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.

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.

4.2 HemaTrace™ may also be performed using the supernatant from PSA / sperm cell extractions, if necessary. If the supernatant is to be used, positive and negative controls (extracted in TE buffer) will be conducted prior to performing daily 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 1mm^2 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 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.