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Evaluation of the Sterility of PRP Obtained with Multi-bag System (PRPBAG®)

Güven Çetin[1], Bilge Sümbül[2], Tuba Özkan[3], Seda Turgut[4], Nang Hseng Kyio[5], Kübra Şen[6], Damla Aslan Kirazoğlu[6]

[1]Bezmialem Vakıf University Hospital, Clinic of Internal Medicine, Division of Hematology, İstanbul, Turkey
[2]Bezmialem Vakıf University Hospital, Clinic of Medical Microbiology, İstanbul, Turkey
[3]İstanbul University-Cerrahpaşa, Cerrahpaşa Faculty of Medicine, Department of Internal Medicine, Division of Hematology, İstanbul, Turkey
[4]University of Health Sciences Turkey, Bakırköy Dr. Sadi Konuk Training and Research Hospital, Clinic of Endocrinology and Metabolism, İstanbul, Turkey
[5]King Hamad University Hospital, Bahrain Oncology Center, Busaiteen, Bahrain
[6]Bezmialem Vakıf University Hospital, Clinic of Internal Medicine, İstanbul, Turkey

ABSTRACT

Introduction: The aim of this study is demonstrating the sterility of PRPBAG®, a multi-bag completely closed PRP preparation system different than PRP kits in the market and determining preservability of the sterility of PRP produced with PRPBAG® up to 5 days similar to other platelet products.

Methods: We recruited 60 participants for increased statistical significance. 150 mL of whole blood was collected in specially produced PRPBAG®. A double-spin preparation method was used for the samples with a refrigerated centrifuge specially manufactured for PRPBAG®. We started centrifugation within 1 h after collecting blood. In the 1st spin of the centrifugation, whole blood was centrifuged. Erythrocytes precipitated in the first bag because of blood component density, and the supernatant plasma was transferred into the second bag using a manual plasma extractor. The third bag was empty after the first spin. We used a hose-closing device to separate the first bag, which contained erythrocytes, from the other bags. In the second spin of centrifugation, the plasma was centrifuged.

Results: PRP product with high-quality was collected in the second bag. We have considered the European guidelines for the preparation, use, and quality assurance of blood components during the whole process. 1 cc of PRP was added into separate pediatric blood culture bottles on days 0, 1, 2, 3, 4, and 5. These samples were sent to the microbiology laboratory and incubated for 5 days. After this incubation period, the blood culture bottles that did not give positive signals were reported as sterile.

Conclusion: Currently, PRP is used immediately after preparation. Our study demonstrates that PRP prepared with a closed triple blood bag system PRPBAG® may be used for 5 days if agitated at room temperature. There was not any bacterial growth in any sample after 5 days.

Keywords: Platelet-rich plasma, PRPBAG, sterility

Introduction

Platelet-rich plasma (PRP) is an autologous blood fraction with increased concentration of platelets and plasma growth factors above a certain threshold (1-3). The most common PRP acquisition methods in the market are kits. However, they differ significantly in terms of platelet concentration because of unstandardized production processes, and these are open methods where the plasma gets in contact with air (4). Sterilization is the process of removing living and viable germs from objects (5). Sterilization, especially of blood products, is important for preventing secondary infections and transmission of diseases (6,7).

PRPBAG® is a patented multi-bag PRP preparation system that uses apheresis bag technology. Its oxygen-permeability prevents platelets from hypoxia, which results in a high-quality PRP product. PRPBAG® is a completely closed and sterile system. It can provide an increase of as high as eightfold in the concentration of platelets compared to plasma (8).

Thrombocytes are delicate blood cells that are typically stored at room temperature for 5 days with gentle agitation (9,10).

The aim of this study is demonstrating the sterility of PRPBAG®, a multi-bag completely closed PRP preparation system different than PRP kits

Address for Correspondence: Güven Çetin MD, Bezmialem Vakıf University Hospital, Clinic of Internal Medicine, Division of Hematology, İstanbul, Turkey
Phone: +90 532 551 47 98 **E-mail:** drgvn20@gmail.com **ORCID ID:** orcid.org/0000-0002-4265-8105

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in the market and determining preservability of the sterility of PRP produced with PRPBAG® up to 5 days similar to other platelet products.

Methods

PRP was prepared using PRPBAG® and with the following the steps:150 mL of whole blood was drawn from the antecubital veins and collected in specially produced PRPBAG®. A double-spin preparation method was used for the samples with a refrigerated centrifuge specially manufactured for PRPBAG® (Large Capacity Refrigerated Centrifuge, Inovia Technology INO-FBC 5000). We started centrifugation within 1 h after collecting blood. In the 1st spin of the centrifugation, whole blood was centrifuged at 1600 (540 x g), 1800 (684 x g) and 2000 (845 x g) rpm for 10 min. Erythrocytes precipitated in the first bag because of blood component density, and the supernatant plasma was transferred into the second bag using a manual plasma extractor. The third bag was empty after the first spin. We used a hose-closing device to separate the first bag, which contained erythrocytes, from the other bags. The first bag was destroyed afterwards, and the process was continued with the remaining two bags. In the second spin of centrifugation, the remaining two bags were placed on the centrifuge. Plasma was centrifuged at 3500 rpm (2587 x g) for 15 min promptly. PRP product with high-quality was collected in the second bag. Supernatant plasma that is platelet-poor was transferred into the third bag using a manual plasma extractor, resulting in 10-18 mL of high-quality PRP in the second bag. We used hose-closing device to separate and destroy the third bag with platelet-poor plasma. We have considered the European guidelines for the preparation, use, and quality assurance of blood components during the whole process. The products used for acquiring PRP are medical devices authorized for clinical use and trade by the responsible institution (PRPBAG) (7).

1 cc of PRP was added into separate pediatric blood culture bottles (Becton-Dickinson, USA) on days 0, 1, 2, 3, 4, and 5. These samples were sent to the microbiology laboratory and incubated for 5 days. After this incubation period, the blood culture bottles that did not give positive signals were reported as sterile. The bottles that gave positive signals were planned to be gram-stained immediately and cultured. For culturing, the samples were to be inoculated into four quadrants of 5% sheep blood agar (BectonDickinson, ABD), Eosin-Methylene Blue agar (BectonDickinson, ABD), and chocolate agar (BectonDickinson, ABD) plates by the dilution method using sterile standard inoculating loop. The agar plates were planned to be incubated in CO_2 incubator aerobically at 35±2 °C for 24-48 hours. In the case of bacterial growth, colonies were projected to be processed for further identification. Bacterial strain typing was to be performed with a VITEK MS MALDI-TOF (bioMérieux, USA) system and VITEK® 2 Compact (bioMérieux, Marcy l'Etoile, Fransa) automatized in accordance with the recommendations of the producer.

Our study was approved by the Bezmialem Vakıf University Local Ethics Committee (approval number: 5/27, date: 06.03.2019), and we acquired individual informed consent from all participants.

Statistical Analysis

Statistical analyses were planned to be performed with SPSS version 20.0 for Windows (SPSS Inc. Chicago, IL, USA). The number of volunteers to be included in this study was calculated by the department of statistics as 40 with a 95% confidence level and 80% power. We determined the final number of participants was 60.

Results

The minimum number of volunteers to be included in this study required for 95% confidence level and 80% power was determined by the statistics department as 40. However, we recruited 60 participants for increased statistical significance. We took 6 samples of PRP from each patient making 360 samples and added them into pediatric blood culture bottles (Becton-Dickinson, USA). The samples were incubated in BACTEC FX (Becton-Dickinson, USA) for 5 days. There was not any bacterial proliferation in any of 360 samples. Therefore, the processes planned for further bacterial identification as explained in methods were not needed.

Discussion

As commonly known, most of the systems that produce PRP are open tubes, and the product has to be used immediately. In this study, we wanted to test the safety of closed triple bag systems in terms of bacterial contamination and safety for 5 days, which is the standard usage time of platelets. Therefore, we added 360 samples from 60 subjects into pediatric blood culture bottles (Becton-Dickinson, USA). There was not any bacterial proliferation in any sample. Therefore, there was no need to conduct a statistical analyzes. These results agree with the findings of blood banking and apheresis systems in terms of sterility and terms of use (Hoots, 2001) (6).

Currently, PRP is used immediately after preparation. Our study demonstrates that PRP prepared with a closed triple blood bag system may be used for 5 days if agitated at room temperature. There was not any bacterial growth in any sample after 5 days (Table 1).

PRP is a treatment option with few side effects and is considered almost harmless. However, it has certain complications as any intervention that include infection. Therefore, the sterility of PRP is important for preventing infections (11). Dincer et al. (12) report a case of a 27-year-old male soccer player who is treated with PRP injections for grade 2 rupture in the gastrocnemius muscle. He developed an ulcer on the leg after being treated with PRP. This article demonstrates the importance of using a method of obtaining PRP scientifically proven to be safe from bacterial contamination.

Conclusion

The use of a standardized closed system, the long-term use of the product, and the standardization of the amount of platelets in the sample have brought a new dimension to the use of PRP, which will be a scientific basis for further clinical studies.

Table 1. Bacterial proliferation on the corresponding day

Patient number	The patient name	Day 0*	Day 1*	Day 2*	Day 3*	Day 4*	Day 5*
1	Ö.B.	None	None	None	None	None	None
2	E.İ.	None	None	None	None	None	None
3	Ö.G.	None	None	None	None	None	None
4	A.T.	None	None	None	None	None	None
5	N.B.	None	None	None	None	None	None
6	S.K.	None	None	None	None	None	None
7	S.A.	None	None	None	None	None	None
8	S.F.P.	None	None	None	None	None	None
9	S.K.	None	None	None	None	None	None
10	O.Ş.	None	None	None	None	None	None
11	S.D.	None	None	None	None	None	None
12	İ.E.	None	None	None	None	None	None
13	O.K.	None	None	None	None	None	None
14	İ.S.	None	None	None	None	None	None
15	S.E.	None	None	None	None	None	None
16	Ö.P.	None	None	None	None	None	None
16	D.Ç.	None	None	None	None	None	None
18	Ç.Ü.	None	None	None	None	None	None
19	H.A.	None	None	None	None	None	None
20	Z.K.	None	None	None	None	None	None
21	N.Ö.	None	None	None	None	None	None
22	Ç.G.	None	None	None	None	None	None
23	B.K.	None	None	None	None	None	None
24	F.G.	None	None	None	None	None	None
25	F.K.	None	None	None	None	None	None
26	S.A.	None	None	None	None	None	None
27	K.D.	None	None	None	None	None	None
28	M.D.	None	None	None	None	None	None
29	M.A.	None	None	None	None	None	None
30	E.Ö.	None	None	None	None	None	None
31	Z.D.	None	None	None	None	None	None
32	N.B.	None	None	None	None	None	None
33	T.P.	None	None	None	None	None	None
34	E.A.	None	None	None	None	None	None
35	U.S.	None	None	None	None	None	None
36	N.T.	None	None	None	None	None	None
37	D.Ş.	None	None	None	None	None	None
38	S.K.	None	None	None	None	None	None
39	Ş.V.	None	None	None	None	None	None
40	S.K.	None	None	None	None	None	None
41	Ö.F.T.	None	None	None	None	None	None
42	A.Ö.	None	None	None	None	None	None
43	B.K.	None	None	None	None	None	None
44	E.Ö.	None	None	None	None	None	None
45	K.S.	None	None	None	None	None	None
46	A.K.	None	None	None	None	None	None
47	B.P.	None	None	None	None	None	None
48	Y.T.	None	None	None	None	None	None
49	A.E.	None	None	None	None	None	None
50	S.G.	None	None	None	None	None	None
51	M.S.	None	None	None	None	None	None
52	S.S.	None	None	None	None	None	None
53	A.V.	None	None	None	None	None	None
54	E.V.	None	None	None	None	None	None
55	S.P.	None	None	None	None	None	None
56	F.Ş.	None	None	None	None	None	None
57	A.K.	None	None	None	None	None	None
58	R.B.	None	None	None	None	None	None
59	Ç.Ö.	None	None	None	None	None	None
60	B.B.	None	None	None	None	None	None

*Bacterial proliferation on corresponding day

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Ethics Committee Approval: Our study was approved by the Bezmialem Vakıf University Local Ethics Committee (approval number: 5/27, date: 06.03.2019).

Informed Consent: We acquired individual informed consent from all participants.

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