Determination of serological markers (blood group markers) of biological fluid (urine) obtained from crime scene for individualization of the donor(s).

Ravindra Pal Verma, National Institute of Criminology & Forensic Science, Delhi, India
Arun Kumar, Department of Anthropology, Dr. H.S. GourVishwavidylaya, (A central university), Sagar-470003(M.P.), India
Dr. Harsh Sharma, Sr. Scientific Officer, In-charge, Regional Forensic Science Lab., Bhadbhada Road, Bhopal, India

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Corresponding author

Ravindra Pal Verma
National Institute of Criminology & Forensic Science, Delhi, India

Phone: +91 8085474901
Email: ravindraverma764@gmail.com

Abstract

Urine samples collected from 20 donors with unknown blood group and secretor status had been determined from saliva. ABO typing on the concentrated samples was successfully performed after 1 month of storage. Urine stained clothing samples are often submitted to forensic science laboratories for ABH blood group antigen determination. The serogenetic markers of urine stains submitted can be used to determine the origin of any of these samples. ABH blood group substances have previously been identified from urine. ABH blood group substance is low in urine in comparison with other body fluids.

Keywords: Crime Scene, serological markers, body fluids.

Introduction

Biological Fluids at the Crime Scene

Crime scenes relating to accidents, homicides or suicides involve murders, sexual assaults, rapes, thefts, burglary, road accidents, etc. Biological fluids commonly encountered as forensic evidence include blood, semen, urine, vaginal fluid, sweat etc. and play an important role in criminal investigations as they may help to link the suspect, victim and crime scene/act in accordance with the Locard's Principal of Exchange.

It is a common observation that (except for blood and urine which may be found in liquid form) these fluids are encountered as wet or dry stains on clothes, cloth pieces, floor, walls etc. or found on the body of suspect/victim which may be collected in the form of swabs. Presumptive and confirmative tests are conducted in forensic science laboratories to first determine the nature of the stains/swabs followed by establishing the identity of the donor, the probability of which among a number of suspects may be a difficult task. Immunological tests based on antigen-antibody reaction for excluding non-donors can include blood typing markers like ABO, MN, Rh, Kell, Duffy, etc. With the advancement of DNA profiling techniques, individualization may be done from the reduced number suspects (1).

Serological markers in body fluids

For identification of the donor, it is important to first exclude non-donors from a number of suspects involved in the forensic investigation so as to reduce the number of samples for individualization using expensive and time consuming techniques. Biological stains forwarded to the biological division of forensic science laboratories may be subject to blood typing for identification of the blood group of the donor, an important step towards the identification of the donor. Of the many blood markers, ABO typing is more commonly done for blood stains in laboratories and less commonly done for urine, semen and saliva (2).

Lattes (1887-1954) became involved in blood grouping in its early years, and even his papers indicate the early techniques, in which the agglutinin in the stain was used for determination of the ABO

group. Tests for isoagglutinin in blood stains are still referred to as "Lattes" tests (3). Syracuse's work introduced the elution method for detecting agglutinogens in dried blood, and it discusses the absorption-inhibition method as well. The two were used parallel to each other in these studies. Franz Josef Holzer (1 903-1 974) was a well-known medico-legal blood grouping specialist who studied in this country for a time with Landsteiner. His 1931 paper introduced an inhibition procedure for grouping bloodstains which was used for many years. In 1937, he discussed the secretor characteristic as a marker in forensic investigations. The 1953 paper reviewed the current status of blood grouping, especially in its medico-legal applications. Dr. Holzer spent much of his career at the University in Innsbruck (3).

In 1986 Chase mentioned in her study that was ABO Typing Studied on Liquid Urines. It was the first study on urine sample to determined ABO Typing from those samples. When she was done with her research on these urine samples, for blind trials, a total of 24 liquid urines were collected. These urines were stored for 48 h at 4^0 centigrade. Absorption-inhibition was then performed. Absorption-inhibition was performed on these samples at different time intervals. Time studies were conducted until the results were no longer reliable. The pH of these samples was also monitored over the storage period (4).

In the year 1990, Reena Roy, studied ABO typing from the concentrated urine samples and she conducted that ABO blood group substance analysis from all of the concentrated samples by absorption-inhibition and absorption-elution tests. The pH values of all the samples were monitored from the time of the urine at 4 weeks, at 8 weeks, and finally, at the end of 12 weeks. Experiments on this sample were repeated, and the same results were obtained (5).

Itaru*et al* (2000) determined ABO Typing from semen samples by using Sandwich ELISA. When semen samples, diluted 250-fold, from 54 individuals were subjected to the sandwich ELISA to determine the ABO blood types, the types of 42 of these samples were demonstrated specially by treatment with the corresponding anti-A, B or H antibodies, but other 12 samples showed no reactivity with these antibodies. The secretor status of former group except one and latter group were secretor (Se) and non-secretor (se), respectively, according to the Lewis system type of their blood. Then these samples were subjected to the indirect ELISA to examine correctly (6).

In 1990 Bunai et al determined the ABO blood group from mixed blood stains. They mentioned Immunocytochemical methods to determine the ABO blood group of each blood of mixed blood stains have been developed. Mixed bloodstains were made on surgical blades and a cedar board. These immunocytochemical methods showed specific immunologic reactions and allowed determination of the blood group of each blood of mixed bloodstains. Further, these methods indicated a possibility to determine who was stabbed first, in cases where two or more victims were stabbed with a single knife (6).

Hamada et al (2002) used dot enzyme-linked immunosorbent assay for ABO typing from biological fluid and stains. A simple dot enzyme-linked immunosorbent assay (Dot-ELISA) using commercially available monoclonal anti-A and anti-B antibodies and biotinylated anti-H lectin was developed for ABO blood typing of biological fluid and stains. Its application to forensic practice was examined with 117 saliva samples and their stains, and practical case samples of 8 seminal, 6 vaginal and 45 aged salivary stains. The simple Dot-ELISA could determine accurately the ABO blood type of a small amount of secretor's and non-secretor salivary samples. In practical tests of seminal, vaginal and salivary stains, all results were confirmed to be identical to those determined by the conventional absorption-inhibition test and the absorption-elution test (7).

Morita & Kimura (1998) described the ABO typing saliva specimen and other biological stains by using immunochromatographic method. A simple immunochromatographic method was used to determine ABO blood groups and secretor status from saliva stains. The results of this test were obtained within 2 to 3 minutes. Moreover, the GP reagents utilized were quite inexpensive. The principles underlying this method might be useful in species and organ identification (8).

Shiono (1996) described in his research personal identification using DNA polymorphism--the identification of forensic biological materials. According to him, personal identification by DNA

polymorphism can be performed using forensic specimens. In sexual assaults against women, one key to identifying the suspect is ABO phenotyping or the typing of other polymorphic markers of the seminal fluid in the victim's vagina. However, ABO phenotyping is frequently unsuccessful, since mixtures of fluids cannot be separated to be subjected to conventional methods for the detection of antibody or antigen material. They therefore studied ABO blood group genotyping of sperm DNA isolated from contaminating vaginal fluid by the PCR-RFLP method. They attempted sex and individual identifications of several forensic specimens by detecting various sex chromosome-specific sequences by the polymerase chain reaction (PCR) method. In this study, they found the usefulness of DYS384 and DYS390 in Japanese subjects. In their opinion, future studies on personal identification by DNA polymorphism will mainly evaluate short tandem repeat (STR) (9).

Zachová*et al* (2004) have done their research on determination of ABO blood group system from degraded blood stains on serological and molecular genetic level. They explained The AB0 blood group system typing remains one of the basic laboratory tasks in a forensic practice. However, problems arise when the analyzed samples are seriously degraded. They attempted to determine the AB0 blood group system after thermal degradation at high temperature, accurately at 200^0 C for 10 min. For the AB0 blood group system typing a Polymerase Chain Reaction method was used to amplify glycosyltransferase gene, when DNA had been isolated from artificially created blood stains, followed by their subsequent artificial thermal degradation. For serological AB0 typing the mixed agglutination and the Therkelsen method were used. The DNA analysis seemed to solve problems with seriously degraded blood stains but they found out that classical serological methods were even better in some cases (10).

In 2004, Zlobina determined the specific research of antigens of the ABO system in samples of decayed blood. Zlobinahad taken Liquid blood and blood stains were examined after storage under different conditions and temperature regimes ranging from 18 to 26^0 C. Blood stains were washed by distilled water or heated to 120^0 C for as long as 4 hours. Then, blood groups were determined by absorption-elution (11).

In 2003 Diadichkina&Burago determined ABO,GM and haptoglobin (HP) phenotype in mixed bloodstains of human and goose. The traditional methods of investigations according to systems AB0, GM and HP were used to define the serological specificity of home gooses' blood. The experimental examinations' results related with mixed bloodstains of man and home gooses are described. A possibility is demonstrated to identify the blood group factors of man in bloodstains with amixture of home-goose blood (12).

Sakharov *et al* (1997) described a rapid poly-cationic method for typing erythrocyte antibodies in liquid blood and stains. The new rapid poly-cationic method for typing erythrocytic antibodies in liquid blood and its smears permits a rapid (within 4-5 min) detection of antibodies of virtually all erythrocytic isoserological systems ABO, MNSs, Rh, P, Lewis, Duffy, and Kidd in liquid blood and the group isohemagglutinins and incomplete Rh antibodies in blood smears. The sensitivity of the proposed method was found to be much superior to that of the most sensitive and routine tests: enzymatic, antiglobulin, and salt. The only exclusion were anti-K antibodies, the majority of which were not detected by modern polycationic (tube and plane) methods(13).

In 1990 Thomsen &Adamzik explained the absorption-elution test and the mixed cell agglutination reaction are both ultimately based on the ability of indicator cells to agglutinate. An immunocytochemical method was presented which permits ABH and MN antigen determination on dried blood traces of nano- liter quantities without special fixation. This method was based on immunocytochemical demonstration of antigens directly on the cell membrane in combination with the use of a coated glass slide that ensures maximum economy of epitopes (14).

Kimura *et al* (1993) determined ABO blood grouping of human bloodstains was performed by a sandwich ELISA using a species-specific monoclonal antibody to the amino-terminal cytoplasmic domain of human red cell membrane band 3. In a blind trial, all A, B and O blood stain (a 1 cm long thread) and AB bloodstains (a 1.5 cm long thread) were accurately typed by this method. Even when bloodstains were contaminated by other body fluids (e.g., semen and saliva), only the ABO blood group epitopes on band

3 of the red cell membrane were detected. Thus, identification of human blood and ABO blood grouping of bloodstains which were contaminated by other body fluids could be simultaneously performed by this method (15).

In 1999, Bunai*et al* explained Immuno-cytochemical methods to determine the ABO blood group of each blood of mixed bloodstains have been developed. Mixed bloodstains were made on surgical blades and a cedar board. The immune-cytochemical methods they have attempted have shown specific immunologic reactions and allowed determination of the blood group of each blood of mixed bloodstains. Further, these methods indicated a possibility to determine who was stabbed first, in cases where two or more victims were stabbed with a single knife (16).

Zhou *et al* (1990) in their research described using ABH enzyme-labeled monoclonal antibodies, the authors could rapidly detect the ABO group from body fluids and body fluid stains by the dot enzyme-linked immunosorbent assay dot-ELISA. The method proposed by them is accurate, simple, direct, rapid, and sensitive; it also produces easily observed results, requires no equipment, and can be completed in 30 min. The test proved to be clearly more sensitive for the detection of the ABO blood group in secretor saliva than the conventional hemagglutination inhibition test. Also saliva diluted 10(-4) to 10(-5) and the ABO group of nonsecretor saliva and urine could be easily detected by this method (17)f

De Soyza (1991) described an ELISA for the detection of the ABO group and secretor status of body fluids and stains other than blood is described, together with the validation procedures employed before its introduction into forensic casework. Criteria for the interpretation of results have been formulated for the method in use in this laboratory. The method was found to be reliable and to have a higher success rate than the haemagglutination techniques previously employed (18).

Pflug*et al* (1989) published their study “A new method for ABO and Lewis typing of body fluids is described.” It combines the advantages of a good antigen binding to nitrocellulose membranes, the need of only very small amounts of stain material and the high sensitivity of an enzyme-linked immunosorbent assay for antigen detection. This is of special interest because conventional ABO and Lewis typing of secretion stains need relatively large stain dimensions. The method is very easy to handle, does not need any expensive equipment and gives a permanent record. Furthermore the high sensitivity offers the possibility of analyzing even sweat and urine stains without the need of concentrating these extracts (19).

De Soyza& Garland (1988) explained that Leb positive individuals may phenotypically express both Lea and Leb in their secreted body fluids. Therefore, the interpretation of a Le(a +, b-), non-secretor result is dependent on the absence of Leb. This study emphasizes the importance of accurate procedure and biased selection of antisera such that Leb is preferentially detected in comparison with Lea. The relationship of the ABO group to the expression of Le is discussed in conjunction with the selection of samples for testing antisera and inclusion as control standards (20) .

In 1986 Bässler described that forensic investigations often demand a clear definition of secretor status. Lewis-typing of secretion stains may help to verify non-secretor results and to identify mixtures of secretions from Le (a-b-) persons and secretors (or non-secretors). Furthermore it gives an additional check on secretor status, determined by ABO-grouping. Few problems may arise, when testing prepared saliva or semen stains. Therefore our interest was focused on the possibility of Lewis-typing in stains appearing in forensic case work such as cigarette tips, stamps and envelope flaps, semen stains and vaginal swabs, nasal secretion, sweat and urine stains. All stains with the exception of sweat and urine were successfully Lewis typed. In saliva stains Lewis substances could be determined even after 5 years and in semen stains for at least up to 40 days (21).

Piner&Sänger (1980) proved that the Lewis group system is intricately related to the secretor system, which in turn controls the presence of ABH factors in human secretions. It has been suggested that Lewis typing of secretion stains could help to verify non-secretor results obtained in ABO typing; however, the literature contains conflicting reports on the presence of Lewis substances in secretions. As a preliminary study in the investigation into the usefulness of Lewis typing in case-work, we examined paired saliva and vaginal stains and paired

saliva and semen stains from laboratory donors. The activity of Lewis substances per se in these secretions and their viability in stains over a ten-week period are described (22).

Pereira (1984) said that it is well known that ABH group specific substances are usually present in high concentrations in body fluids of secretors. In normal circumstances these substances can withstand drying and retain their antigenic activity over a prolonged period. This enables the forensic serologist to assist in the investigation of various crimes by grouping stains of body fluids such as semen and saliva. It is possible, for instance, to group saliva and lip mucosa stains on cigarette ends, gags, masks, postage stamps and envelope flaps etc. but it is the grouping of seminal stains in the investigation of sexual crimes which predominates. The results of such tests can be extremely valuable in either including or excluding suspects(23) .

Lee et al (2011) explained in their research that many different molecular typing methods have been reported to complement routine serological ABO blood typing in forensics. However, these ABO genotyping methods are often time-consuming and call for an initial DNA isolation step that requires the use of expensive kits or reagents. We report here a rapid direct ABO genotyping method that eliminates the need for DNA extraction from fresh blood, hair, and body fluid stains before PCR. Using a fast PCR instrument and an optimized polymerase, the genotyping method-which employs a multiplex allele-specific primer set for the simultaneous detection of three single-nucleotide polymorphism (SNP) sites (nucleotides 261, 526, and 803)-identifies A, B, O01/O02, O03, and cis-AB01 alleles in around 70 min from sample collection to electropherogram. Not only will this ABO genotyping method be efficiently used in forensic practice for rapid screening of samples before full-blown multilocus short tandem repeat profiling, but it will also demonstrate an example of rapid direct genotyping of SNPs that offers the advantages of time- and cost efficiency, convenience, and reduced contamination during DNA analysis (24).

Harrington *et al* (1988) reported that since 1928, hemagglutinins have been known to exist in saliva; however, they have not been utilized as evidence in criminal investigations because in the past, techniques for measuring them have not been sufficiently sensitive. In this paper they describe improved techniques for detecting salivary hemagglutinins and report initial results obtained with these methods. Because salivary hemagglutinins can be used to determine ABO blood type, analyses of this kind can serve as an important confirmatory test which the forensic serologist can use in conjunction with salivary agglutinogen determinations (25).

In 1992 Rao *et al* described that ABH antigens, by virtue of their stability and widespread distribution are of unique significance in forensic examination. The conventional methods of typing often lack sensitivity and specificity. They fail to provide dependable results when samples are of minute size and exposed to harsh climatic conditions. They have developed a simple, rapid and highly sensitive solid phase double antibody dipstick immunoassay for detection of A&B antigens by using A&B antibodies (Human) immobilized on nitrocellulose membrane (NCM) strips. The bound antigens have been probed by enzyme labelled second antibodies (Mouse monoclonal). The dipstick assay successfully detected A&B antigens in stains containing as little as 100 ng of dried blood. Blood stains as old as two years could be correctly typed by this method. The assay has the added advantage of simplicity and rapidity. A and B antigens found in tissues, saliva, urine, or sweat can also successfully be detected by the assay. Contaminated bloodstains that conventional methods failed to detect were also identified by this assay (26).

Yazawa*et al* (1992) explained that a novel and simple method for blood group typing has been developed. The procedure involves the use of type-specific monoclonal antibodies covalently linked to dyed microspheres of polyglycidyl methacrylate. The presence of ABH and Lewis blood group antigens in saliva and plasma could be determined easily and specifically with respective monoclonal antibodies bound to dyed microspheres. The present method could also be applicable for the determination of the presence of invisible antigens for the forensic and diagnostic purpose with visible agglutination reaction (27).

Saga (1990) have mentioned the results of their work based on blood grouping laboratory tests performed with the partially fixed erythrocytes as indicators. Both anti-A and anti-B agglutinins in

normal human sera could be detected without difficulty. Presence of irregular agglutinins, such as anti-H and anti-N, in healthy donors' sera could also be detected, as with the freshly obtained erythrocytes. The partially fixed erythrocyte (stored at 4^0 C for 2.5 to 3 months) were used as indicators of ABO-blood grouping from blood stains, saliva stains and hairs by the agglutinin-inhibition test, absorption-elution test or mixed agglutination test. The results obtained were practically equal to those of the tests with freshly obtained erythrocytes, indicating the availabilities of the partially fixed erythrocytes(28).

In 1989 Takizawa *et al* reported in their research that the detection of A, B and H blood group substances (ABH-BGS) in saliva and in saliva stains has been investigated quantitatively by an indirect ELISA using a horseradish peroxidase conjugate in combination with the use of monoclonal antibodies. Through this method, the reaction specificity to BGS in the saliva was very high and its detection sensitivity was found to be approximately 1,000 times greater than has been achieved in a hemagglutination-inhibition test. The BGS levels in experimentally prepared salivary stains were found to be approximately proportional to the levels in the original saliva. As for actual and aged stains, it was possible to detect BGS in most cigarette butts and in aged stains, however, such detection proved impossible in saliva samples from postage stamp (29) .

Komuro*et al* (1995) gave their report on Medico-legal identification of saliva stains and bloodstains was performed by an enzyme-linked immunosorbent assay (ELISA) using a horseradish peroxidase conjugate in combination with the use of monoclonal antibodies. Activity of alpha-amylase in the stains was measured for an identification of saliva using an anti-human amylase antibody, and secretory IgA was detected for a species identification using an anti-IgA antibody. ABO and Lewis blood group antigens were detected using anti-A, anti-B, anti-H, anti-Lea and anti-Leb antibodies (30).

In 1988, Oepen has given review of proved techniques for detection of individual characteristics of blood stains and seminal stains. Above all the results of German investigators are considered because these have mostly been insufficiently treated in the English-language literature, for instance papers concerning Gm and Km typing (31).

Bolton & Thorpe (1988) reported that aseminal stain, apparently contaminated with a detergent cleanser, was received for examination. The contamination interfered with the normal biochemical reactions of such stains. Treatment of the sample enabled ABO groups to be determined (32).

Baechtel (1985) provided the research on a sensitive microplate hemagglutination-inhibition technique used to ascertain the distributions of secreted blood group substances (BGS) in a population of 176 semen specimens and to characterize the stability of these substances in dried seminal stains. Studies of the stability of BGS in Groups A and O semen suggested that these substances were stable when the semen stains were stored at -20^0 C, 4^0 C, or at ambient laboratory temperature in a dry state. In contrast, stains stored at 37^0 C under humid conditions suffered a dramatic loss in BGS titer, with the half-life of the BGS being on the order of 30 days (33).

Sagisaka*et al* (1984) noted that A antigens of red blood cells and body fluids such as saliva, semen and sweat could be serologically distinguished using rabbit or guinea pig immune anti-A. As for antisera specific for red blood cell A, A+ rabbits were intravenously immunized with A group red blood cells. The resulting antisera were absorbed with O and B red cells and with A. Se saliva. The absorbed anti-A reacted with A red cells (titer 1:32) and was not inhibited with A. Se saliva. Guinea pigs were intramuscularly injected with A. Se saliva. Crude antisera contained agglutinins to human red cells which were abolished by absorption with A red cells. After absorption with O. Se saliva, the antisera were proved to have agglutinin activity with A group saliva using latex coated with A. Se saliva. A antigens from blood or body fluid stains could be distinguished by the elution method with these anti-A sera (34).

Materials and methods

Collection and preservation of samples

Sample from 20 donors are directly collected in sterile containers under normal temperature with their consent. They donate the sample under normal temperature (20-25^0 centigrade) in liquid form. Preparations of stains are done in the laboratory

under room temperature. Preparation of stain is done by directly dip the gauge in individual container separately dipping and drying 10 times. Dried the all sample in the form of stains separately by hanging in a rope within the laboratory condition. The time taken to dry these sample within the laboratory are 20-30 days. At this time these all samples are dried perfectly and are ready to examination.

Methods of examination

1. Checking of all the stains by using U.V. light because each biological fluid gives different florescence under U.V. light. All these samples gave the greenish-yellow coloration under UV light.
2. Use corresponding presumptive test to identify the body fluids such as :
    - Creatinine test.
3. Use of confirmatory test such as:
    - Urea nitrate crystal test.
4. Determination of secretor and non-secretor status of each stain by using double diffusion method.

Table1: Secretor Status of volunteer

S.No.	Samples	Secretor Status
1	Sample No.-1	Non-Secretor
2	Sample No.-2	Secretor
3	Sample No.-3	Secretor
4	Sample No.-4	Secretor
5	Sample No.-5	Secretor
6	Sample No.-6	Secretor
7	Sample No.-7	Secretor
8	Sample No.-8	Secretor
9	Sample No.-9	Secretor
10	Sample No.-10	Secretor
11	Sample No.-11	Secretor
12	Sample No.-12	Secretor
13	Sample No.-13	Secretor
14	Sample No.-14	Non-Secretor
15	Sample No.-15	Secretor
16	Sample No.-16	Secretor
17	Sample No.-17	Secretor
18	Sample No.-18	Secretor
19	Sample No.-19	Secretor
20	Sample No.-20	Secretor

5. Blood typing of different stains by using Absorption-elution and Absorption-inhibition methods separately.

Absorption Elution method

Procedure:

a. Take a cellulose acetate sheet (minimum thickness 0.4mm) and mark three areas as A, B and H. Glue one cm long bloodstained thread with acetone/fingernail polish to each of 3 areas.

b. Allow the threads to fix on the sheet for 15 minutes.

c. Put one drop of the anti- A serum, anti- B serum and anti- H lectin respectively on the fixed threads

d. Place the sheet in a moist chamber and allow it to absorb overnight at 4^0C in the refrigerator.

e. Remove from the refrigerator, rinse off the excess antisera and blot the threads dry with a paper towel by inverting the plate face down on a paper towel and rubbing the back of the glass plate with another towel.

f. Place in the refrigerator at 4^0C for a minimum of 2 hours. Longer wash times will not have a negative effect on the results provided the temperature does not exceed 4^0C. Shorter wash times may result in incomplete rinsing of unbound antibody.

g. Remove from the wash bath and blot dry with a paper towel as previously described.

h. Add one drop of appropriate 0.2-0.5% indicator cells to each thread.

i. Place in a moist chamber and elute in a 56^0C incubator for 20 minutes.

j. Place the sheet in a moist chamber and rotate on a VDRL rotator for 30 minutes.

k. Read results microscopically.

Absorption Inhibition Method

Procedure:

1. Prepare the dilutions of antiserum of its penultimate titer (if the titer is 32 then prepare a 16 dilution by taking one drop of the serum and adding 15 drops of normal saline to it).

2. Take a clean and dry cavity tile and mark three cavities as A, B and H and place one drop of suitable dilution of anti-A serum, anti-B serum and anti-H lectin to the respective cavities.

3. Add one drop of the extract in each cavity, shake and keep at 4^0C for 2 hours.

4. Add one drop of 0.2% indicator cells in the respective cavities and keep at 4^0C for half an hour.

5.Shake the tile and examine the contents for the presence of agglutination both macroscopically and microscopically.

6.Now to note down the ABO blood typing of each biological stains using in the whole processes.

Result and discussion

The present study deals with the secretor and non-secretor status and blood typing of the urine stains of 20 individuals.

As a general rule, in India about 10-20% of the population are Non-secretors and remaining 80-90% being Secretors.

The present study generally fit well within the range previously described out of 20 samples, 2 are non-Secretors and 18 are Secretors.

Table2: Secretor Status & Blood Typing of volunteers

S.No.	Samples	Secretor Status	Blood typing
1	Sample No.-1	Non-Secretor	-
2	Sample No.-2	Secretor	O
3	Sample No.-3	Secretor	AB
4	Sample No.-4	Secretor	A
5	Sample No.-5	Secretor	AB
6	Sample No.-6	Secretor	O
7	Sample No.-7	Secretor	B
8	Sample No.-8	Secretor	AB
9	Sample No.-9	Secretor	O
10	Sample No.-10	Secretor	B
11	Sample No.-11	Secretor	B
12	Sample No.-12	Secretor	AB
13	Sample No.-13	Secretor	O
14	Sample No.-14	Non-Secretor	-
15	Sample No.-15	Secretor	A
16	Sample No.-16	Secretor	B
17	Sample No.-17	Secretor	AB
18	Sample No.-18	Secretor	A
19	Sample No.-19	Secretor	A
20	Sample No.-20	Secretor	O

Table3: Frequency of secretors and non-secretors

S.No.	Number tested	Secretors	Non-secretors
1	20	18 (90 %)	2 (10 %)

These 18 secretors provide their original blood grouping that they have and remaining were left due to lack of their secretor status.

Conflict of Interest

None

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