DIYPRP Leukocyte-poor PRP Documentation

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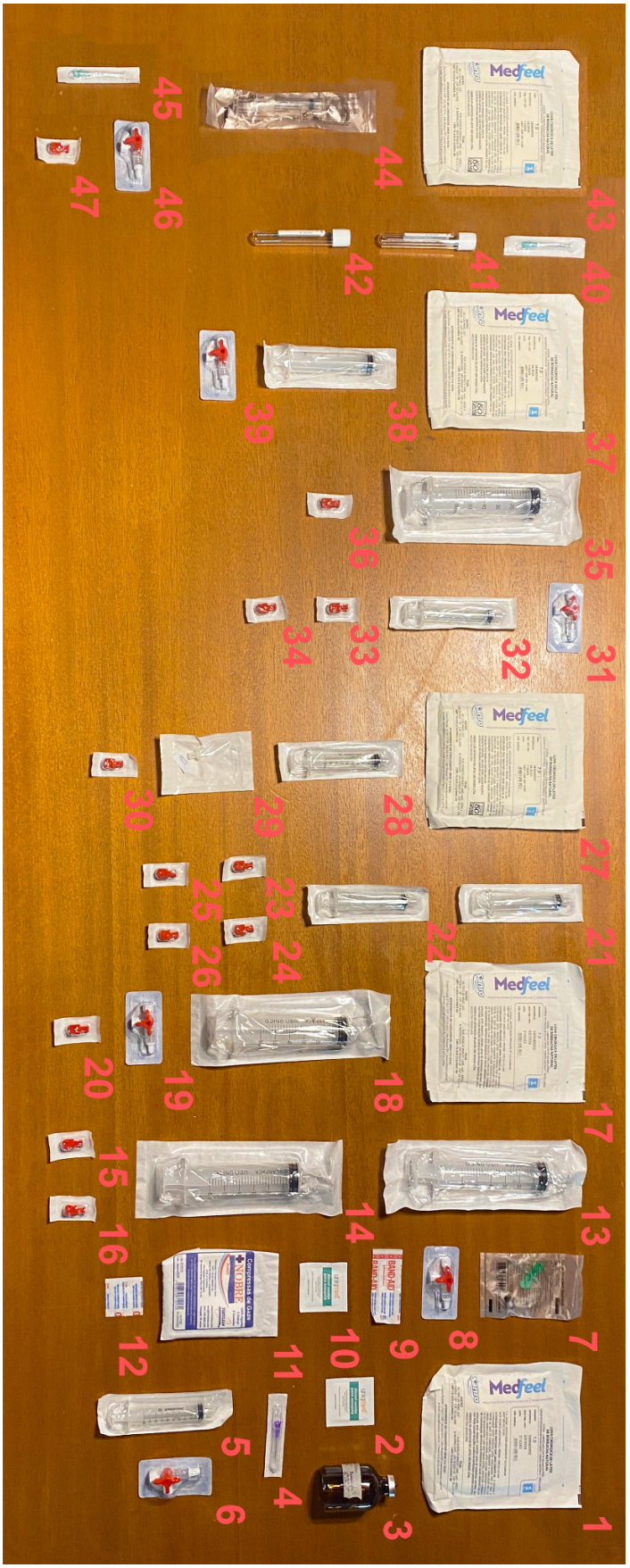
Double Spin & Pellet LP-PRP Preparation
www.DIYPRP.com

NOTE: This documentation is a work-in-progress and shall be expanded upon and modified continuously.

Leukocyte-poor PRP Preparation Materials



Preparation Materials

1. Sterile Gloves
2. Sterile Wipe
3. ACD-A Anticoagulant Solution
4. 20 x 0.55 mm (24G x 3/4) Needle
5. 10 mL Leur Lock Syringe
6. Three-way Stopcock Valve
7. 20 x 0.80 mm (21G x 0.75) Butterfly Scalp Needle
8. Three-way Stopcock Valve
9. Band-aid (Medium)
10. Sterile Wipe
11. Sterile Gauze
12. Band-aid (Small)
13. 60 mL Leur Lock Syringe
14. 60 mL Leur Lock Syringe
15. Leur Lock Cap
16. Leur Lock Cap
17. Sterile Gloves
18. 60 mL Leur Lock Syringe
19. Three-way Stopcock Valve
20. Leur Lock Cap
21. 10 mL Leur Lock Syringe
22. 10 mL Leur Lock Syringe
23. Leur Lock Cap
24. Leur Lock Cap
25. Leur Lock Cap
26. Leur Lock Cap
27. Sterile Gloves
28. 10 mL Leur Lock Syringe
29. Leur Lock-to-Leur Lock Transfer Connector
30. Leur Lock Cap
31. Three-way Stopcock Valve
32. 10 mL Leur Lock Syringe
33. Leur Lock Cap
34. Leur Lock Cap
35. 60 mL Leur Lock Syringe
36. Leur Lock Cap
37. Sterile Gloves
38. 20 mL Leur Lock Syringe
39. Three-way Stopcock Valve
40. 30 x 0.80 mm (21G x 1 1/4) Needle
41. 9 mL Additive-Free Vacutainer
42. 9 mL Additive-Free Vacutainer
43. Sterile Gloves
44. 3 mL Leur Lock Syringe
45. 30 x 0.80 mm (21G x 1 1/4) Needle
46. Three-way Stopcock Valve
47. Leur Lock Cap

Additional Items (not pictured):

1. Tourniquet
2. Paper Towels
3. Permanent Marker
4. Shears
5. 60 mL Buckets
6. 20 mL Buckets + Inserts
7. Vortex Mixer
8. Cup with water
9. Scale
10. Tube Rack

LP-PRP PREPARATION INSTRUCTIONS

1. Ensure the workspace is clean and sterilized.
2. Layout all items to be used in the procedure.
3. Verify the integrity of centrifuge buckets in the centrifuge. The 60 mL syringe buckets are used first, and only two of them. Verify that the 20 mL buckets are standing by.
4. Put on sterile gloves (1).
5. Open the gauze packet (11).
6. Take sterile wipe (2) and clean the lid of the ACD-A solution (3).
7. Take the 24G needle (4) and attach it to the 10 mL syringe (5). Proceed to prime the syringe.
8. Proceed to collect **7.5 mL** of ACD-A anticoagulant. Remove and cap the needle.
9. Attach the three-way stopcock valve (6) to the 10 mL ACD-A collection syringe (5).
10. Prime and attach the first 60 mL syringe (13) to the assembled three-way stopcock valve.
11. Using the first 60 mL syringe, proceed to collect **3.75 mL** of ACD-A anticoagulant from the 10 mL syringe.
    a. Use a **1:8 ratio** of ACD-A to whole blood. (**3.75 mL** of ACD-A to **30 mL** of whole blood)
12. Remove the 60 mL syringe and set aside.
13. Prime and attach the second 60 mL syringe (14) to the assembled three-way stopcock valve.
14. Proceed to collect the remaining **3.75 mL** of ACD-A anticoagulant from the 10 mL syringe.
    a. Use a **1:8 ratio** of ACD-A to whole blood. (**3.75 mL** of ACD-A to **30 mL** of whole blood)
15. Remove the 60 mL syringe and set aside.
16. Prepare the blood collection assembly by attaching the collection butterfly needle (7) to the three-way stopcock valve (8).
17. Remove the leur-lock caps from the three-way stopcock valve (8) and attach the two 60 mL syringes (13) (14) to the device.
18. Set the three-way stopcock valve to collect to the in-series syringe first.

19. Attach the bandaid **(9)** across the wings of the butterfly needle to assist in securing the wings against the skin.

20. Prime the collection tube by pushing the ACD-A anticoagulant from the in-series syringe to the tip of the butterfly needle.

21. Attach the tourniquet to the left bicep and form a fist. Allow the veins to engross.

22. Fold the wings of the butterfly needle inward and then proceed to insert it into the left cephalic vein and fold the wings down allowing the bandaid to stick to the skin.

23. Remove the tourniquet.

24. Begin to collect blood into the first syringe (in-series). **1 mL** per second at max rate. Upon full collection to **34 mL**, gently rotate the syringe a few times to mix the anticoagulant being careful not to bend the collection tube much.

25. Switch the three-way stopcock valve to collect from the unused syringe now.

26. Begin to collect blood into the second syringe (perpendicular). **1 mL per second** at the rate. Upon full collection to **34 mL**, gently rotate the syringe a few times to mix the anticoagulant being careful not to bend the collection tube much.

    a. If intending to collect a sample, collect 35 mL of whole blood.

27. Remove the butterfly needle from the arm and cover with the gauze **(11)** on removal.

28. Place a bandaid **(12)** on the wound.

29. Remove the syringe (in-series) from the three-way stopcock valve and then attach the leur lock cap **(15)**. Gently mix the syringe one more time and set aside.

30. Remove the syringe (perpendicular) from the three-way stopcock valve and then attach the leur lock cap **(16)**. Gently mix the syringe one more time and set aside.

    a. If collecting a 1 mL sample of whole blood, remove the syringe (perpendicular) from the three-way stopcock valve and attach a 21G needle.

    b. Release the vacuum of the vacutainer.

    c. Proceed to collect a 1 mL sample of whole blood into the vacutainer.

    d. Remove the needle and attach the leur lock cap. Gently mix the syringe one more time. Set aside by the centrifuge.

31. Proceed to gently perform the syringe modification using the gardening shears.

32. Set both syringes side-by-side and take a photo of both syringes showing the level of the whole blood within the syringe.

33. Place a rubberband on each syringe at the 12 mL mark.

34. Place a rubberband on each syringe at the 62 mL mark.

35. Place an internal plunger standoff in each syringe.
36. Proceed to place the two syringes into the 60 mL centrifuge buckets and tighten the bucket screws, then secure the lid. Proceed to the first spin configuration.
37. Spin 1) The **Rmax** of the plunger will be **118 mm**. Spin at **2,700 RPM (960 RCF)** for **14 minutes**.
38. Replace gloves with new gloves **(17)**.
39. Upon ceasing of the spinning, carefully remove both syringes and set aside.
40. Allow the syringes to set for **10 minutes**, allowing for the platelets to settle.
41. Set both syringes side-by-side and take a photo of both syringes showing the level of the separated components within the syringe.
    a. Note: Expected result is about a 50/50 split of RBCs and PPP.
42. Prepare the 60 mL syringe **(18)** by opening the packaging and priming the syringe.
43. Open the three-way stopcock valve **(19)**, attaching it to the 60 mL primed syringe and remove the cap from the first 60 mL spun syringe.
44. Attach the assembled syringe-three-way-stopcock-valve combination to the first spun 60 mL syringe.
45. Proceed to aspirate the PPP plasma from the spun syringe, leaving **4 mL** of plasma above the erythrocyte interface.
46. Remove the 60 mL syringe of aspirated PPP together with the three-way-stopcock valve from the 60 mL spun syringe.
47. Unscrew the leur-lock cap of the second spun 60 mL syringe and proceed to attach the PPP aspiration assembly to the second spun syringe.
48. Proceed to aspirate the PPP plasma from the second spun syringe, leaving **4 mL** of plasma above the erythrocyte interface.
49. Remove the PPP aspiration 60 mL syringe from the assembly, and proceed to attach the leur-lock cap **(20)**.
    a. If collecting a 1 mL sample of PPP, attach a 21G needle.
    b. Release the vacuum of the vacutainer.
    c. Proceed to collect a 1 mL sample of PPP into the vacutainer.
    d. Remove the needle and attach a leur lock cap.
50. Prepare a new 10 mL syringe **(21)** by opening the packaging and priming the syringe.
51. Gently attach this 10 mL primed syringe to the assembled three-way stopcock valve and spun syringe combination.

52. Using the 10 mL syringe, collect the remaining **PPP (1-3 ml)**, buffy coat, and **7 ml of WB (10 mL total)**.
53. Remove the 10 mL syringe from the assembly, and proceed to attach the leur-lock cap **(23)**. Set the syringe aside.
54. Prepare a new 10 mL syringe **(22)** by opening the packaging and priming the syringe.
55. Gently attach this 10 mL primed syringe to the assembled three-way-stopcock valve and spun syringe combination.
56. Transfer the syringe and three-way stopcock valve assembly to the second spun 60 mL syringe.
57. Using the 10 mL syringe, collect the remaining **PPP (1-3 ml)**, buffy coat, and **7 ml of WB (10 mL total)**.
58. Remove the 10 mL syringe from the assembly, and proceed to attach the leur-lock cap **(24)**. Set the syringe aside.
59. Remove the three-way-stopcock valve from the 60 mL spun syringe.
60. Place a leur-lock cap **(25)** **(26)** on each 60 mL spun syringe.
61. Proceed to homogenize the blood via a vortex mixer.
62. Proceed to gently modify both syringes to fit within the buckets.
63. Set the 10 mL syringes of whole blood on the table and take a photo of the syringes showing the levels of the components within each syringe.
64. Proceed to place both syringes into the 20 mL centrifuge buckets and verify the bucket screws are tight.
65. Spin 2) **Rmax** of **170 mm** at **1,260 RPM (300 RCF)** for **7 minutes**.
66. Replace gloves with new gloves **(27)**.
67. Upon ceasing of the spinning, remove the 10 mL syringes that contains the PRP then set aside.
68. Take a photo of the syringes showing the level of the separated components within each syringe.
    a. Expected outcome is about **1-3 mL of prepared PRP** in each 10 mL syringe.
69. Prepare a 10 mL syringe **(28)** by opening the packaging and priming the syringe.
70. Open the two-way leur-lock-to-leur-lock transfer device **(29)**, attach it to the new 10 mL syringe.
71. Attach the two-way leur-lock-to-leur-lock transfer device and 10 mL syringe assembly to the 10 mL syringe of PRP.

72. Proceed to aspirate the PRP to the new 10 mL syringe.
    a. Note: For a true LP-PRP, stop just before the erythrocyte interface.
    b. Note: For a LR-PRP, collect into the buffy coat.
73. Remove the 10 mL PRP syringe from the assembly and attach a leur-lock cap **(30)**. Set aside.
    a. If collecting a 0.2 mL sample of PRP, attach a 21G needle.
    b. Release the vacuum of the vacutainer.
    c. Proceed to collect a 1 mL sample of PRP into the vacutainer.
    d. Remove the needle and attach a leur lock cap.

PELLET PREPARATION

74. From the two original 60 mL spun syringes, remove the leur-lock caps.

75. Attach the first 10 mL spun syringe and two-way leur-lock-to-leur-lock transfer device assembly to the first spun 60 mL syringe, and transfer the remaining contents to the 60 mL syringe.

76. Remove the 10 mL syringe from the assembly, ensuring to leave the two-way leur-lock-to-leur-lock transfer device attached to the 60 mL syringe.

77. Attach the second 10 mL spun syringe to the two-way leur-lock-to-leur-lock transfer device and the first spun 60 mL syringe assembly, and transfer the remaining contents to the 60 mL syringe.

78. Attach the second 60 mL spun syringe to the first 60 mL spun syringe assembly, and transfer the contents to the 60 mL syringe.

79. Remove the empty 60 mL syringe and two-way leur-lock-to-leur-lock transfer device from the 60 mL syringe of whole blood.

80. Using the 60 mL syringe of PPP, remove the leur-lock cap and attach a fresh three-way stopcock valve **(31)**.

81. Attach a freshly primed 10 mL syringe **(32)** to the three-way stopcock valve assembly for creating the Autologous Plasma Contrast Technique (APCT) PPP.

82. Aspirate 3 mL of APCT PPP into the 10 mL syringe.

83. Detach the 10 mL syringe of APCT PPP and attach a leur-lock cap **(33)** and set aside.

84. With the combined 60 mL PPP syringe and three-way stopcock valve assembly, attach it to the 60 mL syringe of whole blood and transfer the PPP into the syringe of whole blood.

85. Remove the new recombined PPP + RBC 60 mL syringe from the three-way stopcock valve and attach a fresh leur-lock cap **(34)**.

    a. If collecting a 1 mL sample of whole blood, roceed to attach the vortex mixing cap and homogenize the blood at 850 RPMs.

    b. Detach the leur lock cap and attach a 21G needle.

    c. Release the vacuum of the vacutainer.

    d. Proceed to collect a 1 mL sample of whole blood into the vacutainer.

    e. Remove the needle and attach a leur lock cap.

86. Using the scale, weigh the 60 mL syringe of whole blood, and create an equally weighted counterweight using a 60 mL syringe (35) and the leur-lock cap (36), modifying the syringes and confirming the final weight.

87. Proceed to homogenize the blood via a vortex mixer.

88. Set the 60 mL syringe of whole blood on the table and take a photo of the syringe showing the levels of the components within the syringe.

89. Place a rubberband on each syringe at the 12 mL mark.

90. Place a rubberband on each syringe at the 62 mL mark.

91. Place an internal plunger standoff in each syringe.

92. Proceed to place both syringes into the 60 mL centrifuge buckets and verify the bucket screws are tight.

93. Spin 3) **Rmax** of **165 mm** at **1,500 RPM (420 RCF)** for **7 minutes**.

94. Replace gloves with new gloves (37).

95. Upon ceasing of the spinning, remove the 60 mL syringe that contains the supernatent PRP solution then set to the side.

96. Take a photo of the syringe showing the level of the separated components within the syringe.

97. Prepare a 20 mL syringe (38) by opening the packaging and priming the syringe.

98. Attach a three-way stopcock valve (39) to the 20 mL syringe.

99. Attach the 20 mL syringe and three-way stopcock valve assembly to the 60 mL syringe and aspirate the supernatent PRP solution into the 20 mL syringe, leaving **1-2 mL** behind.

100. Remove the 20 mL syringe of PRP from the assembly and attach a 21G needle (40).

101. Proceed to equally distribute the PRP solution between two 9 mL glass vacutainer tubes (41) (42), setting them aside in the tube storage rack. Cap the needle and set the needle/syringe assembly aside within the sterile field.

102. Take a photo of the two 9 mL vacutainers showing the levels of the internal components.

103. Remove the 60 mL centrifuge buckets and place the 20 mL centrifuge buckets with holder inserts.

104. Place the 9 mL vacutainers into the centrifuge buckets and prepare to spin.

105. Spin 4) **Rmax** of **185 mm** at **1,700 RPM (600 RCF)** for **10 minutes**.

106. Replace gloves with new gloves (43).

107. Upon ceasing of the spinning, remove the two 9 mL vacutainers which contain the pellets and then place them in the tube storage rack.

108. Take a photo of the 9 mL vacutainer tubes showing the levels of the separated components within the tubes.

109. Reutilizing the 20 mL syringe/needle assembly, insert the needle into each 9 mL vacutainer tube, proceed to aspirate the supernatent PPP to the 20 mL syringe, leaving behind 0.5 mL of plasma in each tube and then cap the needle.

110. Using the remaining plasma in each tube, gently homogenize the platelet pellets back into a supernatent PRP suspension.

111. Open a new 3 mL syringe **(44)** and prime the syringe. Attach the 21G needle **(45)** to the 3 mL syringe.

112. Collect the homogenized PRP from each vacutainer into the 3 mL syringe.

113. Detach the 21G needle from the 3 mL syringe, and attach a new three-way stopcock valve **(46)** to the 3 mL syringe.

114. Remove the leur-lock cap from the 10 mL syringe of originally prepared PRP and attach the 3 mL syringe and three-way stopcock valve assembly to the 10 mL syringe of PRP.

115. Proceed to transfer the PRP contents of the 3 mL syringe into the 10 mL syringe of PRP.

116. Remove the assembly and attach a new leur-lock cap **(47)** to the 10 mL syringe of PRP.

    a. If collecting a 0.2 mL sample of PRP, attach a 21G needle.

    b. Release the vacuum of the vacutainer.

    c. Proceed to collect a 1 mL sample of PRP into the vacutainer.

    d. Remove the needle and attach a leur lock cap.