Expired human blood as an alternative substituent of sheep blood for Streptococcus Sp. growth

Siti Juariah1, Darmadi1, Mega Pratiwi Irawan1, Alfin Surya1, Mulyani Puspa dewi 1, Ira Oktaviani Rz2, Isna Wardaniati2, Wahyu Margi Sidoretno2 Deswidya Hutauruk 3

1 Akademi analis Kesehatan Pekanbaru, Riau -Indonesia
2 Universitas Abdurrab Pekanbaru Riau – Indonesia
3 Universitas Evarina, Siantar Sumatera Utara - Indonesia

Abstract. Using of blood on the agar medium made to be obtained usually uses the sheep’s blood, but the sheep’s blood is difficult and high cost, so it was taken on the other alternative which could changes that sheep’s blood such as the human expire blood. The research aimed to determine affectivity of the expired blood as medium from the Streptococcus Sp. growth. The used research method was true experiment by “the post-test only control group design”. Packed red sell by NaCl 0,9 % which is centrifugated caused times and added into 100 ml of medium Blood agar Plates (BAP) and Mueller Hinton Agar (MHA) with the difference of concentrate expired blood. Outcome of the research was on the BAP medium, was the higher human expired blood concentration was resulted the narrower, whereas on the MHA medium, was the higher the resistor zone expired blood concentrate was resulted the higher. Conclusion of the research is the optimal concentrate which can is used on the blood agar medium made is 5%-8%.

1. Introduction

Blood Agar Medium is general medium which is used to isolate Streptococcus Sp. The blood using is needed to view hemolysis which is outcome by that bacterium. The bacteria species is the normal flora for the human. Some of them are pathogen and its pathogens can be seen when the bacteria gives the hemolysis zone on the blood ager.

The blood agar using is as media of the Streptococcus Sp. growth. Usually using the animal’s blood, for the best example shows the bacteria’s growth is the sheep’s blood. Sheep’s blood is determined having a little inhibitor content, so it will not make difficult the Streptococcus Sp. Growth. So the more viral in using the sheep’s blood must notice the available sheep, started from the care and 74 others. This is seen difficult enough, so from this study was taken one of the alternative way which could be used to make the blood agar without using the sheep’s blood such as by using the expired human’s blood. According Yeh and friends (2009), some laboratories generally exploited the human’s blood as the ingredient in making the agar medium, the expired blood which is form the blood bank of the hospital or laboratory donator’s blood, but according to the HIV prevalence, hepatitis and other influenced infection decease, the human’s blood using is considered risk for the laboratory working.

Expired blood is the blood which is saved and passing the saving time limit. The expired blood still shows the red blood so possibly having the nutrient for the Streptococcus Sp growth. According to Almac
and Ince (2007), as long as the blood saving, erythrocytes will have the form change from bikonkaf becoming ecosystem and finally becoming spherophyte.

The erythrocyte from changing is predicted able to increase the Streptococcus SP lysis power through the expired human’s blood. The complete conglutination profile from the blood with citrate phosphate dextrose (CPD) which is saved for twenty one days on four degrees Celsius shows that the activity reduction of factor V and VIII (Hondow and friends, 1982). Reduction of the blood frosting will give the effect to reduce the expired blood Streptococcus Sp growth cannot be used as the medium making alternative for blood agar.

The study was made by Magbojos and friends (2011), proved that the expired human’s blood could still be used to grow the positive kokus gram. According to Clinical and Laboratory Standard Institute (CLSI) (2013), the blood addition on the Agar medium such as Mueller Hinton Agar (MHA) was as many as 5% and according to Oxoid, the blood addition on the blood Agar medium was as many as 5% - 10%.

Agar medium by adding the blood as many as 5% was general medium which is routinely used at the clinic microbiology Laboratory to identify the pathogen bacteria (Murray and friends. 2017). The blood addition as many as 5% certainly had the enough nutrient for the Streptococcus SP growth. But it still showed that the sheep’s blood addition was better, said by Egwuatu and Friends (2014), that ram’s blood using was better to show the colony growth than rabbit’s and chicken’s blood using. Related at it, it was needed the study between both sheep’s blood using of 5% and human’s blood with the different concentration to consider the optimization of the expired blood.

2. Method

The study was operated on November of 2016 until January of 2017 at the Microbiology Laboratory of Academy of the medical analysis of Pekanbaru. The equipment which was used on the study was the expired transfusion blood and S. pyogenes bacteria strain.

Sample Management

As much as 20 ml of the expired blood (packed red cells) which was found from the blood bank washed by NaCl 0,9%. Centrifuged blood as many as twice (Djannatun and friends, 2010) the sample management was operated centrifuged as long as five minutes by 3000 Rpbs. Then, formed plasma was thrown and remaining packed red cells was added NaCl 0,9%, and then centrifuged was back with the same time and speed.

Blood agar medium making

Blood Agar medium was made as much as 700 mLs by dissolving 28 grams of Blood Agar Base (Oxoid) into 700mL of Aquades (Oxoid 2016). Then sterilized by using autoclave with temperature 1210C as long as 15 minutes. The sterilized medium then was added the defibrinated sheep’s blood as much as 5% and washed human’s blood as much as 5%, 5%, 7%, 8%, 9% and 10%. As much as 100mL of the liquid Blood Agar Plate medium was added each 5 mLs, 6 mLs, 7 mLs, 8 mLs, 9 mLs, and 10 mLs of the expired blood, then homogenized and poured on the petri dish.

Inoculation on the blood Agar medium

Hemolytic Strain Streptococcus β (Streptococcus pyogenes) was made suspension with 5 mL. NaCl 0,9% then inoculated on the sheep’s blood agar medium of 5% defibrination as the control and expired human’s blood agar of 5%, 6%, 7%, 8%, 9%, 10% which were washed quadrant scratch technique. According to Ibrahim and friends (2015), technique in operating as quadrant inoculation was by dividing the petri dish into four quadrants such as quadrant one, quadrant two, quadrant three, quadrant four then operated the scratch as zig zag continuing from the quadrant one until quadrant four. The inoculated medium then was incubated by temperature of 370 C as long as 24 hours.
Inoculation on the Mueller Hinton Agar medium

Hemolytic Strain Streptococcus β (Streptococcus pyogenes) was made suspension with 5 mL NaCl 0.9%. Them inoculated on the sheep’s blood agar medium of 5% defibrination as the control and MHA medium and expired human’s blood of 5%, 6%, 7%, 8%, 9%, 10% which were flatten scratch technique. Warbung and friends (2013) added the sterilized cotton fiber into the bacteria suspension until wet and then pressed with using that cotton fiber on the inside tube wall and scratched flattening on the MHA medium.

Bacitracyn Test

Bacitracyn Disk of 10 units was clamped by the sterilized tweezer and patched on the MHA medium edge and incubated on the temperature 370 C as long as 24 hours.

Hemolysis zone observation

Hemolysis zone observation was quantitatively done by establishing the score on the each sample treatments, (- -) so is not formed hemolysis around the bactery colony (+2) hemolysis zone which was formed well enough and (+3) the formed hemolysis zone was well enough according to Cafiso and friends (2012), the score determination on the hemolysis delta zone forming which was resulted by S aureus was by giving scale 0 – 3, 0 ( --) so there is no formed hemolysis zone, 1 (+) showed the hemolysis production was a little 2 (++) or 3 (+++) showed enough or the highness of the hemolysis activity for each.

The inhibition zone measurement on the bacitracyn test

The bacitracyn test was said postitive when formed the inhibition zone around the bacitracyn disc then the inhibition zone diameter which was formed measured by using the ruler and stated in mm.

3. Experimental Result

Inoculation on the blood agar medium

The cultivation on the BAP medium was used to consider the hemolysis from one bacteria one of the β hemolytic resulter bacteria around the colony growth was S. Pyogenes. The growth observation of S. Pyogenes on the BAP medium with the expired blood with concentration of 5% - 8% showed the hemolysis outcome on the value (+2) was good where as on the concentration of 9% - 10% on the value 10% on the value (+1) was unwell. The bacteria colony growth on the limpid zone around that colony could still be seen clearly with eyes on the expired blood concentration of 5% - 8%, where as on the concentration of 9% - 10% the bacteria colocy growth could still be seen but the limpid zone around colony was difficult to see with eyes. S pyogenes’s growth on the BAP medium of the sheep’s blood of 5% was found the colony growth with formin circle, grey with size <1 mm and hemolysis outcome with value (+3) was very good. The growth outcome on the blood agar medium with the expired blood can be seen on the table 1, and the growth outcome on the blood agar medium with the sheep’s blood can be seen on the table 2.

Inoculation on the Mueller Hinton Agar medium

The S. Pyogenes growth on the MHA medium with the concentration expired blood of 5% - 10% showed the positive bacitracyn test outcome with the diameter of the inhibition zone of 30 – 35 mm, the higher the expired blood concentration the inhibition zone which was resulted by S. Pyogenes was the bigger. The S. Pyogenes growth on the MHA medium with the sheep’s blood of 5% showed the positive bacitracyn test outcome with the inhibition zone diametre of 30 mm. The growth on the mueller Hinton Agar medium with the expired blood can be seen on the table 3, and the growth outcome on the Mueller Hinton Agar medium with the sheep’s blood can be seen on the table 4.
### Table 1. S. Pyogenes growth outcome on the BAP medium with the expired blood

| Treatment | prevention | Colony Morphology | Hemolysis |
|-----------|------------|-------------------|-----------|
| 5%        | I          | Forming round, grey with the colony size <1 mm. | (+2)      |
|           | II         | Forming round, grey with the colony size <1 mm. | (+2)      |
|           | III        | Forming round, grey with the colony size <1 mm. | (+2)      |
| 6%        | II         | Forming round, grey with the colony size <1 mm. | (+2)      |
|           | III        | Forming round, grey with the colony size <1 mm. | (+2)      |
|           | I          | Forming round, grey with the colony size <1 mm. | (+2)      |
| 7%        | II         | Forming round, grey with the colony size <1 mm. | (+2)      |
|           | III        | Forming round, grey with the colony size <1 mm. | (+2)      |
| 8%        | II         | Forming round, grey with the colony size <1 mm. | (+2)      |
|           | III        | Forming round, grey with the colony size <1 mm. | (+2)      |
|           | I          | Forming round, grey with the colony size <1 mm. | (+1)      |
| 9%        | II         | Forming round, grey with the colony size <1 mm. | (+1)      |
|           | III        | Forming round, grey with the colony size <1 mm. | (+1)      |
|           | I          | Forming round, grey with the colony size <1 mm. | (+1)      |
| 10%       | II         | Forming round, grey with the colony size <1 mm. | (+1)      |
|           | III        | Forming round, grey with the colony size <1 mm. | (+1)      |

Note: (-) = not hemolysis, (+1) = unwell hemolysis; (+2) = well hemolysis, (+3) = very well hemolysis.

### Table 2. S. pyogenes growth outcome on the BAP medium with the sheep’s blood 5%

| Treatment | prevention | Colony Morphology | Hemolysis |
|-----------|------------|-------------------|-----------|
| 5%        | I          | Forming round, grey with the colony size <1 mm. | (+3)      |
|           | II         | Forming round, grey with the colony size <1 mm. | (+3)      |
|           | III        | Forming round, grey with the colony size <1 mm. | (+3)      |

Note: (-) = not hemolysis (+1) = unwell hemolysis; (+2) = well hemolysis, (+3) = very well hemolysis.

### Table 3. S. pyogenes growth outcome on the MHA medium with the expired blood

| Treatment | prevention | Colony Morphology | BS Test | Zone (mm) |
|-----------|------------|-------------------|---------|-----------|
| 5%        | I          | Forming round, grey with the colony size <1 mm. | (+)     | 30        |
|           | II         | Forming round, grey with the colony size <1 mm. | (+)     | 30        |
|           | III        | Forming round, grey with the colony size <1 mm. | (+)     | 30        |
| 6%        | II         | Forming round, grey with the colony size <1 mm. | (+)     | 35        |
|           | III        | Forming round, grey with the colony size <1 mm. | (+)     | 35        |
|           | I          | Forming round, grey with the colony size <1 mm. | (+)     | 30        |
| 7%        | II         | Forming round, grey with the colony size <1 mm. | (+)     | 34        |
|           | III        | Forming round, grey with the colony size <1 mm. | (+)     | 30        |
| 8%        | I          | Forming round, grey with the colony size <1 mm. | (+)     | 35        |
|           | II         | Forming round, grey with the colony size <1 mm. | (+)     | 35        |
|           | III        | Forming round, grey with the colony size <1 mm. | (+)     | 34        |
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Note: BS Test = Basitrasis

Table 4. S. pyogenes growth outcome on the MHA medium with the sheep’s blood of 5%

| Treatment | prevention | Colony Morphology | Hemolysis | BS Test | Zone (mm) |
|-----------|------------|-------------------|-----------|---------|-----------|
| 5%        | I          | Forming round, grey with the colony size <1 mm | (+3)      | +       | 30        |
|           | II         | Forming round, grey with the colony size <1 mm | (+3)      | +       | 30        |
|           | III        | Forming round, grey with the colony size <1 mm | (+3)      | +       | 30        |

Note: BS Test = Basitrasis.

The study was operated by using the expired blood > 30 days which passed the expire limit as long as three days with the blood type of O⁺, there are six sample treatments which was used such as the expired blood with the concentration 5%, 6%, 7%, 8%, 9% nad 10% which was added to each 100 mL of BAP and MHA. The defibrination sheep’s blood with the concentration of 5% as the control was added to each 100 mL of BAP and MHA> Streptococcus pyogenes is hemolytic β which is used as the expired blood effectivity considerer.

The cultivation on the BAP medium with concentration of 5%, 6%, 7%, 8%, 9% and 10% was to view the hemolysis zone which was formed around the S. pyogenes colony, then compared by the hemolysis zone on the BAP medium with the concentration sheep’s blood of 5%. The S pyogenes cultivation on the BAP medium with the expired blood of 5% - 8% resulted the limpid zone which was narrower but could be clearly seen, it is different to the limpid zone which was resulted on the BAP medium with the expired blood of 9% - 10% which was not almost seen with eyes, but it could be seen by the sunny light.

The hemolysis outcome difference on six expired blood concentrations could be caused by the blood concentration in 100 mL of the Agar medium. The cultivation outcome on the BAP medium with the sheep’s blood of 5% showed the bigger hemolysis zone on the expired BAP medium of 5%,8%. According to Brooks (2013). Streptolysis O which was resulted by S. Syogenes had a role in the hemolysis zone forming on the blood, but when streptolysis O belted as quantitative with the anti streptolysis O (ASO) which was one antibody for the human which appeared after Streptococcus infection of the Streptolysin O producer, so Antibody will inhibit the hemolysis forming for the blood. Besides that, according to Yeh and friends (2009), the morphology and erythrocyte functional changing related to the bacteria hemolysis zone growth and forming between expired blood and fresh blood. Yeh and friends (2009), also stated that the sheep’s erythrocyte had the smaller size than the human’s erythrocyte.

Based on the state, difference of the erythrocyte size and ASO is predicted to be one of the causer of the hemolysis zone different. Predicted that the erythrocyte with the smaller size is easier to illustrate o9n the bacteria and then the more concentration of the expired blood so the hemolysis forming inhibiton by ASO is the bigger. The S.pyogenes growth outcome on the BAP medium with the expired blood and BAP medium with the sheep’s blood of 5% can be seen on the Appendix III.

Cultivation on the MHA medium with the concentration expired blood of 5%, 6%, 7%, 8%, 8% and 10% aimed to view the outcome and test of the S. pyogene bacitracin and inhabitation zone with the concentration sheep’s blood of 5%. The cultivation outcome on the MHA medium of concentration…
expired blood of 5% - 7% showed the positive bacitracin test with inhibition zone diameter of 30 – 35 mm, on the expired blood concentration of 8% - 10% showed the positive bacitracin test with the inhibition zone diameter of 34 – 35 mm. the cultivation outcome on the MHA medium with the sheep’s blood of 4% was found the positive bacitracin test outcome and inhibition zone diameter of 30 mm which was although same with the inhibition zone on the MAH medium and expired blood of 5% - 7%.

The inhibition zone diameter increasing on the higher expired blood concentration then the inhibition zone diameter difference on the MHA medium with the expired blood and sheep’s blood of 5%, can be concluded by the antibody, antibiotic or citrate phosphate dextrose (CPD) on the expired blood. According to Russel and friends (2006), existence of the citrate acid anticoagulant, antibiotic, antibody and other anti-infection agent can inhibit the bacteria growth. The S pyogenes growth outcome on the MHA medium with the expired blood and MHA medium with the sheep’s blood of 5% can be seen on the appendix IV.

4. Conclusion
From the study outcome which is operated then it can be concluded this following:

a. Concentration expired blood of 5% - 8% on the BAP medium shows the well hemolysis zone outcome, whereas on the expired blood concentration of 8%- 10% on the BAP medium shows the unwell hemolysis zone outcome.

b. The concentration expired blood of 5% - 8% on the BAP medium shows the well hemolysis, whereas on the expired blood concentration of 9% - 10% on the BAP medium shows the unwell hemolysis zone outcome.

c. The concentration expired blood of 5% - 10% on the MHA medium shows the positive bacitracin test outcome with diameter of 35 mm.

d. Optimal concentration which can be used the expired blood increasing in making blood agar medium (BAP is 5% - 8%.

For increasing the science especially about clinic bacteriology, it can be suggested that it can be operated the study through the benefit in using the expired blood in the microbiology. Hopefully, no limit benefit as the sheep’s blood alternate of 5% in making the Agar medium.

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