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Research Article

Blood Grouping Made Painless: Detecting ABO from Human Saliva.

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Secretor, Non-secretor, Hemagglutination inhibition technique, Agglutination.

Abstract: *The frequency of ABH secretor status was studied by detecting the ABH blood group substance using the standard tube-hemagglutination inhibition technique in the saliva of 30 students from Varee Chiangmai School, grades 10 to 12. The participants included 11 males and 19 females, accounting for 36.37% and 63.33% respectively. The secretor gene frequency among the students at Varee Chiangmai School was found to be 90%*

1. INTRODUCTION

There are various methods currently used for blood group testing. The Slide technique is widely popular due to its rapid results; however, it serves only as a screening method. In cases where a patient requires a blood transfusion, confirmatory testing is necessary. This includes methods such as the Conventional test tube technique or the Column agglutination test, which are the standard techniques used for ABO and Rh typing. Each technique has its own advantages and limitations. Particularly in cases where ABO discrepancies are found, additional procedures may be required to obtain accurate blood group results—for example, the use of adsorption/elution techniques to separate antibodies from cells. These methods are time-consuming and are not standard practices that can be performed in every laboratory. Therefore, there is a need for supplementary blood group testing methods that require less time and can be performed in all laboratories, serving as an additional option.

Detection of ABH secretion in saliva using the Hemagglutination inhibition test is a method for determining blood groups from other bodily secretions without the need for blood samples. This procedure assesses ABH Secretor Status—that is, an individual's ability to secrete ABH antigens (A, B, and H) into body fluids such as saliva, milk, and other secretions. Individuals who are ABH secretors (Secretors) possess these antigens in their body fluids, whereas those who are non-secretors (Non-secretors) do not. ABH secretion is controlled by the FUT2 (Secretor gene), which encodes the enzyme required for the formation of ABH antigens in body fluids (1). Secretors have the genotypes SeSe or Sese, while Non-secretors carry the genotype sese. In the general population, approximately 80 % are Secretors and 20 % are Non-secretors (2).

The research team recognizes that ABH secretor status plays an important role in blood and body fluid testing, as well as in ensuring safety in medical treatments—especially in blood transfusion and organ transplantation. Knowing one's own blood group helps physicians select compatible blood for patients, reducing the risk of adverse transfusion reactions. Therefore, the research team conducted an experiment to determine ABO blood groups from saliva samples of upper secondary students at Varee Chiangmai School. The aim was to study the method of blood group testing using saliva and to compare the frequency of individuals with ABH secretor status (Secretors) and those without (Non-secretors) with the statistics reported by the Thai Society of Hematology.

2. LITERATURE REVIEW

2.1 Blood Group System

Blood groups are classifications of blood based on the specific characteristics of antigens, which are proteins or carbohydrates present on the surface of red blood cells. Blood group classification can be used for personal identification and to indicate blood compatibility among individuals. At present, there are up to 39 recognized blood group systems. Among these, the ABO blood group system is the most important and the most widely known.

Blood group antigens are inherited genetically and exhibit variations in their expression among different ethnic groups.

2.2 The ABO Blood Group System

The ABO blood group system consists of the ABO gene located on chromosome 9 at position 9q34.3, as well as the FUT1 gene (H gene) and the FUT2 gene (secretor gene), both located on chromosome 19 at position 19q13.3. In the ABO blood group system, the H antigen serves as the precursor for the formation of A and B antigens. The synthesis of the H antigen is regulated by two main mechanisms involving the addition of sugar moieties at specific sites on glycoproteins and glycolipids.

The first mechanism is controlled by the FUT1 gene, which encodes the enzyme 1,2-fucosyltransferase. This enzyme adds fucose to type 2 chain oligosaccharides, resulting in the synthesis of the H antigen as a membrane-bound (sticky) antigen on the surface of red blood cells. The second mechanism is controlled by the FUT2 gene, which also encodes the enzyme 1,2-fucosyltransferase. This enzyme adds fucose to type 1 chain oligosaccharides, leading to the synthesis of the H antigen as a soluble antigen present in body secretions such as saliva, sweat, tears, and semen.

The A and B blood groups are distinguished by the specific addition of sugar residues to the H antigen. The A gene encodes the enzyme 1,3-N-acetylgalactosaminyltransferase (A-transferase), which adds -N-acetylgalactosamine, whereas the B gene encodes the enzyme 1,3-galactosyltransferase (B-transferase), which adds D-galactose. In contrast, the O blood group results from the absence of functional enzymes, leading to no additional sugar modification of the H antigen.

In the Thai population, the frequency of blood group O is the highest at approximately 38%, followed by blood group B at approximately 34%, blood group A at approximately 21%, and blood group AB at approximately 7%.

2.3 Secretor Status

Secretor status is classified into secretors (SeSe or Sese) and non-secretors (sese), and it indicates an individual's ability to secrete ABO blood group antigens into body fluids such as saliva, tears, sweat, and semen. Individuals who are secretors secrete antigens corresponding to their blood group. Specifically, individuals with blood group A secrete A and H antigens; those with blood group B secrete B and H antigens; those with blood group O secrete only the H antigen; and those with blood group AB secrete A, B, and H antigens. In contrast, non-secretors do not secrete A, B, or H antigens into body secretions. It has been suggested that non-secretor status may be associated with an increased risk of reduced immune system function.

In the Thai population, based on data reported in the Journal of Hematology and Transfusion Medicine of the National Blood Centre, Thai Red Cross Society, approximately 70.87% of individuals are secretors, while 29.13% are non-secretors.

2.4 ABO Blood Group Determination from Blood Samples

2.4.1 ABO Blood Group Determination Using the Slide Technique

The slide technique is a simple and rapid method for ABO blood group determination. It requires a small volume of blood and reagents and can be completed within a few minutes. However, a limitation of this method is its inability to detect weakly expressed antigens, which may lead to errors in result interpretation. Therefore, the slide technique is suitable primarily for emergency situations or for preliminary screening purposes only.

2.4.2 ABO Blood Group Determination Using the Test Tube Technique

The test tube technique is a more sensitive and detailed method compared to the slide technique; however, it requires appropriate amounts of reagents. Result interpretation is based on the observation of agglutination. To read the results, the test tube is held at eye level with the cell button positioned at the top. The tube is then gently tilted so that the liquid comes into contact with the cell button, and the wrist is moved gently to allow the liquid to wash the cell button completely. Subsequently, the tube is tilted to allow the cell suspension to spread evenly. The results are recorded by grading the strength of agglutination reactions as 4+, 3+, 2+, 1+, or 0.

2.5 Detection of ABH Substances in Saliva Samples

The detection of A, B, and H substances in saliva is one of the methods used to confirm and determine the ABO blood group. This test is based on the principle of the hemagglutination inhibition test. Substances A, B, or H are antigens that, when present in saliva, can neutralize (inhibit) appropriately diluted anti-A, anti-B, or anti-H antibodies, respectively. As a result, no free anti-A, anti-B, or anti-H antibodies remain to react with subsequently added standard A cells, B cells, or O cells (which contain the highest amount of H antigen). Consequently, no red blood cell agglutination is observed.

In contrast, if substances A, B, or H are absent in the saliva, the added anti-A, anti-B, or anti-H antibodies are not neutralized and remain active. These antibodies can then react with the subsequently added standard A cells, B cells, or O cells, respectively, resulting in visible red blood cell agglutination.

3. METHODOLOGY

3.1 Objectives

- To study the method for detecting ABO blood groups from saliva using the Hemagglutination inhibition test
- To analyze the frequency of ABH secretor status among upper secondary students at Varee Chiangmai School using statistical methods

3.2 Scope of Work

Independent Variable : ABH secretor status

Dependent Variable : Frequency of the population group that secretes ABH substances

Controlled Variables :

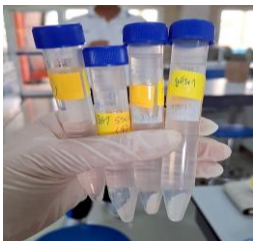
1) Population Selection
   - Students from Varee Chiangmai
   - Upper secondary level students
2) Sample Collection Method
   - Fill out a consent form
   - Saliva collected from the sublingual salivary glands
   - Rinse mouth before saliva collection
3) Laboratory Testing for ABH
   - Temperature
   - Volume of Saliva
   - Volume of Standard Anti-A, Anti-B, and Anti-H Antisera
   - Volume of Standard ABO Cells (Indicator Cells)
   - Volume of Normal Saline (Isotonic Saline Solution)
   - Centrifugation Force (Relative Centrifugal Force, RCF)
   - Centrifugation Time for Sedimentation
   - Centrifugation Time for Reading the Reaction
   - Size of Test Tubes
   - Accuracy/Resolution of Pipettes
   - Incubation Time for Saliva-Antibody Interaction

3.3 MATERIALS AND METHODS

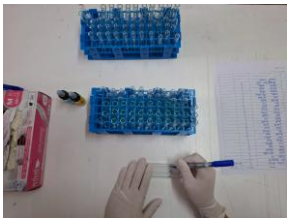
3.3.1 Process

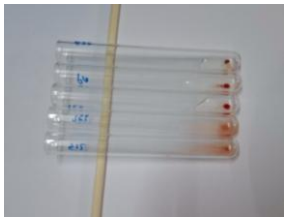
1) Collect saliva from 50 upper secondary students at Varee Chiangmai School.

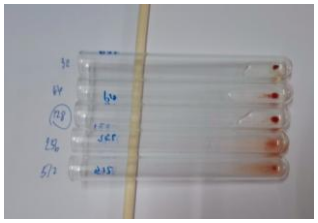
2) Boil the saliva for 10 minutes to inactivate enzymes in the saliva. Centrifuge the boiled saliva at 900–1000 g for 10 minutes to precipitate it. Collect only the supernatant and divide it into 3 tubes per sample.



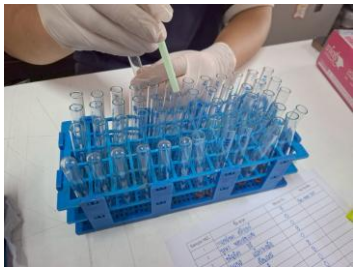
3) Dilute Anti-ABH using NSS with 2-fold dilution using an Autopipette. Test by adding one drop each of standard cell A, cell B, and cell O into the respective tubes according to the type of antibody. Centrifuge and read the agglutination reaction (Agglutination). Select the final dilution titer that gives a 2+ reaction strength.



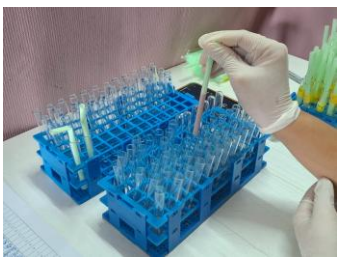


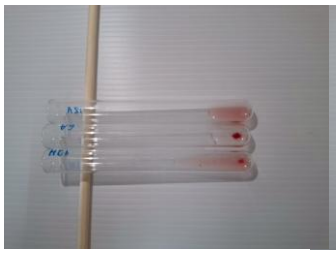
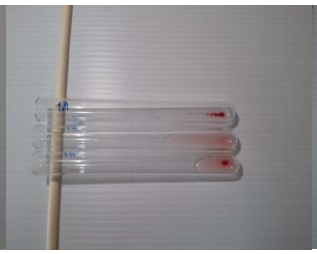
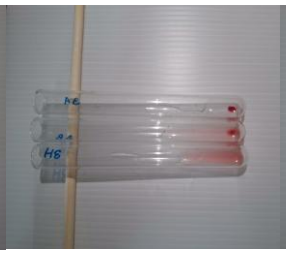
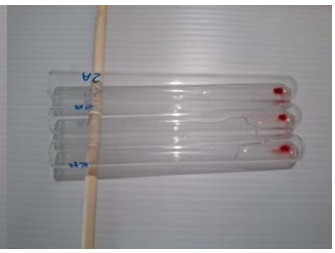


4) Add the Anti-A at the selected dilution titer (from step 6) into the test tubes. Let stand for 10 minutes to allow the antigen in the saliva to bind with the added antibody. (The procedures for detecting substances B and H are the same as for substance A)



5) Add standard cell A into all 3 test tubes, each containing Anti-A, Anti-B, and Anti-H. Gently mix and leave at room temperature for 30–60 minutes. Centrifuge and observe agglutination with the naked eye.



Blood type A Blood type B Blood type O Non-secretor

3.3.2 Equipment

1) Large glass tubes (15×150 mm)

2) Test tubes (12×75 mm)

3) Hot plate/water bath & test tube rack

4) Standard Anti-A Anti-B Anti-H diluted at Titer 1:64 1:128 and 1:32 respectively

5) Standard A B O cell

6) Normal saline solution (NSS)

7) Centrifuge

8) Dropper

9) Beakers

10) Fresh or Frozen saliva from Inactivate with A, B, or H substances [Positive control]

11) Fresh or Frozen Inactivate saliva from individuals without A, B, or H substances [Negative control]

12)Autopipette

4. FINDINGS AND DISCUSSION

4.1 Distribution of secretor status in Student of Varee Chiang Mai School

Secretor(n)	Non-secretor (n)	Total
27 (90%)	3 (10%)	30 (100%)

This study compared the proportion of Secretors and Non-secretors between data obtained from the National Blood Centre, Thai Red Cross Society (n = 206), and a sample of high school students from Varee Chiangmai School (n = 30). Depending on subgroup sizes, either the Chi-square test or Fisher's Exact Test was used to determine whether the difference in proportions was statistically significant. The results indicate a higher percentage of Non-secretors in the student group compared to the reference population. However, due to the relatively small sample size in the student group, further statistical analysis is recommended to confirm the significance of this difference.

4.1.1 Comparison of the Proportion of Secretors and Non-secretors Blood Donation Center, Thai Red Cross Society (2016) and Upper Secondary Students from Varee Chiangmai School

	Secretor(n)	Non-secretor (n)	Total
Blood Donation Center, Thai Red Cross Society 2016	146	60	206
Upper Secondary Students from Varee Chiangmai School	27	3	30

Statistical Analysis Results

The analysis using Fisher's Exact Test (two-tailed) at a 95% confidence level yielded the following results: Odds Ratio = 1.22, p-value = 0.8032

Given that the p-value is greater than 0.05, it can be concluded that there is no statistically significant difference (p = 0.8032) in the proportions of Secretors and Non-secretors between the dataset from the National Blood Centre, Thai Red Cross Society, and the group of upper secondary students from Varee Chiangmai School at the 95% confidence level.

Although the Varee Chiangmai student group showed a slightly higher proportion of Secretors compared to the national dataset, statistical analysis does not support that this difference is significant.

4.2 Distribution of secretor status in A,B,O and AB blood groups

Blood groups	Secretor (n)
A	2 (7.407%)
B	14 (51.8519%)
O	10 (37.0370%)
AB	1 (3.7037%)
Total	27 (100%)

This study aimed to compare the distribution of blood groups A, B, O, and AB between two populations: the general Thai population as represented by data from the National Blood Centre, Thai Red Cross Society (n = 206), and a sample of upper secondary students from Varee Chiangmai School (n = 30). The objective was to determine whether there is a statistically significant difference in the proportions of blood groups between the two groups.

4.2.1 Comparison of Blood Group Proportions (A, B, O, AB) between data from the National Blood Centre (2016) and Upper Secondary Students from Varee Chiangmai School

Blood group	Blood Donation Center, Thai Red Cross Society 2016 (n)	Upper Secondary Students from Varee Chiangmai School (n)
A	27	2
B	50	14
O	64	10
AB	5	1

The Chi-square test was conducted at a 95% confidence level and yielded the following results:

- Chi-square = 9.0134
- Degrees of freedom = 3
- p-value = 0.0293

Since the p-value (0.0293) is less than 0.05, it can be concluded that there is a statistically significant difference in the distribution of blood groups between the dataset from the National Blood Centre, Thai Red Cross Society, and the group of upper secondary students from Varee Chiangmai School.

5. CONCLUSION

The analysis revealed that the experimental group had a higher proportion of Secretors compared to the data from the National Blood Centre, Thai Red Cross Society (3). Fisher's Exact Test was employed for the analysis, as it is appropriate for categorical data presented in 2x2 contingency tables with small sample sizes in certain groups (e.g., only 3 Non-secretors in the experimental group).

	Secretor (n)	**Non-secretor (n)**	**Total**
Blood Donation Center, Thai Red Cross Society 2016	146	60	206
Upper Secondary Students from Varee Chiangmai School	27	3	30

No statistically significant difference was found between the two groups ($p = 0.8032$) at the 95% confidence level. The observed difference in proportions may be attributed to random variation rather than a true difference in the populations.

The data analysis using the Chi-square test for independence revealed a statistically significant difference in the distribution of blood groups between upper secondary students from Varee Chiangmai School and the dataset from the National Blood Centre, Thai Red Cross Society ($\chi^2 = 9.01$, $df = 3$, $p = 0.0293$) at the 95% confidence level.

Blood group	**Blood Donation Center, Thai Red Cross Society 2016 (n)**	**Upper Secondary Students from Varee Chiangmai School (n)**
A	27	2
B	50	14
O	64	10
AB	5	1

This indicates that the composition of blood groups in the experimental group does not conform to the proportions observed in the reference group. Such differences may reflect underlying genetic factors, characteristics of the sampled population, or differences in sampling methods. However, the relatively small sample size in certain subgroups (e.g., blood group AB = 1) should be taken into consideration, as it may affect the accuracy of the results.

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