



## Research Article

# Determination of ABO blood group based on secretors or non-secretors analysis in body fluids

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## ABSTRACT

Criminal acts often leave traces that are analyzed by law enforcement officials, especially the Indonesian National Police (POLRI). Sometimes various objects are found and analyzed to establish a connection between the victim and the perpetrator. Blood, the most common body fluid at a crime scene, consists of cellular and fluid components. The aim of this review is based on ABO blood group analysis, focusing on secretory or non-secretory analysis in forensic medicine. In some cases, no blood or bloodstains are found, but at other times matches with other body fluids are found. Considering that 80% of the population belongs to the secretory gene group, blood group analysis can still be carried out based on the secretory gene (Se). There are two chromosomes involved in blood regulation: chromosomes 9 and 19. The first refers to the ABO blood group, and the second refers to the secretory phenotype. The basic principle of secreted substances is antigens found on the surface of red blood cells and in all body fluids except cerebrospinal fluid. The inheritance pattern of chromosomes 9 and 19 is based on Mendel's law of probability, which states that all genotypes (homozygous dominant recessive, or heterozygous) are passed on to offspring.



## INTRODUCTION

The identification process is a way of recognizing individuals by utilizing characteristics and characteristics to distinguish them from other people, which include dead victims, living victims, or skeletons. The identification process is sometimes related to cases, such as criminal cases (the process of identifying criminals, murderers, perpetrators of abuse, rape, etc.). (Yudianto A, Sispitasri YA, and Margaret N, 2017).

One of the identification processes uses forensic serology examination. The development of Forensic Serology examination is guided by Mendel's laws regarding the mechanism of inheritance of individual traits and the discovery of human polymorphism. Human polymorphism, which in this case is also protein polymorphism, can be used as a basis for the identification process in the forensic field, this is based on the following characteristics: protein polymorphism is eternal, which means that a person's protein polymorphism will remain for the rest of his life, the method for determining protein polymorphisms has been tested and is reproducible, and the process of inheritance from mother and father to child is by utilizing Mendel's laws of inheritance (Kusuma, 2004; Yudianto, 2013)

According to Kusuma, 2004 in his professorial inauguration speech and Putra, 2018 in his thesis said that the identification process using blood type can be used to identify and uphold justice in Indonesia, which until now Indonesia is categorized as a country where there is still a high level of crime, which can be related to with crimes of rape, murder, theft, robbery where at the crime scene (TKP) there must be silent evidence (silent witnesses) which will later be analyzed by Forensic experts to examine the exhibit

(corpus delicti) scientifically so that they can describe the triangular relationship The crime scene consisting of: the alleged perpetrator-victim-and the evidence came from, so that there was a bright spot in the case. (Kusuma, 2004; Putra, 2018)

One of the identification processes can be blood spots, or semen spots whether they are still wet or dry. It is still possible to identify these blood spots because the antigens on the superficies of the red blood cell membrane remain intact even though the cells have been destroyed and a serological examination approach can be used to determine the blood type found in the blood spots. If there are no blood or blood spots at the crime scene, but there is evidence, for example, cigarette butts, glasses, or clothes, then from these materials, a serology-based identification process can be carried out to determine the blood type that may be present in the evidence. According to Yudianto, 2013 and Darmono, 2009 in their book, this is possible considering that determining blood type does not have to come from a blood sample but can be done through non-blood samples (saliva, sperm, sweat) which is because 80% of the population is a secretor group. The principle of the secretor here is that the antigen contained in the red blood cell membrane will be secreted into all materials related to body fluids (except cerebrospinal fluid) so that in these body fluids it is possible to detect blood type even from samples that are not from the sample. blood. Apart from identifying evidence, blood group identification can also be done for individuals who want to have their blood type checked, but are afraid of the invasive procedure of needles. It can also be done through all forms of fluid in their body, especially saliva, as was done in research by Farida in 1993. If the individual is included in the 80% population which is the secretor group, blood type can be detected through body fluids. (Farida *et al*, 1993;



Darmono, 2009; Yudianto, 2013; Woike, 2017; Rajawat *et al*, 2023)

Based on the things explained in the previous paragraph, the writing of this paper will focus on determining the blood group of the ABO system from samples that do not come from blood (from body fluids, except cerebrospinal fluid) by relying on the secretor status inherent in a person, on the basis of The theory of this approach is that 80% of people are secretors and it is possible that no blood or bloodstains will be found at the crime scene, but evidence may be found in the form of cigarette butts, glasses, straws, sperm or sperm stains, and used clothes that are wet because there is sweat inside the shirt (Metgud e al, 2016; Bakhtiari *et al*, 2016).

## LITERATURE REVIEW

### Blood and Bloodstains

Blood is the most important trace evidence that cannot be denied because it is a tool to identify certain individuals compared to other physical evidence. Examination of blood fluid and bloodstains is one of the examinations most frequently carried out in forensic laboratories because blood is easily discovered in nearly all shapes of violent acts, so blood investigations are considered very useful for uncovering criminal acts. Blood examination at crime scenes in criminal cases can provide useful information for the investigation process. By examining the spots, you can determine the approximate location of the floor and the source of the bleeding, the direction of movement of the source of the bleeding from both the victim and the perpetrator of the crime, and the approximate age/oldness of the blood stain. Yang The focus of examination in the forensic world is blood or red blood spots, while white blood is more the focus of examination in the world of immunology. (Idries, 2013; Walpola *et al*, 2024)

Blood is a complicated fluid made up of blood cells hanging in plasma, a yellowish liquid. Blood serves as a means of transportation for all of the constituent parts to reach their respective organs within the body. Red blood cells (erythrocytes), white blood cells (leukocytes), and blood platelets (thrombocytes) are examples of cellular components of blood; non-cellular components of blood include water, proteins, carbohydrates, lipids, amino acids, vitamins, and minerals, among other substances. (Yudianto, 2013; Putra, 2018; Metgud *et al*, 2016)

The main characteristic of blood is the presence of hemoglobin so the tests carried out in the forensic laboratory are based on the presence of hemoglobin or its components. Hemoglobin is a protein that functions to carry oxygen from the lungs to tissues throughout the body consisting of heme which transports oxygen and globin which is a protein component. Fresh blood is liquid and has a fishy smell, which will dry out within 12-36 hours, while the color of the blood will change to brown within 10-12 days. (Soejima & Kode, 2021; Putra, 2018).

### Blood Grouping System

As explained in the previous paragraph, blood grouping is important in the world of forensics after a spot is included in a blood spot and comes from a human. This is important in the judicial process by utilizing blood type analysis from blood sources or blood spots and from non-blood sources. The American Association of Blood Banks defines blood type as a collection of antigens produced by gene alleles. However, blood type is genetically controlled and is a characteristic that can be checked throughout life because it is different for each individual. After Landsteiner discovered the ABO blood group in 1900, a number of different blood grouping systems have been used, including MN, Rhesus Rh, Lewiss Kell, and Duffy



antigens. More recently, protein complexes and hemoglobin have been added. The most established blood grouping method is based on the ABO system and has significant significance in the legal and medicolegal systems. Of the other blood grouping systems, the ABO and Rhesus systems are the most frequently utilized broadly and widely. (Yudianto, 2013; Putra, 2018; BoKheder *et al*, 2020)

Two chromosomes—chromosome number 9 (location 9q34.1-9q34.2), which contains the ABO gene allele, and chromosomal number 19 (19q13.3), which is linked to the secretor gene (Se)—are responsible for the ABO blood group phenotype. It is important to realize that blood types A and B are dominant over blood type O. Blood type A can have either a homozygous (IAIA) or heterozygous (IAIO) phenotype, while blood type B can also have either an IBIB or heterozygous (IBIO) phenotype. Blood type O can be homozygous dominant IHh, heterozygous IHh, or homozygous IhIh. Since the IhIh genotype is frequently present in Bombay demographic groups, blood type O is frequently referred to as such. Mumbai. (Bahram, 2007; Matos, 2016; Woike, 2017; Walpola *et al*, 2024)

Blood type can be used to:

- a. Proving whether bloodstains on weapons, clothing, or crime scenes may or may not come from the suspect or victim,
- b. To help the process of identifying or uniting parts of the human body that were separated due to a train or airplane accident,
- c. Giving signs to newborn babies to avoid the possibility of mistakes in the hospital,
- d. Cases of questionable inheritance are related to paternity-maternity problems.

### Antigens and Antibodies in Blood

Although there are several blood group systems (including Lewis, Duffy, Rhesus, ABO, and others), the ABO and Rhesus blood type systems are the most commonly utilized. Antigens A and B, which are present in the ABO blood group, are characterized by either a sequence of proteins projecting from the bilipid bed or sugar endings that are directly connected to the cell wall. The group of every individual with one of the four major blood groups—O, A, B, or AB—as a sign of the existence of agglutinogens in the blood serves as the foundation for the application of forensics. As was previously discussed, four different types of sugar molecules serve as the antigens in the red blood cell membrane that determine the ABO blood group system are sugar molecules which consist of 4 types, namely: D-galactose, N-acetylgalactosamine, N-acetylglucosamine & L-fucose. (Putra, 2018; Bakhtari *et al*, 2016).

Blood type A has surface antigen A on the surface of the erythrocyte membrane, with surface antigen A consisting of 1 molecule of fucose, 2 molecules of galactose, 1 molecule of N-acetyl galactosamine and 1 molecule of N-acetyl glucosamine. Blood type B has a B surface antigen on the surface of the erythrocyte membrane with the B antigen being slightly different when compared to the A antigen, where this antigen is composed of an N-acetyl galactosamine molecule which is replaced by 1 galactose molecule. Blood type AB has two types of surface antigens on the surface of the erythrocyte membrane, where the antigen is a combination of antigen A and antigen B. Blood type O was initially thought to have no antigens, but after being traced, blood type O still has antigens on the superficies of the erythrocytes. which is in the form of a carbohydrate bond consisting of 1 fucose molecule, 1 N-acetyl glucosamine molecule, and 2 galactose molecules. (Putra, 2018; Groot *et al*, 2020)



The structure of antigens is found in blood groups. In individuals with blood type A, there will be an excess of N-acetylgalactosamine, while in individuals with blood type B, there will be an excess of D-galactose. However, before the D-galactose molecule can accept the carbohydrate monomer that determines A or B activity, it must bind to the carbohydrate monomer fucose. A D-galactose group that binds fucose without active N-acetylgalactosamine A or active D-galactose B has an antigenic activity called H. The glycosyltransferase specified by the A and B genes depends on the presence of the precursor substance H for its activation. The attachment of fucose to D-galactose provides this precursor and is mediated by another enzyme, namely the fucose-transferase enzyme whose existence is determined by the H gene with the location of the H gene being

on chromosome 19 (19q to be precise) and the location of the H antigen at the same time as Se. The H antigen precursor consists of four types of precursor antigen, namely: type I antigen precursor (Galβ1 which becomes 3GlcNAcβ1 found in endodermal cells), type II (Galβ1 which becomes 4GlcNAcβ1 found in mesodermal cells, and erythrocytes), type III (Galβ1 which becomes 3GlcNAcα1 which is O-linked), and type IV (Galβ1 which becomes 3GlcNAcβ1) which is related to glycolipids. Of the four types of antigen precursors, the important ones are type I and type II antigen precursors, type I antigen precursors are found in mucosal secretions and body fluids, while type II antigen precursors are found on the superficies of red blood cell membranes (Bahram, 2007; Woike, 2017; Soejima & Kode, 2021)

**Table 1.** Marriage and possible and unlikely blood types of offspring (based on Mendell's Law).

No	Marriage	Possible blood groups of the child	Impossible child's blood type
1	O X O	O	A, B, AB
2	O X A	Oh, A	CHAPTER
3	O X B	Oh, B	A, AB
4	A X A	Oh, A	CHAPTER
5	A X B	O, A, B, AB	---
6	B X B	Oh, B	A, AB
7	O X AB	A, B	Oh, AB
8	A X AB	A, B, AB	O
9	B X AB	A, B, AB	O

Source: Yudianto, 2013



The ABO blood group inheritance system follows the system of inheritance of traits related to Mendel's law with Mendel's law being based on probability as shown in table 1. Determining paternity cannot be confirmed because the basis of Mendel's law is probability, but it can confirm whether someone is not the father of a child or is the father of a child. Mendel's law states that a blood group cannot arise in a child unless it is present in one or both parents and if an individual is homozygous (for example homozygous AA) for his blood group genes then all of them must appear in the blood group of all children. his son. Another basic principle that must be adhered to is that each agglutinin group (the phenotype that comes out of serological tests) has a pair of genes, where one gene comes from the father and one gene comes from the mother. Blood group A can have a homozygous genotype AA or heterozygous AO, blood group B can also have a homozygous genotype BB or heterozygous BO, while blood group O only has a homozygous OO, so from these results a conclusion can be drawn that the marriage between individual O and individual O is unlikely to produce offspring with blood type A or B or AB, while the marriage of individual AB with individual B is also unlikely to produce children with blood type O. (Gueuning *et al*, 2023)

### Method for Determining Blood Type from Spots or Dried Blood

Determination of blood group from spots or dried blood can be done using two types of methods, namely: indirect method (absorption-inhibition) and direct method (absorption-elution and mixed agglutination). (Groot *et al*, 2020; Rajawat *et al*, 2023)

### 1. Indirect Way

This method is known as the absorption-inhibition method which was first used by Holzer. The principle of this method is that blood spots or dried blood contain antigens that match the antiserum added so that specific absorption occurs. The absorption process causes the antiserum titer to decrease which is determined by comparing it with the initial serum titer. This method is also used in determining blood group substances in body fluids, for example in seminal fluid, saliva, or saliva.

### 2. Direct Method

The method included in the direct method of determining blood group from spots or dried blood is the absorption-elution method which was first used by Sircausa and Fiori. This method is the newest way to identify dry spots, where using the agglutination method is not possible. The technique is based on antibodies adsorbed to antigens bound to blood-stained fibers and then the fibers being washed to remove all adsorbed antibodies. The adsorbed antibodies are then removed from the antigen in a medium containing complementary red blood cell indicators, thereby causing agglutination. Cells that do not undergo agglutination of course do not have antibodies in their suspension.

The second method that can be used to determine blood groups from spots or dried blood is the mixed agglutination method which was first carried out by Coombs, Bedford, and Rouillard in 1956 and is used to determine the presence of antigens in cells such as cheek mucosal cells, skin epithelium, and others.

### Body fluids

#### 1. Saliva

Saliva stains can be discovered on a variety of objects, including handkerchiefs, cigarettes that have been thrown away, spit, glasses, bottles, postal stamps, envelopes, toothpicks,



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and even a piece of fabric that was used as a practical joke. Ptyalin, one of the enzymes found in saliva, hydrolyzes starch when it is introduced. Therefore, adding saliva extract to starch prevents the starch from reacting with iodine to change color. Blood group components can be grouped in secretor saliva. Criminal investigations often find saliva on cigarette butts. Saliva stains now have an equal level of evidentiary value as other bodily fluids like blood and semen thanks to DNA analysis. Combining saliva and food ingredients can cause problems for blood grouping. (Gaensslen, 2009; Sen *et al*, 2015; Harbison & Fleming, 2016; Metgud *et al*, 2016; Woike, 2017)

## 2. Sperm or Semen

Sperm is a complex body fluid and is a male reproductive fluid. Sperm consists of two parts, namely: a liquid part called seminal fluid and a cellular part that contains spermatozoa known as male reproductive cells. In general, spermatozoa secretion amounts to 250 million per milliliter of semen, with milliliters being a unit of measurement for volume. (Gaensslen, 2009)

Semen is *set alp* in liquid form, smears, or stains or can be *set alp* in the vagina, anus, or rectum. Fresh cement is a gel- suchlike liquid, which liquefies when exposed to the atmosphere. Normal exclaim is about 3.5 ml, containing about thirty million sperm. The dry weight is about seven percent of the liquid weight. Sperm has a definite morphological structure. Identification in the smear establishes the presence of cement. The shape and size of mortal spermatozoon are characteristic. But morphology alone doesn't allow individualization. A person's semen doesn't contain spermatozoon so it's called aspermic semen. This may be due to some complaint or may be due to vasectomy surgery. In similar cases, the criteria for semen identification are lost. Immunological testing

using anti-semen becks against seminal tubes is decreasingly accepted as a dependable test for aspermic semen. Electrophoresis is becoming popular for semen identification. (Harbison & Fleming, 2016)

It's a complicated blend of organic and inorganic composites. Important semen ingredients from an identification point of view are proteins including enzymes, blood group factors, choline, fructose, citric acid, uric acid, and zinc. Composition varies from individual to existent. The enzyme, acid phosphatase, *set alp* in semen is in significantly more advanced attention than that *set alp* in other body fluids. Acid phosphatase offers a veritably sophisticated test for the identification and position of semen stains although identification of positive semen isn't grounded on the acid phosphatase test alone. Choline in semen is used to gain a demitasse test. Fructose, citric acid, and zinc are more or less absent in other body fluids and hence their discovery in semen should allow identification but these substances haven't been used to any extent so far. Blood group antigen secretors can be *set alp* in sperm body fluids. (Verma & Arya, 2014; Yamamoto, 2021)

## 3. Urine

Urine is linked by the large quantum of urea in it. The stain is located with ultraviolet light. This is also uprooted with water and tested for urea. Urine stains can now be personalized via DNA profiling. Identification of urine stains is delicate because they're generally verbose, pale, and spread over a large area. Plausible tests are generally grounded on the discovery of urea, urease, or uric acid. This test isn't specific, because sweat and other substances containing high quantities of urea also reply appreciatively. Tests for creatinine have also been used to describe urine. Discovery of Tamm- Horsfall glycoprotein protein( TMP), which is also present in beast urine, has been



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reported preliminarily and was included in the RSID™- Urine test. TMP appears to be suitable as a specific test for urine although the presence of vaginal fluid may hamper the test results and the presence of blood in the sample may make the test delicate to read. (Harbison & Fleming, 2016; Jelinek *et al*, 2022)

#### 4. Sweat

There's no practical webbing test to identify sweat, although DNA is frequently *set alp* and determined from areas of apparel likely to contain sweat, little exploration has been done. ELISA-grounded tests have been developed to describe the sweat-specific proteins G- 81 and dermicidin but haven't been extensively espoused. (Harbison & Fleming, 2016; Walpola *et al*, 2024)

#### 5. Vaginal Fluid Secretions and Menstrual Blood

Menstrual blood and vaginal fluids are fluids with blended ingredients that are delicate to identify. Lugol's staining of glycogen-containing scaled epithelial cells of the vaginal wall, bitsy identification of endometrial cells, and discovery of lactate dehydrogenase isoenzymes 4 and 5 are now considered nonspecific for vaginal cells. Immunochromatographic testing for D-D-dimer, an answerable fibrin declination product clinically sensible for the opinion of thrombosis, is honored as a possible test for menstrual blood. An indispensable approach using ELISA targeting MMP14, estrogen receptor  $\alpha$ , and fibrinogen is used to separate between supplemental blood and period, although no other body fluids were tested for cross-reactivity. (Harbison & Fleming, 2016; Rajawat *et al*, 2023)

The Relationship between Body Fluids and the Determination of Blood Type Based on

Secretor/Non-Secretor

The identification process is sometimes related to cases, such as criminal cases (the process of identifying criminals, murderers, perpetrators of abuse, rape, etc.). (Yudianto A, Sispiasri YA, and Margaret N, 2017). Criminal cases involving criminal acts will leave evidence (trace evidence) either from the victim or the perpetrator or objects around them which must be examined at the crime scene (TKP). At the crime scene, the alleged perpetrator, witnesses, alleged victims, and alleged surrounding accessories that could be owned by either the alleged victim or the perpetrator can be found. The alleged accessory is related to the term trace evidence, which is the most meaningful examination material in uncovering the light of a case.

This evidence can consist of cigarette butts, clothing, and toothbrushes (which are the most commonly discovered at crime scenes), and from these three snippets of the exhibit, an analysis process can be carried out for the presence of blood or bloodstains, semen, saliva, sweat, and hair except for cerebrospinal fluid. From the results of the analysis of these things, blood groups can be identified, where the system for classifying blood consists of the ABO, Rhesus, and Lewis systems. The basis for classifying the three types of systems is chromosome number 9 (9q34.1-9q34.2 to be precise) and chromosome number 19, where chromosome number 1 regulates expression by the enzyme Fucosyltransferase 1 which is related to ABO blood grouping built upon the availability of antigen A, antigen B, AB antigen, and absence of antigen to A-BO in humans. Chromosome number 19, regulates the expression of chromosome number 19, specifically chromosome 19q3.3, where chromosome number 19 is related to the H antigen (found in blood group O) and also the antigen secretor found in the red blood cell (HR)





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antigen which will secretion occurs in tissues and body fluids (sweat, saliva, sperm and other body fluids except cerebrospinal fluid) so that these body fluids or tissues can contain antigens contained in RBCs. Secretors, according to the literature, are found in approximately 80% of people in the population, while the remaining 20% are individuals who are not secretors (non-secretors).

For individuals who constitute 80% of the population where HR antigens are expressed in body fluids, determining blood type does not have to be done using blood subjects, but can use body fluids (sweat, saliva, sperm) considering that these three types of body fluids are often found in at crime scenes, which can include cigarette butts, clothing, and toothbrushes. In cigarette butts and toothbrushes (on the part that comes into contact with the buccal mucosa) saliva can mix with body fluids, and in clothing, it can come into contiguity with body fluids in the form of sweat.

Determination of blood type (either through secretors or non-secretors) is then used and linked to its identification with evidence found at the crime scene, from which conclusions can be drawn from the results of this connection to include or exclude alleged victims and suspects at the crime scene.

### CONCLUSION

Determination of blood groups can be done from blood group sources and non-blood group sources, which is because 80% of the population are “secretors” which are coded by genotype (Se-Se/Se-se) with the purpose of secretors being the secretion of antigens on the superficies of the membrane. red blood cells into body fluids (saliva, sweat, sperm, and other body fluids except cerebrospinal fluid) so that blood group examination can be carried out from blood sample sources and non-blood sample sources.

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