

The Effect of BlueStar® and Luminol on RSID™-Blood Strip Tests

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Abstract

BlueStar® and Luminol are presumptive tests for blood that crime labs use in crime scene investigations (1, 2, 3). Rapid Stain Identification of Human Blood (RSID™ – Blood) is a strip test used by forensic laboratories for the detection of human blood from a variety of samples (4). BlueStar® and Luminol offer presumptive detection of blood but are not specific to human blood (2, 3). In order to test the effect that BlueStar® and Luminol had on detection of blood for the RSID™ – Blood tests, an experiment using blood sprayed onto several tiles and onto fabric which were treated with either BlueStar® or Luminol or were not treated at all. There were four different blood samples used: pure blood sample, bleached, diluted (2, 10, 50, 250 microliters), and fabric. 51 blood samples were tested on the RSID™ - Blood which came out with mixed results. Luminol affected the RSID™ – Blood's ability to detect the blood while BlueStar® did not affect the blood detection.

Methods

Setup of blood stains. A volunteer donated blood. 25 microliters (μL) of whole blood was mixed with 275 μL of Phosphate Buffered Saline (PBS) or 5 % bleach in PBS. A spray apparatus was used to apply blood solutions to sets of 3 floor tiles: Untreated, Luminol, BlueStar® . Blood in PBS was sprayed onto 3 quadrants each of 3 tiles. Blood in 5% bleach solution was sprayed onto 3 quadrants each of 3 tiles. One quadrant of each of the 6 PBS and bleach tiles was left blank as a control. Whole blood was diluted so that an equivalent of 6.25, 1.25, 0.25, and 0.05 μL of blood was applied to one area of a tile divided into 5 sections, with one area left as a control. 25 μL of whole blood was pipetted onto 3 areas of each of 3 pieces of fabric. An area was left blank as a control. The samples were left in a controlled environment for one week (Fig. 1).

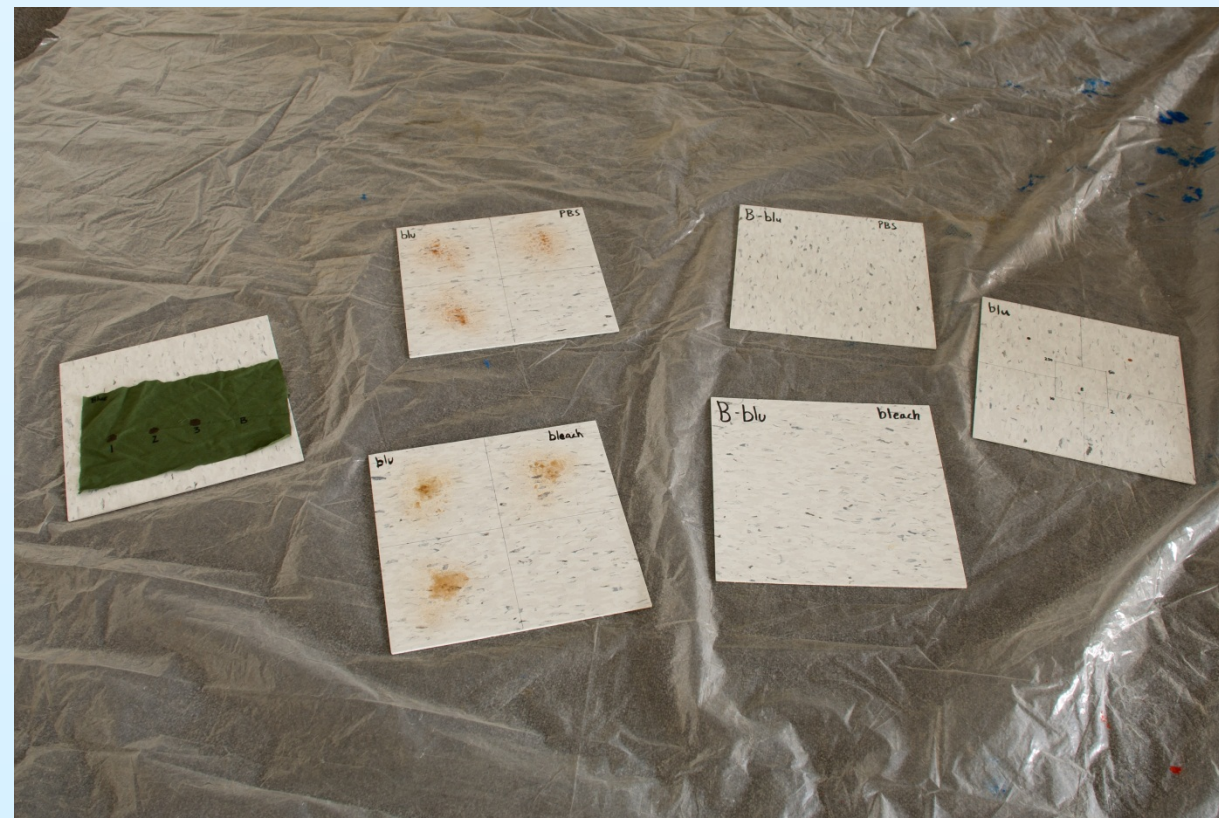
Methods (cont’d.)

Treatment of bloodstains with BlueStar® and Luminol. One set of samples (blood in PBS on tile, blood in 5% bleach on tile, diluted blood on tile, and blood stained fabric swatch) were treated with Luminol reagent and photographed (5). One set of samples were treated with BlueStar® reagent (Fig. 2). One set of samples was left untreated as a control group. The samples were left in a controlled environment for one week.

Collecting of the samples. Every tile sample was swabbed using a sterile cotton swab moistened with high purity water. After the swabs were left to dry for one week, they were transferred into extraction tubes. All of the fabric samples were cut individually and transferred to extraction tubes

RSID™ Testing of samples. Each swab or fabric cutting was extracted one hour at room temperature in 500 μL of RSID™ -Blood extraction buffer. 10 μL of each extract was run with 90 μL of RSID™ -Blood running buffer on RSID™ - Blood strips (except blood dilutions: 20 μL extract with 80 μL buffer). Each RSID™ test was scored and recorded after 10 minutes of run time (Fig. 3).

Fig. 1. Bloodstains on tiles and fabric



Results

Table 1. RSID™ - Blood Strip Test Results

Phosphate Buffered Saline			
Sample	Untreated	Luminol	BlueStar®
1	+	+	+
2	+	+	+
3	+	+	+
B	-	-	-

Bleach			
Sample	Untreated	Luminol	BlueStar®
1	+	-	+
2	+	-	+
3	+	+	+
B	-	-	-

Diluted			
Sample	Untreated	Luminol	BlueStar®
2	-	-	-
10	-	-	-
50	+	+	+
250	+	+	+
B	-	-	-

Fabric			
Sample	Untreated	Luminol	BlueStar®
1	+	+	+
2	+	+	+
3	+	+	+
B	-	-	-

+ = Glycophorin A detected
- = Glycophorin A not detected

Fig. 2. BlueStar® applied on Bloodstains



Fig. 3. RSID™ Strip Test



Discussion

BlueStar® and Luminol utilizes hemoglobin's peroxidase-like activity. RSID™ -Blood uses two monoclonal antibodies to detect the presence of human Glycophorin A. The detection limit for RSID™ blood is less than 1 microliter of blood (~50nL). All diluted sample sets of 2 and 10 microliters tested negative while the dilutions of 50 and 250 were positive with all sample sets. Several bleach samples were observed to be positive but very faint to the point where one staff scientist described them as “shadow bands” or negative (J. Old, pers. comm., March 22, 2012).

Conclusion

Luminol affected RSID™ 's ability to detect the blood in the sample. BlueStar® did not affect the RSID™ blood detection even after being treated with bleach. The bleach and the BlueStar® did not affect the hemoglobin in the blood nor did it affect the presence of Glycophorin A which are abundant in red blood cell membranes. The dilutions of 2 and 10 microliters did not have a high enough blood concentration to be detected on any of the RSID™ strip tests.

References

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