



## Technical Note,

# Technical note: The effects of Bluestar<sup>®</sup> and luminol when used in conjunction with tetramethylbenzidine or phenolphthalein

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## ABSTRACT

There are numerous presumptive tests available in the forensic science field to help identify the presence of blood. While many articles are available on the effects of Bluestar<sup>®</sup> and luminol and potential interactions with subsequent DNA identification, the research field falls short in identifying the effects these two presumptive tests may have on subsequent presumptive tests used to help identify blood. To rectify this ongoing issue in the forensic science field, the chemiluminescence methods of Bluestar<sup>®</sup> and luminol for the detection of blood at a crime scene were tested for their effects when used in conjunction with tetramethylbenzidine (TMB) or phenolphthalein (PT) at the forensic science laboratory. Six different substrates (untreated wood, pressure treated wood, ceramic tile, shag carpet, cement block, and cotton clothing) were stained with varying dilutions (range 1:1 to 1:100,000) of blood. Neither luminol nor Bluestar<sup>®</sup> affect the results of PT or TMB tests at blood dilutions equal to or less than 1:100. However, interactions did occur between agents and substrates with blood dilutions 1:1000 or greater. Bluestar<sup>®</sup> was the only presumptive test that can detect blood dilutions of 1:100,000 on some substrates and luminol was inclusive on pressure treated wood. These findings suggest that forensic science laboratory personnel need to know and understand the details of how the blood was detected by the crime scene investigator and the substrate on which the blood was obtained from for their preparation of presumptive blood testing with PT or TMB.

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## 1. Introduction

The procurement of samples from a crime scene by forensic crime scene investigators can influence forensic science laboratory analysis of evidence. Two primary reagents used in the field by crime scene investigators to assist in the detection of blood are Bluestar<sup>®</sup> and luminol. Bluestar<sup>®</sup> and luminol utilize a chemiluminescence method to detect blood at a crime scene and have been shown to not alter subsequent Short Tandem Repeats (STR) DNA analysis in the laboratory [1,2]. The oxidation of luminol by hydrogen peroxide in the presence of the hem catalyst of hemoglobin emits a chemical luminescence visible in the dark (Fig. 1A; [3]). Bluestar<sup>®</sup> is a luminol based presumptive test that produces a more intense and longer lasting chemical luminescence [2]. Once the evidence is delivered to the forensic science laboratory, color change tests utilizing phenolphthalein (PT; [4])

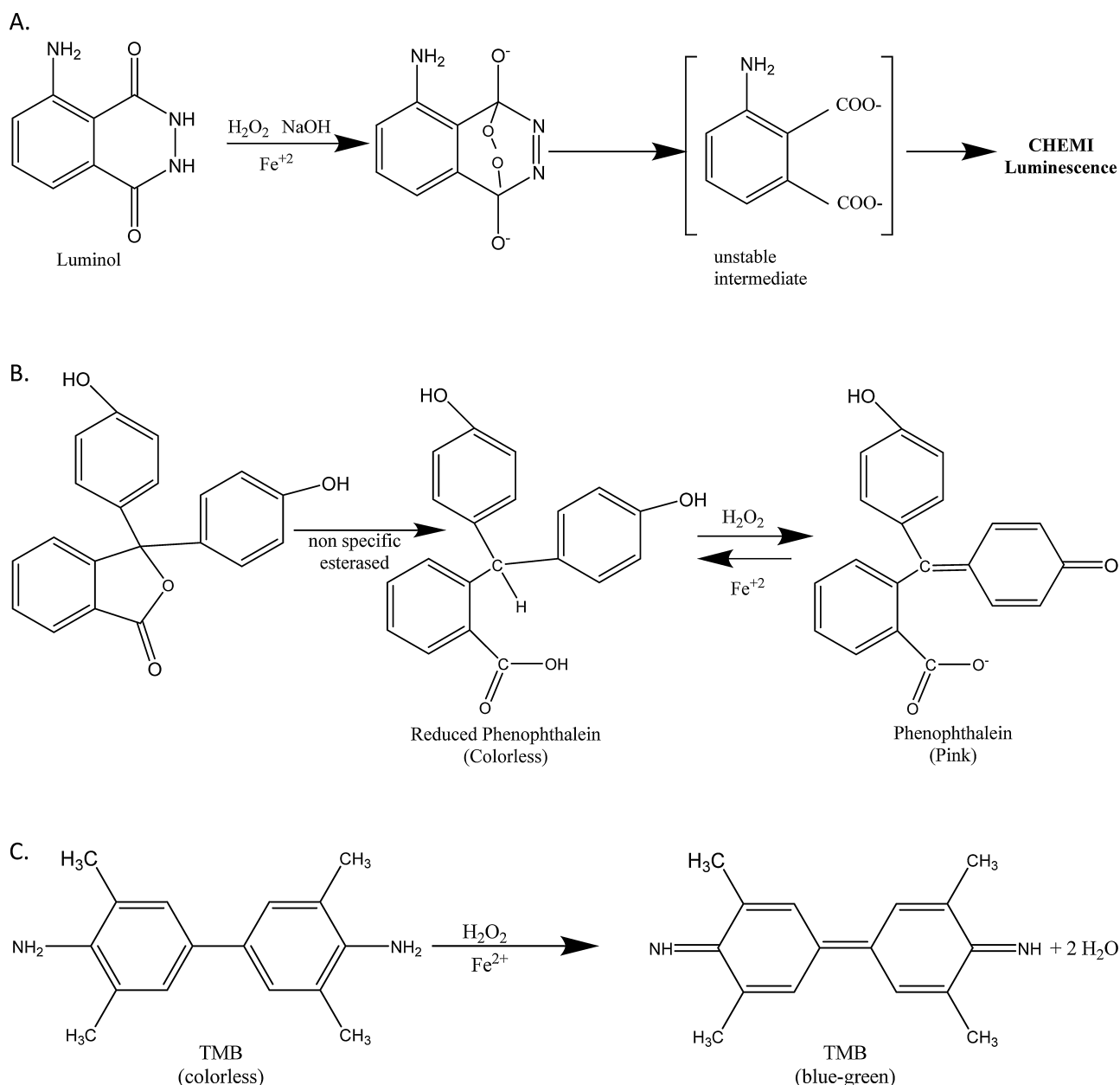
or tetramethylbenzidine (TMB; [5]) are commonly used for presumptive identification of blood. The oxidation of PT and TMB induced by hydrogen peroxide and the iron moiety (hem) of hemoglobin is similar to luminescence produced by luminol (Fig. 1B and C). Due to the similarities in the mechanisms by which these presumptive blood tests work (e.g. peroxidase-like activity), we investigated the effects of TMB and PT in the forensic laboratory analysis of blood when Bluestar<sup>®</sup> or luminol are utilized at a crime scene.

## 2. Materials and methods

### 2.1. Chemicals and reagents

Bluestar<sup>®</sup>, luminol and PT were obtained from Sirchie (Youngsville, NC). TMB and the hemoglobin standard were obtained from Sigma Chemical (St. Louis, MO). The 3% hydrogen peroxide was obtained from a local retail pharmacy. All other reagents were obtained from Fisher Scientific (Hanover Park, IL).

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**Fig. 1.** Presumptive blood tests utilized in the present study. (A) The oxidation of luminol by hydrogen peroxide ( $\text{H}_2\text{O}_2$ ) in the presence of the iron ( $\text{Fe}^{2+}$ ) of hemoglobin results in the production of an unstable intermediate that yields a luminescence. (B) The oxidation of phenolphthalein by  $\text{H}_2\text{O}_2$  in the presence of  $\text{Fe}^{2+}$  results in a pink color change. (C) The oxidation of tetramethylbenzidine (TMB) by  $\text{H}_2\text{O}_2$  in the presence of  $\text{Fe}^{2+}$  results in blue–green color change. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

## 2.2. Substrates and blood dilutions

Six different substrates were used in this experiment: untreated wood, pressure treated wood, ceramic tile, shag carpet, cement block, and cotton clothing. The pressure treated wood products were preserved with the standard copper-based treatments typically used today [6]. Each substrate was wiped clean with a paper towel and had six different human blood dilutions applied: 1:1, 1:10, 1:100, 1:1000, 1:10,000, and 1:100,000 using 100  $\mu\text{L}$  for each stain. The blood dilutions were allowed to dry on their substrates for 12 days before being tested. Each substrate was then tested with the following methods Bluestar<sup>®</sup> only, luminol only, TMB only, PT only, Bluestar<sup>®</sup> + TMB, Bluestar<sup>®</sup> + PT, luminol + TMB and luminol + PT. The order presented here represents the order of testing.

## 2.3. Bluestar<sup>®</sup> and luminol

In a dark room, luminol and Bluestar<sup>®</sup> were sprayed with 8.2 mLs (17 pumps) onto each of the test substrates as specified above. Copper pennies, which emit light upon exposure to luminol based blood tests were placed next to the blood dilutions for ease in identification of the blood treated areas. A positive test was indicated by the emission of light within 60 s of application. All reactions were photographed at 61 cm. away for documentation purposes.

### 2.3.1. Tetramethylbenzidine (TMB)

TMB was prepared and quality controlled by assessing the positive and negative controls to ensure accuracy. A hemoglobin swab was utilized for the positive control while a clean sterile swab

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