the



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negative control are erroneous, the controls and the associated samples must be re-tested.

- 4.5 For aged stains, an alternative extraction may be used:
 - 4.5.1 The stain will be soaked in 2 to 3 drops of 5% ammonia for approximately 2 to 5 minutes.
 - 4.5.2 The ammonia will be allowed to evaporate.
 - 4.5.3 Several (8 to 10) drops of the kit provided extraction buffer will be added.
 - 4.5.4 The pH of the resultant liquid must be between 1 and 9. The pH will be checked with commercially available test strips.
 - 4.5.5 The extract may be added to the sample well immediately
- 4.6 If a sample is confirmed as human blood, it may be transferred to the Forensic Biology Section for DNA analysis.
- 4.7 If the examiner believes that the age or condition of the stain is producing false negative results, the examiner may submit the sample directly to the Forensic Biology Section.