Research Article
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Serological identification of some blood groups of the washed bloodstains by using some common detergents based on micro-elution method

Abstract

Blood grouping of the bloodstains at the scene of the crime may help to connect the criminal with the crime. An assailant attempts to remove the bloodstains from clothing by various procedures such as the use of water or detergents. This study aimed to assess the effect of some commonly used detergents (Ariel automatic, Persil and Soap) on the identification of human blood groups of ABO system which stains the different fabrics (cotton, wool and synthetic material). The study was carried out on 2160 samples of blood that stained fabrics dividing into four different blood groups; each group comprising of 36 dry bloodstains of specific blood group which stained the three mentioned fabrics and washed by water alone and the three mentioned detergents individually along with three control groups. Grouping of bloodstains was done by micro-elution technique before and after the washing by water only or detergents. The study showed that the use of detergents or water in the washing and removing of the bloodstains on the different fabrics affect the identification of blood groups of the bloodstains (A,B,O) except the blood group (AB) which showed that the use of water alone for removing it, did not affect its identification on different fabrics. The negative effect of all detergents on the identification of blood groups of dry bloodstains does not depend on the type of the blood group or the type of fabric or the type of detergent.

Keywords: blood, stains, grouping, detergents, genotypes, fabrics

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Introduction

The examination of biological materials in the scene of the crimes is an important method to connect the criminal with the crime; the biological materials may be stains for the blood, semen or saliva. The task of forensic scientist during the investigation of crimes is stains examination to determine their nature and origin, and carry out tests for identification their blood group markers.[1]

There are different blood groups systems such as ABO, MN, Rh that are established hereditary factors since an early age and unchanged throughout the life, and then it is specific to the individual who cannot possess any antigen which both his parents lack according to the Mandel's laws of the heredity. ABO system consists of a series of triple allelomorphs whereas the genes A and B are dominants while O is recessive to A and B, so an individual derives one genetic factor from each of parents, and then the possible genotypes may be AA, AO, AB, BB, BO, and OO.[2]

Identification of blood stains grouping depends on the presence of antigens in the shrivelled red cells of stains which have the ability to absorb the corresponding antibody specifically. Antigens of ABO blood group system are active enough to be detectable because they display a considerable toughness, thermo stability, durability while the antigens of other blood group are more liable, and then it is active for some months only such as MN and Rh antigens. Blood group antigens are deteriorated in the stains at the different rates based on the conditions to which the stains are exposed before the testing such as the washed stains via using the detergents. Blood stains on some fabrics are usually difficult to locate and may easily escape by the naked eye examination, so the washed stains on the fabrics are also very hard to detect.[3]

Identification of blood groups in the stains depends on the availability of the amount of blood in the stain, agglutinogens, and agglutinins to determine the type of the blood groups. So, the washed clothes by using the usual household products such as detergent may affect the blood stain identification.[4] Therefore, this study aims to determine the effects of some commonly used detergents on the serological identification of some blood groups of bloodstains (ABO) on the different fabrics based on micro-elution method.

Material and methods

2160 dried samples of the human bloodstains from different individuals, 24hours of age, were obtained from the blood bank of Suez Hospital; they were divided into four different blood groups wherein each group comprising of 36 dry bloodstains of specific blood group. The first group represented the bloodstains of blood group A while the second group was the bloodstains of blood group B. The third and fourth groups were the bloodstains of the blood groups AB and O respectively. The bloodstains of the different blood groups were placed on the different types of fabrics (cotton, wool, synthetic material) that were washed by using water only and by using the different types of detergents individually (Ariel automatic, Persil, Soap). There were three control groups to determine the original strength of the antigen city of each stain of known human blood group on the different types of fabrics before washing with water or by using of detergents. There were also another three blank control groups for three different fabrics in each group, they were identified by micro-elution technique without neither staining nor washing by water or detergents. The assessment of the agglutination degree and identification of each dry stain on the different types of fabrics were also done by micro-elution technique after the washing process. The bloodstains of 24hours of age were prepared by depositing one drop

of fresh known human blood group of ABO system on the different types of fabrics wherein the amount of bloodstains on each fabric was 1 ml and occupied nearly an area of (1cm x 1cm) on the fabric. These stains were left to dry in the air away from the dust at a room temperature and then kept in an envelope.

The utilized washing procedure was the same for each detergent where the washing process was done by hand wash for every detergent even Ariel Automatic and carried out by tap water for two minutes at a normal temperature (atmospheric temperature) to avoid the alteration of bloodstains as the result of the effects of heat.[5] The fixation of the variables in this study is attributed to that the identification of dried bloodstains depends upon the quantity and age of the bloodstains, and the type of involved fabric as well as the utilized washing procedure in accordance with Mushtaq et al.[6]

The standard blood corpuscles

1-2ml of the blood was added to an equal volume of 3.8 % tri-sodium citrate dehydrate solution wherein the tubes were inverted frequently to prevent clotting. The red cells were washed to be free of their own plasma, and then the cells were centrifuged to pack them. The supernatant fluid was removed; the cells in the bottom were shaken in a new amount of normal saline and the cells were packed again. This process was repeated three times. One volume of the red cells was added to one volume of the preservative solution "AL server's solution "after a complete washing. The standard red cells were kept at 4ºC for one week.[7]

Micro-elution technique

2mm threads of the blood-stained fabrics were placed in 6x1cm glass tubes. 0.06ml of the suitably diluted antiserum was added to each tube; they were capped and incubated at 4ºC for 16hours regarding ABO groups. After the incubation, the serum was removed from each tube, and the threads were washed five times using ice-cold saline. The tubes were left to stand at 4ºC during this washing. A final wash is carried out using ice-cold 1/100 dilution of 30 % of bovine albumin in saline. This washing process was extended over 2-3hours. At each washing, the care was taken to remove a much liquid as possible as. 1 to 2 volumes of the albumin diluents was added to each tube and the elution was carried out for 10 minutes in a shaking water bath at 55-60 ºC. At the end of this elution process, the tubes were removed from the water bath, and one volume of the appropriate red cells suspended in 0.3% of albumin was added to each tube at once without removing the fabrics. A low concentration of the indicator red cells (0.5percent for ABO) was used to increase the sensitivity. After addition the cells to the tubes, they were incubated for 1-1½hours at a room temperature for ABO blood groups. We removed some of the supernatant fluid before removing the button of the cells which were placed on a slide for a reading microscopically with a gentle rolling of the slide to aggregate the cells assisting the agglutination to facilitate the reading. The degree of agglutination was determined by using a scoring scale of 0–3 for the clumps of cells wherein no agglutination represented (0) and weak agglutination "clumps of 4-10 cells " was (1) while moderate agglutination "clumps of 11-16 cells with more free cells" was (2) and severe agglutination "clumps of more 17cells with few free cells" was (3).[8]

The titration of anti-sera was carried out in the precipitin tubes where the diluents was a buffered saline (PH 7.0) while the age of the standard red cells were less than 24hours when they were used. Double dilutions were prepared by adding an equal volume of anti-sera to the diluents. The same volume of the mixture was transferred to the next volume of the diluents and so on. Anti-H, Anti-A, and Anti-B were obtained from Hoechst Laboratory, Cairo in 5 ml bottles where a titter of Anti-H was 128 while the titter of Anti-A and Anti-B were 256.[9]

It is conventional to add one volume of the red cell suspension 5% to one volume of anti-sera dilution, and hence the final titter will be twice that of the original anti-sera dilution. The titrations are allowed to stand for 2hours at a room temperature. The cell deposit is then agitated by tapping the tube sharply. The titter of the anti-sera is expressed as the reciprocal of the greatest dilution causing agglutination.[10]

Sample size

$$N > \frac{K\left(P1q1+P2q2\right)}{\Delta^2}$$

N is the member of individuals required in each group; P1 and P2 are the presumed proportions in the two groups being compared; q1=1-P1; q2=1-P2; is the minimum difference to be detected; P1is 0.99; P2 is 0.7; Confidence level (α) is 95 %; Power of the test is 80 %.[11]

Ethical considerations

This study doesn't need personal consent because it deals with anonymzed samples without any personal reference in accordance with the required applicable international laws and regulations for the research ethics.

Statistical analysis

Statistical analysis was performed using SPSS version 17. The data analysis was performed using Median test, ANOVA and Nonparametric Kruskal-Wallis test to investigate the difference among the different groups where P value of 0.05 was considered statistically significant.

Results

1- Comparison between the effects of some detergents and water on the agglutination degrees of bloodstains grouping on the different fabrics

Table 1 Shows M±SD values of the agglutination degrees of the grouping of bloodstains on cotton fabrics that were washed using some detergents then by using water only. Mean±SD values of the agglutination degree of the blood groups A, B, AB, and O in the control groups were 2.750±0.4392, 2.944±0.2323, 1.114±1.3234 and 2.750±0.4392 respectively. Mean±SD values of the agglutination degree in the washed samples using Ariel automatic were 0.056±0.3333 for the blood group (A) while they were 0.139±0.4245, 0.917±1.2507 and 0.000 ± 0.000 for the blood groups (B), (AB) and (O) respectively. Mean±SD values of the agglutination degree in the washed samples using Persil were 0.366 + 0.6685 for the blood group (A) while they were 0.472±0.8779, 2.33±1.1952 and 0.000± 0.000 for the blood groups (B), (AB) and (O) respectively. Mean±SD values of the agglutination degree in the washed samples using soap were 0.417±0.7700 for the blood group (A) while they were 0.667±1.1952, 2.611±0.9936 and 0.000±0.000 for the blood groups (B), (AB) and (O) respectively. Mean±SD values of the agglutination degree in

the washed samples using water only were 1.333±1.1212 for the blood group (A) while they were 2.111±1.0359, 0.543±1.1205 and 1.139±1.1251 for the blood groups (B), (AB) and (O) respectively. There were high statistical significant differences in the blood groups (ABO) of blood stains on cotton fabric which were washed using Ariel automatic, Persil, Soap and water only at $p < 0.001$ except for the blood group (AB) of blood stains that were washed by using water only wherein it showed non-significant differences at $p>0.05$.

Table 2 shows M+SD values of the agglutination degrees of the grouping of bloodstains on wool fabrics that were washed using some detergents then by using water only. Mean±SD values of the agglutination degree of the blood groups A, B, AB, and O in the control groups were 2.667±0.5345, 2.917±0.2803, 0.800±1.1832 and 2.833±0.3780 respectively. Mean±SD values of the agglutination degree in the washed samples using Ariel automatic were 0.028±0.1667 for the blood group (A) while they were 0.083±0.2803, 0.889±1.3475 and 0.000±0.000 for the blood groups (B), (AB) and (O) respectively. Mean±SD values of the agglutination degree in the washed samples using Persil were 0.389±0.5989 for the blood group (A) while they were 0.667 ± 0.8944, 1.917± 1.3175 and 0.000 + 0.000 for the blood groups (B), (AB) and (O) respectively. Mean±SD values of the agglutination degree in the washed samples using soap were 0.361±0.6825 for the blood group (A) while they were 0.306±0.8218, 2.556±1.0541and 0.000±0.000 for the blood groups (B), (AB) and (O) respectively. Mean + SD values of the agglutination degree in the washed samples using water only were 1.361±1.1502 for the blood group (A) while they were 2.000±1.1464, 0.514±1.0947 and 1.472±0.9996 for the blood groups (B), (AB) and (O) respectively. There were high statistical significant differences in the blood groups (ABO) of blood stains on wool fabric which were washed using Ariel automatic, Persil, Soap and water only at $p<0.001$ except for the blood group (AB) of blood stains that were washed by using water only wherein it showed non-significant differences at $p>0.05$.

Table 1 Comparison between the effects of some detergents and water on the grouping of bloodstains on cotton fabrics

Detergents groups	Ariel auto. M±SD	Persil M±SD	Soap M±SD	Water M±SD	Control M±SD
A	0.056±0.3333•	0.366±0.6685•	0.417±0.7700•	1.333±1.1212•	2.750±0.4392
B	0.139±0.4245•	0.472±0.8779•	0.667±1.1952•	2.111±1.0359•	2.944±0.2323
AB	0.917±1.2507•	2.33±1.1952•	2.611±0.9936•	0.543±1.1205▲	1.114±1.3234
O	0.000±0.000•	0.000±0.000•	0.000±0.000•	1.139±1.1251•	2.750±0.4392

Number per group was 36; M+SD is Mean+Standard Deviation

• High significant in comparison to the control group at $p<0.001$.

▲Non-significant in comparison to the control group.

Ariel auto., Ariel automatic

Table 2 Comparison between the effects of some detergents and water on the grouping of bloodstains on wool fabrics

Detergents group	Ariel auto. M±SD	Persil M±SD	Soap M±SD	Water M±SD	Control M±SD
A	0.028±0.1667•	0.389±0.598•	0.361±0.682•	1.361±1.1502•	2.667±0.5345
B	0.083±0.2803•	0.667±0.894•	0.306±0.821•	2.000±1.1464•	2.917±0.2803
AB	0.889±1.3475•	1.917±1.317•	2.556±1.054•	0.514±1.0947▲	0.800±1.1832
O	0.000±0.000•	0.000±0.000•	0.000±0.000•	1.472±0.9996•	2.833±0.3780

Number per group was 36; M+SD is Mean+Standard Deviation.

• High significant in comparison to the control group at $p<0.001$.

▲ Non-significant in comparison to the control group.

Ariel auto., Ariel automatic

Table 3 shows M±SD values of the agglutination degrees of the grouping of bloodstains on synthetic material fabrics that were washed using some detergents then by using water only. Mean±SD values of the agglutination degree of the blood groups A, B, AB, and O in the control groups were 2.639±0.5426, 2.833±0.3780, 1.057±1.4130 and 2.861±0.3507 respectively. Mean±SD values of the agglutination degree in the washed samples using Ariel automatic were 0.083 ±0.2803 for the blood group (A) while they were 0.139±0.3507, 0.722± 1.2097 and 0.000 ± 0.000 for the blood groups (B), (AB) and (O) respectively. Mean±SD values of the agglutination degree in the washed samples using Persil were 0.194 ±0.4672 for the blood group (A) while they were 0.222±0.5404, 2.306±1.1667 and 0.000±0.000 for the blood groups (B), (AB) and (O) respectively. Mean + SD values of the agglutination degree in the washed samples using soap were 0.250±0.5542 for the blood group (A) while they were 0.778±1.1979, 2.500±1.0556 and 0.000±0.000 for the blood groups (B), (AB) and (O) respectively. Mean±SD values of the agglutination degree in the washed samples using water only were 1.278±1.1113 for the blood group (A) while they were 1.667±1.3093, 0.600±1.0347 and 1.472±1.2533 for the blood groups (B), (AB) and (O) respectively. There were high statistical significant differences in the blood groups (ABO) of blood stains on synthetic material fabric which were washed using Ariel automatic, Persil, Soap and water only at $p<0.001$ except for the blood group (AB) of blood stains that were washed by using water only wherein it showed non-significant differences at $p>0.05$.

2-Comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (A) of bloodstains on the different fabrics.

Table 3 Comparison between the effects of some detergents and water on the grouping of bloodstains on synthetic material fabrics

Detergents group	Ariel auto. M±SD	Persil M±SD	Soap M±SD	Water M±SD	Control M±SD
A	0.083±0.2803•	0.194±0.4672•	0.250±0.554•	1.278±1.1113•	2.639±0.5426
B	0.139±0.3507•	0.222±0.5404•	0.778±1.197•	1.667±1.3093•	2.833±0.3780
AB	0.722±1.2097•	2.306±1.1667•	2.500±1.055•	0.600±1.034▲	1.057±1.4130
O	0.000±0.000•	0.000±0.000•	0.000±0.000•	1.472±1.2533•	2.861±0.3507

Number per group was 36; M+SD is Mean+Standard Deviation.
• High significant in comparison to the control group at p<0.001.
▲Non-significant in comparison to the control group.
Ariel auto., Ariel automatic

Figure 1 shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (A) of bloodstains on cotton fabric. 97.2% of blood group (A) of bloodstains on cotton fabric shows a negative result due to washing by using Ariel automatic while 80.5% shows a negative result because of washing by using Persil. 75% of blood group (A) of bloodstains on cotton fabric shows a negative result due to washing by using soap but 33.4% shows a negative result due to washing by using water only.

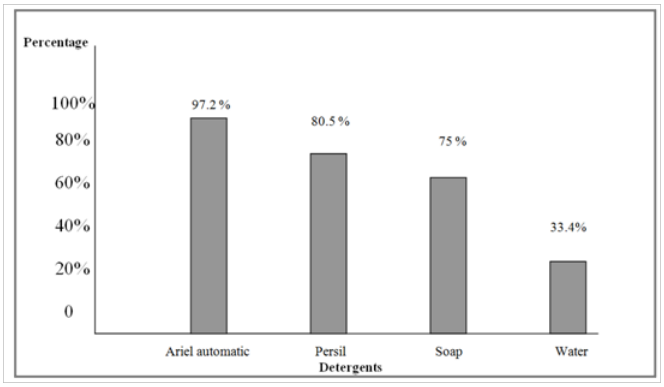

Figure 1 Shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (A) of bloodstains on cotton fabric.

Figure 2 shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (A) of bloodstains on wool fabric. 97.2% of blood group (A) of bloodstains on wool fabric shows a negative result due to washing via Ariel automatic while 63.9% shows a negative result because of washing by using Persil. 72.2% of blood group (A) of bloodstains on wool fabric shows a negative result due to washing by using soap but 36.1% shows a negative result due to washing by using water only.

Figure 3 shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (A) of bloodstains on synthetic material fabric. 91.7% of blood group (A) of bloodstains on synthetic material fabric shows a negative result due to washing via Ariel automatic while 83.3% shows a negative result because of washing by using Persil. 80.5% of blood group (A) of bloodstains on synthetic material fabric shows a negative result due to washing by using soap but 30.5% shows a negative result due to washing by using water only. 3-Comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (B) of bloodstains on the different fabrics.

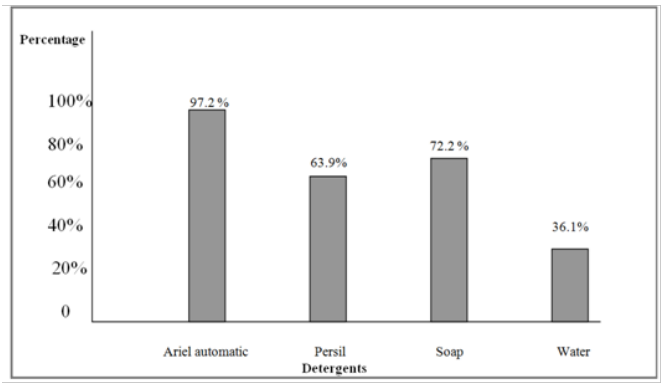

Figure 2 Shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (A) of bloodstains on wool fabric.

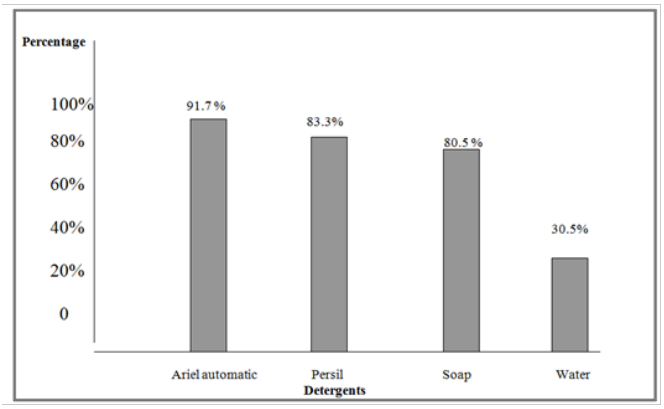

Figure 3 Shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (A) of bloodstains on synthetic material fabric.

Figure 4 shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (B) of bloodstains on cotton fabric. 88.9% of blood group (B) of bloodstains on cotton fabric shows a negative result due to washing by using Ariel automatic while 72.2% shows a negative result because of washing by using Persil. 75% of blood group (B) of bloodstains on cotton fabric shows a negative result due to washing by using soap but 11.1% shows a negative result due to washing by using water only.

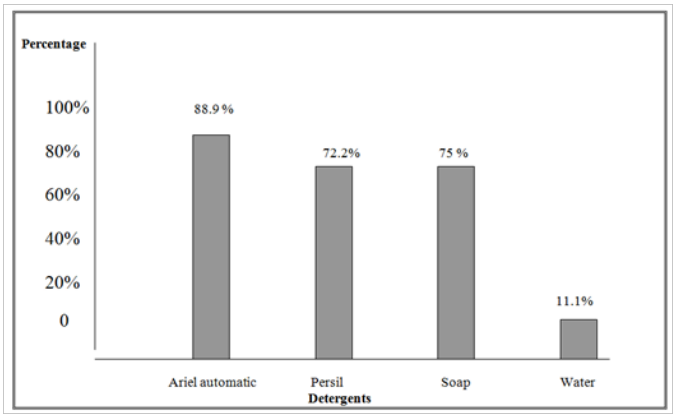

Figure 4 Shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (B) of bloodstains on cotton fabric.

Figure 5 shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (B) of bloodstains on wool fabric. 91.7% of blood group (B) of bloodstains on wool fabric shows a negative result due to washing via Ariel automatic while 52.8% shows a negative result because of washing by using Persil. 86.2% of blood group (B) of bloodstains on wool fabric shows a negative result due to washing by using soap but 16.7% shows a negative result due to washing by using water only.

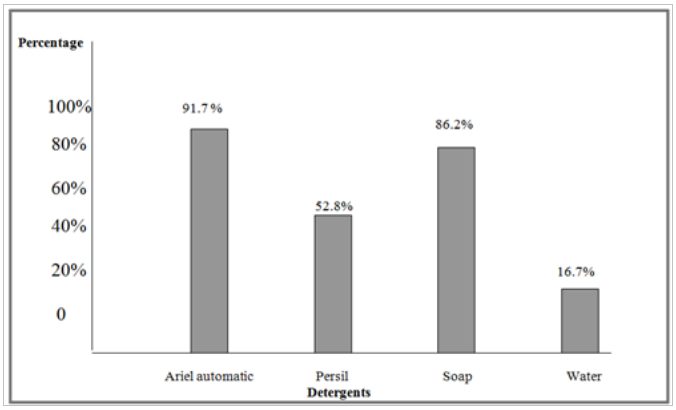

Figure 5 Shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (B) of bloodstains on wool fabric.

Figure 6 shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (B) of bloodstains on synthetic material fabric. 83.3% of blood group (B) of bloodstains on synthetic material fabric shows a negative result due to washing via Ariel automatic while 83.3% shows a negative result because of washing by using Persil. 63.9% of blood group (B) of bloodstains on synthetic material fabric shows a negative result due to washing by using soap but 30.5% shows a negative result due to washing by using water only.4- Comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (AB) of bloodstains on the different fabrics.

Figure 7 shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (AB) of bloodstains on cotton fabric. 91.6% of blood group (AB) of bloodstains on cotton fabric shows a negative result due to washing by using Ariel automatic while 58.3%shows a negative result because of washing by using Persil. 55.5% of blood group (AB) of bloodstains on cotton fabric shows a negative result due to washing by using soap but 8.3% shows a negative result due to washing by using water only.

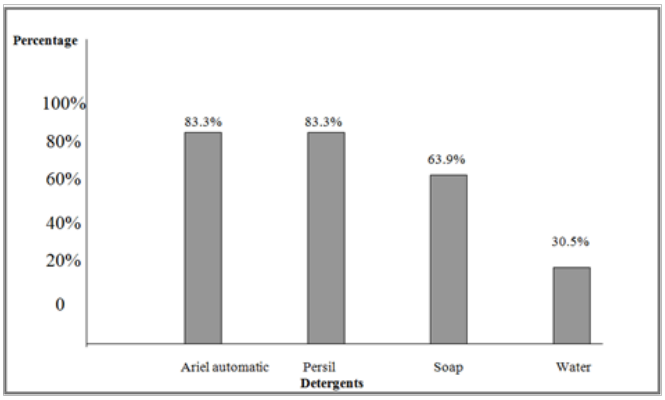

Figure 6 Shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (B) of bloodstains on synthetic material fabric.

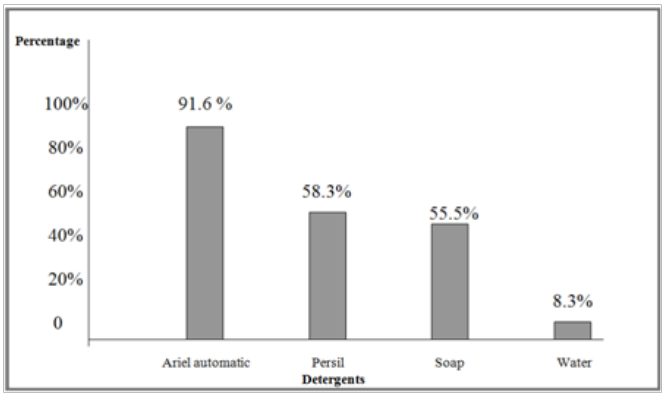

Figure 7 Shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (AB) of bloodstains on cotton fabric.

Figure 8 shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (AB) of bloodstains on wool fabric. 88.9% of blood group (AB) of bloodstains on wool fabric shows a negative result due to washing via Ariel automatic while 77.8% shows a negative result because of washing by using Persil. 61% of blood group (AB) of bloodstains on wool fabric shows a negative result due to washing by using soap but 16.6% shows a negative result due to washing by using water only.

Figure 9 shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (AB) of bloodstains on synthetic material fabric. 83.3% of blood group (AB) of bloodstains on synthetic material fabric shows a negative result due to washing via Ariel automatic while 69.4% shows a negative result because of washing by using Persil. 66.7% of blood group (AB) of bloodstains on synthetic material fabric shows a negative result due to washing by using soap but 8.3% shows a negative result due to washing by using water only. 5- Comparison between the negative results percentage after washing

by using some detergents and water only on the identification of blood group (O) of bloodstains on the different fabrics

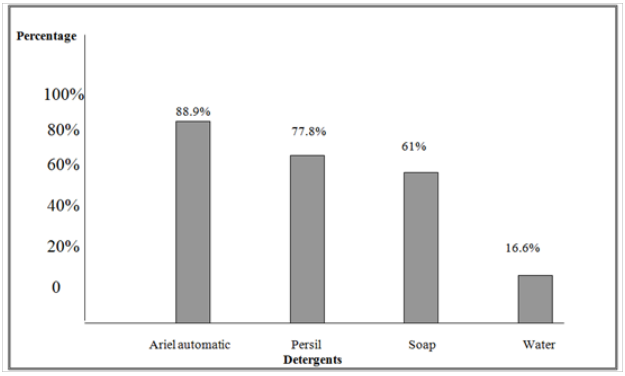

Figure 8 Shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (AB) of bloodstains on wool fabric.

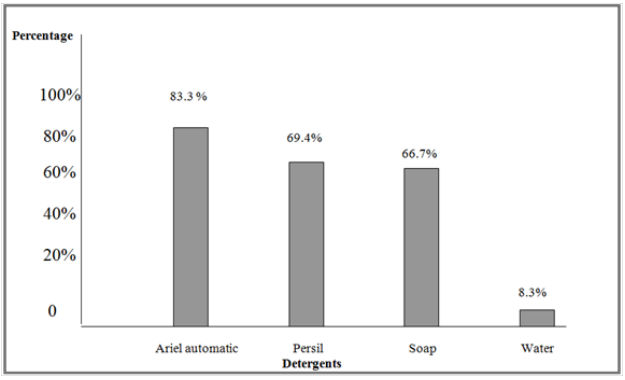

Figure 9 Shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (AB) of bloodstains on synthetic material fabric.

Figure10–12 show a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (O) of bloodstains on the different fabrics. 100% of blood group (O) of bloodstains on the different fabrics (cotton, wool, synthetic material) show negative results due to washing by using all different types of detergents (Ariel automatic, Persil, Soap). Regarding washing by using water alone, 47.3% negative result was on cotton fabric while 16.7% and 41.7 7% negative results were on wool and synthetic material fabrics respectively.

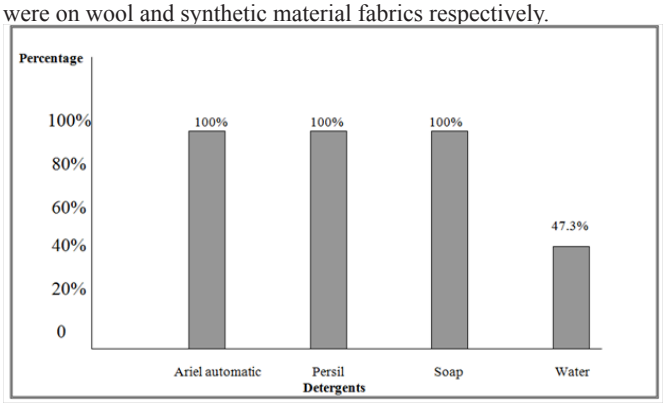

Figure 10 Shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (O) of bloodstains on cotton fabric.

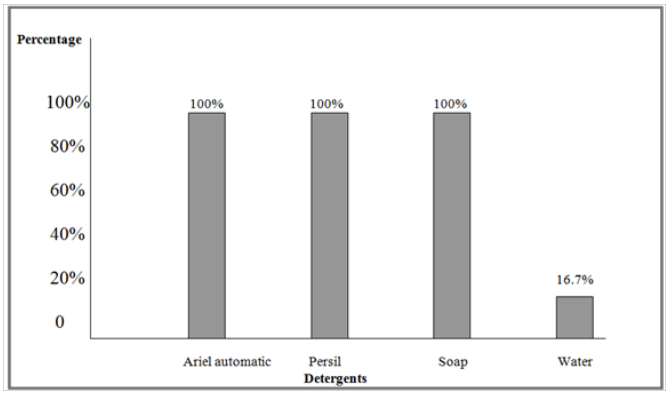

Figure 11 Shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (O) of bloodstains on wool fabric.

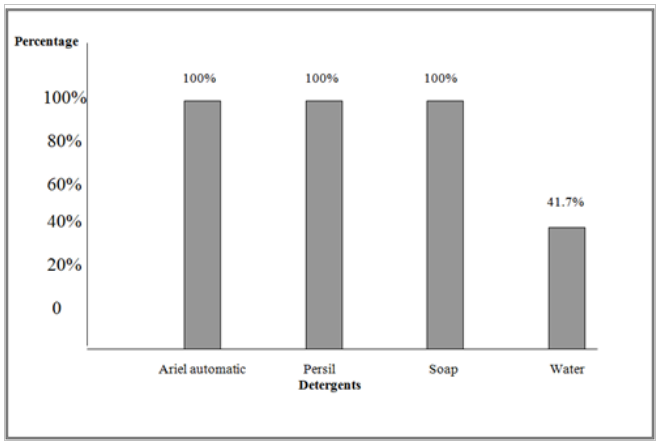

Figure 12 Shows a comparison between the negative results percentage after washing by using some detergents and water only on the identification of blood group (O) of bloodstains on synthetic material fabric.

Discussion

Bloodstains can help to connect the accused with the crime because it bears the marks of fingerprints. Blood group antigens may deteriorate in stains at the different rates because of a wide variation that is attributed to the conditions to which the stains is exposed before testing. Washing of the contaminated clothes with blood stains by water alone or detergents is one of these conditions.[12] So the purpose of this study is to assess the effect of common detergents on washing different fabrics stained by known blood on the identification of ABO blood groups of such stains wherein the ABO blood group system is the most stable blood group associated with a good discrimination power in contrast with Rh that has not stable antigens and MN grouping which has not high titter antisera beside M antigen is a precursor of N antigen, and therefore it is often difficult to confirm the N group in the dried blood.[9]

Identification of blood groups of the blood stains in the current study was carried out using a micro-elution method that depends on agglutination between antigen and antibody because this method attains a high degree of sensitivity, requires only small amounts of stained material, and allowing the stained threads to absorb the eluted antibody particularly if a low concentration of cells is used.[13] On contrary, the inhibition method has limitations wherein it is not

very sensitive and may require the larger areas of stained material per test. Therefore, it has been excluded in the favour of a micro-elution method.[8]

The present study chooses the common detergents in Egypt that have different chemicals compositions such as Ariel automatic, Persil, and Soap wherein this study proved that these detergents have a highly statistically significant negative effect on the identification of blood groups (A, B, AB, O) of bloodstains in comparison with the control groups . This is in agreement with Thomsen and Adamzik[14] who showed that the use of detergents and water to clean or obliterate the bloodstains from the fabrics leads to a low volume of blood traces resulting a small number of antigenic epitopes where the absorption-elution test depends on the agglutination reaction and the ability of indicator cells to agglutinate which requires a large number of antigenic epitopes and a high volume of blood traces.

Our results were consistent with Matsubara et al.[15] James and Edel[16] who confirmed that the different washing procedures by using various detergents cause alteration, or complete removal of the stained areas due to the dissociation of binding between antigens and antibodies where these detergents are chemicals such as polycarboxylates, phosphates, anionic surfactants, oxygen based bleaching agents; these chemicals cause alteration of pH and the nature of antigens, high ionization , and lead to prevention or dissociation of the binding between antigens and antibodies because of the intermolecular forces affection .

In the same context, Kathleen et al.[17] reported that agglutination is the end point of an antigen-antibody reaction which results from the cross linking of cells and insoluble particles by specific antibodies. So, there are many reasons which prevent the occurrence of the agglutination such as an insufficient amount of antibodies, incomplete antibodies or unsuitable temperature. In the ABO system, their agglutinating ability is a function of the antigen rather than the antibody because ABO sites are extremely plentiful and moreover protrude from the red cell surface. In addition to, Knowles[10] referred that antigens and antibodies bind through reversible non-covalent bonds that can be prevented or dissociated by high ionic strength or extreme pH where there are many intermolecular forces which are involved in antigen-antibody binding such as electrostatic bonds which results from the attraction between oppositely charged ionic groups of the side chains of two proteins according to Weir[18]

Our results were also consistent with Karger et al.[19] who showed that bloodstains which are produced by the projected droplets on the fabric at the crime scene are poor impregnation of the fabric resulting in poor blood soakage so the removal of these stains is complete via washing by using the detergents affecting the bloodstains grouping. On the contrary, Kathleen et al.[17] confirmed that agglutination which is caused by the agglutinate and agglutinins of the ABO system is tough and not easily broken down in contrast with the agglutination of the most other blood group systems which is fragile.

The present study indicated that no statistically significant variation for the identification of different types of blood groups (ABO) in the bloodstains after washing by using detergents among the different individuals belonging to different blood groups or the same blood group (the sensitivity of the cells of the same blood group to agglutination). On the contrary, Tokiwa[20] confirmed that A and B blood groups were more sensitive determined than O blood group. In the same context, Waters and Lioyed [21] referred that although all individuals of the same blood group have the same kind of agglutinate in their red cells, the sensitivity of the cells to agglutination varies from individual to individual. Moreover, there are very wide variations in the amount of agglutinin in the serum of different individuals belonging to the same blood group.

Our study showed that no statistically significant difference between the effects of the used detergents on the grouping identification of the bloodstains on the different fabrics according to Kruskal-Wallis, ANOVA and Median tests. Thus, the effect of some detergents on the grouping identification of the bloodstains does not depend on the type of fabric nor the type of detergent because all different types of detergents have the same efficacy on the blood groups identification in the bloodstains. Our results are consistent with Sheithauer and Schilling [22] who explained there is a difficulty in the detection of the blood groups in the stains due to the use of detergents because the grouping identification of bloodstains depends on the amount of blood in the stain. In contrast context, Eckert and James,[23] indicated that there is a difference in the efficacy of different types of detergents on the blood grouping of the bloodstains. In the related context, Gupta et al.[24] suggested that this difference in the efficacy of different detergents may be due to the concentration and component of detergent, and the utilized washing procedure.

The results of the current study were not affected by many factors that may lead to inaccurate results wherein some factors were neutralized such as the use of one specific blood screening test (micro- elution technique), the fixed drying time of bloodstains (24hrs.), and using the same concentration for different detergents. In the related context, Ohmori and Sato[25] showed that the use of micro-elution technique is the best for ABO blood grouping consistent with our study, while Hurley and Pex [26] referred that the drying time of bloodstains has not any effect on grouping of bloodstains after washing by using the detergents. Conversely, Cox[27] used many liable factors and reported that the grouping identification of the blood stains on the washed clothes depends on the specific blood screening test, the particular fiber composition of the fabric, and whether the detergent was used in the wash or not while Sidrov et al.[28] proved that the grouping identification of the blood stains on the washed clothes depends on the drying time of bloodstains and the concentration of detergents.

However, the present study was in agreement with El-Habashi et al.[29] wherein it also used some liable factors such as the use of different fabrics which did not affect identification of blood groups (ABO) in the bloodstains according to Kruskal-Wallis, ANOVA and Median tests whereas the level of significance was $P<0.05$. On contrary, Bevel and Gardner,[30] Arjun and Ashish[31] proved that the use of different fabrics affect the bloodstains grouping because of the different absorption of blood by the different fabrics resulting in different impregnation of the fabrics with the blood.

The current study showed that water has highly statistically significant negative effect on the identification of some blood groups of the bloodstains (A,B,O) except the blood group (AB), thus the type of the blood group (A,B,O) of the bloodstains cannot be determined after the fabric washing by water use in agreement with Stuart and William.[32] Zachová [33] showed that water is more effective than detergents in the removal of the bloodstains but it takes more time, so identification of the bloodstains grouping may be difficult after washing by water use. Moreover and consistent with our study,

Gupta et al.[24] reported that dry stains of ABO system retain their antigen city activity than the wet stains while Knowles[10] indicated that the agglutination reaction of AB blood group is stronger than the agglutination reaction of other blood groups because of the presence of both A and B agglutinogens in the red cells. Furthermore, the effect of water on the grouping identification of the bloodstains is weak in comparison to the effect of all the used detergents (Ariel automatic, Persil, Soap) because water affects the activity of antigen via increasing the humidity while the detergents affect the agglutination reaction by the alteration of pH and ionic strength of the medium according. In contrast context, Stuart and William[32] showed that the effect of water on the grouping identification of the recent stains (24h) is more effective than the detergents. The present study showed that the percentage of negative effect due to the washing of bloodstains on the different fabrics by using all detergents is 100% for the identification of the blood group (O). Tokiwa[20] explained that H agglutinate is less sensitively determined than A and B agglutinogens. Moreover, the O gene cannot produce a specific antigen because it is a silent gene in accordance with Waters and Lioyed.[21]

Conclusion

The use of detergents and water in the washing and the removal of dried bloodstains on the different fabrics affects the grouping identification of bloodstains (A , B , O) except the blood group (AB) which showed that the use of water alone for removing it, did not affect its identification on the different fabrics.

Recommendations

It is recommended to carry out further studies in the future to investigate the effect of detergents on identification of the dried bloodstains using the different concentrations of detergents, the various degrees of temperature and the different ages of the dried bloodstains, and by using the DNA profiling for identification of the dried bloodstains on the different fabrics.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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