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The Role of Biological and Serological Exhibits as Corroboration Evidence in Forensic Investigation

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Abstract: ***The crime scene that is occurred were investigated by officers and that is sent to the forensic laboratory for further examination. The serological methods are very important methods of investigation of crime. Based on highly scientific methods using immunological reaction and various chemical methods, the serological test methods are to be the most important to confirm the offender and offense worldwide. The evident materials left at the crime scene will be having biological materials like blood and other body fluids such as semen, saliva, sweat, etc., along with other non-body fluids like hair, skin & teeth. The material at the crime scene like stained cloth, stained earth samples like soil, the weapons used, etc., are to be collected for sampling. The origin of the sample whether it is of human or animal to be ascertained. Blood grouping tested. In the paper, an effort is made to investigate the crime using serological tests on the biological materials available at the crime scene.***
Keywords: Serological Tests, Crime Scene, Blood, Investigation of Crime, Immunological Reaction

I. INTRODUCTION

Biology is the branch of science dealing with examinations of biological materials encountered in violent crimes such as murder, road accidents, sexual assaults, burglary, robbery, dacoity, etc. The biology section deals with examinations of hair, fibers, diatoms, and other biological substances (plant and animal materials) like maggots, wood, leaves, flower and pollen grains, fruits and vegetables, seeds, etc. For each of these materials, the scientists of the biology section use different procedures for analysis. The branch of science dealing with the study of blood and body fluids is known as ―Serology‖. Serology section deals with the study of properties and reactions of serums, especially blood serum. The characteristics of a disease or organism shown by the study of blood serums; the serology of acquired immune deficiency syndrome; the serology of mammals.

1) *Forensic Serology***:** Serological tests may also be used forensically, generally to link the perpetrator to a piece of evidence (e.g.:- linking a rapist to a semen sample).
2) *Scope:* Forensic science procedures deal with the examination and comparison of biological materials and serological materials derived from human, animal and plant sources. In the following pages, such procedures used item wise are documented.
3) *Categories:* All Serological materials received for examination are broadly grouped into 4 major categories.

a) Bloodstains
b) Body fluids (Semen, Saliva, etc...)
c) Tissues, Teeth, and Bones

All biological materials received for examination are broadly grouped into 4 major categories.

i) Hairs
ii) Fibers
iii) Diatoms
iv) Other Plant and Animal Materials.

4) *Blood:* Blood is the most common, well-known and perhaps most important evidence in the world of criminal justice today. Its presence always links suspect and victim to one another and the scene of violence. Over the years criminals have tried many ingenious ways to hide, clean up, and remove blood evidence, but it‘s an area where criminal justice technology has always stayed one step ahead of them. One might even say that forensic serology is all about ―antigens‖ and ―antibodies‖ but that is the domain of immunology. Identical twins may have the same DNA profile but completely different antibody profiles and we begin to see how promising the field of forensic serology really is.
5) *Scope:* This procedure deals with the examination of serological materials derived from human and animal sources, such as blood, semen, saliva, sweat, urine, feces, and vaginal secretions, tissues, organs, pieces of muscle, skin, teeth, bones, etc.
6) *Theory:* Bloodstain patterns tell a lot about position and movement during the crime, who struck whom First, in what manner, and how many times. This destroys most alibi and self-defense arguments for crime and at the very least, trips most

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suspects up in their explanation of what happened. Over the years, criminals have tried many ingenious ways to hide, clean up, and remove blood evidence, but it's an area where criminal justice technology has always stayed one step ahead of them. Blood is a slightly alkaline fluid made up of water, cells, enzymes, proteins, and inorganic substances that circulate throughout the vascular system carrying nourishment and transporting oxygen and waste. The most fluid portion of blood consists of plasma, which is mostly water and serum, which is yellowish and contains white cells and platelets. The most non-fluid portion of blood consists of red blood cells which outnumber white cells by five hundred to one. A forensic scientist is more interested in red cells and secondly with serum. With serum, the analyst can determine the freshness of a blood sample because of serum clots several minutes after the exposure to air (a centrifuge is necessary to separate clotted material from rest of serum). With red cells, the analyst looks for smaller substances residing on their surfaces, such as antigens, which have important forensic implications. One might even say that forensic serology is all about antigens and antibodies, but that is the domain of immunology. In forensic law, blood has always been considered class evidence, but the potential exists for individualized blood typing and even today, forensic serologists can provide testimony with some strong probability estimates linking a single individual and that individual only, to a blood stain. Consider that identical twins may have the same DNA profile but completely different antibody profiles, and you begin to see how promising the field of forensic serology really is.

7) *Semen:* Identification of characteristics of semen is particularly useful in the investigation of spermatozoa in cases related to sex crimes. Semen is a suspension of spermatozoa in seminal plasma which is a pool of different secretions by different structures and such as vas deferens seminal vesicles, prostate gland, and mucous glands. Invisible stains can be detected by examining the material in question under UV light. Seminal stains florescence bluish-white in color on a dark black ground. This is true of other fluids also such as blood, saliva, pus, milk, etc.

II. REVIEW OF LITERATURE

In 1901, Karl Land Steiner announced one of the most significant discoveries of this century-the typing of blood - a finding that 29 years later was to earn for him a Nobel Prize. It was Land Steiner who first recognized that all human blood was not the same; instead, he found blood to be distinguishable by its group or type. Out of Land Steiner's work came the classification system that we presently call the A-B-O system. Now for the first time, physicians had the key for properly matching the blood of a donor to a correct recipient. One blood type cannot be mixed with a different blood type without disastrous consequences. By 1937, the Rh factor in blood was also demonstrated. Serology is defined as the study of procedures and practices used in the identification and examination of blood and another body (Physiological) fluids. Blood consists of hematopoietic lineage cells, in a proteinaceous fluid known as plasma. The serum is the fluid exuded from the blood once it has clotted and thus comprises the plasma minus the proteins responsible for the clotting process. Karl Landsteiner (1) discovered that certain combination of red blood cell suspensions from different people, mixed with blood serum from other people reproducibly produced a cell dumping or agglutination reaction, whereas other combinations produced on such reaction. Karl Land Steiner (2) describe the ABO grouping system on the basis of the presence of specific antigen in blood, First-time Kastle-Mayer performed Benzidine test and Phenolphthalein test for the preliminary examination of the blood, but not for confirmatory, because many oxygenated compounds also are given this test positive. Sanger, R. et al. (3-5) described blood grouping of the human being by a dried stain. Saferstein R (5-6) described an alternative method for grouping of blood. The two main confirmatory crystal tests for blood are the Teichmann and Takayama test (7-8) named after their developers who initially described the reaction. The haem moiety of hemoglobin has a characteristic absorption spectrum which, it present in a sample is often regarded as conclusive evidence for the presence of blood – Divall G.B. (9-10). Baechtel (11-12) explain how semen analysis performs and individualize.

III. MATERIALS AND METHODS

A. *Samples*

1) Blood stains.

2) Seminal sample.

a) *Blood:* If an individual is type "A" this simply indicates that each red blood cells have "A" antigens located on its surfaces. Similarly, all type "B" antigens and the red blood cells of type. "AB" contains both "A" and "B" persons will have neither "A" nor "B" antigens on their cells. Hence it is the presence or absence of the "A" and "B" antigens on the red blood cells that determine a person‘s blood type in the A-B-O system. Another important blood antigen has been designated as the "Rh" negative. An antibody (which is present in the serum) will react only with its specific antigen and no other. Thus, if serum containing anti - "B" is added to attach itself to the cell. Antibodies are normally bivalent-that is, they have two

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reactive sites. This means that each antibody can simultaneously be attached to antigens located on two different red blood cells. This creates a vast network of cross-linked cells usually seen as clumping or agglutination.

b) *Principle:* The typing of blood for its "A-B-O" group identity and "Rh" factor are done by adding known commercial anti-sera (antibodies) to the blood and confined by the presence or absence of agglutination.

B. Detection of Blood In Stains

1) Preliminary Screening Tests

a) *Principle:* There 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 by peroxides. When a colorless reagent and hydrogen peroxide are added to the blood stain, the hemoglobin in blood will cause the formation of different colors. In a luminal test, the presence of blood is indicated by luminescence. Several methods as detailed below are practiced for detecting blood, present in minute quantities. Among them, Benzidine test is used in the laboratory.

b) *Phenolphthalein Test:* A small piece of stained thread or a small quantity of stained material is taken on a white glazed porcelain tile. One drop of phenolphthalein solution followed by one drop of hydrogen peroxide is added.

c) *Phenolphthalein Reagent:* (Kastle-Mayer Reagent)

Stock: Phenolphthalein -2gm.
Potassium hydroxide - 20gm
Distilled water - 100ml

Reflux the mixture with 20gms. Of zinc powder for the two hours until the solution becomes colorless and should be stored in a dark bottle and refrigerated with some zinc added to keep it colorless.

d) *Conclusion:* The -A positive reaction is indicated by the development of pink to red color within fifteen seconds.

e) *Ortho-Toluidine Test:* A small amount of stained material is taken on a white glazed porcelain tile. Two drops of Ortho-Tolidine solution are added.

f) *Conclusion:* A positive reaction is indicated by the development of an intense blue color.

2) Confirmation Test of Blood: The presence of hemoglobin and its derivatives in bloodstains indicated by the screening test is confirmed by any of the following tests.

a) *Principle:* These tests are based on the formation of crystals by hemoglobin derivatives. Haemin and Haemochoromogen with different salts (acetate and nitrogen bases) in the acidic or alkaline aqueous medium.

Two types of confirmatory tests are in use and are as described as:-

i) *Teichman Test (Haemen Test):* A stain portion is taken in a porcelain dish and socked in normal saline for a few minutes and a few drops of acetone are added. One drop of the extract is taken on the microscope slide. A drop of 10% Hydrochloric acid is added to it. The mixture is covered with a coverslip and observed under the microscope.

1. Conclusion: - Brown rhombohedron shaped acetone chlor-haemin crystals are seen confirming the blood.

ii) *Haemochromogen Test (Takayama Test):* A stained portion is taken in a porcelain dish and socked in normal saline for a few minutes. One drop of the extract is taken on the microscope slide. One or two drops of Takayama reagent are added. The mixture is covered with a coverslip and warmed gently and allowed to cool. Examine under the microscope.

C. Takayama Reagent

1) A saturated solution of Glucose - 3ml.
2) 10% Sodium Hydroxide solution - 3ml.
3) Pyridine (extra pure) - 3ml.
4) Distilled water - 7ml.

a) *Conclusion:* Salmon, pink color middle shaped crystal of pyridine haemochromogen is observed under microscope confirming the presence of blood.

b) *Determination Of Origin Of Species Of Bloodstains: Once* the stain has been confirmed as blood, tests are conducted to determine whether the stain is of human or animal origin. For this purpose, the standard test used is the Precipitin test. Precipitin test is based on the fact that when animals (usually rabbits) are injected with human blood antibodies are formed that react with the invading human blood to neutralize in its presence. The investigator can recover these antibodies by bleeding the

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animals and isolating the blood serum. For this reason, the serum is known as the human antiserum. In this same manner by injecting rabbits with the blood of other known animals, virtually any kind of animal antiserum can be produced. Currently, antiserums are commercially available for humans and for a variety of commonly encountered animals- for example, dogs, cats, deer, etc.

c) *Principle:* For determination of the origin of species the principle of precipitation is employed. Blood globulins are specific to human and different animal species. When specific antisera of human or other animal are added to the bloodstained extract precipitation takes place when the corresponding antigen is present.

D. Gel Diffusion Method (Ouchterlony Double Diffusion)''

The Precipitin Test is Carried Out in Agar Gel Plate.

Preparation of Agar-Gel Plate

1) A glass plate (4‖x 4‖) is taken and placed on an even surface of a table.
2) 300mg of agarose, a sea-weed extract is weighed and placed in a clean test tube and dissolved in 20ml of normal saline by heating the solution on a water bath.
3) Dissolved agarose is carefully poured on the glass plate to make a 1 to 2 mm thick semi-solid layer of gel.
4) A template having two rows of holes are cut in the gel with the hollow cutter or with a metal punch.
5) The size of these holes is 2mm in diameter, & distance between two rows is 5mm.
6) The Agar-gel is to be prepared afresh every time.



Agarose Gel Plate

A portion of bloodstained material is taken in a clear test tube. A small amount of saline is added to it and soaked for about two hours to obtain a concentrated extract. The strength of the stain is standardized if persistent foam develops on slight shaking of the saline extract in a tube (1/1000 dilution). The plate is then kept in a moisturized Petri dish and left in the refrigerator overnight.

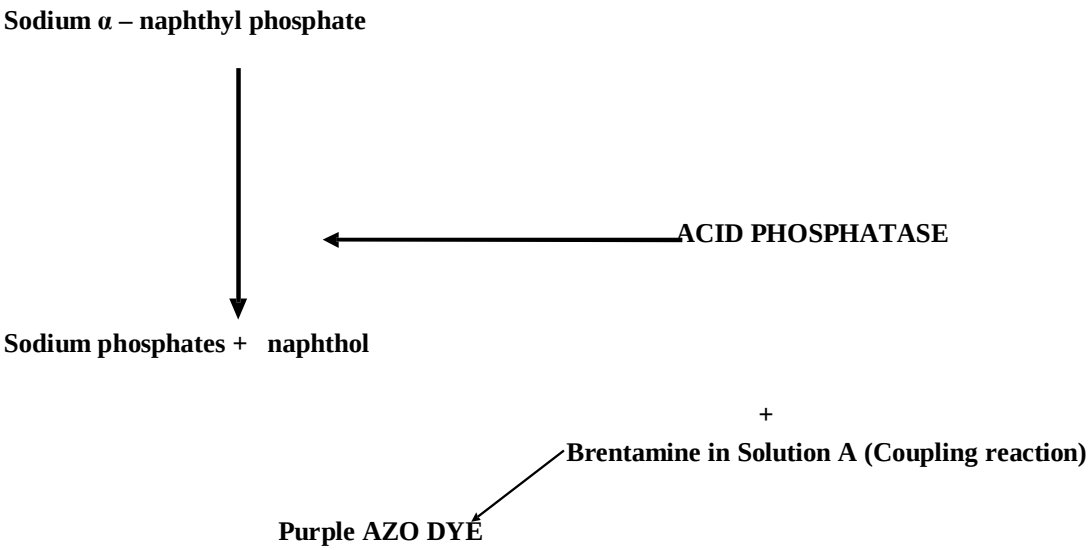
a) *Conclusion:* Presence of precipitin band in between the wells containing blood-stained extract and antihuman serum indicates the presence of human blood. Similar tests will be conducted for determining the presence of different animal blood using a corresponding antiserum
b) *Seminal Stains:* The normal male releases 2.5 to 6 milliliter of seminal fluid during ejaculation. Each milliliter contains 100 million or more spermatozoa the male reproductive cells. The Forensic Examination of articles for seminal stains can actually be considered a two-steps process. First, before any tests can be conducted the stain must be located. Considering the number and soiled condition of outer garments, undergarments and possible bed clothing submitted for the examination this may in itself proves to be an arduous task. Once located the stain will have to be subjected to tests that will prove its identity possible it may even be tested for the blood type of the individual from whom it originated.
c) *Definition:* Semen is the physiological fluid that is released when the male ejaculate.
d) *Available Forms:* Semen is available in the forms of fluid dried stains and smears.
e) *Sources:* Fluid semen is directly collected from the male person. In sexual offences seminal stained clothing‘s of victim & suspect and also bed sheets etc. are collected from the scene of offence swabs & smears(vaginal and cervical), nail clippings & pubic hair of the victim and semen‘s , nail clippings & pubic hair of suspect collected by the medical officer are sent for the examinations.
f) *Sample Preparation:* The stained portion is cut out of exhibits (cloth item) with the help of clean scissors. Smears, swabs, nails & hairs and liquid semen are taken directly.
g) *Control Samples:* The controls are obtained from adjacent to the suspected seminal stains.

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E. Procedure

1) *Physical Examination:* In ordinary light, seminal stains appear as greyish white or yellow-grey having irregular outlines. Stain on black clothing and on pubic hair appears as whitish stains. The stained areas of fabric are stiff to feel. Under ultra-violet light, the stains show bluish white. The suspected area is demarked and their location are noted
2) *Chemical Examination:* The following methods are adopted for the detection of semen in suspected seminal stains.
3) *Acid Phosphatase Color Test:* Acid phosphatase is an enzyme that is secreted by the prostate gland into seminal fluid. Its concentrations in the seminal fluid are up to 400 times greater than those found in any other body fluid. Its presence can easily be detected when it comes in contact with an acidic solution of sodium alpha-naphthyl phosphate and fast blue B dye.
4) *Principle:* The enzyme acid phosphatase of semen hydrolyzes the sodium alpha naphthyl phosphate liberating alpha-naphthol. This couples with the dye forming material (Brent amine fast blue) to give the purple color.
5) *Reaction*

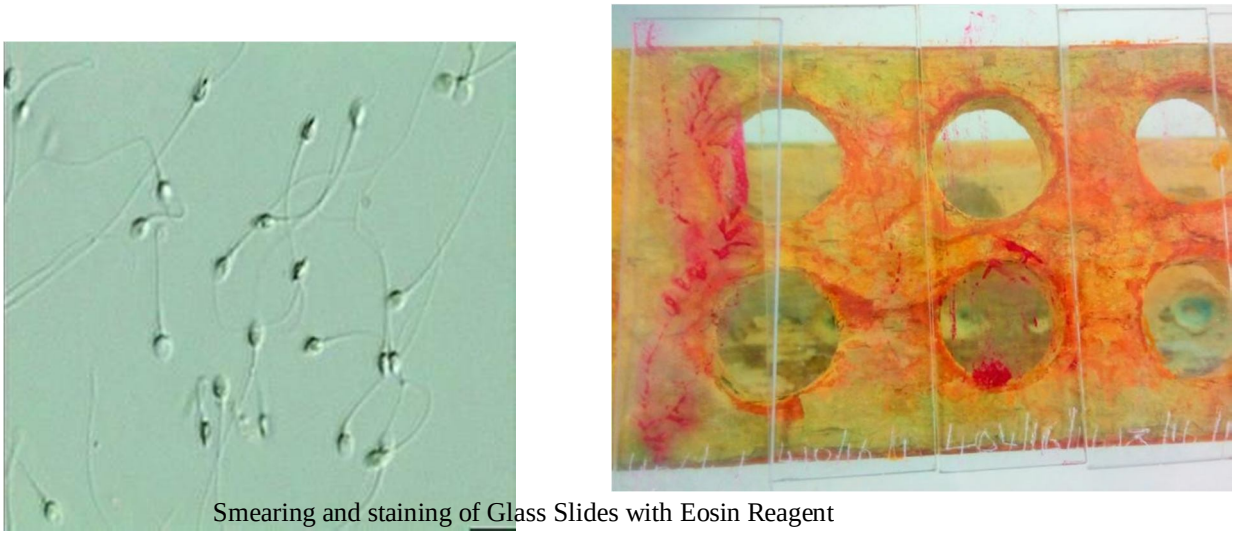


6) *Test:* A small piece of suspected seminal stain is cut out from the garment, crime scene material and is placed in a piece of filter paper kept in a porcelain dish. Some drops of acid phosphatase reagent are added using a clean pipette to the stained area.
7) *Conclusion:* Within a few minutes a purple color develops in the area of the seminal stain. A negative reaction can be interpreted as meaning the absence of semen. Although some vegetable juices (e.g., cauliflower and watermelon) fungi, contraceptive creams, and vaginal secretions do give a week positive response to the acid phosphatase test, none of these substances normally reacts with the speed of the seminal fluid. A reaction time of fewer than 30 seconds is considered a strong indication of the presence of semen. Other tests for semen involve the detection of choline and spermine. Procedures for the identification of choline and spermine utilize microcrystalline tests. The Florence test for choline uses potassium triodide and barberio test for spermine uses picric acid as the crystallizingreagents. Of these two methods, the Florence test which is followed in the laboratory is described below.
8) *Microscopic Identification of Spermatozoa:* Microscopic detection of the spermatozoa is a confirmatory evidence for the presence of semen.
9) *Preparation Of Smear:* A small portion of the seminal stained area from the fabric or swabs are cut out. They are moistened with few drops of 0.01N hydrochloric acid and are kept for about half an hour. Individual threads are then teased apart using a pair of dissecting needles. A drop or two of the fluid is squeezed out on a microscopic slide. A thin smear is prepared on the slide. In the case of fluid semen is placed on a microscope slide and a thin smear is prepared. The smears prepared on the slides are then allowed to dry in shade. Thereafter the smears are stained by the following method and examined under a microscope for the presence or absence of spermatozoa.

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F. Staining Method

1) Malachite Green-Eosin Reagents: Smeared slides are passed through a flame for fixing. The slides are then acidified with dilute sulphuric acid and washed with water. The slides are then stained with eosin for 20-30 minutes. Slides are thoroughly washed using a gentle flow of water. The slides are counterstained with malachite green for one minute. Slides are washed using the gentle flow of water and air dried. The stained slides were observed under a microscope under 100x in oil immersion.



Smearing and staining of Glass Slides with Eosin Reagent

Microscope observation of the stained slides in this manner shows the posterior part of the spermatozoa head stained as purple and anterior part pink in color. Neck and tail of the spermatozoa assume green color. Epithelial cells take uniform pink color and leucocytes remain almost unstained. Morphology of human spermatozoa: Each consists of an oval and flattened head with a nucleus; with a long slender tail connected with the head by means of a very short neck. The total length varies from 50 to 70 microns, the head is 3.5 to 5 microns in diameter

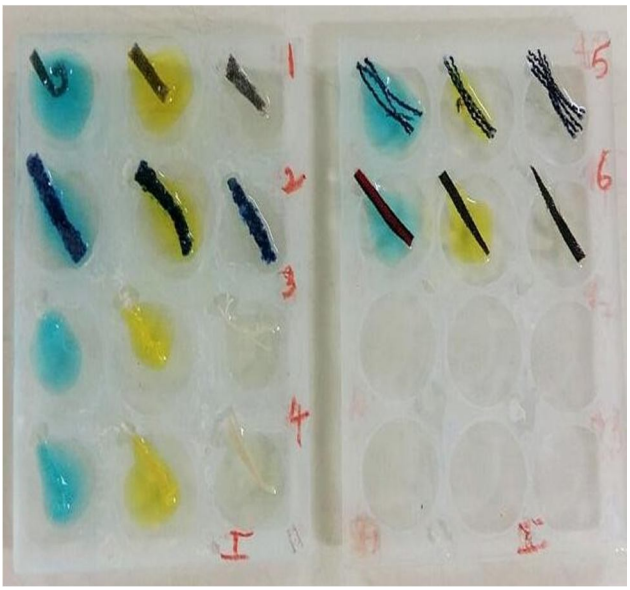
2) Conclusion: Identification of intact spermatozoa with head and tail is the most reliable proof of the presence of semen.

3) Grouping Of Blood for Blood Stains and Seminal Stains

a) Absorption-Elution: Method: It is based on the direct testing of the absorbed antibody after washing away of unbound serum. The absorbed antibody is eluted and is tested with R.B.C's of corresponding blood groups. Agglutination indicates the presence of the corresponding antibody in the elute stain. The stained material contains the same blood group of antigens as that of R.B.C's.

b) Procedure: A small portion of the blood stain is taken. The sample is then glued using a colorless adhesive material like nail polish at the top of each of the cavities in a row of clean V.D.R.L slide marked as A, B&O along with exhibit numbers with glass marking pencil, the sample should be in such a way that it occupies $3/4^{th}$ of the cavity. A set of control samples are also taken in the same way. A drop of Anti-A serum, Anti-B serum, and Anti-H lectin are taken in the 1^{st}, 2^{nd}, 3^{rd} cavities respectively. Precaution is taken to see that the threads are immersed in the corresponding anti-sera & Anti H lectin. The slides are placed in a moist chamber, kept in a refrigerator at 4^0C overnight absorption. The slides are taken out and threads are thoroughly washed 3 to 4 times with ice- cold saline to remove unbound serum and are air dried. One drop of 0.5% red cells suspension of each A, B&O groups treated with 1% Bovine Albumin is added to the threads of respective cavities. Slides are placed in a moist chamber and kept in an incubator at 56^0C for 40 minutes the slides are removed from the incubator and allowed to stand till they attain the room temperature. The slides are then agitated by placing them on a mechanical shaker and then viewed under a microscope.

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V.D.R.L Slides With Anti A, B, H Serum

4) Conclusion: If agglutination is observed in cavity-A, the stain is of ″A" blood group. If agglutination occurs in cavity B, the stain is of "B" blood group. If agglutination occurs only in the cavity O, it confirms that the blood group is "O". In the case of agglutination both the cavities A& B, it indicates "AB" blood group.

IV. RESULT AND DISCUSSIONS

A. Case study 1

During the course of investigation, the IO along with the available staff rushed to the spot located near, beside the state bank of Hyderabad, wherein the dead body of deceased lying in a pool of blood with one dagger remained in his stomach and one hero Honda passion pro motorcycle, lying near the body, as there was a traffic jam on the road immediately the IO shifted the deceased to the mortuary of Osmania hospital for preservation. The SI examined the scene minutely and conducted the scene observation cum-seizure-panchanama in presence of mediators and one Hero Honda Passion Pro motorcycle undercover of panchanama and collected the control earth as well as blood sampled from the scene. I visited the mortuary of Osmania hospital, secured the presence of two mediators and conducted the inquest panchanama over the dead body of deceased in their presence, wherein found several stab injuries all over the body of deceased (as per the inquest panchanama) and send the body for postmortem examination, and at the time of Autopsy the IO seized the blood-stained clothes of deceased such as:

1) Blue color full sleeves T-shirt
2) Blue color shaded Jeans Pant
3) White color Banyan
4) Blue color full underwear together with the blood-stained dagger

In presence of same mediators under cover of seizure report for sending them to FSL for analysis and report. During the inquest, the IO examined the complainant and other witnesses thoroughly and recorded their detailed statements, in which they collaborate with the contents of the complaint, and after completing the Autopsy, the dead body of the deceased was handed over to the blood relatives for performing last rites under proper acknowledgment. During the course of an investigation, the IO arrested accused persons. One sword, three daggers and three cell phones, brought them all to police station interrogated them thoroughly wherein they all voluntarily admitted their guilty of committing the offense, as such the IO secured the presence of two mediators(Govt. officials) and recorded the confession-cum-seizure panchanama of A1 to A4 and seize one sword, and one cell phone from A1, one dagger and two cell phones from A2, one dagger, two cell phones from A3, and one dagger, one cell phone from A4 under cover of panchanama, the IO also recorded the confession statement of A5 in presence of same mediators. The accused persons A1 to A5 were remanded to judicial custody.

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a) *List of Articles Forwarded for Examination:* Blood stained dagger, measuring the 32cm length (blade length 19cms, Handle with plastic grip 13cms, one side curved blade with three teeth another side, Sharp edge with 4 holes in between). There is an eagle with USA flag emblem, words engraved as –USA SABER‖, which was collected from the body of deceased at the mortuary of OGH –marked as S1. Blood stained blue color (one side cut) full sleeves T-shirt-marked as S2. Blood stained blue color shaded Jeans pant-marked as S3. Blood stained white banyan (one side cut)-marked as S4. Blood stained blue color full underwear- marked as S5.

b) *Report*

i) The item numbers 1 to 11 is examined.

ii) Human blood is detected on item nos. 1 to 10.

iii) The blood group of blood stain on item nos. 1, 2 and 3 are of a ‗A' blood group.

iv) The blood group of blood stain on item nos 4 to 10 could not be determined.

v) Blood is not detected on an item no. 11 which is received as a control for item no. 10.

B. Case NO 2

Case details as the motive for the offense when and how the offense was committed. Occurred on 05/02/2015 at 20:00 hours victim A (1) was alone and watching TV in her house. The accused B-1 came to her house and dragged her into the kitchen room and forcibly raped her by covering her mouth with his hand.

1) List of article forwarded for examination at a forensic science laboratory

a) Green color Langa (petty wat) with gold color flower border (the above petty what was seized from the victim girl under cover of seized panchnama).

b) Foreign hair for detection.

c) Two seized vaginal swabs preserved by the medical officer.

2) The exact nature of the examination required

a) Examine item No: - 1 and specify whether the blood or seminal stain are found on it or not.

b) If found, specify whether they belong to human or not and its group.

c) Whether item No: - 1 and 3 are identical or not.

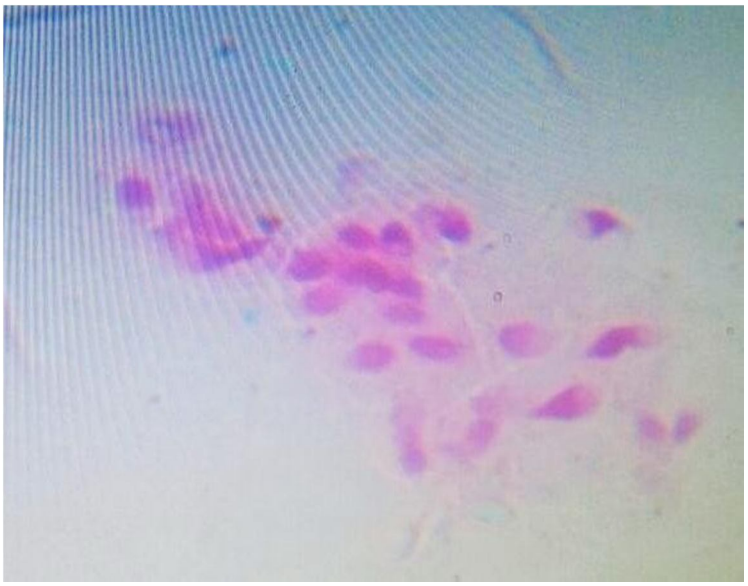
3) Report: Item No: -1, 2 and 3 are examined:

a) Semen and spermatozoa are detected on item No:-1 and 3.

b) Blood is not detected on item No: - 1 and 3.

c) The blood group of seminal stain on item No: - 1,3is of "B" group.

d) Foreign hair is not found in item 2



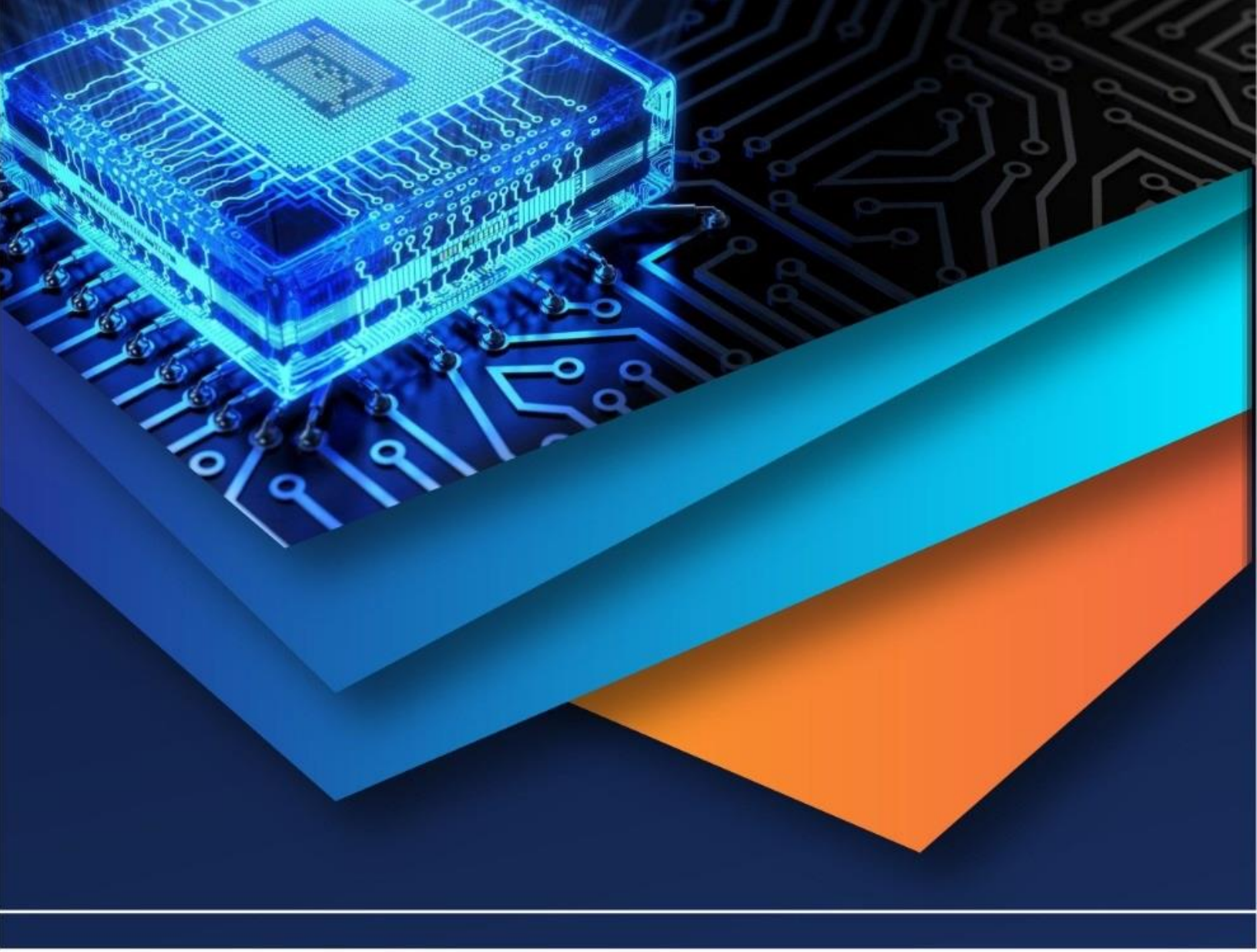
Microscopic view of spermatozoa at 100X

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