SCREENING TESTS FOR BLOOD

8.1 PURPOSE

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

A. Theory

1. The Kastle–Meyer (KM) test is a chemical screening test for the presence of blood in which the chemical indicator phenolphthalein is used to detect the possible presence of hemoglobin. In the presence of an oxidant (H_2O_2), phenolphthalein relies on the peroxidase-like activity of the heme component of hemoglobin in blood to catalyze phenolphthalin (colorless reduced form) into phenolphthalein (oxidized form), which is visible as a bright pink color.

2. The o-Tolidine chemical screening test for the presence of blood is similar to the KM test. The o-Tolidine test also relies on the peroxidase-like activity of the heme component of hemoglobin in blood to catalyze the solution's transparent beige color (reduced form) to a blue/blue-green color (oxidized form) in the presence of an oxidant (H_2O_2).

B. Limitations

1. Chemical oxidants will cause a color change before the addition of the hydrogen peroxide. Therefore, it is important to employ the two-step method.

2. False positive results may occur if the result is not read within 10 seconds.

3. False positive results may occur in the presence of plant peroxidases.

4. These tests can neither determine nor detect the difference between human blood and animal blood.

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 (however titled) 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 (Appendix 3) located in the FB folder on the shared drive.

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

A. KM: Kastle-Meyer
B. o-Tol: o-Tolidine
C. QRW(s): Quality Record Worksheet(s) (Appendix 1)

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 being examined.

B. A sample is considered limited when it appears to be of low quantity and compromised when it appears to be 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. For previously tested items (examples: field tested, cold case samples, Latent Print blood enhancement reagent):
   1. Swabs: Blood screening tests may be omitted.
   2. Other evidence:
      a. If the item of evidence has visible reddish-brown stains, then blood screening tests may be performed.
      b. If an area is designated as positive on an item of evidence and no reddish-brown staining is visible, then collect a swabbing of the indicated area. Do not perform blood screening tests on the swabbing. Forward the swabbing for DNA analysis.
   3. Evidence previously examined by the Latent Prints Unit, see FB SOP-03 Section 3.4.2.

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 appropriate QRW.

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 QRW 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 to try to determine the root cause.

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 in place of dH_2O, record on the appropriate QRWs.

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 QRW.

H. If the quantity of stain is insufficient to perform a confirmatory test, or the case scenario does not warrant further serological testing, a positive chemical screening test is sufficient to forward the stain directly for DNA analysis.

8.5.3: Results and Conclusions

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

The description of a positive result may vary between examiners. This variation is acceptable since all descriptions designate a positive result.

Recording the description of a positive result may be helpful in interpreting the result(s) obtained from additional serological testing and/or DNA analysis.

3. Suggested Report Wording:
a. When no blood confirmatory test conducted:

Testing Performed	**Results**	**Conclusion**
Screening – Blood	Positive	Blood indicated[1]

Appendix:
[1]No further serological testing was conducted at this time.

b. When confirmatory test conducted:

Testing Performed	**Results**	**Conclusion**
Screening – Blood	Positive	Blood indicated

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

B. Negative

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

2. Suggested Report Wording:
a.

Testing Performed	**Results**	**Conclusion**
Screening – Blood	Negative	Blood not detected

b. *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).

2. Suggested Report Wording:
   a.

Testing Performed	Results	Conclusion
Screening – Blood	Indeterminate	Inconclusive[1]

Appendix:
[1]Due to an indeterminate result and/or substrate interference, this test was determined to be inconclusive.

   b. 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.

3. Record the reason a result is determined to be inconclusive on the appropriate QRW.

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.

2. Suggested Report Wording:
   a.

Testing Performed	Results	Conclusion
Screening – Blood	Failed Test	No conclusion possible

   b. 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 QRW.

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

The control date will be one (1) year from the date of preparation.

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 on the control date, 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

The control date will be six (6) months from the date of preparation.

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 to try to determine the root cause.

E. If the reagent is acceptable 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.

The KM reagent is acceptable for use when a positive result is obtained with the blood control and a negative result is obtained with the blank/negative control.

F. Discard on the control date (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

The control date will be one (1) year from the date of preparation.

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 to try to determine the root cause.

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

The o-Tol reagent is acceptable for use when a positive result is obtained with the blood control and a negative result is obtained with the blank/negative control.

E. Discard on the control date, 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 to try to determine the root cause.

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

The 3% Hydrogen Peroxide is acceptable for use with KM and o-Tol reagents when a positive result is obtained with the blood control and a negative result is obtained with the blank/negative control.

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)
The control date will be six (6) months from the date of preparation.

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 to try to determine the root cause.

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

The 0.5% ammonia must be tested with KM, o-Tol and HemaTrace tests and found acceptable with each test, before placing into service.

a. The ammonia is acceptable for use with KM and o-Tol reagents when, with each test, a positive result is obtained with the blood control and a negative result is obtained with the blank/negative control.
b. The 0.5% ammonia is acceptable for use with HemaTrace when a positive result is obtained with an extract made from the blood control and a negative result is obtained with an extract made from the blank/negative control (see FB SOP-11 RIAs for blood).

E. Discard the 0.5% ammonia on the control date, 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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