DNA Analyst Training
Forensic Biology Screening Workshop

Protocol 2.19
Tetramethylbenzidine Presumptive Test for Blood

This laboratory protocol (or part thereof) has been provided as an example of a laboratory SOP, courtesy of the National Forensic Science Technology Center. It has been included for training and example purposes only.

PRESIDENT'S DNA INITIATIVE

INTRODUCTION

The tetramethylbenzidine (TMB) presumptive test for blood is a catalytic test which is based on the peroxidase-like activity of hemoglobin. Hemoglobin has the ability to cleave oxygen molecules from H_2O_2 and catalyze the reaction from the reduced form of 3,3',5,5'-tetramethylbenzidine to the oxidized blue-green colored product.

SAFETY CONSIDERATIONS

1. Hydrogen Peroxide 30% - Danger! Corrosive!
2. Glacial Acetic Acid – Danger! Corrosive
3. TMB – Danger! Possible carcinogen

PREPARATIONS

Tetramethylbenzidine (TMB) reagent

1. Weigh out 0.2 grams of TMB
2. Add to a 50 ml beaker
3. Measure out 10 ml glacial acetic acid and add to the TMB
4. Mix thoroughly until the TMB is dissolved
5. Store refrigerated – expiration date 1 week

Hydrogen Peroxide 3%

1. Measure out 10 ml of 30% hydrogen peroxide
2. Add 90 ml of deionized water
3. May be stored at room temperature or refrigerated - expiration date one year.

INSTRUMENTATION

- Top loading balance

MINIMUM STANDARDS & CONTROLS

- Positive control – known blood stain
- Negative control

PROCEDURE OR ANALYSIS

1. Swab suspected blood stain with clean filter paper or a swab, which may be moistened if necessary with deionized water, ethanol or saline.

2. Apply 1-2 drops of the TMB reagent.

3. Note any blue-green color change. A blue-green color change at this step indicates a chemical oxidant and the test should be considered inconclusive. If there is no color change, proceed to the next step.

4. Add 1-2 drops of 3% hydrogen peroxide.

5. Note any immediate blue-green color change

6. An immediate blue-green color change indicates a positive result. No color change indicates a negative result. A negative result indicates that either no blood is present or is below the limit of detection of the test.

Return to Protocol Index