

Section F	TMB (Tetramethylbenzidine)	Subsection 6
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Name of Procedure:

TMB (Tetramethylbenzidine)

Suggested Uses:

TMB may be utilized to develop latent impressions in blood. This technique may be used on porous or non-porous surfaces. TMB is a colorless solution that will oxidize the hemoglobin present in blood. The oxidation which occurs with the treatment of TMB changes color and the latent impressions will become greenish-blue in color.

Equipment Needed to Perform Procedures:

- A - Rubber apron and rubber gloves
- B - Magnetic stirrer, magnetic follower and magnetic retriever
- C - Glass beakers
- D - Dark shatter proof storage container (four (4) liter)
- E - Measuring cylinders
- F - Camera (35 mm, 2 1/4, MP-4, CU5, TC III)
- G - Fume hood
- H - Mist sprayers
- I - Filter

Chemicals Needed For Preparation of Chemical Solution(s):

- A - Five (5) grams of Sodium Acetate
- B - Forty-three (43) ml of Glacial Acetic Acid
- C - 0.4 grams of 3, 3 ' , 5, 5 ' Tetramethylbenzidine

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D - Thirty (30) ml of Collodion

E - Fifteen (15) ml of ethanol

F - One-hundred twenty (120) ml of Ethyl Ether

G - 0.5 grams of Sodium Perborate

Formula/Directions for Preparation of Chemical Solution(s):

Acetate Buffer Solution:

1. Place forty-three (43) ml of Glacial Acetic Acid in a large large beaker with a magnetic stirrer.
2. Add five (5) grams of Sodium Acetate to the Glacial Acetic Acid and stir.
3. Add fifty (50) ml of distilled water to the solution with continual stirring until the Sodium Acetate has completely dissolved.
4. Place the solution in a clearly marked spray bottle or dark container until needed. The Acetate Buffer Solution can be stored at room temperature.

TMB-Acetate Buffer Solution:

1. Place twenty (20) ml of Acetate Buffer solution in a clean beaker with a magnetic stirrer.
2. Add 0.4 grams of Tetramethylbenzidine to the buffer solution and stir for approximately five (5) minutes.
3. Filter the solution through a standard filter to remove any undissolved particles.
4. Place the solution in a clearly marked spray bottle or dark container until needed. The TMB-Acetate Buffer Solution should be stored in a refrigerator until needed.

Collodion-Ethanol-Ether Solution:

1. Place thirty (30) ml of Collodion in a clean large beaker.

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2. Add fifteen (15) ml of Ethanol to the Collodion. Let the solution stand for five (5) minutes until the chemicals are completely dissolved.
3. **Slowly** add one-hundred twenty (120) ml of Ethyl Ether to the solution and place a magnetic stirrer in the solution and stir until all the solutions are completely dissolved.

TMB Spray Reagent:

1. Place six (6) ml of the TMB-Acetate Buffer Solution in a large clean beaker with a magnetic stirrer.
2. Add 0.5 grams of Sodium Perborate to the solution and stir the solution until thoroughly mixed.
3. Add one-hundred twenty (120) ml of the Collodion-Ethanol-Ether Solution to the solution and continue to stir until thoroughly mixed.
4. Place the solution in a mist sprayer for application to items of evidence.

Note: The Collodion-Ethanol-Ether Solution should be freshly made when needed.

Processing Procedures for Application to Item(s) of Evidence:

Prior to spraying the item of evidence with the solution, the bloody impression must be dried or cured. This procedure is necessary to keep the print from dissolving when the solution is applied. Photograph all visible impressions prior to applying the solutions.

1. Carefully spray the item of evidence with the solution at a distance of approximately ten (10) inches from the surface. The item should be sprayed two (2) to three (3) times with the solution.
2. The impression(s) will start to turn greenish-blue in color and the spray technique may be applied as needed to achieve proper contrast. Care should be taken during this step as the impression can be over developed.

Note: If the impression disappear in a few days after being sprayed, reapply the solution

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to the item of evidence to make the impression visible again.

Over-spraying an item may result in a dark blue pattern which may mask the fine areas of the impression. If this occurs, try heating the item to reverse the TMB enhancement reaction.

Steps to Preserve Developed Impressions:

The most appropriate methods to preserve developed impressions is through photography, using the proper techniques (See Photographic Equipment/Procedures) and/or electronically recording the impressions (See Image Processing).

Safety Concerns:

TMB has been found to be safe and non-carcinogenic; however, this does not preclude it from being included on a list of hazardous chemicals in the future.

Ethanol and Ethyl Ether are extremely flammable.

All solutions should be mixed in a fume hood and handled with rubber gloves at all times to avoid injury. Handle all solutions with extreme care.

Storage and Location of Chemicals and Solutions:

TMB, Sodium Acetate, and Sodium Perborate should be stored in the original shipping containers until needed.

Ethanol and Ethyl Ether should be stored in a flammable cabinet until needed.

The Acetate Buffer Solution may be stored in a dark shatterproof container at room temperature until needed.

The TMB-Acetate Buffer Solution should be stored in dark shatterproof containers in a refrigerator.

The TMB Spray Reagent and the Collodion-Ethanol-Ether Solutions should be stored in temporary use containers only.

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Shelf Life:

TMB, Sodium Acetate, and Sodium Perborate - Indefinite if stored properly.

Acetate Buffer Solution - Up to six (6) months.

The TMB-Acetate Buffer Solution - Six (6) months if refrigerated.

The TMB Spray Reagent and the Collodion-Ethanol-Ether Solutions are for immediate use.

Other Information:

Ninhydrin may be used on the item of evidence after the use of TMB to further enhance the impression(s).

Serological samples should always be taken prior to treatment with TMB. TMB is sensitive to bloody impressions; however, it is not blood specific. Plant materials such as tomatoes, onions, garlic and cucumbers may give false color reactions with treatment of the solution. Animal substances such as saliva and mucous may also yield the same color change as will occur with blood.