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EDVO-Kit

192

Forensics Antigen Detection: Can A Dead Cat Tell Us If The Owner Was Murdered?

See Page 3 for storage instructions.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to introduce to the students the principles of antigen-antibody interactions for forensic identification of blood using the Ouchterlony procedure. Samples were obtained from the blood around the dead cat and from the blood stains on the bed sheets to determine if it was human or cat blood.

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All components are intended for educational research only. They are not to be used for diagnostic or drug purposes, nor administered to or consumed by humans or animals.

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This experiment is designed for 10 student groups.

No actual blood or blood products are used in this experiment.

Experiment Components

Store components A - F in the refrigerator.

	Component	Storage
A	Cat Anti-serum	Refrigerator
B	Human Anti-serum	Refrigerator
C	Control Cat blood sample	Refrigerator
D	Control Human blood sample	Refrigerator
E	Questioned Cat blood from crime scene	Refrigerator
F	Questioned blood from T-shirt	Refrigerator
G	Powdered buffer	Room temp.
•	UltraSpec-Agarose™	Room temp.
•	Practice loading solution	Room temp.
•	Transfer pipets	
•	Petri plates	
•	Well cutters	
•	Microtest tubes	

Requirements

- Micropipet and tips
- Pipet pump
- Plastic container or Pyrex baking dish
- Plastic wrap
- Distilled Water
- Pipets - 5 ml or 10 ml
- Marking pen
- Measuring spatula or toothpicks
- Heat plate, Bunsen burner, or microwave
- Paper towels
- Waterbath

This experiment does not contain components which have been prepared from human sources.

Background Information

Securing and Handling the Evidence

Forensic Scientists collect and analyze evidence from a crime scene in order to identify the nature of the evidence and its source. While this collection process takes place, the scientist cannot make any definitive statements about the nature of the evidence. One cannot assume that a red stain on the floor or a latent (not currently visible) stain found by another detection method is actually blood. The first step when dealing with any biological evidence is to correctly identify the material. It may seem obvious that the red stain on a knife found lying next to a murder victim is blood, yet it still must be tested to confirm if the stain is indeed blood. Only when you have determined the exact nature of the evidence, can further testing be done to produce additional information.

Determining the nature of the evidence is a complex multi-step process. Forensic scientists use various assays to quickly and accurately determine the identity of a substance that must also satisfy the following criteria: the test must be quick, inexpensive, and most importantly, it must minimally affect the evidence. It is important that the initial testing be performed quickly and inexpensively in order to determine the direction of the investigation. A lengthy, expensive test would waste time and money if the sample being tested turns out to be something other than what it's thought to be.

Maintaining the Integrity of the Evidence

In addition to collecting and testing evidence, the Forensic Scientist is also one of many people responsible for maintaining the integrity of the evidence itself. Steps must be taken to ensure that nothing is done to the evidence that would minimize or diminish its value, thus making it less useful in an important situation, as in a courtroom or trial setting. When evidence is collected, it is placed into a collection bag that is then sealed and taped shut, with the initials of the collector and collection date written across the tape. This is the first line of defense against any potential evidence tampering. In order to access the evidence, the seal on the bag/container must be broken. The evidence is then brought to a secure evidence room where signed records of it's arrival are documented. Access to the room is restricted to only a few people who keep track of all the items. Any scientist who wishes to perform a test on the evidence must sign for the items they remove, noting the date and time as well. Eventually, these records provide a detailed picture of when the evidence was collected and every time it was moved or accessed for testing.

Testing procedures are affected in a variety of ways. After retrieving the evidence from the storage room a forensic scientist will note the condition of the container is in. Is the container properly sealed? Does the evidence tape sealing the openings look undisturbed? Are there any new openings in the container? All these questions must be answered and the condition of the container must be noted in the scientist's lab notebook before testing can begin. Next, the scientist must open the container without disturbing the prior sealing done by others. If at all possible, new openings should be made. This allows others who have made openings and sealed them to be able to say that their seal was undisturbed afterwards. This is important to show that the evidence wasn't tampered with and to document everyone who has tested the evidence. Often, by the end of its life, the evidence bag will have numerous openings that have been resealed and signed. Even when testing the items, the scientist should take absolute care to ensure that evidence is never compromised to a point where contamination can occur. Contamination is

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Background Information

the transfer of minute amounts of material from one piece of evidence to another and it can drastically alter the value of the evidence and can result in a complete loss of value. For example, if two articles of clothing are laid on a lab bench together. One is a reference sample collected from a suspect. The other was found on the victim of a crime. By placing the items in the open air in the same room at the same time, you run the risk of material from one article transferring over to the other. This could result in an innocent suspect being punished for a crime he or she did not commit. When working on a crime case, only one piece of evidence should be unsealed at a time. With all items sealed in their respective bags, contamination cannot occur. After testing is complete, all items are then signed back into the evidence room by the scientist who removed them. This procedure is used to document all of those who had access to the evidence and what was done to the evidence.

Presumptive and Confirmatory Tests

These quick tests have to use very little sample so that further testing can be done. Often the amounts of evidence collected are very small. We can't use up an entire piece of evidence in one test. It is imperative that as a forensic scientist, you do your best to preserve as much of the evidence as possible. That is why simple chemical tests are used for the initial testing of possible biological evidence. There will of course be times when the amount of evidence is so small that the entire sample would be consumed in a test. In only these cases, the evidence is saved for the most conclusive tests. In all other cases, you will conduct both Presumptive and Confirmatory tests.

The first level of testing consists of Presumptive Tests. You have found a substance you think will contribute to your investigation and you want to know what the substance is. Common sense may tell you that the red liquid on the ground near a knife is probably blood but that doesn't mean you have scientifically proven it to the degree that it can be used as evidence in court. You instead perform simple tests to screen evidence in order to determine if further testing is necessary.

These initial tests are called "presumptive" because of their potential results. Presumptive tests can, at best, only strongly indicate that the tested substance is what you think it is. This is because there are other substances which could give a positive result to the test, making you think that your sample is one thing when it is actually another. These are called "false positives". It is very likely that the positive result is correct and with experience you can learn to tell a true positive from some of the false one. But, because there is a chance of a false positive, you can't say for sure that you have confirmed the identity of the tested substance.

That is where aptly named Confirmatory Tests come in. Presumptive testing has indicated that the substance is probably what you think it is. Now, you have to perform a test that conclusively confirms this substance is what you think. As with the presumptive tests, it is the results of a confirmatory test that place it in this category. Confirmatory tests do not give false positives. A positive result from a confirmatory test for blood means that the substance you tested is blood. It is only after you have gotten a correct, positive result from a confirmatory test that you can state the identity of a substance.

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The Ultimate Purpose of Forensic Testing: The Courtroom

Why do we go through all of these steps? The first reason is simply that it is part of doing a good job. As a Forensic Scientist, you should feel proud of the quality of the work you do and should always be striving to perform as well as you possibly can. That means you should be taking all the necessary actions to make sure that your results are accurate, repeatable and timely. In short, you should strive to be an exemplary scientist.
The second and more practical reason is that this evidence ultimately has a purpose and if you don't do a good job, you will make the evidence unfit for that purpose. And that purpose is of course the use of the evidence in a courtroom setting. Regardless of whether or not you are working for a law enforcement agency or at a private lab doing work for clients, your work is eventually going to end up being used in the justice system to help assign responsibility for a crime. Sometimes, these crimes are very serious and the judgment of the court could seriously affect the lives of the people involved in the case. Incorrect results on your part can allow guilty people to go free and can condemn the innocent to horrible fates. Any time a forensic scientist might be annoyed with the myriad rules in place to govern their actions, they would do well to remember the far-reaching consequences of possible mistakes.

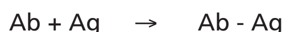
A CONFIRMATORY TEST: SPECIES IDENTIFICATION USING THE OUCHTERLONY DOUBLE DIFFUSION TEST

Species Identification

As previously stated, you cannot assume anything about the nature of a possible biological stain found at a crime scene. Even if you can show that it is blood, you can't immediately assume that it is human blood. The first steps that the forensic scientist would take after collection would be to determine if the substance was in fact blood. This is accomplished through several types of presumptive and confirmatory tests. A popular presumptive test is the Kastle-Meyer test. And increasingly, tests that exploit the Antigen-Antibody interaction have been used as confirmatory tests. These confirmatory tests show only that the substance is blood, relying on the testing on antibodies common to all blood types and common among many mammalian species.

Often after these confirmatory tests, the samples will be processed using PCR based DNA fingerprinting techniques. Obtaining a forensic profile from the sample is also confirmatory for blood, particularly human blood as the DNA primers used in the PCR process are specific to certain regions of human DNA. If the sample does produce a full profile, it is absolutely a human sample. But this test is time consuming and expensive and results may not be back from the lab for weeks. A simple chemical test that only tests for the species of the source of the blood is often needed. In that instance, a more specific version of an Antibody-Antigen interaction is used.

The interactions of an antibody (Ab) with an antigen (Ag) is the fundamental reaction of immunology.



Most antigens are proteins. The exact identity of the groups that react with the antibody are usually not known. Macromolecular antigens and antibodies form complexes that become insoluble and precipitate from solution. This property makes it possible to perform

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qualitative and quantitative assays on the antibody-antigen system.

Precipitation occurs with most antigens because the antigen is multivalent, i.e., has several antigenic determinants per molecule to which antibodies can bind. Antibodies have at least two antigen binding sites, thus large aggregates or lattices of antigen and antibody are formed. Experimentally, an increasing amount of antigen is added to a constant amount of antibody in solution. Initially at low antigen concentration, all of the antigen is contained in the precipitate. This is called the antibody-excess zone. As more antigen is added, the amount of protein precipitated increases until the antigen and antibody molecules are at an optimal ratio. This is called the equivalence zone, or equivalence point, where maximum precipitation occurs. When the amount of antigen in solution exceeds the amount of antibody, the amount of precipitation will decrease. This is known as the antigen-excess zone (Figures 1 and 2).

When antibodies and antigens are inserted into different areas of an agarose gel, they diffuse toward each other and form opaque bands of precipitate at the interface of their diffusion fronts. Precipitation reactions of antibodies and antigens in agarose gels provide a method of analyzing various antibody-antigen reactions.

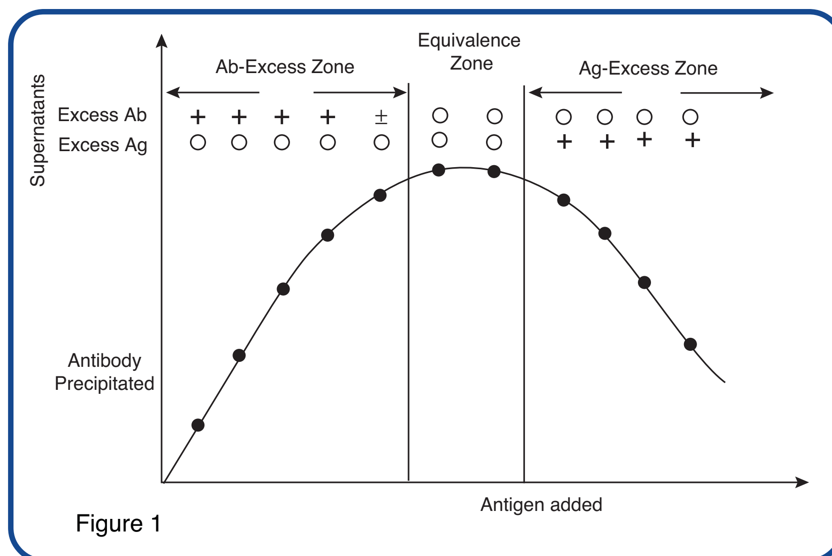

Figure 1

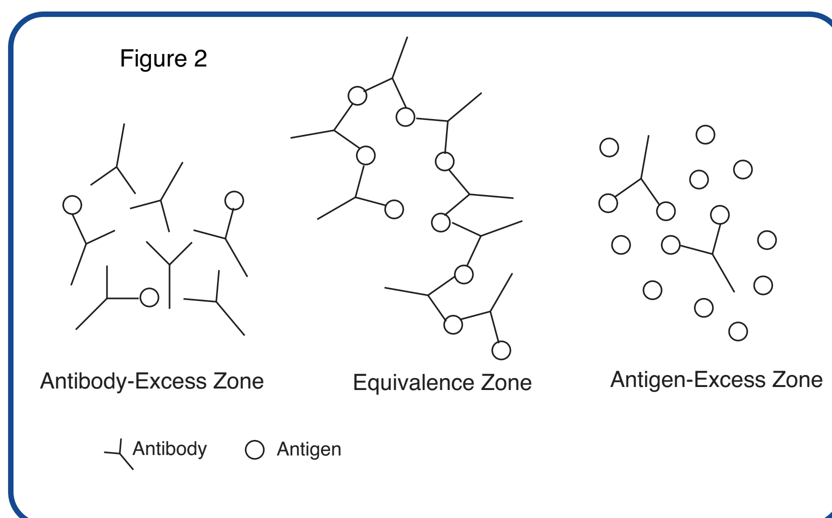

Figure 2

THE OUCHTERLONY PROCEDURE

Double diffusion in two dimensions is a simple procedure invented by and named after the Swedish scientist, Örjan Ouchterlony. Antigen and antibody solutions are placed in separate wells cut in an agarose plate. The reactants diffuse from the wells toward each other and precipitate where they meet at equivalent proportions. A single antigen will combine with its homologous antibody to form a single precipitation line. When two antigens are present, each behaves independently of each other. Thus, the number of precipitin bands indicates there are at least that many antibody-antigen pairs present (see Figure 3). Arrows indicate diffusion patterns of antigens and antibodies.

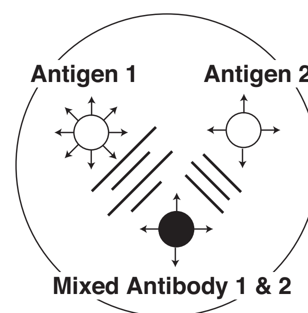

Figure 3

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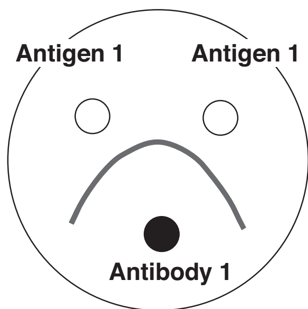

Figure 4:
Reaction of Identity

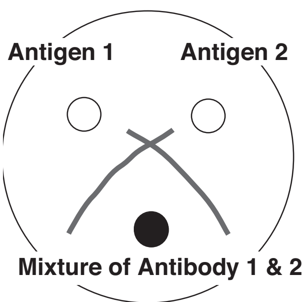

Figure 5
Reaction of Non-identity

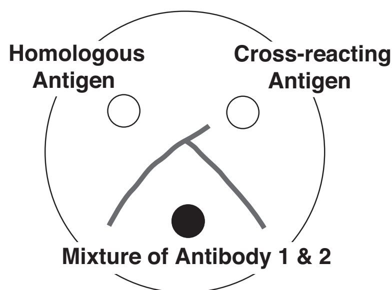

Figure 6
Reaction of Partial Identity

The patterns shown in Figures 3 - 6 are the ideal representation. Under experimental conditions, the spurs are often difficult to visualize.

Double diffusion in two dimensions is a useful technique for comparing antigens for the number of identical or cross-reacting determinants. If a solution of antigen is placed in two adjacent wells and the homologous antibody is placed in the center well, the two precipitin bands that form will join at their closest ends and fuse. This is known as a reaction of identity (Figure 4).

When unrelated antigens are placed in adjacent wells and the center well is filled with antibodies for each antigen, the precipitin bands will form independently of each other and will cross. This is known as a reaction of non-identity (Figure 5).

If two purified antigens cross-react, then placing them in adjacent peripheral wells with antibody to one in the central well will give a single band with the homologous and cross-reacting antigen. Since the cross-reacting antigen lacks some of the antigenic determinants present in the homologous antigen, it is not able to precipitate all of the antibody. The remaining antibody will diffuse beyond the line of cross-reacting precipitate to react with the homologous antigen to produce a spur. The spur that forms projects toward the antigen with the fewer determinants, i.e., the cross-reacting antigen. This is called a reaction of partial identity. Since these non-cross-reacting antibodies often are only a fraction of the total antibody involved in the homologous precipitin reaction, the spur is usually less dense (often difficult to visualize) than the precipitin band from which it projects (See Figure 7).

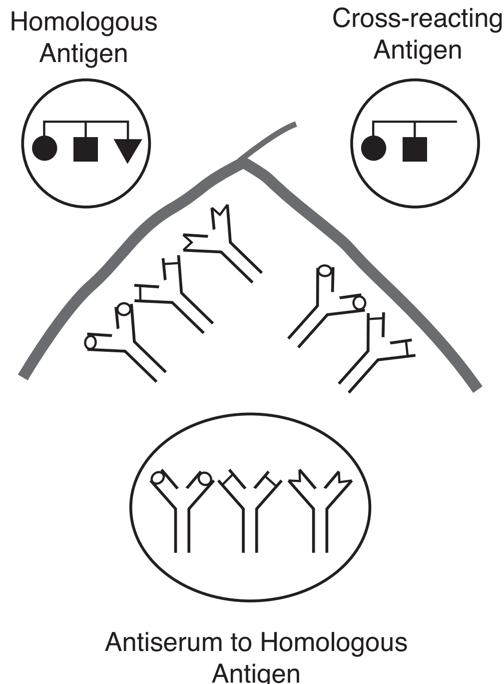

Figure 7

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Experiment Overview and General Instructions

BACKGROUND:

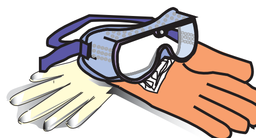
A young woman who lives with her cat was last seen on her daily run. After two days, she was reported missing to the police by her friends. Upon entering her penthouse, her cat was found in a pool of blood, shot in the head. Tiny drops of bloodstains were found trailing from her bed sheets to the blood by the cat. The detective in charge concluded that the woman and the cat were brutally murdered, her body was removed from the site of the crime, and the tiny stains of blood were overlooked when the area was cleaned. Samples were obtained from the blood around the dead cat and from the blood stains on the bed sheets to determine if it was human or cat blood. Students will determine the validity of the hypothesis set forth by the detective.

EXPERIMENT OBJECTIVE:

The objective of this experiment is to introduce to the students the principles of antigen-antibody interactions for forensic identification of blood using the Ouchterlony procedure. Samples were obtained from the blood around the dead cat and from the blood stains on the bed sheets to determine if it was human or cat blood.

LABORATORY SAFETY

No human materials are used in this experiment. Gloves and safety goggles should be worn as good laboratory practice.



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Student Experimental Procedures

A. PREPARATION OF AGAROSE & POURING OF OUCHTERLONY PLATES

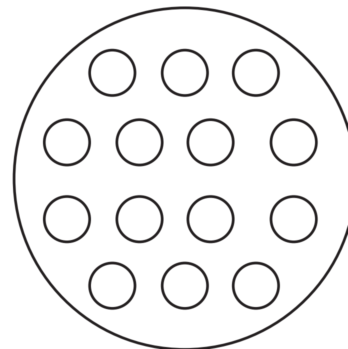
1. Each group requires 3 plates: 1 practice loading plate and 2 experimental plates. Using a 5 ml or a 10 ml pipet, carefully pipet 5 ml of the cooled agarose (65°C) into each plate, rotating the plate to cover the bottom with agarose. Repeat with the remaining plates.

2. If the molten agarose contains bubbles, gently swirl to remove the bubbles.

3. Allow the agarose to solidify. This will take approximately 10-15 minutes, at which time the gel will appear slightly opaque.

4. If the plates are not to be used that day, the plates can be wrapped with plastic wrap and stored inverted in the refrigerator for two weeks.

B. PRACTICE WELL LOADING (OPTIONAL)



This experiment contains practice loading solution. This solution is included to allow instructors and students to practice loading the sample wells before performing the actual experiment . Use a micropipetting device, or one of the plastic transfer pipets included in your experiment kit to practice loading the sample wells with the practice loading solution.

1. One practice plate should be prepared for each group. Enough reagents have been provided for this purpose.

2. Using the well cutters provided, cut several rows of wells as shown in the diagram above.

3. Practice loading the sample wells with the plastic, disposable transfer pipets. (See "Sample Loading of Wells with Transfer Pipets").

4. If you are using an automatic micropipetting device, the amount of sample that should be loaded is 30 microliters.

Sample Loading of Wells With Transfer Pipets

1. Squeeze the pipet stem, not the bulb, to slowly draw a portion of the sample up into the pipet. The sample should remain in the lower portion of the pipet.

If the sample is overdrawn and becomes lodged in the bulb or on the walls, tap until the sample moves down into the lower stem of the pipet. Eject it back into the tube. Try step 1 again.

2. While holding the pipet tip above the tube, slowly squeeze the pipet stem until the sample is nearly at the opening of the pipet tip.

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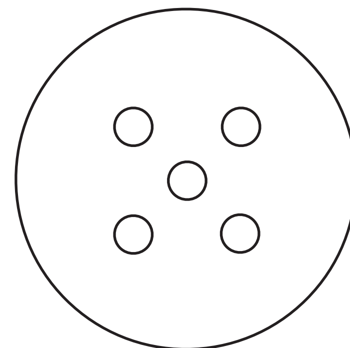
Student Experimental Procedures

3. Place the pipet tip just over, not inside, the sample well. Maintain steady pressure on the pipet stem to prevent sample from being drawn back up into the pipet.
4. Slowly squeeze the pipet bulb to eject two (2) drops of sample. The well should appear full, but be careful not to overfill the wells and cause spillage on the agarose surface. Put any remaining sample in the pipet back into the tube.

C. PREPARATION OF SAMPLE WELLS



1. Make several copies of the template (at right) for your lab group.

2. Place the template under one of the plates so that the pattern is in the center of the plate. The distances between the wells is important. Try to follow the template as accurately as possible.

3. Cut the five wells using the well cutter (provided in the kit) in a gentle punching motion. Remove the agarose plugs with a flat-edged toothpick or spatula.

4. If well placement is not accurate, there should be enough room on the plate to re-cut the wells using the template.

5. Repeat steps 2 and 3 with the remaining plates.

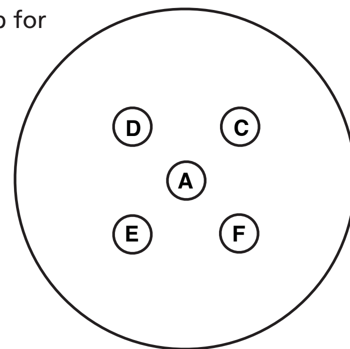
D. LOADING THE SAMPLES

1. Orient your lab number or group designation at the top before loading samples.

2. Using the same pipet, fill the center well of plate 1 with 30 microliters (2 drops with a transfer pipet) of the Cat antiserum from Tube A. Wells should appear full, but be careful not to overfill the wells and cause spillage on the agarose surface. This may affect your results.

3. Fill the outer wells with 30 microliters of blood samples using a clean pipet tip for each sample as follows:

   Plate 1
   Center well: Cat antiserum (Tube A)
   Left upper well: Control Human blood sample (Tube D)
   Right upper well: Control Cat blood sample (Tube C)
   Left lower well: Questioned Cat blood from crime scene (Tube E)
   Right lower well: Questioned blood from T-shirt (Tube F)



4. Using a new pipet, fill the center well of plate 2 with 30 microliters (2 drops with a transfer pipet) of the Human antiserum from Tube B. Wells should appear full, but be careful not to overfill the wells and cause spillage on the agarose surface. This may affect your results.

Experiment Procedure

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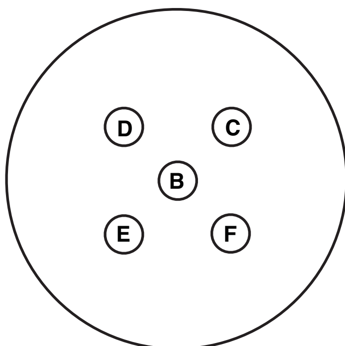
Student Experimental Procedures



5. Also fill the outer wells with 30 microliters of blood samples using a clean pipet tip for each sample as follows:

   Plate 2
   Center well: Human antiserum (Tube B)
   Left upper well: Control Human blood sample (Tube D)
   Right upper well: Control Cat blood sample (Tube C)
   Left lower well: Questioned Cat blood from crime scene (Tube E)
   Right lower well: Questioned blood from T-shirt (Tube F)

E. INCUBATION

Replace lids onto plates. Carefully place the covered plates in the incubation chamber on top of the wet paper towel layer. Do not invert the plates. Cover the chamber with plastic wrap and let incubate at room temperature 24-48 hours to allow precipitin lines to form or the chamber can be placed in a 37°C incubation oven.

F. READING THE RESULTS

The precipitin lines will be visible in 24-48 hours. Carefully hold a plate up so that the overhead room lights shine through it. You should be able to see opaque white arcs in each side of the plate where the antibody and antigen precipitated. A drawing of the results should be made.

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Student Experimental Results

LABORATORY NOTEBOOK RECORDINGS:

Address and record the following in your laboratory notebook or on a separate worksheet.

Before starting the experiment:

- Write a hypothesis that reflects the experiment
- Predict experimental outcomes

During the Experiment:

- Record (draw) your observations, or photograph the results.

Following the Experiment:

- Formulate an explanation from the results.
- Determine what could be changed in the experiment if the experiment were repeated.
- Write a hypothesis that would reflect this change.

Study Questions

Answer the following study questions in your laboratory notebook or on a separate worksheet.

1. In what situation would you expect to see more than one precipitation line between wells on an Ouchterlony plate?
2. What three principle type of reactions may be observed in Ouchterlony gel diffusion?

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Notes:

Instructor's Guide

Notes to the Instructor & Pre-Lab Preparations

If you do not find the answers to your questions in this section, a variety of resources are continuously being added to the EDVOTEK® web site. In addition, Technical Service is available from 9:00 am to 6:00 pm, Eastern time zone. Call for help from our knowledgeable technical staff at 1-800- EDVOTEK (1-800-338-6835).

APPROXIMATE TIME REQUIREMENTS

Your individual schedule and time requirements will determine when the Ouchterlony plates should be prepared. It takes approximately 20 to 30 minutes to prepare the plates (generally 10 minutes of this time is required for solidification). Students can prepare the plates, if time allows.

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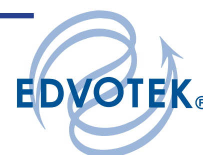
Please have the following information ready:

- **Experiment number and title**
- **Kit lot number on box or tube**
- **Literature version number** (in lower right corner)
- **Approximate purchase date**

Visit the EDVOTEK web site often for continuously updated information.



Notes to the Instructor & Pre-Lab Preparations

A. PREPARATION OF AGAROSE AND POURING OF OUCHTERLONY PLATES

1. In a 500 ml Erlenmeyer flask or beaker, add the entire contents of powdered buffer package (Component G) to 225 ml of distilled water. Swirl the flask or beaker to dissolve the powder.

2. Add the entire contents of the agarose package to the flask or beaker. Swirl to disperse large clumps. With a marking pen, indicate the level of solution volume on the outside of the flask or beaker.

3. You must boil the solution to dissolve the agarose. This can be accomplished with a hot plate or Bunsen burner. Cover the beaker with foil. Heat the mixture to boiling over a burner with occasional swirling. Wear safety goggles and a hot glove. Boil the mixture until all the gelatinous agarose is dissolved. During heating, occasionally remove the beaker from the heat and check to see that there are no small, clear particles of agarose. Continue heating with occasional swirling. The final solution should be clear.

A microwave can also be used to melt the agarose (no foil cover) on high in 30 sec. pulses for 2 minutes if required. Swirl the flask in between pulses and microwave for an additional 1-2 minutes. Check to see that there are no small clear particles of agarose. The final solution should be clear.

4. If detectable evaporation has occurred, add distilled water to bring the solution up to the original volume as marked on the flask or beaker in step 2. Total volume should be a minimum of 230 ml.

5. Cool the agarose solution to 65°C in a waterbath. Swirl to promote even dissipation of heat.

6. Aliquot 20 ml of the agarose solution (65°C) for each of the ten groups.

Proceed to A-Preparation of Agarose and Pouring of Ouchterlony Plates on page 10 promptly to prevent solidification of agarose solution. If the solution happens to solidify prior to pouring of Ouchterlony plates, quickly warm it up in a microwave to dissolve.

B. PREPARATION OF INCUBATION CHAMBER (PREPARE DAY OF LABORATORY)

Line the bottom of a plastic container or glass container (such as a Pyrex® dish) with several paper towels. Soak the paper towels with distilled water. There should not be any layer of liquid above the paper towels. All liquid should be absorbed into the paper toweling. Cover the entire chamber with plastic wrap.

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Notes to the Instructor & Pre-Lab Preparations

C. PREPARATION OF ANTIBODY AND ANTIGENS

Four Blood samples and two antisera have been supplied in bulk. It is recommended that the blood samples and antisera are aliquoted for each group.

1. Label 10 microtest tubes "A".
2. Label 10 microtest tubes "B".
3. Label 10 microtest tubes "C".
4. Label 10 microtest tubes "D".
5. Label 10 microtest tubes "E".
6. Label 10 microtest tubes "F".
7. Aliquot 40 µl of Cat antiserum (component A) into each tube "A".
8. Aliquot 40 µl of Human antiserum (component B) into each tube "B".
9. Aliquot 80 µl of Control Cat blood sample (component C) into each tube "C".
10. Aliquot 80 µl of Control Human blood sample (component D) into each tube "D".
11. Aliquot 80 µl of Questioned Cat blood from crime scene (component E) into each tube "E".
12. Aliquot 80 µl of Questioned blood from T-shirt (component F) into each tube "F".
13. Each group requires one each of tube A, B, C, D, E and F.
14. Aliquot 10 microtest tubes 100 µl of practice solution per group.

AVOIDING COMMON PITFALLS

1. Follow instructions carefully when preparing the gel for the plates. Make sure the agarose is completely dissolved.

2. Make neat, clean wells with the well cutters. Take measures to ensure that the wells are properly spaced according to the template on page 11.

3. Add samples to the wells carefully and precisely. Avoid overfilling the wells.

4. Do not tip or invert plates when transferring to the humidity chamber.

5. Placing the humidity chamber in a 37° C incubation oven will expedite the formation of precipitin arcs.

6. Absence of precipitin lines is usually due to disproportionate pipetting between antiserum and blood sample wells, or distance between antiserum and blood sample wells.

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Please refer to the kit insert for the Answers to Study Questions

EDVOTEK® Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200. Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): **Simulated Blood Samples / 192 - Human Simulated Blood**

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK	Emergency Telephone Number: 202-370-1500
Address (Number, Street, City, State, and ZIP Code): 1121 5th Street NW, Washington DC 20001	Telephone Number for information: 202-370-1500
	Date Prepared: 08/25/11
	Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identity Information

Hazardous Components (Specific Chemical Identity; Common Name(s))	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
BSA CAS#9048-46-8				

Section III - Physical/Chemical Characteristics

Boiling Point	NA	Specific Gravity (H_2O = 1)	NA
Vapor Pressure (mm Hg.)	NA	Melting Point	NA
Vapor Density (AIR =1)	NA	Evaporation Rate (Butyl Acetate =1)	NA

Solubility in Water: soluble

Appearance and Odor: red liquid

Section IV - Fire and Explosion Hazard Data

Flash Point (Method Used) | Flammable Limits | LEL | UEL

Extinguishing Media: Water spray, alcohol-resistant foam, dry chem or carbon dioxide

Special Fire Fighting Procedures: Wear self contained breathing apparatus if necessary

Unusual Fire and Explosion Hazards

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	

Incompatibility (Materials to avoid): Strong oxidizing agents.

Hazardous Decomposition or Byproducts: Under fire conditions - carbon oxides, nitrogen oxides

Hazardous Polymerization		
	May Occur	
	Will Not Occur	X

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes | Skin? Yes | Ingestion? Yes

Health Hazards (Acute and Chronic): May be harmful or irritating to eyes, skin, respiratory, or if swallowed.

Carcinogenicity: NTP? | IARC Monographs? | OSHA Regulation?

Signs and Symptoms of Exposure

Medical Conditions Generally Aggravated by Exposure

Emergency and First Aid Procedures: In case of eye contact immediately flush with water, with skin wash with soap and water. If breathed in, remove to fresh air. If swallowed, rinse mouth with water.

Section VII - Precautions for Safe Handling and Use

Steps to Be Taken in case Material Is Released or Spilled: Use absorbant material and keep in suitable, closed container for disposal.

Waste Disposal Method: Follow federal, local, and state laws.

Precautions to be Taken in Handling and Storing: Keep container tightly closed in a dark, dry and well-ventilated place. Non-hazardous for travel.

Other Precautions

Section VIII - Control Measures

Respiratory Protection (Specify Type)

Ventilation		
Local Exhaust		Special
Mechanical (General)		Other

Protective Gloves: Yes | Eye Protection: Safety goggles

Other Protective Clothing or Equipment

Work/Hygienic Practices: General Indrustrial hygiene practice.

EDVOTEK® Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): **Powdered Buffer**

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.	Emergency Telephone Number: 202-370-1500
Address (Number, Street, City, State, Zip Code): 1121 5th Street NW, Washington DC 20001	Telephone Number for information: 202-370-1500
	Date Prepared: 08/25/11
	Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)

Section III - Physical/Chemical Characteristics

Boiling Point	100C	Specific Gravity (H_2O = 1)	1.017
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: soluble

Appearance and Odor: solid

Section IV - Physical/Chemical Characteristics

Flash Point (Method Used): Noncombustible | Flammable Limits | LEL | UEL

Extinguishing Media: Use extinguishing media appropriate to surrounding fire

Special Fire Fighting Procedures: Wear SCBA and protective clothing to prevent contact with skin and eyes

Unusual Fire and Explosion Hazards: Emits toxic fumes under fire conditions

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable		

Incompatibility: Strong acids

Hazardous Decomposition or Byproducts: Nature of decomposition products not known

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur		

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes | Skin? Yes | Yes | Ingestion?

Health Hazards (Acute and Chronic): Cause eye & skin irritation, material is irritating to mucous membranes and upper respiratory tract. The toxocological properties have not been thoroughly investigated.

Carcinogenicity: NTP? | IARC Monographs? | OSHA Regulation?

Signs and Symptoms of Exposure

Medical Conditions Generally Aggravated by Exposure

Emergency First Aid Procedures: Swallowed - wash out mouth with water provided person is conscious. Skin/eye contact - flush with water Inhalation - remove to fresh air

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Wear respirator, chemical safety goggles, rubber boots and heavy rubber gloves, sweep up, place in a bag and hold for waste disposal.

Waste Disposal Method: For small quantities - cautiosly add to a large stirred excess of water. Adjust pH to neutral

Precautions to be Taken in Handling and Storing: Wear appropriate NIOSH/MSHA approved respirator, chemical resistant gloves, safety goggles safety shower and eye bath.

Other Precautions: Non-hazardous for travel.

Section VIII - Control Measures

Respiratory Protection (Specify Type): NIOSH/MSHA approved respirator

Ventilation			
Local Exhaust	N/A	Special	N/A
Mechanical (General)	N/A	Other	N/A

Protective Gloves: Yes | Eye Protection: Yes

Other Protective Clothing or Equipment

Work/Hygienic Practices: Do not ingest. Avoid contact with skin, eyes and clothing. Wash thoroughly after handling.

EDVOTEK® Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200 Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): **UltraSpec-Agarose**

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK, Inc.	Emergency Telephone Number: 202-370-1500
Address (Number, Street, City, State, Zip Code): 1121 5th Street NW, Washington DC 20001	Telephone Number for information: 202-370-1500
	Date Prepared: 08/25/11
	Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identify Information

Hazardous Components [Specific Chemical Identity; Common Name(s)]	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)

This product contains no hazardous materials as defined by the OSHA Hazard Communication Standard.

CAS #9012-36-6

Section III - Physical/Chemical Characteristics

Boiling Point For 1% solution	194 F	Specific Gravity (H_2O = 1)	No data
Vapor Pressure (mm Hg.)	No data	Melting Point	No data
Vapor Density (AIR = 1)	No data	Evaporation Rate (Butyl Acetate = 1)	No data

Solubility in Water: Insoluble - cold

Appearance and Odor: White powder, no odor

Section IV - Physical/Chemical Characteristics

N.D. = No data

Flash Point (Method Used): No data | Flammable Limits | LEL N.D. | UEL N.D.

Extinguishing Media: Water spray, dry chemical, carbon dioxide, halon or standard foam

Special Fire Fighting Procedures: Possible fire hazard when exposed to heat or flame

Unusual Fire and Explosion Hazards: None

Section V - Reactivity Data

Stability			Conditions to Avoid
	Unstable		
	Stable	X	None

Incompatibility: No data available

Hazardous Decomposition or Byproducts

Hazardous Polymerization			Conditions to Avoid
	May Occur		
	Will Not Occur	X	None

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes | Skin? Yes | Ingestion? Yes

Health Hazards (Acute and Chronic): Inhalation: No data available. Ingestion: Large amounts may cause diarrhea

Carcinogenicity: NTP? | IARC Monographs? | OSHA Regulation?

Signs and Symptoms of Exposure: No data available

Medical Conditions Generally Aggravated by Exposure: No data available

Emergency First Aid Procedures: Treat symptomatically and supportively

Section VII - Precautions for Safe Handling and Use

Steps to be Taken in case Material is Released for Spilled: Sweep up and place in suitable container for disposal

Waste Disposal Method: Normal solid waste disposal

Precautions to be Taken in Handling and Storing: Non-hazardous for travel.

Other Precautions: None

Section VIII - Control Measures

Respiratory Protection (Specify Type): Chemical cartridge respirator with full facepiece.

Ventilation		
Local Exhaust		Special
Mechanical (General)	Gen. dilution ventilation	Other

Protective Gloves: Yes | Eye Protection: Splash proof goggles

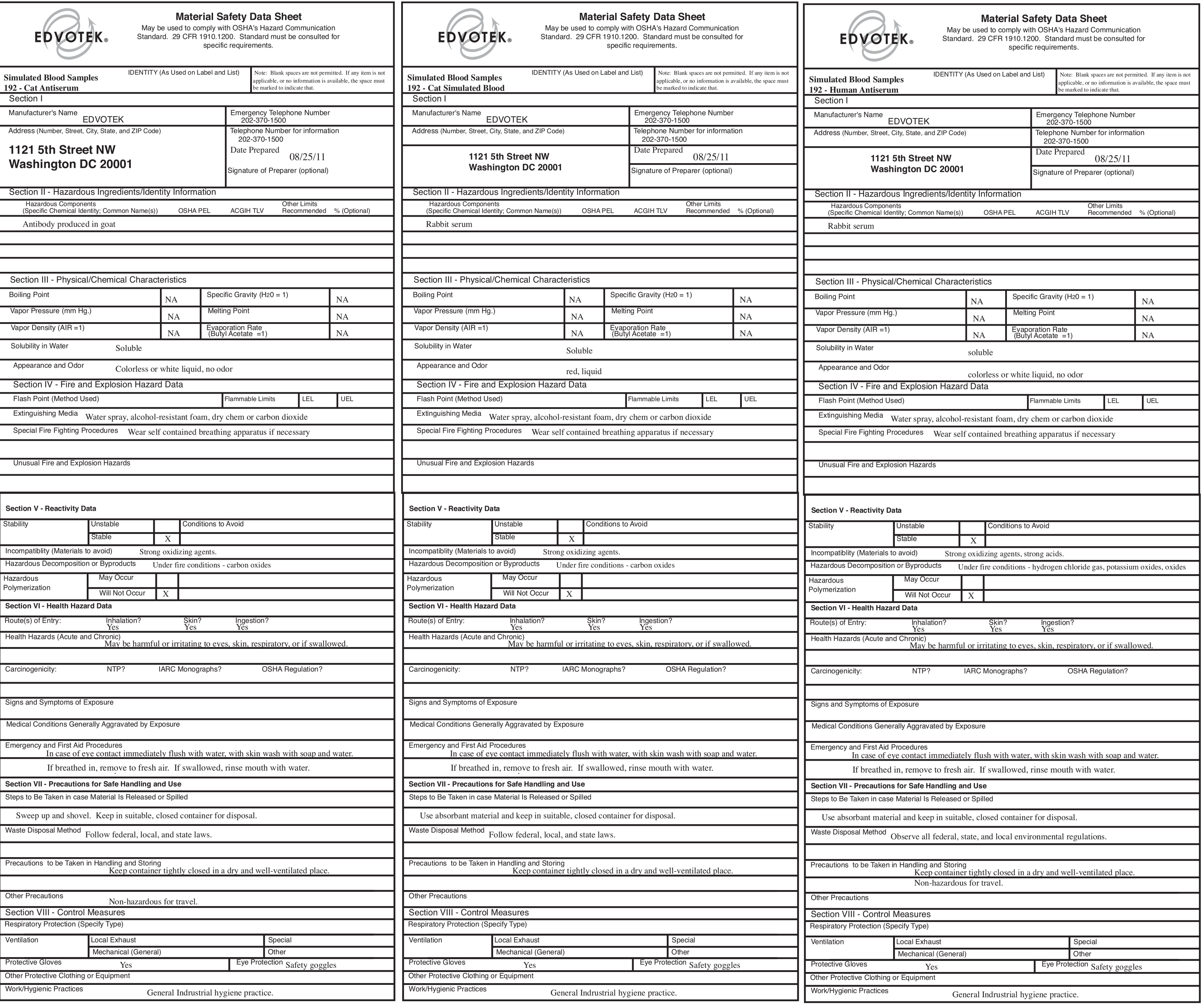
Other Protective Clothing or Equipment: Impervious clothing to prevent skin contact

Work/Hygienic Practices: None

192 Experiment

Material Safety Data Sheets

Full-size (8.5 x 11”) pdf copy of MSDS is available at www. edvotek.com or by request.



EDVOTEK® Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200. Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Simulated Blood Samples 192 - Cat Antiserum

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK
Emergency Telephone Number: 202-370-1500
Address (Number, Street, City, State, and ZIP Code): 1121 5th Street NW Washington DC 20001
Telephone Number for information: 202-370-1500
Date Prepared: 08/25/11
Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identity Information

Hazardous Components (Specific Chemical Identity; Common Name(s))	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Antibody produced in goat				

Section III - Physical/Chemical Characteristics

Boiling Point	NA	Specific Gravity (H_20 = 1)	NA
Vapor Pressure (mm Hg.)	NA	Melting Point	NA
Vapor Density (AIR =1)	NA	Evaporation Rate (Butyl Acetate =1)	NA

Solubility in Water: Soluble
Appearance and Odor: Colorless or white liquid, no odor

Section IV - Fire and Explosion Hazard Data

Flash Point (Method Used) | Flammable Limits | LEL | UEL
Extinguishing Media: Water spray, alcohol-resistant foam, dry chem or carbon dioxide
Special Fire Fighting Procedures: Wear self contained breathing apparatus if necessary
Unusual Fire and Explosion Hazards

Section V - Reactivity Data

Stability: Unstable | Stable X | Conditions to Avoid
Incompatiblity (Materials to avoid): Strong oxidizing agents.
Hazardous Decomposition or Byproducts: Under fire conditions - carbon oxides
Hazardous Polymerization: May Occur | Will Not Occur X

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes
Health Hazards (Acute and Chronic): May be harmful or irritating to eyes, skin, respiratory, or if swallowed.
Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?
Signs and Symptoms of Exposure
Medical Conditions Generally Aggravated by Exposure
Emergency and First Aid Procedures: In case of eye contact immediately flush with water, with skin wash with soap and water. If breathed in, remove to fresh air. If swallowed, rinse mouth with water.

Section VII - Precautions for Safe Handling and Use

Steps to Be Taken in case Material Is Released or Spilled: Sweep up and shovel. Keep in suitable, closed container for disposal.
Waste Disposal Method: Follow federal, local, and state laws.
Precautions to be Taken in Handling and Storing: Keep container tightly closed in a dry and well-ventilated place.
Other Precautions: Non-hazardous for travel.

Section VIII - Control Measures

Respiratory Protection (Specify Type)
Ventilation: Local Exhaust | Special | Mechanical (General) | Other
Protective Gloves: Yes | Eye Protection: Safety goggles
Other Protective Clothing or Equipment
Work/Hygienic Practices: General Indrustrial hygiene practice.

EDVOTEK® Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200. Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Simulated Blood Samples 192 - Cat Simulated Blood

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK
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Address (Number, Street, City, State, and ZIP Code): 1121 5th Street NW Washington DC 20001
Telephone Number for information: 202-370-1500
Date Prepared: 08/25/11
Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identity Information

Hazardous Components (Specific Chemical Identity; Common Name(s))	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Rabbit serum				

Section III - Physical/Chemical Characteristics

Boiling Point	NA	Specific Gravity (H_20 = 1)	NA
Vapor Pressure (mm Hg.)	NA	Melting Point	NA
Vapor Density (AIR =1)	NA	Evaporation Rate (Butyl Acetate =1)	NA

Solubility in Water: Soluble
Appearance and Odor: red, liquid

Section IV - Fire and Explosion Hazard Data

Flash Point (Method Used) | Flammable Limits | LEL | UEL
Extinguishing Media: Water spray, alcohol-resistant foam, dry chem or carbon dioxide
Special Fire Fighting Procedures: Wear self contained breathing apparatus if necessary
Unusual Fire and Explosion Hazards

Section V - Reactivity Data

Stability: Unstable | Stable X | Conditions to Avoid
Incompatiblity (Materials to avoid): Strong oxidizing agents.
Hazardous Decomposition or Byproducts: Under fire conditions - carbon oxides
Hazardous Polymerization: May Occur | Will Not Occur X

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes
Health Hazards (Acute and Chronic): May be harmful or irritating to eyes, skin, respiratory, or if swallowed.
Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?
Signs and Symptoms of Exposure
Medical Conditions Generally Aggravated by Exposure
Emergency and First Aid Procedures: In case of eye contact immediately flush with water, with skin wash with soap and water. If breathed in, remove to fresh air. If swallowed, rinse mouth with water.

Section VII - Precautions for Safe Handling and Use

Steps to Be Taken in case Material Is Released or Spilled: Use absorbant material and keep in suitable, closed container for disposal.
Waste Disposal Method: Follow federal, local, and state laws.
Precautions to be Taken in Handling and Storing: Keep container tightly closed in a dry and well-ventilated place.
Other Precautions

Section VIII - Control Measures

Respiratory Protection (Specify Type)
Ventilation: Local Exhaust | Special | Mechanical (General) | Other
Protective Gloves: Yes | Eye Protection: Safety goggles
Other Protective Clothing or Equipment
Work/Hygienic Practices: General Indrustrial hygiene practice.

EDVOTEK® Material Safety Data Sheet

May be used to comply with OSHA's Hazard Communication Standard. 29 CFR 1910.1200. Standard must be consulted for specific requirements.

IDENTITY (As Used on Label and List): Simulated Blood Samples 192 - Human Antiserum

Note: Blank spaces are not permitted. If any item is not applicable, or no information is available, the space must be marked to indicate that.

Section I

Manufacturer's Name: EDVOTEK
Emergency Telephone Number: 202-370-1500
Address (Number, Street, City, State, and ZIP Code): 1121 5th Street NW Washington DC 20001
Telephone Number for information: 202-370-1500
Date Prepared: 08/25/11
Signature of Preparer (optional)

Section II - Hazardous Ingredients/Identity Information

Hazardous Components (Specific Chemical Identity; Common Name(s))	OSHA PEL	ACGIH TLV	Other Limits Recommended	% (Optional)
Rabbit serum				

Section III - Physical/Chemical Characteristics

Boiling Point	NA	Specific Gravity (H_20 = 1)	NA
Vapor Pressure (mm Hg.)	NA	Melting Point	NA
Vapor Density (AIR =1)	NA	Evaporation Rate (Butyl Acetate =1)	NA

Solubility in Water: soluble
Appearance and Odor: colorless or white liquid, no odor

Section IV - Fire and Explosion Hazard Data

Flash Point (Method Used) | Flammable Limits | LEL | UEL
Extinguishing Media: Water spray, alcohol-resistant foam, dry chem or carbon dioxide
Special Fire Fighting Procedures: Wear self contained breathing apparatus if necessary
Unusual Fire and Explosion Hazards

Section V - Reactivity Data

Stability: Unstable | Stable X | Conditions to Avoid
Incompatiblity (Materials to avoid): Strong oxidizing agents, strong acids.
Hazardous Decomposition or Byproducts: Under fire conditions - hydrogen chloride gas, potassium oxides, oxides
Hazardous Polymerization: May Occur | Will Not Occur X

Section VI - Health Hazard Data

Route(s) of Entry: Inhalation? Yes Skin? Yes Ingestion? Yes
Health Hazards (Acute and Chronic): May be harmful or irritating to eyes, skin, respiratory, or if swallowed.
Carcinogenicity: NTP? IARC Monographs? OSHA Regulation?
Signs and Symptoms of Exposure
Medical Conditions Generally Aggravated by Exposure
Emergency and First Aid Procedures: In case of eye contact immediately flush with water, with skin wash with soap and water. If breathed in, remove to fresh air. If swallowed, rinse mouth with water.

Section VII - Precautions for Safe Handling and Use

Steps to Be Taken in case Material Is Released or Spilled: Use absorbant material and keep in suitable, closed container for disposal.
Waste Disposal Method: Observe all federal, state, and local environmental regulations.
Precautions to be Taken in Handling and Storing: Keep container tightly closed in a dry and well-ventilated place. Non-hazardous for travel.
Other Precautions

Section VIII - Control Measures

Respiratory Protection (Specify Type)
Ventilation: Local Exhaust | Special | Mechanical (General) | Other
Protective Gloves: Yes | Eye Protection: Safety goggles
Other Protective Clothing or Equipment
Work/Hygienic Practices: General Indrustrial hygiene practice.