



ATF-LS-FB27 BlueStar Presumptive Test for Blood	Published Online: March 2018
Authority: Technical Leader	
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1. Scope

This protocol is to be used when examining an exhibit of evidence or a crime scene for the presence of blood and is only a presumptive test. A presumptive test is one that is highly sensitive but not specific to the body fluid in question. The BLUESTAR[®] test is a presumptive test for the presence of hemoglobin, a component of the red blood cells in blood. The BLUESTAR[®] test is based on the peroxidase activity of the iron in heme or heme derivatives, which catalyzes the oxidation of the BLUESTAR[®] reagent. A positive reaction is observed when the colorless BLUESTAR[®] solution is oxidized and produces a chemiluminescent blue color. Other substances such as plant peroxidases, chemical oxidants, and some metal ions may also cause a positive reaction; therefore, a positive BLUESTAR[®] test only indicates the presence of blood and does not confirm the presence of blood.

2. References

- 2.1. Manufacturer's insert for the BLUESTAR[®] Forensic test kit, May 2014.
- 2.2. R.E. Gaensslen, Identification of blood, in: Sourcebook in Forensic Serology, Immunology and Biochemistry, U.S. Department of Justice, Washington, D.C., 1983, pp. 73-133.
- 2.3. R.C. Shaler, Modern forensic biology, in: R. Saferstein, Forensic Science Handbook, second ed., Prentice Hall, Upper Saddle River, 2002, pp. 531-535.

3. Equipment

- 3.1. Disposable gloves
- 3.2. Eye protection
- 3.3. Lab coat
- 3.4. Sterile swabs
- 3.5. Sterile water
- 3.6. Presumptive blood test kit (BLUESTAR[®] Forensic tablets (reagent and catalyst) – E.g. BLUESTAR Forensic: Product #BL-FOR-TAB4)
- 3.7. Known positive control (dried blood)
- 3.8. Known negative control
- 3.9. Spray bottle with adjustable spray nozzle
- 3.10. Scissors
- 3.11. Forceps
- 3.12. 70% ethanol or alcohol wipes
- 3.13. 10% bleach solution
- 3.14. Bench paper

4. Safety/Quality Assurance

- 4.1. Any utensils used to cut or manipulate swabs or other types of evidence must be cleaned between uses with 10% bleach solution followed by 70% ethanol or alcohol wipes.
- 4.2. Disposable gloves shall be worn when handling kit reagents and evidence.
- 4.3. Record the lot number of each reagent used in notes. Do not use the reagents after the expiration date.
- 4.4. Best results are obtained within 3 hours of combining the BLUESTAR® tablets with water.
- 4.5. At a minimum, each day the reagents are to be used, a negative control and positive control must be tested and documented in the analyst's notes, along with the manufacturer, lot number, and expiration date. If either of the controls fail, a different lot of reagents shall be used.
- 4.6. This test is only a presumptive test. It is not human specific. Other means to confirm the presence of human blood are available.
- 4.7. Lab coat and eye protection must be worn at all times while performing this procedure.
- 4.8. When practical, only one item of evidence shall be open at a time.
- 4.9. The laboratory bench surface shall be cleaned before and after use with 10% bleach solution or other sanitizing agent and may be followed by 70% ethanol. Fresh bench paper shall then be placed on the surface prior to examination.
- 4.10. Minor deviations from the protocol may be made at the analyst's discretion based on the analyst's training and experience and shall be indicated in the analyst's notes. Significant deviations from the protocol must be approved by the DNA Technical Leader.

5. Reagent Preparation

- 5.1. Add 125 ml of sterile water to a spray bottle and add one beige reagent tablet and one white catalyst tablet from the BLUESTAR® tablet packet (if more than 125 ml is required, the solution may be scaled-up; one pair of tablets for every 125 ml of sterile water).
- 5.2. Allow the tablets to dissolve for 1-2 minutes and gently swirl the spray bottle to mix (do not invert the bottle).

6. Procedure

- 6.1. Darken the environment as much as possible.
 - 6.1.1. Indoors: Turn off lights and block outside light from windows.
 - 6.1.2. Outdoors: If possible, wait until night or block out as much light as possible.
- 6.2. Adjust the spray nozzle for finest spray (mist) possible and spray the test area with the prepared reagent at a distance of ~2 feet in a side to side motion (over-spraying will not produce better results and may dilute the sample).

- 6.3. An immediate faint to intense blue colored luminescence indicates a positive reaction. No luminescence indicates a negative reaction. Interpretation of results may be made immediately (within 5 seconds). Luminescence should persist for 30 seconds to 1 minute.
 - 6.3.1. Positive Result: Visualization of a luminescent blue color
 - 6.3.2. Negative Result: No luminescence
- 6.4. Record results by noting the luminescence or lack of luminescence and the conclusion of the test.
- 6.5. A cutting or swabbing of an unstained area adjacent to the stained area may be tested as a substrate control at the analyst's discretion.
- 6.6. A positive BLUESTAR® result, alone, cannot be reported as an indication of the presence of blood. This result must be followed by a positive phenolphthalein test result in order to report the indication of the presence of blood.