



## 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 Laboratory Training Manual User Guide](#)