

Standard Operating Procedures for Latent Print Processing with Diaminobenzidine

1 Scope

Diaminobenzidine is used by latent print personnel to develop latent prints and enhance visible prints deposited in blood.

2 Equipment/Materials/Reagents

Beakers or graduated cylinders

Balance

Spatula

Distilled water

Squirt bottles, sprayers, tissues, brushes, or glass trays

Magnetic stirrer and stir bar(s)

Freezer

3,3'-Diaminobenzidine Tetrahydrochloride (purity $\geq 97\%$)

Hydrogen Peroxide (30% Solution)

1M Phosphate Buffer Solution (pH 7.4)

5-Sulfosalicylic Acid (purity $\geq 99\%$)

3 Standards and Controls

Not applicable.

4 Sampling or Sample Selection

Not applicable.

5 Procedures

5.1 Solution Preparation

a) Solution A (Fixer solution)

Combine:

- 5-Sulfosalicylic Acid - 20 g
- Distilled water - 1000 ml

Stir until 5-Sulfosalicylic Acid dissolves.

b) Solution B (Buffer solution)

Combine:

- 1 M Phosphate Buffer Solution - 100 ml
- Distilled water - 800 ml

c) Solution C (Diaminobenzidine solution)

Combine:

- 3,3'-Diaminobenzidine Tetrahydrochloride - 1 g
- Distilled water - 100 ml

Stir until 3,3'-Diaminobenzidine Tetrahydrochloride dissolves.

d) Developer solution

Combine:

- Solution B (Buffer solution) - 180 ml
- Solution C (Diaminobenzidine solution) - 20 ml
- Hydrogen Peroxide (30% solution) - 1 ml

Mix thoroughly.

5.2 Application

- Apply Solution A (Fixer solution) to specimen by spraying, dipping, squirting, painting, or tissue method.
- Leave Solution A (Fixer solution) on specimen for 3 to 5 minutes.
- Apply distilled water to specimen by spraying, dipping, squirting, painting, or tissue method.
- Leave distilled water on specimen for 30 to 60 seconds.
- Apply Developer solution to specimen by spraying, dipping, squirting, painting, or tissue method.
- Leave Developer solution on specimen to achieve maximum contrast and development – do not exceed 5 minutes.

- g) Apply distilled water to specimen by spraying, dipping, squirting, painting, or tissue method to stop development process.
- h) Allow specimen to dry.
- i) For digital capture and photography, see FBI Latent Print Units Processing Manual Preamble.

5.3 Storage

- a) Solution A (Fixer solution) and Solution B (Buffer solution) may be stored in any type of laboratory accepted receptacle.
- b) Solution C (Diaminobenzidine solution) must be stored frozen in a container that can withstand extreme cold.
- c)..Storage is not applicable to developer solution. It is prepared as needed.

5.4 Shelf Life

- a) Solution A (Fixer solution) and Solution B (Buffer solution) have indefinite shelf lives provided the reagent checks are satisfactory.
- b) Solution C (Diaminobenzidine solution) has a 6 month shelf life when frozen provided the reagent checks are satisfactory.
- c) Developer solution is not retained, prepare as needed.

5.5 Reagent Checks

See FBI Latent Print Units Processing Manual, Preamble.

6 Calculations

Not applicable.

7 Measurement Uncertainty

Not applicable.

8 Limitations

Not applicable.

9 Safety

See FBI Laboratory Safety Manual for appropriate information.

10 References

Allman, D. and Pounds, C. A. "Diaminobenzidine: A Simple, Safe, and Sensitive Method for the Enhancement of Blood Marks at a Scene of Crime and in the Laboratory". *CRSE Report*. No. 733.

FBI Laboratory Safety Manual, Federal Bureau of Investigation, Laboratory Division. Latest Revision.

FBI Latent Print Units Processing Manual, Preamble, Federal Bureau of Investigation, Laboratory Division. Latest Revision.

Trozzi, T. A., Schwartz, R. L., and Hollars, M. L. *Processing Guide for Developing Latent Prints*, FBI Laboratory, Washington DC, 2001.

<u>Rev. #</u>	<u>Issue Date</u>	<u>History</u>
0	01/13/14	Original document issued. Derived from Discontinued Latent Print Operations Manual, Standard Operating Procedures for Processes Used to Develop Latent Prints. The original LPU Processing Manual consisted of a single document with a preamble and procedures for all processes. The current document separates each into its own separate document.
1	10/02/17	Specific section numbers referenced in Preamble removed throughout document. Section 1, latent print personnel added. Section 4 removed and remaining renumbered. Titles for Section 4 and Section 7 modified. Section 9, generalized. Updated for Biometrics Analysis Unit. References updated. Abbreviations addressed.

Approval

Redacted - Signatures on File