CRM343
Experiment A: Presumptive Blood Testing

The purpose of this lab is to give you practical experience in the method of applying various presumptive blood testing kits to suspected stains. Based on the reactions you observe, you will be able to categorize each stain as one of the following:

a) One that indicates blood may be present (a positive reaction);
b) One that gives no indication for the presence of blood (a negative reaction); and
c) One that gives a *false-positive* reaction.

Upon completion of the experiments, you will record the outcome of each in the results chart below and as well as a two-page report documenting your observations and whether the tests were effective. Submit the assignment to the Dropbox and Turnitin.com **no later than Sunday 11:59 PM EST/EDT.**

Caution! The following presumptive chemicals can be dangerous. Please follow all safety instructions and/or MSDS information. These chemicals should not be swallowed or inhaled; they may also damage or stain clothing.

Supplies from your CRM343 bodily fluids kit:

- Phenolphthalein (KM) reagent presumptive test kit
- Tetramethylbenzidine (TMB) reagent presumptive test kit
- Leucocrystal violet (LCV) reagent presumptive test kit
- Synthetic blood sample (1)
- Cotton tip swab/applicators
- Gloves
- Face mask

Supplies from home:

- Red craft paint
- Fresh mashed potato (with skin)
- Cooked tomato sauce
- Red food coloring
- Fresh raw beet juice

Remember that all of these tests are a multi-step process, with a waiting period between steps to allow for a false-positive to occur (if it is going to). Make certain you read the instructions first before beginning the testing process to understand how each test will work. You can refer back to the textbook for a quick primer on why presumptive tests are conducted.

NOTE: While you may not have enough of the reagents in your criminalistics kit to do so, on the job training will advise that you should always test the reagents before using the test on the evidence.

These tests have a reagent and hydrogen peroxide, an oxidant that gives off a free-radical oxygen in the presence of the heme portion of blood. Most likely, a false positive would occur **between** the *reagent* being applied and the *hydrogen peroxide* being applied.

Tips

- **Avoid cross contamination!** Do not touch the dropper bottle tips to the swabs at any time. Do not use the same swab on different samples.
- Do not apply the reagents directly onto the evidence or onto the swab which you are holding over the evidence; you could potentially contaminate the evidence if the reagents should drip from the swab tip.
- If needed, use de-ionized or distilled water to moisten the swab tip in order to better transfer some of the suspect stain.

- Normally, when deciding which item/area to test, it is generally best to test a peripheral area of the stain. This allows the main (or concentrated) area of the stain to remain undisturbed for any future lab testing.
- **If you want to "save" or store your test swabs, you can use the swab boxes contained in your criminalistics kit to preserve the samples. Over time, exposure to ambient air will discolor the swabs.**

Phenolpthalein (KM) presumptive pest kit instructions:

- Use the *known bloodstain control* card to test the reagents **prior** to testing the evidence stains. Ideally, this control test should yield a positive reaction and is called a *positive control.*
    - To conduct the *positive control* test, you will follow the directions below, getting the listed result. Then you would repeat these steps to conduct the actual presumptive tests on the samples listed in the chart.
- First, use a cotton tip swab to "retrieve" a sample of the suspected reddish stained sample. If the sample stain is dry, you can moisten the cotton tip swab with the distilled water from your kit.
- Then, add one drop of alcohol to the swab with the suspected stain. Remember to not add the reagent to the swab *over* the evidence; twist to the side as you squeeze a drop of the reagent onto the swab tip.
    - Observe any color changes. If you get a pinkish color developing on the swab tip, you might have a contaminated reagent.
- Next, add a drop of the phenolphthalein reagent to the swab tip ~ it may be necessary to clear the first drops from the reagent bottle to remove any oxidized solution from the tip.
    - Observe any color changes. If you get a pinkish color developing on the swab tip, you might have a **false positive** result or "bad" test.
- Finally, add one drop of hydrogen peroxide to the swab tip.
    - Blood is most likely present/indicated if a **bright pink color** is observed within several seconds. If a pink color develops after the addition of the phenolphthalein reagent but *before* the addition of the hydrogen peroxide, this indicates that a contaminant is present and the test should be considered invalid.
    - The possibility also exists that you can have a **false negative** result, meaning that blood was actually present in the sample but the concentration may be diluted or compromised, thus not producing a color reaction.
- *Note It is suggested that an area adjacent to the sampled evidence stain be tested using the same procedure described above. This test should yield a negative reaction and is called a negative control.*

Leucocrystal Violet (LCV) presumptive pest kit instructions:

- Use the *known bloodstain control* card to test the reagents **prior** to testing the evidence stains. Ideally, this control test should yield a positive reaction and is called a *positive control*.
    - To conduct the *positive control* test, you will follow the directions below, getting the listed result. Then you would repeat these steps to conduct the actual presumptive tests on the samples listed in the chart.
- First, use a cotton tip swab to "retrieve" a sample of the suspected reddish stained sample. If the sample stain is dry, you can moisten the cotton tip swab with the distilled water from your kit
- Then, add one drop of LCV reagent to the swab with the suspected stain. Remember to not add the reagent to the swab *over* the evidence; twist to the side as you squeeze a drop of the reagent onto the swab tip.
    - If a bright blue/purplish color develops after the addition of the LCV reagent but **before** the addition of the hydrogen peroxide, this indicates that a contaminant is present and the test should be considered invalid (also sometimes known as a "false positive" reaction)
- Next, add one drop of hydrogen peroxide to the swab tip.
    - Blood is most likely present/indicated if a **bright blue/purplish color** is observed within several seconds. The possibility also exists that you can have a **false negative** result, meaning that blood was actually present in the sample but the concentration may be diluted or compromised, thus not producing a color reaction.

- *Note: It is suggested that an area adjacent to the sampled evidence stain be tested using the same procedure described above. This test should yield a negative reaction and is called a negative control.*

Tetramethylbenzidine (TMB) presumptive pest kit instructions:

- Use the *known bloodstain control* card to test the reagents **prior** to testing the evidence stains. Ideally, this control test should yield a POSITIVE reaction and is called a *positive control*.
    - To conduct the *positive control* test, you will follow the directions below, getting the listed result. Then you would repeat these steps to conduct the actual presumptive tests on the samples listed in the chart.
- First, use a cotton tip swab to "retrieve" a sample of the suspected reddish stained sample. If the sample stain is dry, you can moisten the cotton tip swab with the distilled water from your kit.
- Then, add one drop of TMB reagent to the swab with the suspected stain. Remember to not add the reagent to the swab *over* the evidence; twist to the side as you squeeze a drop of the reagent onto the swab tip.
    - If a bright blue/greenish color develops after the addition of the TMB reagent but before the addition of the hydrogen peroxide, this indicates that a contaminant is present and the test should be considered invalid (also sometimes known as a "false positive" reaction)
- Next, add one drop of hydrogen peroxide to the swab tip.
    - Blood is most likely present/indicated if a **bright blue/greenish color** is observed within several seconds. If a bright blue/purplish color develops after the addition of the TMB reagent but BEFORE the addition of the hydrogen peroxide, this indicates that a contaminant is present and the test should be considered invalid (also sometimes known as a "false positive" reaction).
    - The possibility also exists that you can have a **false negative** result, meaning that blood was actually present in the sample but the concentration may be diluted or compromised, thus not producing a color reaction.
- *Note: It is suggested that an area adjacent to the sampled evidence stain be tested using the same procedure described above. This test should yield a negative reaction and is called a negative control.*

Results Chart

Sample	Interpretation			Test Used		
Red paint	Positive	False Positive	No Reaction	KM	LCV	TMB
Fresh potato (smashed)	Positive	False Positive	No Reaction	KM	LCV	TMB
Cooked tomato sauce	Positive	False Positive	No Reaction	KM	LCV	TMB
Red food coloring	Positive	False Positive	No Reaction	KM	LCV	TMB
Fresh, raw beet	Positive	False Positive	No Reaction	KM	LCV	TMB
Blood Sample from Kit	Positive	False Positive	No Reaction	KM	LCV	TMB

Tests Used

KM	Phenolphthalein (Kastle-Meyer)
LCV	Leuco-Crystal Violet
TMB	Tetramethylbenzidine

LCV	Phenolphthalein	TMB