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Effect of incubation time on blood group changes in blood stains contaminated with *Aspergillus flavus*

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ABSTRACT

Introduction: The change of blood group on the spot is when the non-O blood groups, namely A, B and AB, are found to be blood group O (false), this can occur one of them because of the long time of scattered blood (blood stains) found at the scene of the crime, so it can cause blood stains to be contaminated by mycoorganisms. One microorganism known to easily contaminate bloodstains is Aspergillus flavus. This can lead to the degradation of contaminated bloodstains because the contents in the blood can be used by microorganisms as their metabolic material. Objective : This study examines whether there is an effect of incubation time on changes in blood group in blood spots contaminated with Aspergillus flavus, this study uses blood spots of blood group A and blood group B contaminated with Aspergillus flavus. Method : This study was conducted in vitro and is an experimental study using a one-group pretest-posttest design. Blood spots contaminated with Aspergillus flavus were treated with an incubation period of 1 week, 2 weeks, 3 weeks and 4 weeks. Changes in blood group were then identified using the absorption-elution method. Results : The results of the study as many as 30 units of blood spots of blood group A and blood group B contaminated with Aspergillus flavus did not change the blood group because it can still be identified antigens that match the blood group of the insect at week 4 seen from the occurrence of agglutination. Conclusions : The conclusion that can be drawn from this study is that blood spots of blood groups A and B contaminated with Aspergillus flavus do not change blood groups during incubation times of 1 week, 2 weeks, 3 weeks and 4 weeks or 28 days.

INTRODUCTION

Bloodstains have an important role in the detection of various legal and criminal cases (Suryadi 2015).Doty et al (2016) mentioned that bloodstains at crime scenes can provide important information in criminal cases (Doty, McLaughlin, and Lednev 2016).

Identifying ABO blood groups in fresh blood samples is easier than in dried blood samples. This is because the cells in dried blood are damaged. However, it is still possible to identify blood groups from dried blood samples. This is because the antigens on the surface of red blood cells remain intact even though the cells have been destroyed (Li 2015).

Proteins from antigens on the surface of the red cell membrane can be used

by microorganisms in their metabolism. Microorganisms growing on red blood cells accelerate the degradation of these antigens. Enzymes produced by microorganisms can convert non-O blood groups (blood groups A, B and AB) into O blood groups (fake O blood groups) (Masyrur, Junitha, and Proborini 2019). This is in line with the opinion of Susilo et al. (2020), who state that the conversion of blood groups A, B and AB into fake O blood groups may be caused by the degradation of antigens on the surface of red blood cells. This process can be caused by enzymes produced by microbes that contaminate the blood.(Susilo, Junitha, and Ramona 2020)

Research by (Indah, Bastian, and Pathia 2022) shows that human dried blood changes its classification after being stored for 25 days. The results of research by Susilo (2020) showed that blood group A on iron and ceramic media started to change to wrong O on day 25, while blood groups B and AB on iron media started to change to wrong O on day 30 (Susilo et al. 2020).

Blood groups in the ABO system are classified according to the structure of the erythrocyte surface antigens. There are four (4) blood group determining molecules in the ABO system, namely D-galactose, N-acetylgalactosamine, N-acetylglucosamine and fucose.

The antigens that make up blood groups A and B, which are specific proteins in the erythrocyte membrane, are altered by the activities of microorganisms so that they are identified as the wrong blood group O due to the activity of certain enzymes produced by microorganisms. This is supported by the results of research (Kubo 1989) and (Kachuri et al. 1995) ,which identified several types of fungi that produce the enzymes alpha-nasethylgalactosaminidase and alpha-galactosidase, which are able to change the blood group. One such fungus is Aspergillus flavus.

The substrates of these enzymes are alpha-nasethylgalactosamine and alpha-galactose, which are part of the agglutinogen component of red blood cells. The activity of these enzymes will damage red blood cell antigens and cause non-O blood groups to change to false O blood groups after a period of exposure to microbes that produce these enzymes (Clausen et al, 1985).

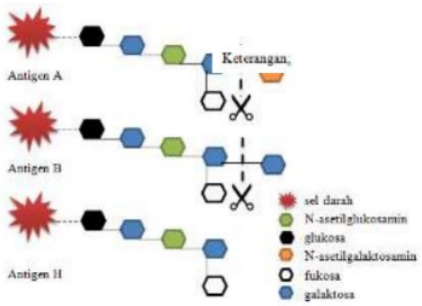

Figure1. *Overview of blood group changes.*
(Olsson and Clausen 2007)

One of the micro-organisms, fungi, plays a role in changing the nature of the dried blood scattered at the crime scene because the nutrient content of the blood is very supportive of fungal growth; one of the fungi thought to degrade antigens found on the surface of red blood cells is *Aspergillus flavus*. According to Mansyur (2019) in his research, the results of Koch's postulate test from *Aspergillus flavus* show that the Aspergillus flavus fungus has the ability to change the blood type to a false O group, the *Aspergillus flavus* fungus also has a high ability to degrade antigens of all blood groups (Masyrur et al. 2019).

With this in mind, this study was conducted to determine the effect of incubation time on ABO system blood group changes in *Aspergillus flavus*-contaminated blood stains.

MATERIALS/METHOD

This research was conducted at Poltekkes Kemenkes Mataram Laboratory from October to December 2023. This study is an experimental study using a one-group pretest-posttest design. In this case, the symptoms or effects resulting from the incubation time of blood group A and B blood spots contaminated with *Aspergillus flavus* are blood group changes with treatment of incubation period (T), namely T0: 0 weeks, T1: one week, T2: two weeks, T3: three weeks, T4: four weeks, each with 3 replicates.

The research materials were blood spots of blood groups A and B, Aspergillus flavus fungus, antisera reagents A and B, physiological solution, 2% erythrocyte suspension. Microscope, Ose, oven, micropipette, cotton stopper, test tube, centrifuge.

The first stage is the preparation of blood spots, blood groups A and B that have been identified are dripped on a glass preparation as much as 150 uL and added with Aspergillus flavus fungal culture as much as 1 ose, the second stage of preparing a 2% erythrocyte cell suspension blood groups A, B and O and covered with plastic film then stored in a refrigerator at 4 ° C until the time of blood group examination. The third stage of blood spots contaminated with Aspergillus flavus was incubated for 1 week, 2 weeks, 3 weeks and 4 weeks. After incubation, the blood spots are identified for blood group changes using the absorption elution method. The third stage of bloodstain identification using the Absorption-Elution Blood Group Test Elution Method (Kind 1960) by means of a gauze sample that has been cut into strands of yarn and then placed in a test tube labelled A, B, O and added antisera A for test tube A, antisera B for test tube B and anti-H for tube O, then soaked and placed in a refrigerator at 4°C for 24 hours. Antisera A is added to tube A, antisera B to tube B and anti-H to tube O, which are then soaked and placed in the refrigerator at 4°C for 24 hours. The next step is washing or elution. The samples are washed 7 times with 0.98% NaCl to remove impurities not bound by antibodies and antigens. Washing also removes excess antisera. The samples were then placed in an oven at 56°C for 20 minutes to remove antisera and antibody binding to the thread. The sample was then cooled to room temperature or placed in a refrigerator for 10 minutes, and the thread in the test tube was removed and discarded. The next step was to add 2-3 drops of the sample, depending on the blood group being tested, to the A, B and O red cell suspension prepared 2 days before the blood group test. The tube is then left to stand for about 10 minutes and centrifuged at 1000 rpm for 1 minute.Observations were then made by gently shaking the tube to see if agglutination had formed. Observations were made qualitatively. If agglutination occurs only in tube A, then the sample is blood group A; if agglutination occurs only in tube B, then the sample is blood group B; if agglutination occurs in tubes A and B, then the sample is blood group AB; and if agglutination occurs only in tube O, then the sample is blood group O.

RESULTS AND DISCUSSION

After determining the blood group change by the abasorbtion elution method, every week for 4 weeks. Positive results for blood group changes occur when agglutination occurs, the results of the study are shown in the table below:

Table 1 Blood group A change identification results

Treatment / perlakuan	Replikasi		
	1	2	3
T0	A +	A +	A +
T1	A +	A +	A +
T2	A +	A +	A +
T3	A +	A +	A +
T4	A +	A +	A +

Table 2 Blood group B change identification results

Treatment / perlakuan	Replikasi		
	1	2	3
T0	B +	B +	B +
T1	B +	B +	B +
T2	B +	B +	B +
T3	B +	B +	B +
T4	B +	B +	B +

Table 4.1 shows that 15 units of A contaminated with *Aspergillus flavus* still give positive results for blood group A from week 1, week 2, week 3 to week 4, this shows that there is no change from blood group A to group O because the antigen A is still detected in the A antigen is still present in the test unit in the form of blood spots contaminated with Aspergillus flavus by the illusory absorption method. And from Table 4.2 it can be seen that 15 units of blood group B samples contaminated with Aspergillus flavus still give positive results from blood group B from week 1, week 2, week 3 to week 4, this shows that there is no There is no change from blood group B to group O, because the B antigen is still detectable in the blood spot. using the illusory absorption method. Blood groups in the ABO system can undergo blood group changes if the where the intended

blood group changes are when non-O blood groups, namely A, B and AB, are detected to be are found to be O (false). In this study, blood groups A and B were contaminated with contaminated with *Aspergillus flavus* did not change blood group until week 4 or 28. The results of this study are very different from previous studies by Utami (2011) and Putri (2015), which concluded that blood groups (A, B and AB) had on day 16 when stored on ceramic media, on day 32 when stored in aluminium media, and on day 25 when
day 25 after storage in iron media and day 30 after storage in wood media. This shows that Storage in different media results in different blood group change times, The media used in this study are glass slides. The glass slides are made of borosilicate glass, which is resistant to temperature changes and chemicals. Borosilicate Borosilicate glass also has heat resistant properties and is resistant to sudden changes in temperature.
temperature changes, so glass preparations can withstand the degradation of blood group antigens.

The more antigen-antibody that binds, the more obvious the agglutination and the stronger the reaction, and the stronger the reaction that occurs. (Faruq 2015). The amount of blood used is proportional to the amount of antigen in the blood. Research carried out 8 pieces of gauze for identification using the elution-absorption method. method and there was a change in blood group on day 25 (Indah et al. 2022). In this study, the gauze was cut into 1x1 cm lengths and widths so that more than 8 pieces of gauze were used. than 8 pieces of gauze were used, resulting in more blood spots that could be absorbed and the antigens contained in the blood spots, this would increase the time it would take to identification of the blood groups.

In this study, blood spots contaminated with *Aspergillus flavus* were incubated at room temperature or around 25°C, (Kulkarni, Gosavi, and Kulkarni 2016) which shows that different temperature treatments on old dried shows that antigens A and B can still act as agglutinogens for up to as agglutinogens for up to two years, while antigen O lasts for a shorter period, but still long enough for one year. so that the same room temperature or different places where bloodstains are are incubated, blood groups can still be identified by the blood group and blood group antigens. group and blood group antigens, so that there is no change in blood group within a certain period of time. over a period of time.

In this study, blood spots contaminated with *Aspergillus flavus* still did not occur. because the time taken for the Aspergillus flavus fungus to break down the erythrocytes was still was still not optimal, so erythrocyte cells were not being degraded. antigens that matched the blood group in the experimental unit.

CONCLUSIONS

At the end of this study, it can be concluded that there were no changes in blood group in blood spots contaminated with contaminated with Aspergillus flavus for 1 week, 2 weeks, 3 weeks and 4 weeks. blood group, characterised by the detection of antigens corresponding to the blood group using the absorption-elution method.

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