

TENNESSEE BUREAU OF INVESTIGATION
Forensic Services Division

Violent Crime Response Team Standard Operating Procedures
TMB



10. VCRT Procedures

10.4 Tetramethylbenzidine (TMB) Test

10.4.1 Scope

The TMB test is used as a screening or presumptive method for blood and is based on the peroxidase-like activity of hemoglobin. Extremely sensitive (1 part in 300,000 to 500,000), it is of great value as a negative test. A negative test is conclusive evidence of the absence of blood in quantities sufficient for further examination. Plant peroxidases and/or other substances such as rust or grease or gun oil can give a false positive result.

10.4.2 Definitions

Refer to VCRT 11.0 Definitions and Abbreviations

10.4.3 Chemicals and Reagents

Tetramethylbenzidine ($C_{16}H_{20}N_2$)
3% Hydrogen peroxide (H_2O_2)

Mixing Procedure

This product is commercially purchased (Doje's 307A)

Shelf Life

One year unless specified by the manufacturer

10.4.4 Equipment and Supplies

Sterile cotton-tipped applicator (swab)
Filter paper (Whatman 1, 90mm)
Dropper bottles

10.4.5 Test Procedure

When there is a sufficient amount of questioned material present, presumptive tests should be conducted on suspected bloodstains prior to collection. Positive and negative controls must be analyzed before testing a suspected bloodstain at a crime scene. A positive control consists of a piece of filter paper used to transfer a portion of a known bloodstain for reagent testing. A negative control consists of a piece of filter paper used for reagent testing in the absence of bloodstain transfer. If a color change is noted for the negative control, either the

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control is contaminated or there is a problem with one of the reagents. Do not proceed until this is resolved.

1. Apply a sterile cotton swab to the suspected stain, assuring visually that some material has transferred to the swab. The swab may be moistened before application with 1 – 2 drops of sterile water. If the stain is still “wet”, simply swabbing the material is sufficient.
2. Add 1 – 2 drops of TMB reagent to the filter paper after stain transfer from the swab. Monitor for any color change. If color change occurs at this stage, a non-specific oxidation of TMB is occurring or another color producing substance is interfering.
3. Add 1 – 2 drops of 3% hydrogen peroxide to the filter paper stain. Monitor for color change (no more than 30 seconds).

The development of an immediate, within 5 seconds, blue-green color indicates a positive test. No color will be observed on a negative test.

10.4.6 Results and Conclusions

If the filter paper stain turns blue-green rapidly, it is said to test presumptive positive for blood. This test has the same reaction with blood from any animal, so further testing would be required to determine whether it originates from a human.

Precaution

Waiting for periods over 30 seconds will result in most stains turning blue-green naturally as they oxidize on their own.

10.4.7 Reporting

Record the results, both positive and negative, along with the results of the control testing in the notes. Additionally, the expiration date or lot numbers of the chemicals used will be recorded in the notes.