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Determination of ABO Blood Grouping from Different Body Fluids (Saliva)

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Abstract

The ABO blood group typing is the primary serological technique to be performed after collecting the blood sample from the crime scene. Cases like murder, rape, hit and run has the crime scene where we can easily find blood stains. But in some cases criminals remove those stains in order to conceal the evidence.

80% of people are secretors, meaning they secrete blood group antigens in other body fluids like saliva, semen which can be used to determine the blood group of the person. These body fluids can be obtained from coffee cup, cigarette butt, bite marks on victim, vaginal swabs and so on. Among these fluids saliva shows the more accurate results. Absorption inhibition and Absorption elution assays are such method which can be used to detect the blood group from body fluids like saliva where we use A and B antisera solution to determine the presence of antigen. And the positive results were obtained from presence or absence of clumping between antigen and antibody.

This leads us to the conclusion that saliva shows 100% accuracy as blood in finding the blood group of the person for both males and females.

Keywords: ABO Blood Grouping; Saliva; Body Fluids; Absorption Inhibition

Introduction

Blood plays a significant role in Forensic investigation. While DNA profiling has become the principal technique for individualization of biological evidences, ABO blood grouping is still a useful test method in the initial stages of crime investigation [1]. Blood was used to detect the blood group antigens present in the surface of red blood cells (RBCs) and helped in narrowing down the suspects. ABO Blood group system was discovered by Karl Landsteiner in mid-1900, which is used in crime scene investigation for the identification of blood group till date [2]. As there are three different types of blood group antigens (A, B and O) present in the human blood group system their presence were detected by agglutination assays where the antigens clump with antibodies.

Yamakami discovered the presence of A and B antigens in saliva in 1926 [3]. Laterally it was also found that 80% of people secrete ABO blood group antigen also in other bodily fluids like saliva, semen, sweat [2]. In 1930, individuals were classified as "secretors" and "non-secretors" according to their ability to secrete ABO blood group antigens in saliva [4]. Although the blood stains can easily found at the crime scene of violent offences in some cases where the criminals remove the evidence or where the stains were contaminated it becomes difficult to perform the blood grouping techniques. In such cases other body fluids can be used. As per studies among all the body fluids saliva shows 100% accuracy in determining the blood group regardless of blood group types. Saliva can be found in the cases like hanging, rape, robbery and so on. In 1928 saliva was first examined for the presence of anti-A and anti-B hemagglutinins but, due to lack of proper techniques it was not used as evidence in medico-legal cases [5]. As the techniques where modified and advanced in nowadays saliva can be used as evidence and also can be presented in court of law. Studies revealed that hemagglutinins present in saliva may be stable for several days at room temperature [6]. Absorption inhibition method was introduced in 1923 by Vittorio Siracusa in Italy [7]. This method works by reducing the strength of antigens present in the stains [8]. Absorption inhibition and absorption elution assay are the methods which detects the blood group not only from blood but also from other body fluids where we use anti-A and anti-B antisera solution to detect the presence of antigens.

Materials and Methods

Materials

This study is based on how body fluid like saliva helps us in determining the blood group of the suspect in the cases where blood is not available as evidence. For this study samples of body fluids were taken from different individuals including both secretors and non-secretors. Blood samples from same subjects were taken to compare and find out the accuracy of the results.

Sample collection

Sterile cotton swabs were placed below their tongues for around 3 min. The swabs were then taken out and squeezed into sterilized glass bottles [9].

Methods

Preliminary test for alpha-amylase

5 mL of distilled water was added in test tubes and gauze containing saliva sample was kept in it undisturbed for overnight. Then 0.5 mL of the resultant solution and 3.5 mL of buffered starch were added and incubated for 30 min at 37°C. And 0.75 mL of conc. Sulfuric acid and 0.25 mL of sodium tungstate were added. And then the solution was centrifuged for 10 min. 2 mL of the resultant supernatant and 2mL of copper sulphate alkaline were added and boiled in a water bath for 10min. After cooling, 2mL of phosphomolybdic acid was added. The presence of alpha-amylase was observed from the formation of blue colour and the remaining extract was used as fresh saliva sample if positive [2].

Absorption Inhibition assay

Saliva sample was taken in two test tubes. The dilution of 1:10 of A and B antisera solution were added to each test tube, and then labelled respectively. A singe drop of saliva was added to both the test tubes and shaken thoroughly and then kept for incubation at 37° for 10 min. After 10 min, a drop of freshly prepared RBC of known blood group was added to the respective test tubes and shaken well and again incubated at 37° for 15 mins and checked for agglutination. In this test, the positive result was obtained from the absence of agglutination [2].

Absorption Elution assay

Here, the saliva was taken in two test tubes and labelled A and B and then the anti-sera A and B were added respectively. The test tubes were thoroughly shaken and kept in incubator for 5 hrs so that adequate antigen-antibody reaction could occur. After the incubation the excess amount of antibody was removed using cold saline solution and that were repeated for 5 times. Then the test tubes kept in a hot water at 56°c temperature which leads to the break down or elution of the antigen – antibody complex. Then a single drop of red blood cells (RBC) of known group was added to the respective test tubes and shaken well and then kept for incubation at 37°c for 15 min. Then both the test tubes were centrifuged at 2000 rpm for 1min. The positive result was observed from the presence of agglutination [10].

Result

The sensitivity of absorption inhibition method was 81% while for absorption elution it was 86%. As per kappa coefficient absorption elution detected 6% more cases than absorption inhibition and the sensitivity were 5% more than absorption inhibition [10]. A slightly higher percentage of secretor status was observed in females (100%) than in males (90%) [5]. Groups A and O revealed 100% secretor status for both males and females, while groups B and AB revealed 95% secretor status [5]. Saliva sample from secretors shows 100% accuracy in determining the blood group type as compared to blood.

Discussion

Blood group type plays an important role in excluding or including the suspect and also acceptable in court of law. Bodily fluids like saliva, semen, sweat, vaginal samples can be found from cigarette butt, bite marks on victims, coffee mugs, bedsheets, victims clothing and private body parts and so on. In such cases it may be possible that blood stains were not be found as evidence. The source of blood group antigens is the water soluble substances that are present in most body fluids and organs of a secretor [5].

Blood group	From blood	Match in saliva	Percentage
A	32	26	81.25
B	18	15	83.33
AB	3	1	33.33
O	24	20	83.33
Total	77	62	80.51

Table 1: The percentage of positive matches between blood group in saliva and blood using absorption inhibition [10]

Blood group	From blood	Match in saliva	Percentage
A	32	29	90.62
B	18	18	100
AB	3	2	66.66
O	24	21	87.5
Total	77	70	90.90

Table 2: The percentage of positive matches between blood group in saliva and blood using absorption elution [10]

In 1978 a study was conducted and observed that 17 (68%) among 25 vaginal samples were secretors of blood group antigens [11]. Then in 1988 a study was conducted on both saliva and sweat stains and observed that 87.5% were secretor in saliva out of 72 samples tested [12]. And the recently in 2014 a study was done to know the secretors and non-secretors in people of Karachi and the concluded that 64.4% out of 101 healthy adult student were secretors and among them blood group B has the highest frequency of 79.5% while blood group AB has the lowest frequency of 45.5% [5,13]. Blood group would be determined with 100% accuracy from the saliva of those subjects who were secretors of antigens in their saliva [14]. Once a blood group is established in an individual it remains unchanged throughout his life, the use of blood group substances is based in medico legal examination [5].

The use of saliva in forensic science is based on the presence of ABH blood group substances in the saliva of secretors in fairly high concentration [5]. Among all other body fluids saliva shows more accurate results in determining the blood group of a person. Absorption elution assay detects more cases as compared to absorption inhibition assay. Errors in the result may be observed if the sample was not collected in proper or in proper amount from the crime scene. As 100% of people are not secretors the limitations may occur in reports.

Conclusion

The present study concludes that blood group of a sample found at the crime scene is a helpful tool for forensic investigation. And body fluids like saliva make easier to determine the blood group and also shows 100% accurate results. Using latest techniques which are more sensitive helps to reveal more reliable result. This makes saliva as the crucial evidence to be collected and preserved in forensically sound manner. And secretory status plays an important role in these findings.

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