# Procedure for Blood Analysis

**1.0 Purpose** - This procedure specifies the methods for performing blood analysis in forensic casework.

**2.0 Scope** - This procedure applies to those Forensic Scientists who have been released, to the extent of their training, to do blood analysis in forensic casework.

**3.0 Definitions** - N/A

## 4.0 Equipment, Materials and Reagents

- Ethyl alcohol, 200 proof, anhydrous, 99.5+%
- Phenolphthalein (see QC procedure)
- 3 % hydrogen peroxide ($H_2O_2$) (see QC procedure)
- Whatman 55 mm filter papers/swabs
- Known blood stain
- Sterile disposable scissors or scalpel blade
- RSID kits which contain the test cards and universal buffer
- 1.5 mL centrifuge tube

## 5.0 Procedure

### 5.1 Kastle-Meyer Test

**5.1.1** The Kastle-Meyer Test (KM) shall be performed on stained areas that appear reddish brown in color. If multiple stains are present, the Forensic Scientist shall determine which stains shall be tested based upon his/her training and experience. If the item is light in color so that bloodstains could be seen easily, but no stains are seen with visual inspection, then no further testing is needed and shall be documented in the notes as such. If blood examination is requested and the item is colored or patterned in such a way as to obscure the bloodstain from being visualized easily, a general rubbing shall be made of the entire item and tested. If this general rubbing is negative, pictures are not required to be taken and notes shall reflect that a general rubbing was made and the item was negative. If the general rubbing is positive, but no visible stain can be located, the notes in FA shall reflect that a general rubbing was positive, but no stains were isolated. A photograph shall be taken of the item to show the general area that tested positive. If latent or touch DNA has been requested or ridge detail is noted, swabbing the item could compromise possible prints or DNA. In this situation, only a visual examination shall be done on this item.

**5.1.2** Each stain that is tested shall be numbered and a photograph shall be taken to show the area(s) that were examined. The only exception is stained swabs. The results of each area tested shall be noted in FA as positive or negative.

**5.1.3** Rub the area to be tested with a folded piece of filter paper or a clean cotton swab.

**5.1.4** Add the following reagents onto the filter paper or swab in order:

- 1-2 drops of ethanol (cover rubbed area).
- 1-2 drops of phenolphthalein (cover rubbed area).
- 1-2 drops of 3 % $H_2O_2$ (cover rubbed area).

**5.1.5** If a rubbing from a suspected area tests negative for KM and the analyst believes, as a result of his/her training and experience that there could be interference from the substrate or that the sample is of a low concentration, then further testing shall be done. Take a small cutting with a sterile disposable scissors or scalpel blade and place on a filter paper. Add the chemicals directly to the sample.

**5.1.6 Results**

**5.1.6.1** A positive reaction is indicated by the development of a pink color within 5 seconds of adding the $H_2O_2$.

**5.1.6.2** A negative result is indicated if no color change occurs within 5 seconds after the addition of $H_2O_2$. In all situations, a color change will eventually occur after the addition of $H_2O_2$. This color change does not indicate a positive reaction; therefore, any color changes that appear after 5 seconds should not be interpreted as a positive result.

**5.1.6.3** An inconclusive result is indicated if a reaction occurs before the addition of the $H_2O_2$ or if color transfers from the item onto the filter paper or swab that interferes with the ability to note a color change. If this occurs, a RSID blood test shall be performed if enough material is present to perform this test.

**5.2 RSID-Blood Test**

**5.2.1 Procedure**

**5.2.1.1** Cut a small sample, approximately 0.5 $cm^2$ (depending on the concentration of the stain), from the evidence sample using sterile disposable scissors or a sterile scalpel blade and place the cutting into a 1.5 mL centrifuge tube.

**5.2.1.2** Add a minimum of 150 µL, up to 1 mL, of RSID universal buffer to each sample and mix well. (The amount of buffer added will depend on the sample size; buffer should cover the sample completely.)

**5.2.1.3** Allow the sample to extract for a minimum of 15 minutes. For weaker or older samples, Forensic Scientists should use a larger quantity of material and/or an extended extraction time to include overnight (not to exceed 24 hours).

**5.2.1.4** After completing the extraction process, pipette 100 µL of the extracted sample into the sample well on the RSID card.

**5.2.2 Results**

**5.2.2.1** A positive reaction will have two lines appear in the test window. One line will appear in the area marked "C" for control and one line will appear in the area marked "T" for test. A positive result may be recorded as soon as both of these lines appear, but after no longer than 10 minutes. The lines must be reddish in color.

**5.2.2.2** If a line does not appear in the "T" area within ten minutes, then the test is considered negative. A line must appear at the area marked "C" to ensure that the test is working properly.

**5.2.2.3** If no line appears at the area marked "C," the test shall be repeated. If no line is seen in the "C" window in the repeated test, the Body Fluid Technical Leader shall be notified as soon as possible. Refer to Forensic Biology Section Administrative Policy and Procedure.

**5.2.2.4** If visual observation fails to reveal a positive result, then based on the training and experience of the analyst, the RSID reader may be used to read the test card immediately following the 10 minutes.

**5.2.2.4.1** Turn on the RSID Reader using the power button. Note: The stylus must be used to make selection choices on the reader screen.

**5.2.2.4.2** Select RSID icon on the Main Menu screen.

**5.2.2.4.3** Select RSID-Blood on the Select Test Screen. Then select Run Tests.

**5.2.2.4.4** Using the alpha or numeric keys, enter the name of the sample. Select Done. Review the sample name. If name is correct, select Proceed. If name is incorrect, select Back and enter the correct name.

**5.2.2.4.5** Insert test card into the reader until the green status light is engaged. The Test Area of the cassette must enter the reader's port first and face out to ensure proper positioning. The message, "Analyzing Cassette. Please Wait," will appear on the screen. Both the positive and negative controls shall be read first, followed by the unknown card(s).
Note: Take care to insert the card completely and do not remove it prior to results being obtained by the reader.

**5.2.2.4.6** Read the results from the reader. These results are the results that shall be reported.

Note: The reader notes inconclusive as invalid. If the reader reports an invalid result, the test shall be repeated. If the repeated test is inconclusive (invalid), the Body Fluid Technical Leader shall be notified as soon as possible.

**5.2.2.4.7** Select Finish. Continue with 5.2.2.4.3 to continue testing any additional cards. When all cards are read, select the home icon.

**5.2.2.4.8** Turn the RSID Reader off using the power button.**5.2.3 Requirements for testing** - The RSID Blood Test shall be performed if human blood identification is requested specifically, species origin is in question, or the Kastle-Meyer test is inconclusive, as provided in **5.1.6.3**. The only exception would be if there is a limited amount of sample and DNA testing is requested.

**5.3 Reporting guidelines** – The results statements shall reflect only the work that is performed. Portions of the statements may be omitted to address the testing actually performed. This interpretation may include or build upon one (1) or more of the following responses depending on the circumstances of the case and the nature of the examination.

**5.3.1** This phrase shall be used when a negative result is indicated for a Kastle-Meyer Test.

Examination of a sample(s)/general rubbing(s) taken from_____ (Item(s) ___), using the Kastle-Meyer Test, failed to reveal chemical indications for the presence of blood.

**5.3.2** This phrase shall be used when a positive result is indicated for a Kastle-Meyer Test.

Examination of a sample(s)/general rubbing(s) taken from _____ (Item(s) ___), using the Kastle-Meyer Test, gave chemical indications for the presence of blood.

**5.3.3** This phrase shall be used when an inconclusive result is indicated for a Kastle-Meyer Test.

Examination of a sample(s)/general rubbing(s) taken from _____ (Item(s) ___), using the Kastle-Meyer Test, was performed. Due to possible interference of the substrate, no interpretable results were obtained.

**5.3.4** This phrase shall be used if no confirmatory blood testing was done.

No confirmatory blood testing was performed.

**5.3.5** This phrase shall be used if no confirmatory blood testing was done due to limited sample.

Due to the limited quantity of sample, no confirmatory blood testing was done.

**5.3.6** This phrase shall be used when the confirmatory RSID blood test yields a negative result:

Further examination of the sample(s) taken from _____ (Item(s) ____), using the RSID Blood Test (and the RSID reader), failed to reveal the presence of human blood.

**5.3.7** This phrase shall be used when the confirmatory RSID Blood Test yields a positive result:

Further examination of the sample(s) taken from _____ (Item(s) _____), using the RSID Blood Test (and the RSID reader), gave conclusive results for the presence of human blood.

**5.3.8** This phrase shall be used when the confirmatory RSID Blood Test reads invalid:

Further examination of the sample(s) taken from ____ (Item (___)), using the RSID Blood Test (and the RSID reader), failed to give conclusive results for the presence of human blood.

**5.4 Controls**

**5.4.1** Kastle-Meyer Controls: A known blood stain shall be used as a positive control and a clean filter paper or swab shall be used for a negative control. These controls shall be tested prior to analysis once each day this test is performed for each lot used and the results shall be recorded as positive or

negative in the case notes in FA for each case worked that day. The controls must react appropriately.

**5.4.2** RSID-Blood Controls: A positive control (applicable body fluid standard), and a negative control (100 µL of universal buffer) shall be run with every case or every batch of cases and the results will be recorded in the case notes as a positive or negative. If a reddish line is seen in the negative control "T" area, the test shall be rerun. If a reddish line appears again in the negative control "T" area, the test shall be considered inconclusive. If this occurs, the Technical Leader shall be notified immediately.

**6.0 Limitations –** Limitations include, but are not limited to, the following: The Kastle-Meyer test is a presumptive test for the presence of blood. There are some substances that will cross-react giving a false positive. If a stain yields a positive result and the analyst suspects the result may be a false positive based on training and experience, the area may be rubbed with filter paper and placed in an incubator at 100 °C for 30 minutes. The filter paper shall then be tested as indicated in **5.1.4.** The result of this test shall be recorded in the FA worksheet.

**7.0 Safety -** See Safety documents.

**8.0 References**

Forensic Biology Section Body Fluid training documents

Forensic Biology Section Procedure for Aseptic Technique and Contamination Control

Forensic Biology Section Procedure for Calibration and Maintenance

**9.0 Records -** N/A

**10.0 Attachments -** N/A

| **Revision History** | | |
|---|---|---|
| Effective Date | Version Number | Reason |
| 10/26/2012 | 1 | Original Document - Combined Procedure for Kastle-Meyer test and RSID-blood portion of the RSID Procedure; added reporting guidelines and allowed for changes to be made by the Forensic Scientist to address the testing actually performed; 5.2.3 added requirement to perform RSID-blood testing. |
| 02/01/2013 | 2 | 5.1.1 – added requirement for testing item with multiple stains present; 5.4.1 – added requirement for QC testing each lot used |
| 02/15/2013 | 3 | 5.3.4, 5.3.5 – changed "no further confirmatory" to "no confirmatory" 6.0 – clarified wording |
| 05/03/2013 | 4 | 5.3.3 – clarified wording |
| 09/13/2013 | 5 | 5.1.1, 5.1.5 – clarified when general rubbings for Kastle-Meyer testing is performed |

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|---|---|---|
| 12/18/2013 | 6 | Header – added issuing authority |
| 08/29/2014 | 7 | 5.2.1.3 – add maximum extraction time; 5.2.2.3 – clarified wording; 5.2.2.4 – added RSID-reader procedure; 5.3.8 – added inconclusive results statement for RSID blood |