A PRACTICAL FIELD METHOD FOR BLOOD AND TISSUE IDENTIFICATION

BARRY COOK, Wildlife Inspector, Law Enforcement Division, Florida Game and Fresh Water Fish Commission, Tallahassee, FL 32304

Abstract: As wildlife law enforcement practitioners, situations are frequently encountered in which identification of blood and tissue is required or otherwise desirable. A simplified technique has been developed for determining the species composing a blood or tissue sample through the use of immunological techniques. Using proper procedures and a minimum of equipment, positive identification of blood and tissue can be obtained within at 24 hour period. Testing procedures are straightforward and uncompleted. However, a thorough understanding of the basic principles of blood and tissue examination is necessary especially for courtroom application.

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For several years the Florida Game and Fresh Water Fish Commission had submitted samples of blood and tissue to the State Crime Laboratory or to the F.B.I. Laboratory for identification. This procedure hampered enforcement efforts due to difficulities in maintaining the chain of evidence, proper shipment of the evidence, slow response time, and the inconvenience, plus expenses incurred when it was necessary for the analyst to appear and testify as an expert witness in criminal proceedings.

The need for an accelerated method of testing samples of blood and tissue was evident particularly with various violations involving illegal taking and commercialization of deer and alligator. A newly developed, simplified method is now being used by wildlife officers to test samples of evidence for use in prosecuting criminal violations involving a wide variety of wildlife species.

Results have been obtained from meat portions, blood stained clothing, knives, whetrocks, floor board residues, etc., and have positively identified which animal species comprised the unknown samples. This enables officers to distinguish between those individuals dealing in legal or illegal meats and to do so quickly.

CASE NO. 1.

During the afternoon of October 30, 1977, a tip was received that a subject was seen getting out of a truck carrying a shotgun and walking in the direction of a wildlife management area. The area around where the truck was parked was placed under surveillance by a wildlife officer. Another wildlife officer heard shots fired approximately 1½ miles from the location of the truck.

Late that night after no one showed up at the truck the wildlife officer started to leave the area when he spotted a subject approximately 100 yards from the truck. The subject was crouched in the bushes, and appeared to have blood on his hands and wrists. Closer examination showed stains on his shirt, trousers, and shoes. The freshness of blood was evident by the odor about the subject.

Another wildlife officer found 3 dead deer (1 buck and 2 doe) just inside a fence about ¼ mile from the truck. The deer were already field dressed with the feet tied together for easy carrying. The subject denied any knowledge of the deer and advised that the blood on his clothes was from a steer he had butchered that afternoon.

The subject was taken into custody and booked. His clothing was seized as evidence for blood and tissue examination.

The subject had 9 shotgun shells and 1 .308 cartridge in his pockets; however, no guns were ever found.

In less than 24 hours, a positive identification of blood on the subject's clothing revealed that it was deer blood. This led to a complete confession of his having taken the deer during the closed season.

He served 30 days in jail with 1 year supervised probation and paid $600.00 for replacement of 3 deer. In addition, the subject lost his hunting privileges for 1 year.

CASE NO. 2.

This case involved the killing of an alligator and removal of the tail for use as food. When wildlife officers approached the subject, he was able to throw the alligator tail from

his truck without being seen. However the sand in the floor of the truck had blood in it, as did a rubber boot found in the truck, and a pocket knife seized from the subject appeared to have blood on it.

Testing gave a positive reaction with all evidence seized (knife, boot and sand) and enabled investigators to tie the presence of the dead alligator to the truck.

CASE NO. 3.

This case involved the identification of venison and beef purchased by undercover agents from commercial operations involving cattle rustling and selling of game mammals.

CASE NO. .4

This case involved analysis of blood stains on old newspapers found in the trunk of a vehicle which was seized while being used to hunt with a gun and light at night. Tests were run to provide insurance against a claim in court that the defendant did not hunt, never possessed a deer, and that deer was the farthest thing from his mind, etc.

CASE NO. 5.

An informant observed a subject with an antlerless deer during closed season. The subject forced the informant to leave the scene at gunpoint until the deer was moved away from the area. Investigators returned to scene with the informant and obtained a dried drop of blood on an oak leaf. Blood tests were positive and added to credibility of informant in obtaining arrest warrants for the taking of deer during closed season.

METHODS

The basis of this examination is related to how man and other animals defend themselves agains disease and invasion of foreign substances (i.e. immunology). People who recover from diseases are usually somewhat resistant to those diseases. Immunity or partial resistance is caused by a substance in the blood of the immune animal. This substance is called antibody and is produced in the blood after a body is invaded by a foreign substance. For example, partial resistance to snakebite is brought about following recovery of an initial snakebite.

Antibody production can be stimulated by injection of a foreign substance into an animal. For example, a goat is injected with deer albumin, 2 weeks later, the same goat is again injected with deer albumin. (This will increase the concentration of antibodies in the blood serum and acts as a booster dose to obtain a greater immunity for a longer time period.) Two weeks later a blood sample is collected from the same goat that was injected. The red and white cells from the blood are discarded leaving serum containing antibodies. This leaves an antiserum (blood containing antibodies against invasion of deer albumin) called goat-anti-deer albumin (Figs. 1 & 2).

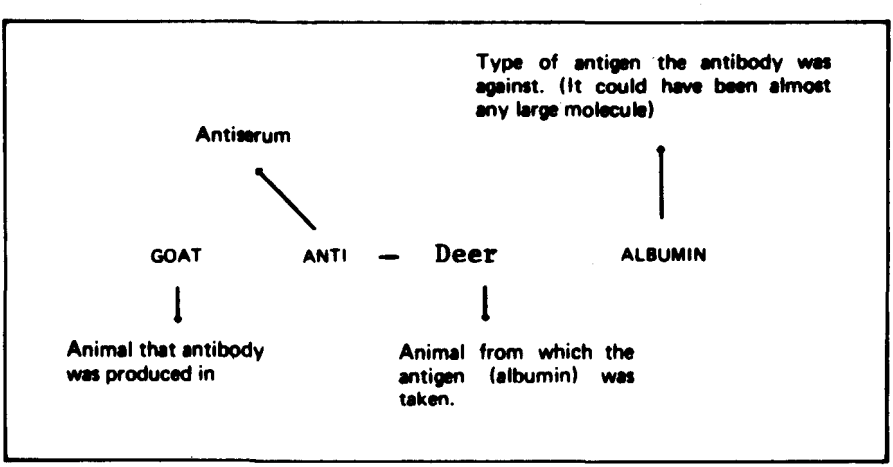

FIGURE 1.

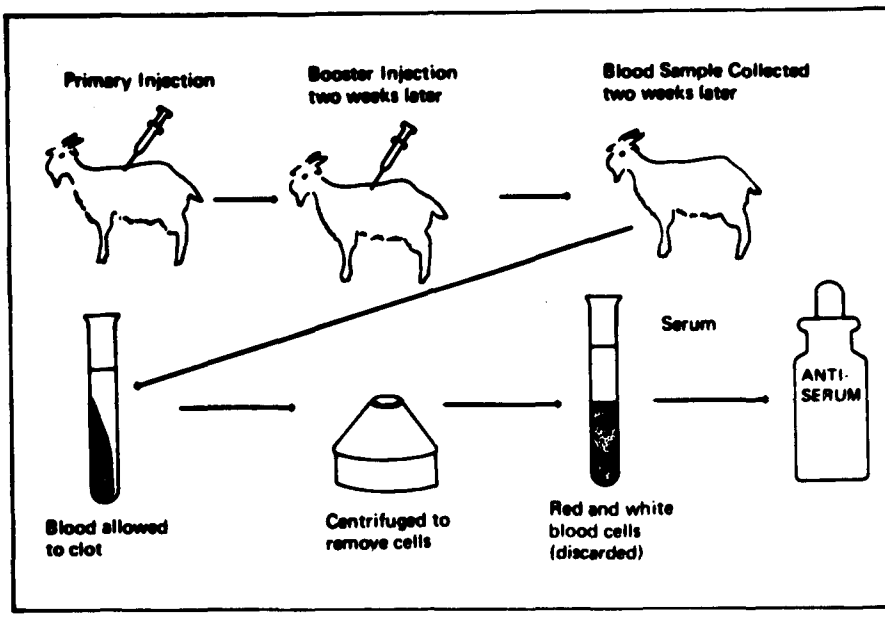

FIGURE 2 • Production of an antiserum.

Antisera are produced in animals by immunization with an antigen and will react only with the same antigen of the species used to produce the antisera. For example, goat-anti-deer albumin will react with deer albumin only, and alligator anti-serum will react with alligator blood only.

If antibody and antigen are simply mixed in solution a precipitate will not necessarily form. This can be shown by taking deer serum albumin and adding increasing amounts of a constant concentration of antibody (goat-anti-deer albumin). The reason for this is that some over concentrated molecular combinations are not insoluble and are invisible to the eye, yielding no precipitate.

A double diffusion in gel (Agar) eliminates this problem so that somewhere between the wells antibody and antigen meet at proper concentrations.

If antibodies diffusing from the antisera in one well meet an antigen diffusing from the other well from that particular species, a fine precipitate is formed. This indicates that particular species is present in the sample being tested (Photo 1).

This system of double diffusion through Agar is called ouchterlony analysis and is named after Dr. Ouchterlony, developer of this technique. The ouchterlony analysis provides a method so that blood and tissue may be analyzed to determine the animal species composing an unknown sample of uncooked, rare, or smoked meat or blood residue and whether or not the sample is composed of more than one species. The determination is made by using an extract of the unknown sample in question in the center well of an Agar filled petri dish with known antisera containing antibodies of various animal species placed in surrounding wells. A precipitin line will form between the central well and the well or wells which contain antiserum to the species represented in the unknown sample (Diagram 1).

Fish species may be specifically isolated in this manner providing a useful means of detecting illegal commercialization or possession, etc. especially when portions of meat or filets are the only parts of the fish available for analysis.

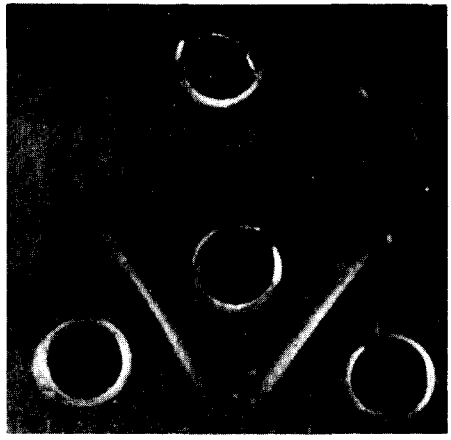

Photo 1.

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PROCEDURES FOR BLOOD AND TISSUE EXAMINATION

1. Place a small amount of unknown meat or blood sample in mortar. (Fingernail scrapings, knife residue, dried blood, etc.) Smoked meat, rare meat, or jerky will usually work in these tests. If the meat is well cooked, heat denatures the proteins and the test will not work.

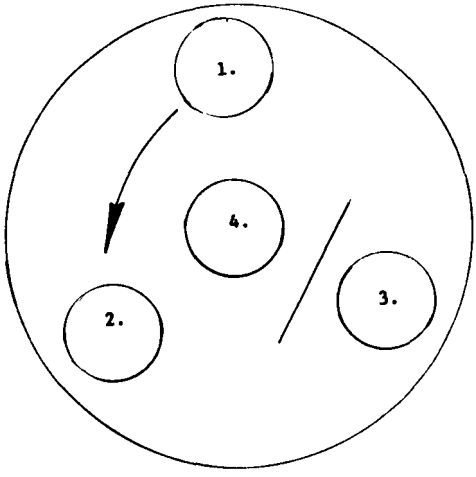
2. Add a few drops of saline. Use just enough to liquefy blood (do not exceed 3/8 total volume). Be careful not to use too much. For blood stained clothing, cut out stained area if possible and add small amount of saline, (the smaller amount of saline used, the better). Mash or squeeze the cloth in order to get heavy blood concentrations. If blood is extremely dry allow to soak approximately 5 minutes.

3. Using a pestle or glass rod, mash the meat or dried blood sample in order to acquire a liquid for testing. Mash the sample as much as possible in order to get a heavy concentration of blood.

4. Carefully punch 5 or 6 wells (holes) from the agar in the petri dish with an eyedropper. Squeeze the eyedropper bulb and gently touch the eyedropper tip to the surface of the agar, while releasing the bulb push the eyedropper tip through the agar to the bottom of the petri dish, and then lift the dropper vertically leaving a well (hole) in the agar. It is recommended that wells be approximately 1/4 inch apart.

5. Mark the petri dish with an indelible pen using an arrow to indicate the starting position and number each well. On a piece of paper draw a corresponding diagram with an arrow indicating the starting position and number each well of the petri dish (Diagram II.)

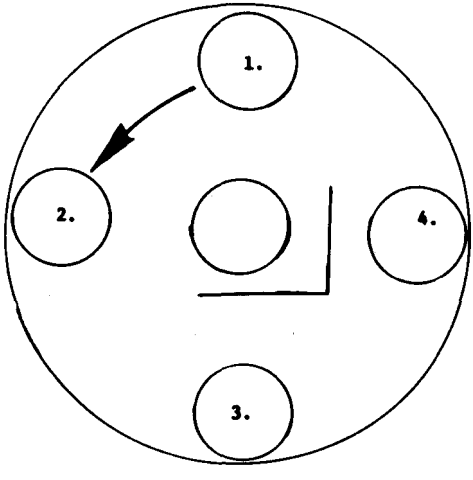
DIAGRAM I



1. Goat-anti-horse albumin
2. Goat-Anti-swine albumin
3. Goat-anti-deer albumin
4. Unknown sample*

*Note: In this case the unknown sample was identified as deer.

DIAGRAM II



1. Sample 1
1. Sample 2
1. Sample 3*
4. Known deer

Center - Goat-anti-deer albumin

*Note: In this case Sample 3 was identified as deer.

6. Add anti serum such as goat-anti-deer albumin in the center well (or the unknown, whichever you prefer). Do not overfill. Insert a capillary pipette into the sample of unknown blood. Allow the blood to enter the pipette then put the index finger over the top of the pipette. Transfer the blood to a well in the petri dish and slowly release the finger pressure on the pipette to allow the blood to fill the well. Again, do not overfill.

7. Fill remaining wells with known blood samples or known anti serum samples (i.e., goat-anti-swine albumin, goat-anti-bovine albumin). The placement of extracts and anti sera in the petri dish is largely a matter of preference. When only one unknown sample is being tested, it may be preferable to place it in the center well and the anti-serum samples in the surrounding wells. When more than one unknown sample is to be tested, it saves petri dishes and simplifies matters if the unknowns are placed in surrounding wells and the anti sera placed in the center well.

8. After the first ½ hour of testing adding more unknown blood and anti serum to wells will decrease the reaction time and form a more obvious precipitate. Place the lid on the petri dish.

9. After 24 hours observe the petri dish for lines of precipitation between unknown samples and anti sera. As these reagents diffuse the antibody and antigen will meet somewhere between the wells and a fine white line of antibody-antigen precipitate is formed. The analysis is qualitative only and therefore does not measure the percentage of the unknown present.

10. When observing results it is usually best to use sunlight against a dark background rather than artificial light. The petri dish may have to be positioned at just the right angle before precipitate lines are visible (Photo 1.)
When a precipitate is formed it indicates a positive reaction between antibodies and the reagent in question and verifies the presence of that species.

11. Always keep unused petri dishes and saline refrigerated. When the saline supply is depleted, it may be mixed by adding 1 teaspoon of table salt to 1 quart of distilled water. Commercially, saline is usually .85 - .9% NaCl with thimersol which is used in very small amounts. Anti sera should be frozen, however, as it is a blood-like substance and constant thawing and refreezing will accelerate its deterioration.

12. Always test anti sera by using known samples of blood or tissue. Diagram III shows a control test verifying that goat-anti-deer albumin does react with deer and does not react with other species.

13. Test results may be diagramed, photographed (with enlarger), or stained with protein dyes for ease of visibility and to preserve results visually.

Scientists and police laboratories use ouchterlony analyses to determine if blood stains are human or non-human and to determine whether or not substances are present in a solution.

Ouchterlony analyses may also be used to indicate contaminated meats such as sausage where venison may be mixed with pork or other meats (Diagram IV.)

DISCUSSION

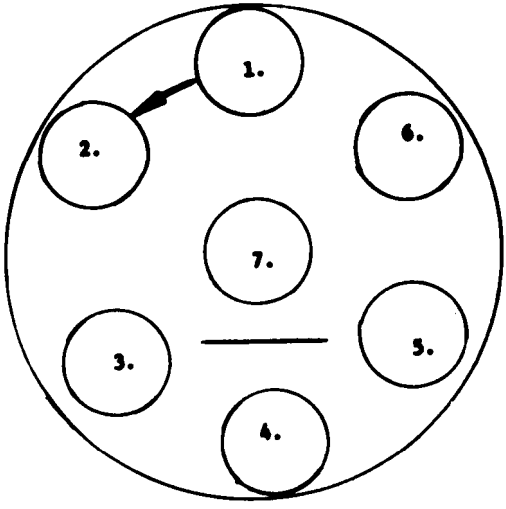
The original cost to implement this program was approximately 200-300 dollars. This provided 5 test kits containing the equipment necessary to complete examinations of blood/tissue of cow, pig, deer, alligator, and goat. An eight hour training program was conducted for wildlife officers to learn the basics of blood and tissue examination.

The test kit is a valuable aid to Game and Fish Agencies and to police investigators such as Sheriff's offices in determining what species are involved when blood/tissue samples are encountered in the course of an investigation.

The only problems encountered have been the unavailability of test kits since there were only five test kits in the state of Florida.

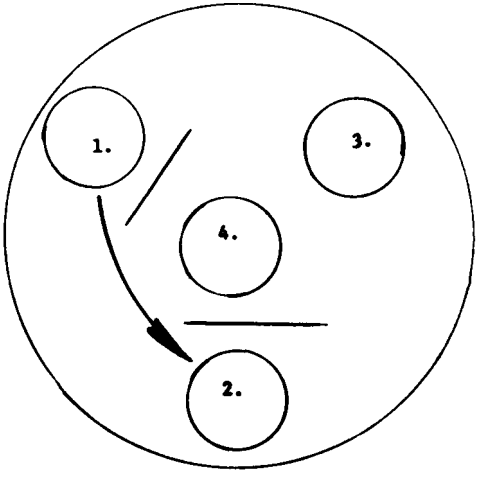
Future plans include obtaining more test kits so that each investigator will have a kit readily available as needed and to establish a correspondence course in cooperation with the University of Florida and the training office of the Game and Fresh Water

DIAGRAM III



1. Cow
2. Pig
3. Horse
4. Deer
5. Goat
6. Sheep
7. Center well is goat-anti-deer albumin.

DIAGRAM IV



Deer - Pork Mix

1. Goat-anti-deer albumin
2. Goat-anti-swine albumin
3. Goat-anti-bovine albumin
4. Hamburger-sausage-unknown

Fish Commission for certification in conducting ouchterlony analyses. This certification will help establish investigators as expert technicians and expert witnetsses in court proceedings.

Those agencies interested in having representatives certified should contact Capt. E. W. Lawrence, Training Coordinator, Florida Game and Fresh Water Fish Commission, Tallahassee, Florida.

GLOSSARY

Agar	An extract made from certain seaweeds that forms a semi-solid transparent gel when heated with water and allowed to cool.
Albumin	The most prevelent protein in an animal's serum.
Antibody	A protein in the blood produced when a foreign substance or antigen is introduced into the body.
Antigen	Any large molecule that causes the production of antibody when injected into a living animal. An antigen can combine with an antibody that is specific for that antigen only, as is often evidenced by the formation of a precipitate.
Antiserum	Serum from an animal that has been immunized with an antigen and which therefore contains antibodies to that antigen.
Bovine Albumin	Albumin found in cow serum (see albumin).
Deer Albumin	Albumin found in deer serum.
Denature	To cause structural damage to a protein by shaking, heating, or chemical treatment.
Diffusion	The way molecules physically spread themselves out to occupy the space that is available to them.
Goat Anti-Bovine Albumin	An antiserum to bovine albumin produced in the body of a goat.
Goat Anti-Deer Albumin	An antiserum to deer albumin produced in the body of a goat.
Goat Anti-Horse Albumin	An antiserum to horse albumin produced in the body of a goat.
Goat Anti-Swine Albumin	An antiserum to swine albumin produced in the body of a goat.
Immunology	The science dealing with the capacity to resist disease.
Precipitate	An insoluble compound which results from a chemical reaction between two substances in solution.
Petri Dish	A small, shallow dish of thin glass or plastic with a cover.
Reagents	Substances or solutions used in chemical reactions.
Serum	The yellow fluid which remains when blood has clotted and red and white blood cells have been removed. Serum makes up approximately 50% of the volume of blood.
Wells	Cylindrical holes cut in agar into which reagents are placed.

TABLE I. *Equipment needed for blood and tissue examination:*

Equipment needed for blood and tissue examinations may be obtained from S.R.S. Sera Company, 3454 N.W. 12th Avenue, Gainesville, Florida 32605.

1. Petri dishes with agar (kept refrigerated until ready for use).
2. Mortar and pestle
3. Eyedropper
4. Capillary pipettes
5. Saline
6. Anti-serum
7. Known blood/meat control samples

NOTE: Clean equipment is used with each reagent in order to avoid contamination.

TABLE II. Anti serum supply source

Antiserums, controls, and other immunochemicals may be commercially purchased from Cappel Laboratories, Inc., Thudridge Farm, Cochranville, Pennsylvania, 19330.

Those readily available antiserums of particular use include;

anti alligator serum
anti bear serum
anti bovine serum
anti cat serum
anti deer serum
anti duck serum
anti elk serum
anti goat serum
anti human serum
anti moose serum
anti pigeon serum
anti rabbit serum
anti swine serum
anti turkey serum
anti turtle serum

Custom antiserums may be special ordered.