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Washed Erythrocyte (WE) Bag Bacteria *Brevundimonas vesicularis* Identification at the Blood Donor Unit PMI DKI Jakarta

Widia Rahmatullah[1], Rina Oktaviyanti[2], Resmi Aini[1], Rudina Azimata R.[1], Reska Handayani[3]
[1]Yogyakarta's Bhakti Setya Indonesia Health Polytechnic, Indonesia.
[2]Indonesian Red Cross DKI Jakarta, Indonesia.
[3]Nursing Faculty Psychology and Health Department, Universitas Negeri Padang, Indonesia.

Corresponding author*
widiarahmatullah@poltekkes-bsi.ac.id

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Abstract

A Packed Red Cell (PRC) component known as a washed erythrocyte (WE) has undergone two to three washings with sterile physiological saline to get rid of 99% of the plasma protein, antibodies, and some leukocytes. The goal of this study was to use Bact/alert media to detect the presence or absence of bacteria (anaerobes or aerobes) in WE blood components because there is a chance that germs from the environment could contaminate WE processing because it is still an open system and the manufacturing is still exposed to the elements. Blood samples come from WE blood products that have undergone quality checks for bacterial contamination in a lab dedicated to product quality. Checking for bacterial contamination in the blood involves utilizing the Bact / Alert tool to examine bacterial contamination. Blood product aerobic (BPA) and blood product anaerobic (BPN) media are used in the culture of blood product sample on Bact/alert. Aside from 1 (2.44%) component sample of WE blood that was identified as bacteria in BPA media, the results of the 41 samples examined in 2020 showed that there was aerobic bacterial contamination in BPA media but no anaerobic bacterial contamination in BPN media. This was confirmed by the examination of samples on BPN media, which yielded negative results in 41 samples (100%) and positive results from none of the 41 samples. The results of identification at the Microbiology Laboratory at the University of Indonesia showed that the aerobic bacteria that contaminated washed erythrocyte (WE) blood products on BPA microbiology media were *Brevundimonas vesicularis.*

Keywords: BACT/Allert 3D; Washed Erythrocyte Blood Components; Bacterial Contamination.

INTRODUCTION

Currently, blood transfusion therapy plays a significant role in medicine, both for life-saving emergency treatment and for common conditions that require ongoing transfusion. For instance, according to (Herlinah, 2016), packed red cell (PRC) components are most frequently requested in blood service units. In 2013, from August to November, Wahidin Hospital in Makassar saw a demand for PRC blood transfusions of 1392, or 36.4%, compared to 2.2% for TC and 0.1% for plasma. PRC transfusion therapy is the cornerstone of treatment for individuals with thalassemia, aplastic anemia, and haematological malignancies. Blood transfusions can also result in reactions, ranging from minor symptoms like chills or a mild fever to moderate symptoms like shortness of breath, fever, and allergic reactions, as well as severe symptoms like shock, severe hypotension, or severe shortness of breath. The likelihood of transfusion responses increases with frequency of blood transfusions (Akbar *et al.,* 2014).

WE blood products, specifically PRC components, which have been washed two to three times with sterile physiological saline to eliminate 99% of plasma proteins, antibodies, and toxins, are one strategy to reduce the risk of transfusion reactions. Some leukocytes Because it only contains a minimal amount of leukocytes and plasma, the type of washed erythrocyte (WE) component is a modified kind of PRC blood component that is thought to be capable of minimizing responses associated with blood transfusion. Because the processing of WE components is still done in an open system, or more specifically, because the manufacturing is still exposed to an open environment, it is done in an airflow laminator. Although this process is referred to as a sterile open system, there is a chance that it will become contaminated with bacteria from the environment because the WE manufacturing already comes into contact with open spaces (Ministry of Health of the Republic of Indonesia). The development of germs in blood components raises questions regarding a transfusion-related response, a According to a Chinese study (He *et al.,* 2018), 44 out of 28,711 TC bags contained bacterial contamination that can lead to transfusion reactions.

Bacterial contamination cases have a higher risk of Transfusion Transmitted Infections (TBI) than viral infections(Haass et al., 2019). In addition, bacterial contamination is the second leading cause of death due to the risk of bacterial sepsis from transfusion. The source of bacterial contamination in TC products can come from the donor's skin surface when the needle enters the blood vessel (Rahmatullah et al., 2024 *cit* He et al., 2018). This is related to previous research showing that 196 blood products were known to be contaminated with Gram-positive and Gram-negative bacteria. The results of the identification showed that more than 50% of the bacteria detected in TC blood products were gram-positive bacteria, while gram-negative bacterial contamination was usually less, but if gram-negative bacterial contamination occurs, there is a risk of transfusion infection to death (Rahmatullah et al., 2024 *cit* Agzie et al., 2019). Asymptomatic, low-grade fever, acute sepsis, hypotension, and even death are possible clinical manifestations brought on by bacterial infection(Astuti & Ayu Maharani, 2014). Bacteria are extremely small organisms that are invisible to the unaided eye. Both inside and outside of the human body, bacteria can exist in a number of conditions.

The term "bakteri aerob" refers to bacteria that require oxygen to function in order to provide energy. Examples of such bacteria include Nitrosococcus and Nitrobacter. Streptococcus, Aerobacter aerogenes, Escherichia coli, lactobacillus, and Alcaligenesis are examples of anaerobic bacteria that do not require oxygen to function (European Committee (Partial Agreement) on Blood Transfusion (CD-P-TS), 2020). According to research conducted in California (Horth et al., 2017), around 9.2% of 196 different types of dried fruit were found to contain bacteria. The results of the identification show that the product for making darah simpan contains the bacteria Staphylococcus aureus, Bacillus sp., Pseudomonas, and Streptococcus pneumonia. Transfusion reactions in therapeutic patients are frequently brought on by bacterial contamination of red blood cell products. Bacterial transfusion responses are frequently swift and violent (Amalia Wahidiyat & Basant Adnani, 2016). For instance Transfusion of red blood cells results in sepsis due to bacterial infection. During blood transfusions, patients frequently develop a high fever (high temperature of 40–43 degrees Celsius) and chills. It is essential to check for bacterial contamination as soon as blood is collected in order to avoid responses brought on by bacteria. To screen for bacterial contamination in the blood, utilize the Bact/Alert instrument. Blood product samples are cultured on bacteria and alert. blood product anaerobic (BPN) and aerobic (BPA) medium, with signs seen from BPN and BPA media that changed color from gray (negative result, no bacteria) and yellow (there were no bacteria). discovered bacterium). Based on the detection of color changes at the bottom of the BPA/BPN tube, the examination's results are displayed on the monitor screen with either positive or negative information about bacterial contamination (He et al., 2018).

WE component production is processed in an air flow laminator because the processing is still in an open system, namely the production is still exposed to the open environment. This processing is called a sterile open system, but because WE production already has contact with an open space, there is a possibility of bacterial contamination from the surrounding environment that affects bacterial growth. This study aims to determine whether or not there is bacterial contamination (anaerobic and aerobic) in WE (Washed Erythrocyte) blood components with Bact/Alert 3D media

MATERIALS AND METHODS

Descriptive research techniques with a quantitative approach were used to conduct this kind of study. This study was carried out at PMI DKI Jakarta Province's Blood Donor Unit (UDD). The Washed Erythrocyte (WE) blood components used in this study were examined for bacterial contamination at the Blood Donor Unit (UDD) PMI DKI Jakarta Province. The goal of this study is to identify the aerobic and anaerobic bacteria found on the Bact/Alert 3D device at PMI DKI Jakarta Province's Blood Donor Unit (UDD). Bacterial Identification The Bact/alert tool is an automatic microbial detection system based on BPN (Anaerobic Blood Product) and BPA (Aerobic Blood Product) media which are incubated to produce a bacterial growth graph if there is a positive sign on the monitor screen. BPN media is used to detect anaerobic bacteria while BPA media is used to detect bacteria in WE products.

At the PMI Blood Donor Unit (UDD) DKI Jakarta Province in 2020, a sample of WE blood component was analyzed for bacterial quality based on the detection of aerobic and anaerobic bacteria on the Bact/Allert 3D instrument. Random sampling was used as a sample approach, and component quality tests' secondary data were used as the basis. WE as many as 41 sample data in the UDD PMI DKI Jakarta quality test laboratory. Using the Excel application, the sample data were calculated to determine the average amount of bacteria found from the total samples of WE components found to contain bacteria.

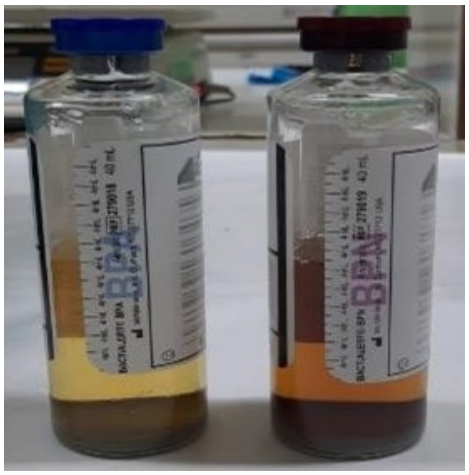

Figure 1. BPA and BPN microbiology media.

RESULTS AND DISCUSSION

The quality control data (QC) of washed erythrocytes (WE) in the blood product quality testing laboratory were used to generate the processed sample data. A sample of the WE component that has been washed with NaCl a couple of times makes up the sample data. The collected information consists of inspection sample data from January to December 2020. The year 2020 saw the collection of 41 samples of data. Taking samples and flowing them into the Bact/alert 3D media, specifically the Blood Product Anaerobic (BPN) media, which will identify the type of anaerobic bacteria, and the Aerobic Blood Product (BPA), which will identify aerobic bacteria, is how the anaerobic and aerobic bacteria in the WE component are identified. The BPN or BPA media provide the result indicator. Gray (negative results, no bacteria) and yellow (positive results, bacteria present) are the two colors of the BPN or BPA media that change color as a result of the test (identified bacteria).

Figure 2. Bact/Alert 3D Culture Tool.

In table 1. the results of the examination of the identification of 41 samples of WE components using the Bact/alert 3D tool with BPN media and BPA media are presented during 2020. Identification of bacteria using the Bact/3D alert tool with BPN and BPA media.

Table 1. The percentage of bacteria that were identified

	BPN Media		**BPA Media**		**Bacterial QC**	
	Negative	Positive	Negative	Positive	Graduated	Not pass
	41	0	40	1	40	1
Percentase (%)	100	0	97,56	2,44	97,6	2,44

Information:
Positive = contaminated with bacteria
Negative = not contaminated with bacteria

The table above demonstrates that the BACT/Allert device was used to evaluate 41 samples of WE blood components for bacterial contamination. Two different media were employed, namely BPN media to identify anaerobic bacteria and BPA media to identify aerobic bacteria in the sample. Table 1's findings reveal that all 41 samples contained in the BPN media had negative results, indicating that no anaerobic bacteria were found in the sample. The outcomes of the BPA media identification are likewise displayed in Table 1. According to the statistics, 1 (2.44%) of the 41 samples evaluated, which were WE blood components, were found to contain bacteria in BPA medium. These findings suggest that the WE component contained aerobic bacterial contamination. Aerobic bacteria can multiply and need unrestricted oxygen to function. Achmad Tjiptoprajitno & Ketut Sudiana, 2012 study discovered that 8 samples out of 120 donor samples were infected with aerobic microorganisms.

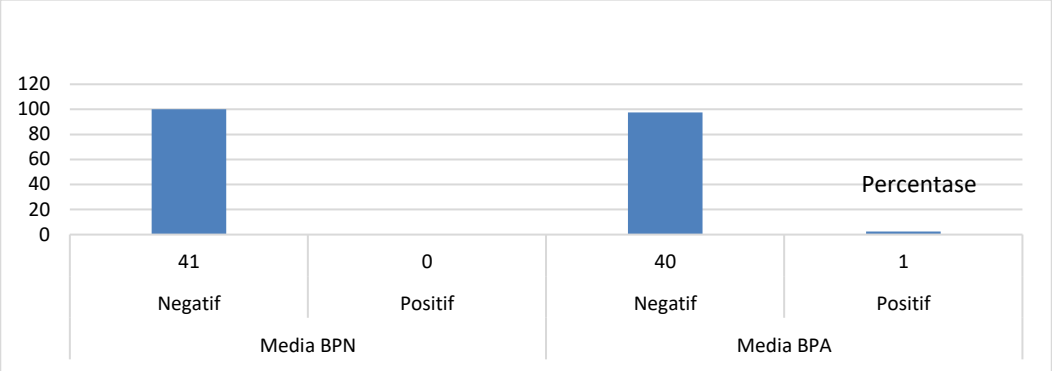

Figure 3. Percentage of bacterial contamination in BPN and BPA microbiological media.

By dividing the total number of samples that were identified by the total number of examination samples, a percentage representing the distribution of the WE component sample data for which the bacteria were identified was calculated. A total of 41 samples were collected between January and December 2020. These samples were each tested using BPN and BPA media, with either negative or positive outcomes. According to the statistics, 41 samples were found to be negative during the evaluation of samples on BPN medium, and 41 samples yielded no positive results. 41 samples were tested using BPA media, with up to 40 samples yielding negative results and just one sample yielding positive results. The data showed that 100% of the samples from BPN medium did not identify bacteria, indicating that there was no chance of anaerobic bacterial contamination, while in BPA media, just 1 sample (2.44%) had positive results for bacteria identification and 40 samples (97.56%) did not. This indicates that one sample of WE blood components included aerobic microorganisms. After a third party, specifically the bacteriology lab at the University of Indonesia, completed the bacterial culture process, it was discovered that the type of bacterium was *Brevundimonas vesicularis*.

There are 41 samples of WE component data in the research findings that have been processed. BPN and BPA culture media were used for the study of bacteria. The presence of bacteria might grow and leave endotoxin, which can produce clinical symptoms in recipients, according to (Astuti & Ayu Maharani, 2014). One BPA media sample yielded a favorable outcome. This suggests that an element of the WE contains aerobic microorganisms. One aerobic cocci bacterium and no anaerobic bacteria were detected in the study by (Astuti & Ayu Maharani (2014). The University of Indonesia's bacterial microbiology lab conducted additional testing, and it was discovered that the germs that tainted WE's blood were *Brevundimonas vesicularis* bacteria. Gram negative *Brevundimonas vesicularis* bacteria are free-living, bacilli-shaped, yellow-pigmented on blood agar, and found in the environment in water, dirt, and dust. Although these bacteria are also discovered in clinical patient samples and are known to cause serious sickness, their presence in the environment plays a part in maintaining the ecosystem's equilibrium (Resmi et al., 2010). The illnesses brought on can range from abscesses to sepsis or meningitis. Infections brought on by the bacteria *Brevundimonas vesicularis* are made worse by the extent of antibiotic resistance (Ryan & Pembroke, 2018). This bacterium's high rate of survival in an unfavorable environment is another intriguing feature. It is known that several species of *Brevundimonas vesicularis* can grow on Despite having a negative effect that causes the size of the cells to decrease, medium with few nutrients.

Brevundimonas sp. are not currently considered major pathogens. However, this should be re-evaluated in light of our investigations, where forty-nine examples of Brevundimonas spp. infections have been found in the literature. These species have characteristics, such as the ability to pass through sterilising filters, which may allow them to cause potentially harmful infections and even death on occasion. Although it is of low virulence and not as big a risk as other non-fermenting Gram-negative bacteria such as Burkholderia etc., it should not be over looked as a possible cause of nosocomial infections and should be considered for inclusion in hospital screening and prevention programs. These programs should consider investigation of possible Brevundimonas spp outbreaks if these bacteria are clinically isolated in more than one patient (Ryan & Pembroke, 2018).

Brevundimonas vesicularis or also called *Pseudomonas vesicularis* is a gram-negative, aerobic, non-spore and non-fermented bacteria. *Brevundimonas vesicularis* bacteria in several studies are human pathogenic bacteria. *Brevundimonas vesicularis* bacteria attack humans who are not immunocompetent and cause sufferers to experience Bacteraemia, Pneumonia, Botryomycosis, Progressive leukocytosis, Liver Abscess, and Febrile neutropenia (Nurfitriani Siska, 2018 *cit* Ryan and Pembroke, 2018).

The outcomes of these investigations are contrasted with those of prior research projects. Finding research on bacteria in WE blood components is challenging; studies on bacterial contamination have been conducted, but they

have used various blood components. The Thrombocyte Concentrate (TC) component was discovered to be contaminated with bacteria in as many as three bags of the 60 TC bags studied at UDD Surabaya, according to research by Tjiptoprajitno Achmad & Ketut Sudiana (2012). The study indicated that commensal bacteria were the most common type of bacteria found. Furthermore, no contaminating microorganisms were discovered in the TC products analyzed at UDD Yogyakarta according to the investigation of (Btari Christiyani Kusumaningrum & Sepvianti, 2020). Although there are discrepancies across the research that have been compared, Although the results of earlier research have been compared, the analysis of the origin of the contamination process has remained consistent. According to a review of earlier studies, the contamination process might happen during phlebotomy and blood processing and could not be distinguished from the likelihood of a bacteremic donor.

This study lists a number of causes for bacterial contamination, including when donor blood is collected, germs from donors, and most recently, the production of WE components. The features of these bacteria suggest that they may have come from the environment, which suggests that the process of contamination may have occurred during manufacturing WE components from donor blood. According to Astuti & Ayu Maharani (2014) study, bacterial contamination might start to happen before a stabbing. The unwashed donor's arm, the blood-taking officer's hand, or the staff members' work tools can all harbor bacteria. Environmental bacteremia or contamination from the surrounding environment at the moment of blood collection are two possible sources of bacterial contamination. Research has shown that contamination can occur when blood collection circumstances are not aseptic (Btari Christiyani Kusumaningrum & Sepvianti, 2020). The phlebotomy procedure was improperly carried out because contaminated staff hands or equipment were not cleaned and disinfected before stabbing the donor. Some liquid disinfectants applied to the skin's surface do less well at lowering the danger of microbiological infection. *Brevundimonas vesicularis* bacteria, on the other hand, can be seen to survive during storage at a storage temperature of 2–6°C in the blood refrigerator based on their features. This is why it is essential to first disinfect the Due to this, it is essential first to clean the room where the blood is taken in order to ensure that it is sterile from the setting where the donor is from. Additionally, it is important to focus on cleaning the donor's arm first. Both when the arm was washed and when the retrieval officer used an alcohol swab to clean the area last.

Bacterial contamination can potentially be a result of the manufacturing process for WE components. Contamination may be a possibility in the insulator's surroundings. Or the area of the insulator where the creation of WE components takes place. This makes it important for officers to do routine cleaning. In the manufacture of WE components, it is necessary to perform pre- and post-process cleaning of insulators, production equipment, and the surrounding area. The pollution on work tools is not removed by the disinfectant solutions utilized.

CONCLUSIONS

Based on research on the identification of bacteria in the 2020 Washed Erythrovyte (WE) component at UDD PMI DKI Jakarta using the Bact/Alert 3D tool with Blood product anaerobic (BPN) and blood product aerobic (BPA) media, it can be said that one sample, or 2.44% of the total 41 samples of WE, were contaminated with aerobic bacteria in 2020. The outcomes also indicated that there was no bacterial contamination. Anaerobes from 41 WE samples were found utilizing the Bact Alert 3D technique on BPN media (100%). It is advised to identify the bacteria in the satellite bag with the same donor for additional research, and donor blood identity must be added. The results of identification at the Microbiology Laboratory at the University of Indonesia showed that the aerobic bacteria that contaminated washed erythrocyte (WE) blood products on BPA microbiology media were *Brevundimonas vesicularis.*

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Competing Interests: The authors declare that there is a conflict of interest.

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