Forensic Characterization of Bloodstains

The criminalist must answer the following questions when examining dried blood: (1) Is it blood? (2) From what species did the blood originate? (3) If the blood is human, how closely can it be associated with a particular individual?

Color Tests

The determination of blood is best made by means of a preliminary color test. For many years, the most common test was the benzidine color test. However, because benzidine has been identified as a known carcinogen, its use has generally been discontinued, and the chemical phenolphthalein is usually substituted in its place (this test is also known as the Kastle-Meyer color test).

Both the benzidine and Kastle-Meyer color tests are based on the observation that blood hemoglobin possesses peroxidase-like activity. Peroxidases are enzymes that accelerate the oxidation of several classes of organic compounds when combined with peroxides. For example, when a bloodstain, phenolphthalein reagent, and hydrogen peroxide are mixed together, oxidation of the hemoglobin in the blood produces a deep pink color.

The Kastle-Meyer test is not a specific test for blood; some vegetable materials, for instance, may turn Kastle-Meyer pink. These substances include potatoes and horseradish. However, such materials will probably not be encountered in criminal situations, and thus from a practical point of view, a positive Kastle-Meyer test is highly indicative of blood. Field investigators have found Hemastix strips a useful presumptive field test for blood. Designed as a urine dipstick test for blood, the strip can be moistened with distilled water and placed in contact with a suspect bloodstain. The appearance of a green color indicates blood.
Luminol and Bluestar

Another important presumptive identification test for blood is the luminol test. Unlike the benzidine and Kastle-Meyer tests, the reaction of luminol with blood produces light rather than color. By spraying luminol reagent onto a suspect item, investigators can quickly screen large areas for bloodstains. The sprayed objects must be located in a darkened area while being viewed for the emission of light (luminescence); any bloodstains produce a faint blue glow (see Figure 9–6).
A relatively new product, Bluestar, is now available to be used in place of luminol (www.bluestar-forensic.com). Bluestar is easy to mix in the field. Its reaction with blood can be observed readily without having to create complete darkness. The luminol and Bluestar tests are extremely sensitive—capable of detecting bloodstains diluted to as little as 1 in 100,000. For this reason, spraying large areas such as carpets, walls, flooring, or the interior of a vehicle may reveal blood traces or patterns that would have gone unnoticed under normal lighting conditions (see Figure 9–7). It is important to note that luminol and Bluestar do not interfere with any subsequent DNA testing.\(^3\)

**FIGURE 9–7** (a) A section of a carpet under normal light showing a faint footprint in blood. (b) Same section of the carpet after spraying with luminol. Courtesy Sirchie Finger- print Laboratories, Youngsville, NC, www.sirchie.com

**Microcrystalline Tests**

The identification of blood can be made more specific if microcrystalline tests are performed on the material. Several tests are available; the two most popular ones are the Takayama and Teichmann tests. Both depend on the addition of specific chemicals to the blood to form characteristic crystals containing hemoglobin
derivatives. Crystal tests are far less sensitive than color tests for blood identification and are more susceptible to interference from contaminants that may be present in the stain.

**Precipitin Test**

Once the stain has been characterized as blood, the serologist determines whether the blood is of human or animal origin. The standard test is the precipitin test. Precipitin tests are based on the fact that when animals (usually rabbits) are injected with human blood, antibodies form that react with the invading human blood to neutralize its presence. The investigator can recover these antibodies by bleeding the animal and isolating the blood serum, which contains antibodies that specifically react with human antigens. For this reason, the serum is known as human antiserum. In the same manner, by injecting rabbits with the blood of other known animals, virtually any kind of animal antiserum can be produced. Antiserums are commercially available for humans and for a variety of commonly encountered animals—for example, dogs, cats, and deer.

Several techniques have been devised for performing precipitin tests on bloodstains. The classic method is to layer an extract of the bloodstain on top of the human antiserum in a capillary tube. Human blood, or, for that matter, any protein of human origin in the extract, reacts specifically with antibodies present in the antiserum, as indicated by the formation of a cloudy ring or band at the interface of the two liquids (see Figure 9–8).
Gel Diffusion

Another method, called gel diffusion, takes advantage of the fact that antibodies and antigens diffuse or move toward one another on a plate coated with a gel medium made from a natural polymer called agar. The extracted bloodstain and the human antiserum are placed in separate holes opposite each other on the gel. If the blood is human, a line of precipitation forms where the antigens and antibodies meet (see Figure 9–9).

Similarly, the antigens and antibodies can be induced to move toward one another under the influence of an electrical field. In the electrophoretic method (examined in detail in Chapter 10), an electrical potential is applied to the gel medium; a specific antigen–antibody reaction is denoted by a line of precipitation formed between the hole containing the blood extract and the hole containing the human antiserum (see Figure 9–10).

**FIGURE 9–9**
A gel diffusion plate showing an extracted bloodstain in the central well and four animal antisera in the peripheral wells. Courtesy Jerome G. Beuscher, Ph.D.

**FIGURE 9–10** Antigens and antibodies moving towards one another under the influence of an electrical potential.
The precipitin test is very sensitive and requires only a small amount of blood for testing. Human bloodstains dried for 10 to 15 years and longer may still give a positive precipitin reaction. Even extracts of tissue from mummies four to five thousand years old have given positive reactions with this test. Furthermore, human bloodstains diluted by washing in water and left with only a faint color may still yield a positive precipitin reaction (see Figure 9-11).

Once it has been determined that the bloodstain is human, an effort must be made to associate or disassociate the stain with a particular individual. Until the mid-1990s, routine characterization of bloodstains included the determination of A-B-O types; however, the widespread use of DNA profiling or typing has relegated this subject to one of historical interest only.