TECHNICAL NOTE

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Field Technique for the Identification of Deer Blood

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ABSTRACT: A latex suspension sensitized with deer antiserum has been prepared, placed on plastic cards, dried, and packaged for field use. The product was tested against bloodstains from 22 species (including *Homo sapiens*). Strong agglutination reactions occurred only with bloodstains from deer and elk.

KEYWORDS: pathology and biology, blood, deer

Current methods of blood identification generally are time-consuming and require some simple equipment and an assortment of antisera and known specimens. Many state wildlife agencies lack either the materials or trained personnel necessary for bloodstain identifications. They must rely upon assistance from universities or state crime laboratories for this service. These laboratories may provide this service [*1,2*], but not as their primary function. Consequently an investigation may be seriously handicapped by a significant time lapse that may occur between specimen collection and specimen identification. A simple test that can be run in the field could at least alleviate if not eliminate this problem.

For many years bloodstains have been identified through the interpretation of immunoprecipitates resulting from the reaction of blood proteins with their corresponding precipitating antiserum. The earliest of these techniques, the precipitin test, was employed by Nuttall [*3*]. More recently Brohn and Korschgen [*4*] and Keiss and Morrison [*5*] used this procedure to differentiate several big game species from common domestic animals. Oates and Weigel [*6*] used immunodiffusion and immunoelectrophoresis in agar gels to differenti-

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ate 26 species of game and domestic animals. One of the most recent techniques uses the agglutination of antibody-sensitized latex particles to visualize a specific antigen-antibody reaction [*7,8*]. The principle of this technique has been used extensively for 20 years in clinical medicine for tests such as pregnancy [*9*] and rheumatoid arthritis [*10*]. Latex particles sensitized with deer antiserum have provided a fast, simple, and sensitive method for identification of cervid blood.

Materials and Methods

Glycine-saline buffer was used as the stock solution. One litre of this solution contained 15.0 g of glycine, 20.0 g of sodium chloride, and 2.0 g of sodium azide. The pH was adjusted to 8.2 with 1.0*N* sodium hydroxide.

Polystyrene latex particles were acquired from Dow Chemical Co. (Indianapolis, IN) as a 10% suspension of uniform 0.22-μm particles. A 1.0% solution was prepared by dilution with the glycine-saline buffer.

Lyophilized deer antiserum (Lot 136662) was obtained from Cappel Laboratories, Inc. (Cochranville, PA). The stock solution was prepared by dissolving 0.12 g of lyophilized antiserum in 1.0 mL of glycine-saline buffer. This solution was then either used immediately or frozen.

Lyophilized bovine serum albumin was diluted to a 0.1% solution using glycine saline buffer and stored at 4°C.

Dri-Dot® cards and testing plates were supplied by Organon Diagnostics. Black plastic cards with two 25-mm flat-bottomed wells were commonly used for storage of the dried, sensitized latex.[5]

Latex particles were sensitized by several methods; however, we found the following modification of a technique described by Itol [*11*] to be the most successful.

The sensitized latex was prepared by adding 0.03 mL of stock deer antiserum to 2 mL of the 1.0% latex and incubating for 20 to 30 min in a water bath (56°C). One drop of 0.1% bovine serum albumin was then swirled by hand into the solution. When the solution reached ambient temperature, it was centrifuged for 10 min and the supernatant was decanted. The resulting latex pellet was suspended in 2 mL of glycine buffer and stored at 4°C until ready for use.

Tests for specificity and sensitivity were then conducted on black-backed glass plates. One drop of diluted dry blood (straw yellow to light pink in color) was added to one drop of the sensitized latex and mixed together with a toothpick so that the material covered an area approximately the size of a quarter. The glass plate was then rocked very slowly by tilting and rocking in a figure-eight motion for approximately 2 min. Presence of aggregates indicated a positive result (Fig. 1).

Following the addition of a 6% sucrose solution to the sensitized latex (2:1),[6] the latex can be dried. The sucrose-latex solution (0.1 mL) was placed either in the center of the Dri-Dot card or in each well of the plastic card. Adequate drying occurred in 2 to 3 h in a forced-air oven set at 30°C. Two of the plastic cards were placed and sealed in a 0.0015 gauge 51- by 102-mm (2- by 4-in.) plastic bag. A second bag containing a small amount of silica gel or anhydrous calcium chloride was then sealed around the first bag. Four tests could be run with each packet.

Field kits were prepared containing plastic vials of known dry deer and bovine blood, four packets of plastic cards, distilled water, screwcapped test tubes, eye droppers, stirs, and an instruction sheet. A drop of distilled water or physiological saline will reconstitute the dried sucrose-latex and allow for identification tests directly on the cards.

[5]G. I. Hoilien, personal communication, Iowa Conservation Commission, 1980.
[6]R. Prodell, personal communication, Organon Diagnostics, West Orange, NJ, 1979.

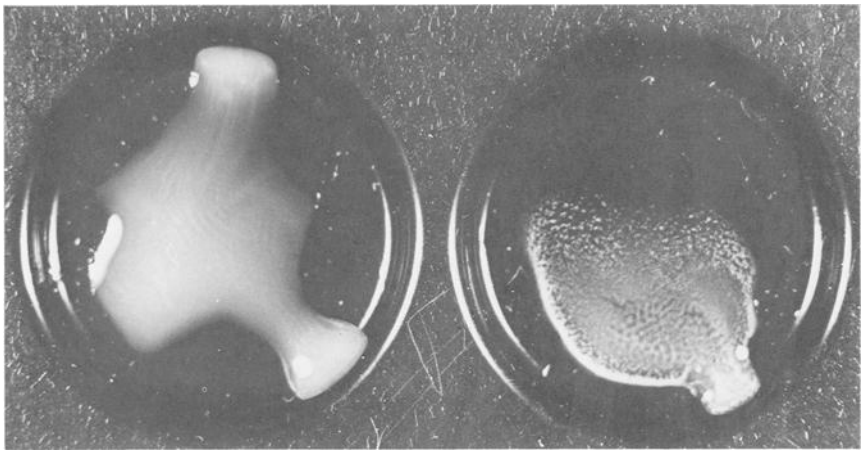

FIG. 1—*Tests were performed on a plastic card using latex sensitized to deer antiserum. Reaction on the right was a strong agglutination reaction typical of reactions with deer and elk blood. Reaction on the left illustrates a typical milky, no agglutination reaction observed when blood from species other than deer or elk were tested.*

Results

Specificity and sensitivity of the sensitized latex was not only related to the specificity and titer of the available antiserum, but also the latex-antibody ratio. Our goal was to produce a product that would give a fast visual agglutination reaction with deer blood and no agglutination with blood from other species. Optimum conditions were determined by varying the latex-antibody ratio until no agglutination occurred with bovine blood. The sensitized latex was then tested against dilute solutions of dry blood from several species (Table 1) and especially those that had previously demonstrated cross-reactivity with deer antiserum [*6,8*]. Of the species examined only white-tailed deer (*Odocoileus virginianus*), mule deer (*O. hemionus*), and elk (*Cervus elaphus*) produced a strong agglutination reaction; mountain goat (*Oreamnos americanus*) occasionally gave a weak agglutination.

Latex particles sensitized with raccoon (*Procyon lotor*) and ring-necked pheasant (*Phasianus colchicus*) antisera have also been prepared in our lab. We used our own rabbit produced raccoon and pheasant antisera which were not as species specific as Cappel's anti-deer serum. The latex sensitized with our antisera agglutinated very strongly with raccoon and pheasant blood, respectively, but the raccoon also agglutinated with blood from other carnivores and pheasant agglutinated with blood from other avian species. They still have served as valuable screening tools to detect the presence of carnivoral or avian blood in an unidentified blood stain. In human forensic science, latex sensitized with human antiserum could also be valuable in screening for primate blood.

Discussion

Like Abu Salik et al [*12*], we also found that the sensitized latex solution could be stored for several months at 4°C. The normal shelf life of the dried form was at least four to five months when it was stored at around 24°C and protected from moisture and direct sunlight. Both the liquid (at 4°C) and the dry (at 4 and 24°C) forms have been successfully stored in the laboratory for 18 to 24 months. Unlike the liquid form, the dry form can be frozen, however, shelf life may be diminished.

Simulated field testing of the dried product showed: (1) the rate of the agglutination reaction was temperature dependent (therefore cold weather or cold test solutions or both can

TABLE 1—*Species examined by latex sensitized with deer antiserum.*

Common Name	Scientific Name	Test Results[a]
	WILD ANIMALS	
Badger	*Taxidea taxus*	—
Beaver	*Castor canadensis*	—
Bobcat	*Felis rufus*	—
Buffalo	*Bison bison*	—
Coyote	*Canis latrans*	—
Deer, mule	*Odocoileus hemionus*	+
Deer, white-tailed	*Odocoileus virginianus*	+
Elk	*Cervus elpahus*	+
Fox, red	*Vulpes vulpes*	—
Goat, mountain	*Oreamnos americanus*	+—
Mink	*Mustela vison*	—
Porcupine	*Erathizon dorsatum*	—
Pronghorn	*Antilocapra americane*	—
Raccoon	*Procyon lotor*	—
Sheep, bighorn	*Ovis canadensis*	—
Skunk, striped	*Mephitis mephitis*	—
	DOMESTIC ANIMALS AND MAN	
Cow	...	—
Dog	...	—
Horse	...	—
Human	...	—
Pig	...	—
Sheep	...	—

[a]Strong agglutination is +, weak agglutination is +—, and no agglutination is —.

inhibit the agglutination reaction) and (2) adverse storage conditions for example, moisture contamination, high temperatures, and so forth) could shorten shelf life.

It would not be practical for field personnel to store the dried sensitized latex at 24°C in a car. They could, however, store it at home or in the office or only carry it when the weather was cool. It could be carried to investigate complaints or tips. A fellow officer could also be radioed to bring a test kit in emergency situations.

Since the sensitized latex has a limited shelf life, two preliminary tests should be run as quality checks. Product breakdown is monitored by testing with dilute solutions of dried deer and bovine blood. Failure to agglutinate with solutions of deer blood or agglutination with bovine blood indicates product breakdown. Four tests can be performed with each individual packet. To ensure that agglutination is not due to a "nonspecific" reaction, a blank consisting of an extract of the material around the bloodstain can also be tested. The fourth test is for the unknown. Specimens should still be sent to the lab both for confirmation purposes and identification of noncervid blood. The technique appears to be simple and expedient for lab use, in addition to being functional in the field. It may be an invaluable tool for field personnel for establishing "probable cause" and perhaps allowing the search of a vehicle or premises without a search warrant.

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