

Is the manual preparation of platelet rich plasma safe?

O preparo manual do plasma rico em plaquetas é seguro?

¿Es segura la preparación manual de plasma rico en plaquetas?

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Abstract

The safety and sterility of Platelet Rich Plasma (PRP) produced manually concerns the scientific community, since there is a huge gap in the literature on the subject. Understanding the risks inherent to sterile handling and incorporating standard procedures is essential for patient safety, with an increasing demand for safely and effectively producing PRP without the prohibitive costs of commercial kits. The purpose of our research project is to show that through adequate training and qualification of health professionals, we can produce a low-cost, extremely safe, and effective product that fits perfectly into the reality of underdeveloped and emerging countries. We have developed a detailed step-by-step guide for standardizing the procedure for manual preparation of PRP, which ranges from the venipuncture procedure, including equipment cleaning, to the preparation of the PRP. Twenty-two PRP samples were produced and sent to an independent analysis laboratory, for identification and isolation of microorganisms through culture media. No microorganisms were identified and isolated from the samples, resulting in a contamination percentage of 0%, proving the effectiveness of the training and method in producing PRP in a safe and sterile way. Through the training of health professionals and the suggested protocols, it was possible to confirm our hypothesis that it is feasible to manually prepare contaminant-free PRP, which is safe for use in interventions where the product is recommended, without the need of commercial kits.

Keywords: Platelet-rich plasma; Sample preparation; Biological safety; Biological contamination; Practice Patterns.

Resumo

A segurança e esterilidade do Plasma Rico em Plaquetas (PRP), quando ele é produzido através de um método manual é algo que preocupa a comunidade científica, uma vez que existe uma enorme lacuna na literatura com relação ao tema. Compreender os riscos inerentes à manipulação estéril e incorporar procedimentos padrões é algo essencial para a segurança do paciente, havendo uma demanda crescente para a produção do PRP com segurança e eficácia sem os custos proibitivos dos kits comerciais. O intuito do nosso projeto de pesquisa é mostrar que através de um treinamento adequado e capacitação dos profissionais da área da saúde, podemos produzir um produto de baixo custo, extremamente seguro e eficaz e que se encaixa perfeitamente na realidade de países subdesenvolvidos e emergentes. Desenvolvemos um passo a passo detalhado para a padronização do procedimento de preparo manual do PRP, que vai desde a técnica de venopunção, incluindo a higienização dos equipamentos, até o preparo do PRP. Foram produzidas 22 amostras de PRP, que por sua vez foram encaminhadas para um laboratório de análises independente para a realização de identificação e isolamento dos microrganismos através de meios de cultura. Não foi possível identificar e isolar qualquer tipo de microrganismos das amostras, resultando em uma porcentagem de contaminação de 0%, o que comprova a eficácia do treinamento e do método em produzir PRP de forma segura e totalmente estéril. Através da capacitação de profissionais da área da saúde e dos protocolos sugeridos, foi possível confirmar a nossa hipótese de que é possível preparar manualmente PRP livre de contaminantes, sendo seguro para a utilização em possíveis intervenções onde o produto é recomendado, sem a necessidade de utilização de kits comerciais.

Palavras-chave: Plasma rico em plaquetas; Preparação de amostras; Segurança biológica; Contaminação biológica; Padrões de prática.

Resumen

La seguridad y esterilidad del Plasma Rico en Plaquetas (PRP), cuando se produce de forma manual, es algo que preocupa a la comunidad científica, ya que existe un gran vacío en la literatura sobre el tema. Comprender los riesgos

inherentes a la manipulación estéril e incorporar procedimientos estándar es esencial para la seguridad del paciente, con una creciente demanda de producción segura y eficaz de PRP sin los costos prohibitivos de los kits comerciales. El propósito de nuestro proyecto de investigación es demostrar que, a través de una adecuada formación y calificación de los profesionales de la salud, podemos producir un producto de bajo costo, extremadamente seguro y efectivo, que se ajuste perfectamente a la realidad de los países subdesarrollados y emergentes. Hemos desarrollado una guía detallada para la estandarización del procedimiento de preparación manual del PRP, que abarca desde la técnica de venopunción, pasando por la limpieza del equipo, hasta la preparación del PRP. Se produjeron 22 muestras de PRP, que a su vez fueron enviadas a un laboratorio de análisis independiente para la identificación y aislamiento de microorganismos a través de medios de cultivo. No fue posible identificar y aislar ningún tipo de microorganismo de las muestras, resultando un porcentaje de contaminación del 0%, lo que demuestra la efectividad del entrenamiento y el método para producir PRP de forma segura y totalmente estéril. A través de la capacitación de profesionales en el área de la suavidad y los protocolos sugeridos, fue posible confirmar nuestra hipótesis de que es posible preparar manualmente PRP libre de contaminantes, siendo seguro para su uso en posibles intervenciones donde se recomienda el producto, sin la necesidad de uso de kits comerciales.

Palabras clave: Plasma rico en plaquetas; Preparación de la muestra; Seguridad biológica; Contaminación biológica; Estándares de práctica.

1. Introduction

Platelet-rich plasma (PRP) has been recognized as a hemostatic agent since 1970, and as a potent source of autologous growth factors since 1990 (Castillo et al., 2010; Slater et al., 1995; Whitman et al., 1997). The levels of platelet-derived growth factors (PDGF), transforming growth factor (TGF- β), and vascular endothelial growth factor (VEGF) found in PRP are known to play a critical role in cell proliferation, chemotaxis, cell differentiation, and angiogenesis (Werner & Grose, 2003). Consequently, there has been strong clinical interest for the use of PRP as a growth factor delivery medium to assist in tissue regeneration. PRP was initially recognized as an effective agent for bone and tissue repair in the field of dentistry and oral maxillofacial surgery (Grageda, 2004). Its applicability then spread to the fields of plastic surgery, demonstrating evidence of improved healing of skin grafts.

In the field of orthopedic surgery, the potential role of PRP in enhancing the healing of bones, muscles, ligaments, and tendons has resulted in several studies in almost all orthopedic subspecialties. PRP has been reported to improve recovery from joint replacement (Berghoff et al., 2006), spine surgery (Marlovits et al., 2004), and fracture healing (Gandhi et al., 2006; Sanchez et al., 2009). Also in Sports Medicine, PRP has caused interest, as the recovery time for the athlete is often a critical issue for the care of this type of patient (Creaney & Hamilton, 2008; Hall et al., 2009). Several studies have reported favorable clinical conditions also in chronic tendinopathies (De Castro et al., 2020; Sánchez et al., 2007), muscle injuries, and knee arthrosis (De Castro et al., 2020; De Carli et al., 2009; Hammond et al., 2009; Menetrey et al., 2000). However, in addition to using different PRP separation systems which perpetuates the lack of standardization and hinders the reproducibility of the products, these studies are not concerned with the standardization of a preparation method focused on safety and sterility of the samples, being an extremely worrying factor.

CERT (Portuguese for Center for Tissue Regeneration Studies) promotes courses, where experts teach how to produce PRP manually, which, due to its low cost, has proved to be effective for various treatments of patients in underdeveloped and emerging countries, such as Brazil. Given that there is a large gap in the literature regarding the safety and sterility of PRP produced by a manual method, the purpose of our research project is to show that through adequate training and qualification of students, we can produce a low-cost, extremely safe, and effective product that fits perfectly into the reality of underdeveloped and emerging countries.

Our hypothesis is that the courses, “Main PRP preparation methods” and “How to safely prepare PRP,” carried out by CERT, provide students with the ability to produce a safe and contamination-free product. Although many preparation techniques have been already proposed in the literature (Gupta, Parihar, Pathak, & Sharma, 2020; Hart et al., 2013; Jo et al., 2013; Kemmochi, 2022; Oh et al., 2015; Sánchez et al., 2012; Ziegler et al., 2019), the safety of PRP produced by

professionals who have received adequate training has not yet been evaluated. The standardization regarding the PRP handling steps is extremely important for the scientific community, ensuring a sterile product at the end of the manual preparation process. Taking this issue into account, we have developed a step-by-step guide to ensure the sterility of the samples.

The objective of this work is to evaluate the qualification of health professionals trained in the courses “Main PRP preparation methods” and “How to safely prepare PRP”, carried out by CERT, regarding the efficiency of preparing PRP manually, guaranteeing the sterility of the samples.

2. Methodology

2.1 Ethical Aspects of the Research Project

According to the need for venipuncture and manipulation of human blood samples by the students involved in the research project, the work was submitted for approval by the ethics committee of Hospital Ifor Ltda. CAAE: 63961416.8.0000.5625 and all participating students provided written consents prior to study enrollment.

2.2 Study Design

The study was conducted with students, who were health professionals and participated in the CERT courses on “Main methods of preparing the PRP” (online) and also participated in the course “How to prepare the PRP safely” (in person), totaling approximately 20 hours of class, with discussion sessions at the end of each class. After receiving adequate training, 11 of the 65 students who took the courses were randomly selected, through the method of drawing opaque and sealed envelopes, to participate in the research project. The students were divided into four pairs, and a group consisted of three members. Before starting the venipuncture and plasma preparation techniques, each group received a briefing, with the main points of each stage, which will be shared in our research project so that the scientific community can have access to this information, ensuring the production of a safe and sterile PRP. To start the project, each student donated approximately 20 ml of blood, which was divided in two tubes containing the anticoagulant acid citrate dextrose (BD Vacutainer™ ACD solution A yellow cap tubes), for the preparation of the PRP, the students only and exclusively manipulated their own blood. The 20 ml of blood were prepared using a single spin method, the plasma was withdrawn from just above the red blood cells (RBC) layer, including the buffy coat, and being classified as a leukocyte rich PRP. Each student generated a total of 2 samples (duplicate) of PRP with approximately 2 ml each, which were sent to an independent analysis laboratory (Dms Burnier Laboratório de Análises Clínicas Ltda) for identification and isolation of microorganisms through culture media. Since the goal is to verify the absence or presence of microorganisms in the PRP samples, GraphPad Prism 9.0 was used to analyse this categorical data which was expressed as percentage. At the end of the procedures, all PRP samples were appropriately discarded by the analysis laboratory.

2.3 Student Inclusion Criteria

Students who took the courses “Main methods of plasma preparation” and participated in the course “How to safely prepare the PRP” were eligible for the study. In addition, all students should have a complete blood count exam, completed within a period of three months, and a result within acceptable parameters was mandatory.

2.4 Student Exclusion Criteria

Pregnant women, students with malignant neoplasms, severe liver or kidney diseases, autoimmune diseases, inflammatory or infectious, acute, or chronic diseases, presence of severe systemic pathology and any other condition that could put the students at risk during the study were excluded.

2.5 Venipuncture

The eleven students were divided into pairs, except for a group that consisted of three members. Before performing the venipuncture procedure, all of them underwent a theoretical and practical venipuncture training with a nurse in charge. Each student performed the procedure with his/her respective pair under the supervision of the nurse, though no intervention was necessary.

The venipuncture procedure was performed according to the steps described below.

- The student responsible for performing the venipuncture procedure sanitized his hands before starting the procedure. If necessary, the hand sanitizing was repeated during the procedure.
- The materials to be used were separated: supplies on the tray, other materials, and equipment on the table/bench.
- The student wore personal protective equipment (PPE): surgical mask, protective goggles, long-sleeved apron, and procedure gloves.
- The furniture was sanitized.
- The student responsible for performing the venipuncture procedure summoned the other student in his group by his/her full name.
- The student was positioned comfortably and appropriately for the procedure.
- The student responsible for performing the procedure introduced himself to the patient and advised him on the procedure to be performed.
- A Nursing Anamnesis was carried out and registration was done in a specific form:
 - o Vital data verification: Blood Pressure, Pulse, Temperature, Oximetry.
 - o Questioning about health history (Table 1).
 - o According to the nursing anamnesis and vital signs, the conduction was discussed with the responsible physician when necessary.
- Both the patient and the ACD tubes were identified with bracelets and tags, respectively, containing the student's full name, and then a double check of the identification information was performed.
- Performing venipuncture:
 - o The limb to be punctured was chosen, preferably the limb opposed to the procedure and non-dominant, avoiding limb and regions compromised by injuries, allergic processes, edema, plegia, burns, lymph node dissection (mastectomy), arteriovenous fistula and other compromises.
 - o Next, the region was chosen, preferably distant from the joints, avoiding flexion region, giving preference to the forearm region, posteriorly the cubital fossa and, if strictly necessary, the wrist region and back of the hand.
 - o Superficial peripheral vessels of large caliber and straight lines were chosen, preferably cephalic, basilic and median veins in the distal to proximal direction.
 - o The tourniquet was performed only for visualization of the veins, being loosened at the time of collection of the samples, not used as pressure for bleeding, which can compromise the quality of the sample. The closing of the hand was requested when more venous filling was necessary for better visualization of the vein, and the opening was requested to perform the puncture.
 - o Antisepsis was performed with gauze soaked in alcoholic solution (70% ethyl alcohol or 0.5% alcoholic chlorhexidine), in a circular and spiral motion, removing dirt from the center of the puncture site outside the perimeter to be punctured, waiting for spontaneous drying of the solution for antiseptic action.
 - o The No Touch technique was used to avoid contaminating the puncture site after antisepsis.

- o The patient' skin was pulled (towards the distal portion of the limb) with the non-dominant hand, positioning the thumb approximately 2.5 cm below the selected puncture site.
- o The patient was informed of the moment of the puncture, and he/she was asked to take a deep breath.
- o The skin was perforated, and the catheter was introduced into the vessel with the needle bevel facing upwards, until blood reflux was observed.
- o The mandrel was removed after puncturing with a catheter over a needle, pressing above the tip of the catheter with the index finger of the non-dominant hand.
- o The luer lock adapter plug was connected.
- o The catheter was fixed to the patient's skin, using a sterile transparent film dressing with fenestra and fixative (Kangli Film IV) in a way that it was firm, visually aesthetic and so as not to interfere movements.
- Comments:
 - o When necessary, a new catheter was used at each new attempt, never reusing the same catheter.
 - o In case of visible dirt at the puncture site, it was removed with soap and water (the student was asked to wash the arm).
 - o It was limited to two peripheral puncture attempts per student, considering that multiple punctures cause pain, delay the start of treatment, compromise the vessel, increase costs, and has risk of complications.
 - o Patients with difficult access require evaluation and multidisciplinary management, use of ultrasound or puncture with a vein viewer.
- Performing the collection of samples (Figure 1):
 - o The adapter plug was perforated with a needle connected to the vacuum collection device ("cannon"), connecting the collection tubes, which were previously sterilized with 70% alcohol or 0.5% alcoholic chlorhexidine, and waiting for the volume to be filled, for flow cessation.
 - o When removing the ACD collection tubes from the venoclysis, they were slowly moved to homogenize the anticoagulant in the blood, then they were placed in a proper grid and in a vertical position.
 - o The samples were immediately delivered to the PRP preparation process.
- Cautions when removing the catheter:
 - o The catheter was removed as soon as its use was no longer necessary.
 - o The sterile transparent film dressing with fenestra and fixative (Kangli Film IV) was carefully removed so as not to injure the skin.
 - o Immediately after catheter removal, compression was performed at the site with dry gauze for 1 to 5 minutes, promoting hemostasis and preventing bruising.
 - o Coverage was performed with blood stop.
 - o The student was instructed not to exert effort with the limb on the day.
- The disposal of sharp materials was carried out in an appropriate place.
- The rest of the material used was discarded also in an appropriate place.
- Procedure gloves were removed.
- The patient's well-being was certified.
- Records were made regarding the collection and expenditure of materials in a specific form.
- The furniture was sanitized.

Table 1: Health history questionnaire.

NURSING HISTORY / PRP PROCEDURE	
NAME:	IDENTIFICATION:
DATE: / /	
PROCEDURE / LIMB:	
HEALTH BACKGROUND: ()DM ()SAH ()OUTHERS	
CONTINUOUS USE MEDICATIONS:	
HAS BEEN USED CORRECTLY IN THE LAST DAYS ()YES ()NO, WHY:	
ALLERGIC REACTIONS: ()NO ()YES, WHICH?	
LAST FEEDING TIME:	
SATISFACTORY WATER INTAKE? ()YES ()NO () ADVISED TO INGEST LIQUIDS AFTER COLLECTION	
BP: mmHg	PULSE: bpm
TEMPERATURE: °C	SATURATION: %
() DOUBLE CONFERENCE WITH THE PATIENT OF LABELS AND IDENTIFICATION BRACELET	
NOTES:	
NURSING:	TIME:

Source: Authors.

Figure 1: Carrying out the collection of samples. In the image, it is possible to observe the student performing the venipuncture technique, leuc lock adapter tube, catheter fixed using a sterile transparent film dressing with fenestra and fixator and ACD tube being perforated by a needle connected to the vacuum collection device.



Source: Authors.

2.6 Centrifuge sanitization

The centrifuge cleaning process was always carried out before and after using the equipment, and the following step-by-step procedure below was performed.

- Materials and PPE needed for cleaning:
 - o Water.
 - o Neutral detergent. Sprayer
 - o Wet cloth.
 - o Dry cloth.
 - o Autoclavable forceps.
 - o Procedure gloves.
 - o Surgical mask.
 - o Long-sleeved apron.
 - o Disposable cap.
- After putting on the necessary PPE, the student washed his hands and forearms with soap and water, before starting the centrifuge cleaning process.
 - When starting the cleaning process, the centrifuge was turned off, removed from the socket, all its components were removed from its interior and placed on a bench that had previously been sanitized with 70% ethyl alcohol.
 - A spray bottle was prepared with a solution containing 30% neutral detergent and 70% water.
 - One of the cloths was moistened with water.
 - The solution, containing detergent and water, was sprayed on the damp cloth, which in turn was rubbed all over the inside of the centrifuge. Soon after, with a clean and dry cloth, the excess of the solution was removed.
 - Again, spraying the solution on the damp cloth, the centrifuge lid was cleaned, and the excess solution was removed with a clean, dry cloth.
 - Following the same principle, the centrifuge panel and its outer portion were cleaned.
 - With the help of autoclavable forceps, the wet cloth was introduced into the bucket holes of the tilting rotor, where the tubes are placed, so that they could be sanitized (Figure 2).
 - After cleaning all the centrifuge components, it was reassembled and kept closed until the next use.
 - Never use volatile chemical products such as alcohol and benzene to sanitize the centrifuge.
 - Never use a sponge to sanitize the centrifuge.

Figure 2: Centrifuge cleaning. Autoclavable forceps, helping to sanitize the bucket holes of the tilting rotor of the centrifuge, where the tubes are allocated.



Source: Authors.

2.7 Vertical laminar flow cabinet sanitization

The cleaning process of the laminar flow cabinet (Filterflux FLV-656/3) was always carried out before and after using the equipment, and the following step-by-step procedure was performed.

- Materials and PPE needed for cleaning:
 - 70% ethyl alcohol.
 - Sprayer.
 - Paper towel or gauze.
 - Mayo table.
 - UV lamp.
 - Procedure gloves.
 - Surgical mask.
 - Long-sleeved apron.
 - Disposable cap.
- After wearing the necessary PPE, the student washed his hands and forearms with soap and water, before starting the process of sanitation.
- To start the cleaning process, the student turned on the light and the cabinet motor.
- The sprayer was filled with 70% ethyl alcohol, which was also used to sanitize the external surface of the sprayer itself.
- The Mayo table was completely sanitized with 70% ethyl alcohol, so that it could be used as a support for paper towels or gauze and the sprayer bottle during the cleaning process.
- The sprayer, previously sanitized, was introduced into the laminar flow cabinet and 70% ethyl alcohol was sprayed on the entire interior surface of the cabinet, including its walls.
- With the aid of a paper towel or gauze, the excess of 70% ethyl alcohol was removed, with a single movement and always in the same direction (from inside to outside the cabin), not using the same paper towel or gauze more than once.
- Once the entire interior surface of the cabin was sanitized, its glass door was closed, and its engine kept running.
- After closing the door, the handles responsible for opening it were also sanitized with 70% ethyl alcohol.
- After all the steps described above, the UV lamp was turned on, for a minimum period of 15 minutes, keeping the engine running.
- In case of accidents or spillage of biological risk agents while using the cabinet, at the end of use, the lower portion of the cabinet must be removed and the interior also sanitized with 70% ethyl alcohol, keeping the engine running.
- When removing the lower portion of the cabinet, support it on a bench previously sanitized with 70% ethyl alcohol.
- After sanitizing the interior of the cabinet, return the lower portion to its proper place and repeat the entire cleaning step already described.

2.8 Preparation of Platelet Rich Plasma (PRP)

Before performing the procedure, all eleven students underwent theoretical and practical PRP preparation training with a biologist in charge. The entire process was supervised by the professional during its execution, though no intervention was necessary.

After performing the standard venipuncture procedure described above, the two blood samples contained in the ACD tubes were directly destined for the preparation of the PRP according to the following steps.

- PPE and equipment necessary for the preparation of the PRP:
 - o Sterile surgical glove.
 - o Sterile surgical gown (with a compress inside).
 - o Disposable cap.
 - o PFF2 mask.
 - o 70% ethyl alcohol.
 - o Brush-sponge set, containing preoperative topical antiseptic with 2% chlorhexidine digluconate.
 - o ACD tubes.
 - o Support grid for ACD tubes.
 - o 10 ml sterile syringe.
 - o Simple intravenous silicone catheter.
 - o Falcon tube.
 - o Support grid for micro tube.
 - o Sterile 2 ml micro tube (responsible for sending the sample to the analysis laboratory).
 - o Labels containing the student's name.
 - o Vertical laminar flow cabinet (HEPA filter class H13/14 and UV lamp).
 - o Benchtop centrifuge with tilting rotor and acceleration and deceleration program.
- After its cleaning, the interior of the laminar flow cabinet (Filterflux FLV-656/3) was equipped with all the materials necessary for the preparation of the PRP, namely:
 - o Sterile surgical glove.
 - o Support grid for ACD tubes and Falcon tubes.
 - o 10 ml sterile syringe.
 - o Simple intravenous catheter.
 - o Falcon tube.
 - o Support grid for micro tube.
 - o Sterile 2 ml micro tube (for sending the sample to the analysis laboratory).
 - o Labels containing the student/patient's name.

All the materials described above were sanitized with 70% ethyl alcohol sprays before being introduced to the laminar flow cabinet, which in its turn had been previously sanitized according to its cleaning process. After all materials were allocated in their respective places, the UV lamp was turned on and kept on for at least 15 minutes.

- During the UV lamp sterilization process, the samples contained in the two ACD tubes were immediately sent to the centrifuge (Fanem Excelsa i-2206), which had been previously sanitized, using the configuration of 1000g for 10 minutes (Harrison, Bowler, Levins, Cheng & Reeves, 2020; Peterson & Reeves, 2014) for separation of the plasma.
- Also, during the centrifugation period, the student responsible for preparing the PRP sanitized his hands with a brush-sponge set containing preoperative topical antiseptic with 2% chlorhexidine digluconate.
- After hand sanitization, the student was dressed in a sterile surgical gown, which contained a compress, also sterile, in its packaging for drying the hands. The biologist helped the students to close the sterile surgical gown (Figure 3).
- At the end of the gowning and 15 minutes of UV lamp sterilization, the student could introduce their arms into the laminar flow cabinet and then put on the sterile surgical gloves. To put on the gloves, the students followed these

steps:

- o Put on your dominant hand first, without touching the outer portion of the glove.
- o Put on the non-dominant hand, with the help of the dominant hand, which was already wearing the glove, touching only the outer portion of the glove. Thus, it is possible to guarantee the sterility of the material.
- o Gloves have a sterile paper inside, responsible for packing them, this paper was used to create a field inside the laminar flow cabinet, ensuring even more sterility and safety during preparation.
- After the student was fully dressed and after the centrifugation was completed, the biologist in charge removed the ACD tubes from the centrifuge and sprayed 3 to 5 sprays of 70% ethyl alcohol in the tubes, before they were introduced into the vertical laminar flow cabin, ensuring that were free of any possible contaminants. The process of introducing the tubes was also carried out by the biologist so that the students could keep their arms inside the cabin and consequently inside the sterile field.
- The biologist was dressed in a long-sleeved lab coat and procedure glove. Before touching the tubes, his/her hands were sanitized with soap and water, and after putting on their gloves, they were sanitized with 70% ethyl alcohol.
- Before the student began to prepare PRP, the falcon tube was opened so that it could be used for discarding platelet-poor plasma (PPP).
- The two ACD tubes were slightly opened just to remove the initial resistance of the caps, caused by the vacuum, but the caps were kept on top of the tubes to avoid any type of contamination.
- The students opened the simple intravenous catheter, removing the needle and its other components, separating only the silicone portion that was attached to the 10 ml syringe, to start the plasma aspiration. Before starting the aspiration process, the initial resistance of the syringe plunger was broken to increase accuracy during the preparation process.
- The PPP was aspirated slowly and gradually, keeping the catheter tip on the surface of the plasma to ensure that the highest concentration of platelets was not aspirated. After this process, the PPP from the two samples was discarded in the falcon tube (Figure 4).
- After the PPP was discarded, the PRP was aspirated slowly and gradually (avoiding platelet lysis and increasing the student's precision), keeping the catheter tip as close as possible to the buffy coat, ensuring the aspiration of the largest number of platelets (Figure 5).
- The students were instructed not to pass their arms and hands over the ACD tubes when they were open, as this could lead to possible contamination of the sample.
- A total of approximately 2 ml of PRP per ACD tube was obtained, resulting in a total of approximately 4 ml per student.
- The volume was deposited in two sterile micro tubes of 2 ml, which were identified with labels 1 or 2, containing the name of the donor student of the sample, since the analysis of the samples for identification and isolation of microorganisms was performed in duplicate.
- At the end of the PRP preparation procedure, all materials used were properly disposed of in an appropriate place.
- During the preparation of the other students, the samples that had already been prepared were stored inside an aluminum box, previously sanitized, avoiding contact with light and under constant refrigeration at 3 – 5°C.
- After the preparation of all samples, they were sent to an independent laboratory, responsible for carrying out the analysis of identification and isolation of microorganisms, inside a thermal box, keeping them at a temperature of 3 – 5°C.
- Upon arrival at the analysis laboratory, the samples were kept under the same refrigeration temperature and the analysis was performed the following day.

Figure 3: Student attire before preparing the PRP. Student being dressed in a sterile surgical gown, with the help of the biologist responsible for closing the gown.



Source: Authors.

Figure 4: Disposal of PPP in falcon tube.



Source: Authors.

Figure 5: PRP aspiration being performed slowly and gradually (avoiding platelet lysis and increasing student accuracy).



Source: Authors.

2.9 Identification and Isolation of Microorganisms Through Culture Media

The samples were collected and prepared by each student, using the methods described above, and stored under refrigeration (2 – 5°C), including during shipment to the microbiological analysis laboratory, to ensure their stability.

- Eight types of culture media and reagents were used for identification and isolation of microorganisms, namely:

- o Blood agar.
- o Chocolate agar.
- o Mac Conkey Agar.
- o Muller Hinton Agar.
- o Brain Heart Infusion (BHI) broth.
- o Gram stain.
- o Means of identification.
- o Antibiotic discs.

The procedure of identification and isolation of microorganisms through culture media was carried out according to the step-by-step procedure described below.

- All microbiological procedures were performed inside the laminar flow cabinet.
- All culture media were at room temperature before use.
- Preparation method:
 - o The sample contained in the sterile micro tube was homogenized.
 - o Seeding (1st repeat):
 - With the aid of a sterile and disposable loop, the material was seeded on Blood Agar, Chocolate Agar, Mac Conkey Agar and BHI broth.
 - Two slides were prepared for Gram staining.

- Two slides were prepared for Ziehl-Neelsen staining.
- The BHI broth and Mac Conkey Agar were incubated in a laboratorial oven at 35 ± 1 °C, while the blood agar and Chocolate Agar plates were kept in a CO₂ atmosphere for 48 hours.
 - o Seeding (2nd repeat):
 - Growth identifications were performed on blood agar and chocolate agar plates, according to the microorganism.
 - As all samples were negative for the growth of microorganisms, they were kept for another 2 days in a CO₂ atmosphere, in a laboratorial oven at 35 ± 1 °C, and were inspected daily.
 - o BHI broth:
 - Exhaustion repeating was performed on Blood Agar and Mac Conkey Agar media.
 - The plates were incubated in a laboratorial oven at 35 ± 1 °C for 24 hours.
 - The BHI broth was kept for another 24 hours at room temperature.
 - As the BHI broth remained clear, it was kept in a laboratorial oven at 35 ± 1 °C for another 24 hours.
- Identification and automated antibiogram were performed using the Vitek system.

2.10 Statistical analysis

Since the goal is to verify the absence or presence of microorganisms in the PRP samples, GraphPad Prism 9.0 was used to analyse this categorical data which was expressed as percentage. No microorganisms were found in the 22 samples (duplicate of the samples of the 11 students), representing a success rate of 100%.

Considering there is no reliable data available regarding microbial contamination of PRP when prepared manually, no sample size estimation could be performed, and so the number of samples was set arbitrarily.

3. Discussion

The purpose of our study was to analyze the rate of contamination of the PRP when performed by a manual preparation method following the protocol of the course “Main PRP preparation methods” and “How to safely prepare PRP”. The understanding of the inherent risks in sterile handling and the establishment of appropriate standards are essential for the patient’s safety. Manipulating drugs without the guidance of rigid principles can result in subpotent, superpotent or contaminated final products, exposing patients to significant risk of adverse events and even death (United States Pharmacopeia Convention [USP], 2008).

The production of a safe and effective PRP, without the prohibitive costs of the available commercial kits is crucial in a country with a deficit in its public health system, which serves approximately 70% of the estimated Brazilian population according to data from the Brazilian Institute of Geography Statistics in 213,317,639 inhabitants (Instituto Brasileiro de Geografia e Estatística [IBGE], 2021).

Several articles related to the effectiveness of PRP have been constantly published, still, there is a large gap in the literature regarding the sterility of samples that are produced through manual methods of preparation and commercial kits. The focus of these articles resides mainly on the recovery rate. Different preparation methods can result in different degrees of sterility of the product, and it is important to standardize a procedure that guarantees the absence of contaminants in all samples produced. Our work was aimed at creating a step-by-step guide and making it accessible to everyone who wants to safely produce PRP. The proposed methodology for the identification and isolation of microorganisms through culture media is more complete and rigid than the usual standards of microbiological analysis necessary for Brazilian regulatory bodies. Also, the idea is that the method as well as the results obtained can be replicated in countries that wish to use the same method.

In our proposed protocol, we were concerned with conducting safety procedures regarding the sterility of the sample from venipuncture to delivery of the prepared product, since in most of the comparative literature only the preparation and recovery protocols are covered.

In the comparative literature (Di Martino et al., 2022; Krief et al., 2021; Zhang, 2021), studies consider the safety of the method through the rate of adverse effects, whereas in our work we objectively focus on the absence of contaminants, being this paper the first report with this type of analysis.

The choice of the single spin PRP protocol was due to the smaller amount of manipulation of the ACD tubes, since the greater the manipulation, the greater the chance of contamination. Another crucial factor is the use of the laminar flow cabinet, since the current cabinets allow a good view of the manipulation of the tubes, including the delimitation of the buffy coat.

Regardless of the method chosen to produce PRP, if the proposed step-by-step procedure is strictly followed, the probability of contamination of the samples is minimized. Concerning the cost, the single spin method is faster for production, which saves time of use of the team, being also competitive in terms of preparation time of commercial kits (Peterson & Reeves, 2014). In the current literature, no work has analyzed the safety of PRP produced manually in a course for physicians. A study from Peterson and Reeves (2014) dealt with the effectiveness of training, similar to the one used in this paper, reported a limitation of their results due to the lack of microbiological analysis of the hematological product produced. In our study we used a laminar flow cabinet and a different safety step-by-step procedure, and we had no contamination. On the other hand, Peterson and Reeves study (2014) analyzed platelet recovery, which is not necessary in our study, since this rate is already established in the literature (Harrison et al., 2020; Peterson & Reeves, 2014).

4. Results

Eleven students who took the training courses “Main methods of preparing the PRP” and “How to prepare the PRP safely”, were randomly selected from a group of 65 students, using the drawing method from opaque and sealed envelopes. All of them were carefully followed during the whole procedure (venipuncture, centrifuge sanitization, laminar flow cabinet sanitization and preparation of platelet-rich plasma), producing 22 PRP samples (duplicates), which in turn were sent to an independent analysis laboratory to conduct the identification and isolation of microorganisms through culture media. No microorganisms were identified and isolated from the samples, resulting in a contamination percentage of 0%, proving the effectiveness of the training and the method for producing PRP in a safe and sterile way.

5. Conclusion

The use of the protocols presented in the courses “Main PRP preparation methods” and “How to safely prepare PRP”, offered by CERT, confirmed our hypothesis that it is possible to manually prepare PRP free of contaminants, being safe for use in possible interventions where PRP is recommended, without the need of using commercial kits.

We suggest that further studies regarding the safety of manual preparation of PRP, when performed by trained health professionals, should be conducted so that this knowledge is further consolidated in the literature.

Fundings

This work was funded by CERT.

Conflict of interest

The authors declare receipt of fees as professors of the courses “Main methods of preparing the PRP” and “How to

prepare the PRP safely”. The authors did not receive any fees for authoring this article.

Limitations

Although it is known that the cost of manually prepared PRP is lower than the cost of commercial kits, our work did not analyze the cost of the material produced due to logistical problems, as the PRP was conducted in an academic environment. We suggest that further studies must be conducted on the cost of manual PRP preparation in Brazilian health system. Another limitation of our work is the lack of analysis of platelet recovery since the respective protocol is already sedimented in the literature.

References

- Berghoff, W. J., Pietrzak, W. S., & Rhodes, R. D. (2006). Platelet-rich plasma application during closure following total knee arthroplasty. *Orthopedics*, 29(7), 590–598.
- Castillo, T. N., Pouliot, M. A., Kim, H. J., & Drago, J. L. (2011). Comparison of growth factor and platelet concentration from commercial platelet-rich plasma separation systems. *The American journal of sports medicine*, 39(2), 266–271.
- Creaney, L., & Hamilton, B. (2008). Growth factor delivery methods in the management of sports injuries: the state of play. *British journal of sports medicine*, 42(5), 314–320.
- De Castro R. L. B., Antonio B. P., de Castro M. F. C., & de Castro G. C. (2020). Clinical improvement and signs of healing in the treatment of partial rupture of supraspinatus tendon using platelet rich plasma: A prospective study. *Sports medicine and rehabilitation journal*, 5(1): 1051.
- De Castro R. L. B., Antonio B. P., de Castro M. F. C., & de Castro G. C. (2020). Is the association of platelet rich plasma and hyaluronic acid useful for severe knee arthrosis?. *Fortune journal of rheumatology*. 090-106.
- De Carli, A., Volpi, P., Pelosini, I., Ferretti, A., Melegati, G., Mossa, L., Tornese, D., de Girolamo, L., & Scarpignato, C. (2009). New therapeutic approaches for management of sport-induced muscle strains. *Advances in therapy*, 26(12), 1072–1083.
- Di Martino, A., Boffa, A., Andriolo, L., Romandini, I., Altamura, S. A., Cenacchi, A., Roverini, V., Zaffagnini, S., & Filardo, G. (2022). Leukocyte-Rich versus Leukocyte-Poor Platelet-Rich Plasma for the Treatment of Knee Osteoarthritis: A Double-Blind Randomized Trial. *The American journal of sports medicine*, 50(3), 609–617.
- Gandhi, A., Dumas, C., O'Connor, J. P., Parsons, J. R., & Lin, S. S. (2006). The effects of local platelet rich plasma delivery on diabetic fracture healing. *Bone*, 38(4), 540–546.
- Grageda E. (2004). Platelet-rich plasma and bone graft materials: a review and a standardized research protocol. *Implant dentistry*, 13(4), 301–309.
- Gupta, V., Parihar, A. S., Pathak, M., & Sharma, V. K. (2020). Comparison of Platelet-Rich Plasma Prepared Using Two Methods: Manual Double Spin Method versus a Commercially Available Automated Device. *Indian dermatology online journal*, 11(4), 575–579.
- Hall, M. P., Band, P. A., Meislin, R. J., Jazrawi, L. M., & Cardone, D. A. (2009). Platelet-rich plasma: current concepts and application in sports medicine. *The Journal of the American Academy of Orthopaedic Surgeons*, 17(10), 602–608.
- Hammond, J. W., Hinton, R. Y., Curl, L. A., Muriel, J. M., & Lovering, R. M. (2009). Use of autologous platelet-rich plasma to treat muscle strain injuries. *The American journal of sports medicine*, 37(6), 1135–1142.
- Harrison, T. E., Bowler, J., Levins, T. N., Cheng, A. L., & Reeves, K. D. (2020). Platelet yield and yield consistency for six single-spin methods of platelet rich plasma preparation. *Platelets*, 31(5), 661–666.
- Hart, R., Safi, A., Komzák, M., Jajtner, P., Puskeiler, M., & Hartová, P. (2013). Platelet-rich plasma in patients with tibiofemoral cartilage degeneration. *Archives of orthopaedic and trauma surgery*, 133(9), 1295–1301.
- Instituto Brasileiro de Geografia e Estatística. (2021). Estimativas da população residente no Brasil e unidades da federação com data de referência em 1º de julho de 2021. Rio de Janeiro: IBGE.
- Jo, C. H., Roh, Y. H., Kim, J. E., Shin, S., & Yoon, K. S. (2013). Optimizing platelet-rich plasma gel formation by varying time and gravitational forces during centrifugation. *The Journal of oral implantology*, 39(5), 525–532.
- Kemmochi M. (2022). Consecutive injections of leukocyte-rich platelet-rich plasma are effective in not only mild but also severe knee degeneration. *Journal of orthopaedics*, 29, 31–37.
- Krief, S., Berrebi-Bertrand, I., Nagmar, I., Giret, M., Belliard, S., Perrin, D., Uguen, M., Robert, P., Lecomte, J. M., Schwartz, J. C., Finance, O., & Ligneau, X. (2021). Pitolisant, a wake-promoting agent devoid of psychostimulant properties: Preclinical comparison with amphetamine, modafinil, and solriamfetol. *Pharmacology research & perspectives*, 9(5), e00855.
- Marlovits, S., Mousavi, M., Gäbler, C., Erdős, J., & Vécsei, V. (2004). A new simplified technique for producing platelet-rich plasma: a short technical note. *European spine journal: official publication of the European Spine Society, the European Spinal Deformity Society, and the European Section of the Cervical Spine Research Society*, 13 Suppl 1(Suppl 1), S102–S106.

- Menetrey, J., Kasemkijwattana, C., Day, C. S., Bosch, P., Vogt, M., Fu, F. H., Moreland, M. S., & Huard, J. (2000). Growth factors improve muscle healing in vivo. *The Journal of bone and joint surgery. British volume*, 82(1), 131–137.
- Peterson, N.S., & Reeves, K.D. (2014). Efficacy of one day training in low-cost manual preparation of high cellular platelet rich plasma. *Journal of prolotherapy*, 6:e922-e927.
- Oh, J. H., Kim, W., Park, K. U., & Roh, Y. H. (2015). Comparison of the Cellular Composition and Cytokine-Release Kinetics of Various Platelet-Rich Plasma Preparations. *The American journal of sports medicine*, 43(12), 3062–3070.
- Sánchez, M., Anitua, E., Azofra, J., Andía, I., Padilla, S., & Mujika, I. (2007). Comparison of surgically repaired Achilles tendon tears using platelet-rich fibrin matrices. *The American journal of sports medicine*, 35(2), 245–251.
- Sanchez, M., Anitua, E., Cugat, R., Azofra, J., Guadilla, J., Seijas, R., & Andia, I. (2009). Nonunions treated with autologous preparation rich in growth factors. *Journal of orthopaedic trauma*, 23(1), 52–59.
- Sánchez, M., Fiz, N., Azofra, J., Usabiaga, J., Aduriz Recalde, E., Garcia Gutierrez, A., Albillos, J., Gárate, R., Aguirre, J. J., Padilla, S., Orive, G., & Anitua, E. (2012). A randomized clinical trial evaluating plasma rich in growth factors (PRGF-Endoret) versus hyaluronic acid in the short-term treatment of symptomatic knee osteoarthritis. *Arthroscopy: the journal of arthroscopic & related surgery: official publication of the Arthroscopy Association of North America and the International Arthroscopy Association*, 28(8), 1070–1078.
- Slater, M., Patava, J., Kingham, K., & Mason, R. S. (1995). Involvement of platelets in stimulating osteogenic activity. *Journal of orthopaedic research: official publication of the Orthopaedic Research Society*, 13(5), 655–663.
- United States Pharmacopeia Convention. (2008). 797 Guidebook to pharmaceutical compounding-sterile preparations.
- Werner, S., & Grose, R. (2003). Regulation of wound healing by growth factors and cytokines. *Physiological reviews*, 83(3), 835–870.
- Whitman, D. H., Berry, R. L., & Green, D. M. (1997). Platelet gel: an autologous alternative to fibrin glue with applications in oral and maxillofacial surgery. *Journal of oral and maxillofacial surgery: official journal of the American Association of Oral and Maxillofacial Surgeons*, 55(11), 1294–1299.
- Ziegler, C. G., Van Sloun, R., Gonzalez, S., Whitney, K. E., DePhillipo, N. N., Kennedy, M. I., Doman, G. J., Evans, T. A., Huard, J., & LaPrade, R. F. (2019). Characterization of Growth Factors, Cytokines, and Chemokines in Bone Marrow Concentrate and Platelet-Rich Plasma: A Prospective Analysis. *The American journal of sports medicine*, 47(9), 2174–2187.