SCREENING TEST FOR BLOOD

8.1 PURPOSE

8.1.1: To perform screening tests for the presence of blood in Forensic samples.

8.1.2: To prepare reagents for blood screening tests and to perform quality control on prepared reagents.

8.2 RESPONSIBILITY

8.2.1: Test Procedure – Forensic Science Examiners from the Division of Scientific Services who have been trained in the discipline of blood screening according to FB SOP-26 (Training Manual and Checklist).

8.2.2: Preparation/QC Procedure – Forensic Science Examiners in the Forensic Biology Unit. Ordering information is maintained in a log book in the Forensic Biology Unit. New chemicals and reagents are purchased according to GL-6 (Purchasing). For additional information, refer to the Biological Inventory in Appendix 2.

8.3 SAFETY

Use appropriate measures for the proper handling of biohazardous material, o-Tolidine solution, ethanol, glacial acetic acid and potassium hydroxide according to GL-2 (Safety Manual) and the Safety Data Sheets.

8.4 DEFINITIONS

KM: Kastle-Meyer

8.5 TEST PROCEDURE

These tests will be performed at the discretion of the examiner, with input from the Unit Lead(s), based on the submitting agency requests, case information and the condition of the evidence.

A. The KM test is conducted routinely. The o-Tolidine test may be used if the sample is limited/compromised and/or due to the color of the item being examined.

B. A sample is considered limited/compromised when it appears to be of low quantity and/or in poor condition. The conditions the evidence may have been exposed to prior to submission shall be considered when assessing the sample tested and/or collected.

C. If the case scenario indicates that a sample collected by the submitting agency (i.e. swabs) was previously treated with a field test, then the above tests may be omitted.

8.5.1: Materials

A. Phenolphthalin solution (for the KM test)
B. o-Tolidine solution
C. 3.0% Hydrogen peroxide
D. Controls: positive (known bloodstain) and negative (blank substrate)
E. dH_2O

F. 0.5% ammonia
G. Cotton swabs or spot plates

8.5.2: Procedure

A. Record the reagent(s) used on the General Reagent Sheet (FBQR-09) and the appropriate Quality Record Worksheet.

B. Test a positive control and negative control according to the following procedure (steps 8.5.2.C - 8.5.2.F) prior to testing the questioned sample. If using dH_2O for the testing of questioned samples, the dH_2O must be tested with the positive and negative controls.

1. If the controls yield the appropriate results, record on the appropriate Quality Record Worksheet and test the questioned samples.

2. If the controls do not yield the appropriate results, review the procedure and retest the controls prior to beginning analysis on casework samples. If the controls still do not yield the appropriate results, then inform the Unit Lead, determine the root cause and correct.

C. Prepare sample for testing as follows:
1. Moisten a cotton swab with dH_2O and lightly swab the questioned stain for testing.
2. Cut a piece of fabric, swab or other substrate for direct testing without dH_2O.
3. Scrape the stain from a hard surface for direct testing without dH_2O.
4. 0.5% ammonia may be used for testing samples that are difficult to remove.
   a. Both controls must be tested with 0.5% ammonia according to steps 8.5.2.B - 8.5.2.F prior to testing the questioned sample.

   b. If 0.5% ammonia is used, record on the appropriate Quality Record Worksheet and the General Reagent Sheet (FBQR-09).

D. Add one drop of phenolphthalin or o-Tolidine solution to the portion of questioned sample. Caution: Testing with o-Tolidine solution should be performed under the hood.

E. If no color change occurs, add one (1) drop of 3% H_2O_2.

F. Observe any color change within 10 seconds.

G. Record the results on the appropriate Quality Record Worksheet.

H. If the quantity of stain is insufficient to perform a confirmatory test, a positive chemical screening test is sufficient to forward the stain directly for DNA analysis.

8.5.3: Results and *Suggested Report Statements*

A. Positive

1. If blood or "other peroxidase-type material" is present, a color change will occur after the addition of 3% H_2O_2. Phenolphthalin (KM test) will turn pink; o-Tolidine will turn blue/blue-green.

2. Positive results may be designated and recorded as follows:
↓/vw(+) = very weak positive = very light pink or blue/blue-green
w(+) = weak positive = light pink or blue/blue-green
(+) = positive = pink or blue/blue-green
↑/s(+) = strong positive = dark pink or blue/blue-green

   Variation between examiners calling a positive result is acceptable since these are not critical designations and will not change downstream testing. The result will still be considered positive.

3. *[ ] gave a positive result(s) with a color screening test(s) for the presence of blood.*

B. Negative

A negative reaction will show no color change within 10 seconds of the addition of 3% H_2O_2, indicating no blood was detected.

A color screening test(s) for the presence of blood was/were performed on [ ]. Blood was not detected with this/these test(s).

C. Inconclusive

1. If a pink or blue/blue-green color change could not be determined after the addition of 3% H_2O_2 (i.e. when there is interference from the substrate).

   A color screening test(s) for the presence of blood was/were performed on [ ]. Due to indeterminate results and/or substrate interference, this/these test(s) was/were determined to be inconclusive.

2. Record the reason a result is determined to be inconclusive on the appropriate Quality Record Worksheet.

D. Failed

1. The appearance of a pink or blue/blue-green color change prior to the addition of 3% H_2O_2 indicates the presence of a chemical oxidant in the stain.

   A color screening test(s) for the presence of blood was/were performed on [ ]. Due to the failure of this/these test(s), no conclusion(s) is/are possible.

2. Record the reason the test failed on the appropriate Quality Record Worksheet.

8.6 PREPARATION/QC PROCEDURE

Manufacturer's expiration dates with only month and year indicated (i.e. 04/2014) expire the last day of the month noted.

8.6.1: Phenolphthalin Stock Solution

Materials

A.	Phenolphthalin	2g
B.	Potassium hydroxide	20g
C.	dH_2O	100ml
D.	Granular Zinc	20g

Procedure

A. Dissolve potassium hydroxide in dH_2O. Note: The solution will be warm from the reaction.

B. Add granular zinc followed by phenolphthalin. Solution turns pink immediately.

C. Swirl solution until it becomes colorless.

D. Place stock solution in a brown bottle containing zinc.

E. Discard after one (1) year or sooner if the colorless reagent turns pink.

8.6.2: Phenolphthalin Working Solution

Materials

A.	Phenolphthalin stock solution	1 part
B.	Ethanol	4 parts
C.	Granular Zinc	~15g

Procedure

A. Dilute stock solution 1:5 with ethanol (1 part plus 4 parts).

B. Place working solution in brown dropper bottles containing zinc.

C. Test each new batch of the working solution before use according to the test procedure and the KM Reagent Log Sheet. Record the required information.

D. If the appropriate results are not obtained, discard the reagent, review the procedure, make new reagent and retest. If the reagent still does not yield the appropriate results, then inform the Unit Lead, determine the root cause and correct.

E. If the reagent is suitable for use, record the solution, lot # (date of preparation), control date and examiner's initials on the stock and dropper bottles and store in the refrigerator.

F. Discard after six (6) months (not to exceed the control date of the stock solution) or sooner if the colorless reagent turns pink.

8.6.3: o-Tolidine Solution

Materials

A.	o-Tolidine	1.6g
B.	Ethanol	40.0ml
C.	Glacial acetic acid	30.0ml
D.	dH_2O	30.0ml

Procedure

A. Mix all materials together and place into a brown dropper bottle.

B. Test each new batch of reagent before use according to the test procedure and the o-Tolidine Reagent Log Sheet. Record the required information.

C. If the appropriate results are not obtained, discard the reagent, review the procedure, make new reagent and retest. If the reagent still does not yield the appropriate results, then inform the Unit Lead, determine the root cause and correct.

D. If the reagent is suitable for use, record the solution, lot # (date of preparation), control date and examiner's initials on the dropper bottle and store in the refrigerator.

E. Discard after one (1) year or sooner if the reagent changes from transparent beige to a dark brown color.

8.6.4: 3 % Hydrogen Peroxide

A. This chemical is purchased from an approved outside vendor.

B. Test the new manufacturer's lot before use in casework according to the test procedure and the KM and o-Tolidine Reagent Log Sheets. Record the required information.

C. If the appropriate results are not obtained, review the procedure and repeat the test. If the reagent still does not yield the appropriate results, then inform the Unit Lead, determine the root cause and correct.

D. If the lot is suitable for use, record the date received, date opened and examiner's initials on the stock bottles and store in the refrigerator.

E. Aliquot into dropper bottles labeled with the chemical, lot #, manufacturer's expiration date, fill date and initials on the dropper bottles.

F. Discard according to the manufacturer's expiration date or sooner if a decrease in reaction activity is observed while testing the controls.

8.6.5: 0.5 % Ammonia Solution

Materials

A. 5% Ammonia solution 1 part
B. dH_2O 9 parts
C. Dropper bottles (30ml)

Procedure:

A. Dilute the 5% ammonia solution 1:10 in dH_2O and place into a dropper bottle.

B. Test the diluted solution before use according to the test procedure above, FB SOP-11 (Rapid Immunoassay Tests for Human Blood) and the 0.5% Ammonia Reagent Log Sheet. Record the required information.

C. If the appropriate results are not obtained, discard the 0.5% ammonia solution, review the procedure make a new dilution and retest. If the reagent still does not yield the appropriate results, then inform the Unit Lead, determine the root cause and correct.

D. If the 0.5% ammonia is suitable for use, record the solution, lot # (date of preparation), control date and examiner's initials on the dropper bottles and store in the refrigerator.

E. Discard the 0.5% ammonia after six (6) months or earlier according to the 5% ammonia manufacturer's expiration date.

F. Discard the 5% ammonia according to the manufacturer's expiration date.

8.7 REFERENCES

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D. Ruttan,R.F. and R.H.M. Hardisty. A new reagent for detecting occult blood. Can. Med. Associat. J. Vol.41(n.s.2), 1912, pp. 995-998.
E. Metropolitan Police Forensic Science Laboratory. Biology Methods Manual. 1978, pp. 2-88 to 2-90.
F. GL-2 (Safety Manual)
G. GL-6 (Purchasing)
H. Safety Data Sheets