ISSN 1507-661X

DERMATOLOGIA ESTETYCZNA

PERFORMANCE EVALUATION OF THREE **PRP/PRF KITS**

2018, vol. 20, 5-6 (118-119): 281-288

STUDY DESIGN

This study compares three different PRP/PRF kits: EZPRF kit, PRP kit from Provider A and PRP kit from Provider B, along with their designated centrifuges. For EZPRF tubes, 2 different centrifugation speeds were tested: 700RPM and 900RPM. For each test scenario, baseline (whole blood) and PRP/PRF platelet count, white cell count, red cell count and volume will be measured. The fibrinogen concentration in the PRP/PRF will be measured. These measurements will be used to compare several important metrics, including platelet count, platelet enrichment ratio, platelet harvest ratio, WBC count, monocyte count and fibrinogen concentration.

Due to procedural limitation, this study only used blood from five healthy donors.

STUDY CASE	GEL/ NO GEL	RPM	RCF (xg)	TIME AT TOP SPEED
EZPRF+700 RPM	NO GEL	700	60	5 MIN
EZPRF+900 RPM	NO GEL	900	100	5 MIN
PRP PROVIDER A	GEL	3500	1500	9 MIN
PRP PROVIDER B	GEL	3500	1500	10 MIN

Table 1. The centrifugation cycles of test cases

EQUIPMENT AND PROTOCOL

An Abbott Cell-Dyn® Emerald hematology analyzer was used to measure the RBC, WBC with 3-part differential, platelet count of the whole blood and PRP/PRF.

The fibrinogen concentration was obtained by:

1. Measuring total protein concentration of PRP using total protein assay by diluting 150uL sample in 3000uL total protein reagent (Total Protein Reagent with Biuret Method, Sigma Life Science), incubating 10min, and reading in Cecil CE2021 Spectrophotometer at 460nm. The absorbance at 460nm was compared to standard absorbance curve obtained using protein concentration standards (Protein Standard Set, Sigma-Aldrich), and total protein concentration was obtained using linear interpolation.
2. Precipitating out fibrinogen.
3. Removing fibrinogen by centrifugation at 3200g for 3min and collecting the supernatant.
4. Measuring total protein concentration of the supernatant using total protein assay.
5. Calculating the fibrinogen concentration by taking the difference between the total protein concentration of PRP and the supernatant.

For each donor, venipuncture was made. Two EZPRF tubes, one Provider A tube and one Provider B tube were filled. The tubes were marked for volume measurement later. The cell counts were obtained for each of the four tubes immediately after manual inversion. Each tube was centrifuged according to respective protocol, tabulated in Table 1. After centrifugation, PRP or PRF was extracted according to tube insert. Cell counts were obtained from each of the extracted PRP or PRF samples. 1mL to 2mL of the PRP/PRF was transferred out to a 2mL micro centrifuge tube. 150uL was taken from each tube to perform total protein assay. They were incubated in a water bath at 60°C for 3min, followed by 3min centrifugation at 3200g. After centrifugation, 150uL was taken from supernatant and total protein assay was performed. The fibrinogen concentration was then calculated.

RESULTS

1. Enrichment of Platelets

For each sample, the platelet concentration of the PRP or PRF was tested. As seen in Fig. 1, the EZPRF tube centrifuged at 700RPM and 900RPM have significant higher platelet concentration than Provider A PRP kit and Provider B PRP kit.

Another metric researchers and doctors have been using to characterize platelet enrichment of the PRP/PRF is fold of increase from the baseline platelet count. The baseline refers to the platelet count of the whole blood. As in Fig. 2, the EZPRF tube produced around 2.1-fold platelet concentration from the baseline whole blood, while the Provider B tube has 1.2x and 0.5x (decreased) for Provider A.

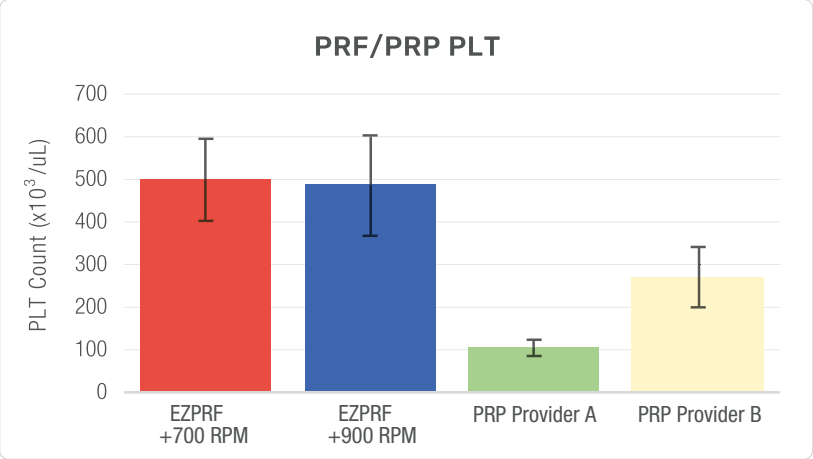

Figure 1. The platelet concentrations in the obtained PRP or PRF (MEAN±SD)

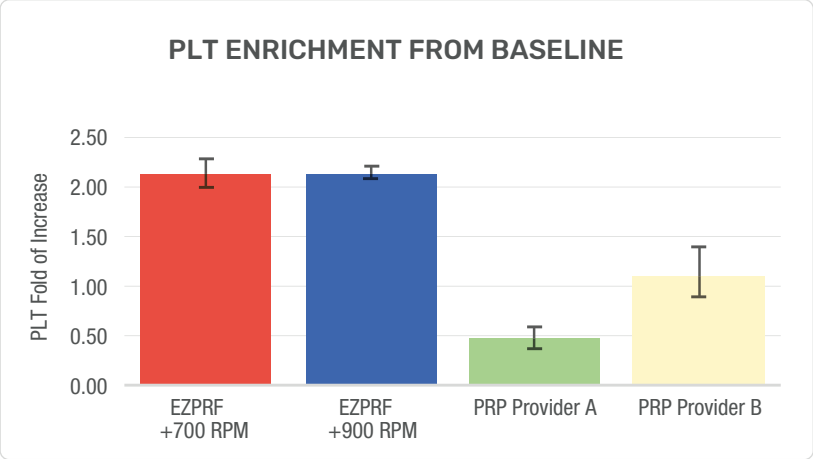

Figure 2. The enrichments of platelet (fold increase) from the Baseline (whole blood) (MEAN±SD)

2. Harvest Ratio of Platelets

For different methods of making PRP/PRF, harvest ratio tells how much total platelets are being captured in the PRP/PRF. The harvest ratio is calculated by the following equation and the ratio should be between 0% and 100%. The harvest ratios are plotted in Fig. 3.

$$(PLT_{PRP|PRF} \times Volume_{PRP|PRF})/(PLT_{Whole\ Blood} \times Volume_{Whole\ Blood})$$

The EZPRF centrifuged at 900rpm has the highest harvest ratio of 51%. The Provider A has the lowest harvest ratio of 26%.

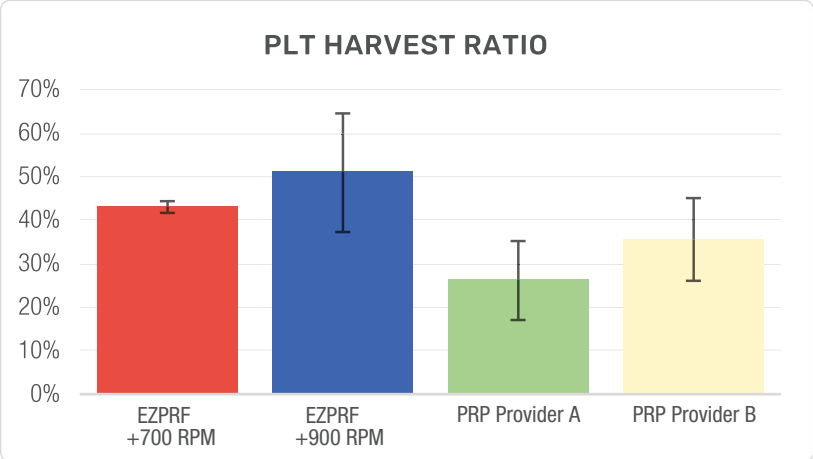

Figure 3. Platelet harvest ratios (MEAN±SD)

3. Fibrinogen in PRP/PRF

Fibrinogen concentrations of obtained PRP/PRF were plotted in Fig. 4. All PRP/PRF kits increased the fibrinogen concentration from whole blood. EZPRF Tube centrifuged at 900RPM produced the highest fibrinogen concentration, over 2300mg/dL. Provider B tube has the lowest fibrinogen concentration around 670mg/dL. For reference, the normal fibrinogen concentration in whole blood ranges from 150mg/dL to 400mg/dL.

It was also observed, between the two EZPRF tubes, the one centrifuged at 900RPM formed fibrin clot much faster than the one centrifuged at 70RPM. This may be attributed to the higher concentration of the fibrinogen.

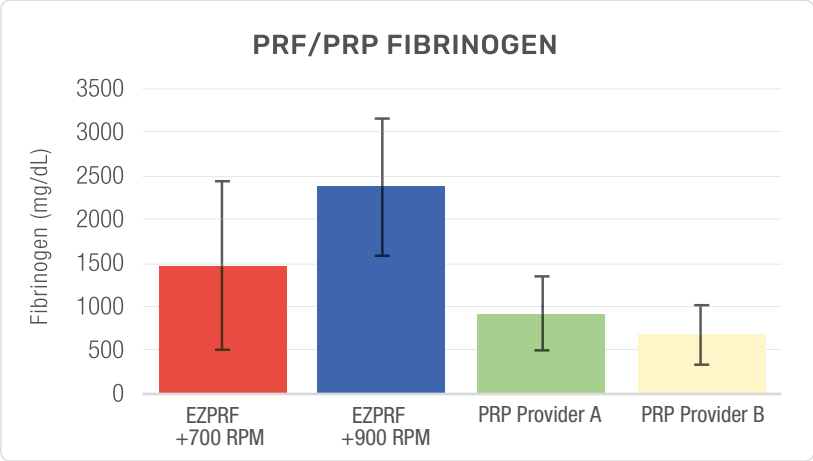

Figure 4. The Fibrinogen concentrations of the PRP/PRF (MEAN±SD)

4. WBC in PRP/PRF

The WBC counts of the PRP/PRF were obtained and plotted in Fig. 5. EZPRF tubes have significantly higher WBC count, while Provider A and Provider B tubes reduced WBC from the baseline. For reference, the normal range for human WBC count in whole blood ranges from $4.5x10^3$/uL to $11.0x10^3$/uL

The standard deviations of WBC count are also much higher in the EZPRF tubes. This is due to techniques of extracting PRP/PRF. The EZPRF tube does not use gel separator as in Provider A tube or Provider B tube, user is free to extract more or less of the buffy in the centrifuged blood tube. This subjectivity will impact the WBC in the PRP/PRF, thus demands proper training for consistency.

The monocyte counts of the PRP/PRF were plotted in Fig. 6. The normal range for monocyte in human whole blood is between 0.2 $x10^3$/uL and 1.0 $x10^3$/uL.

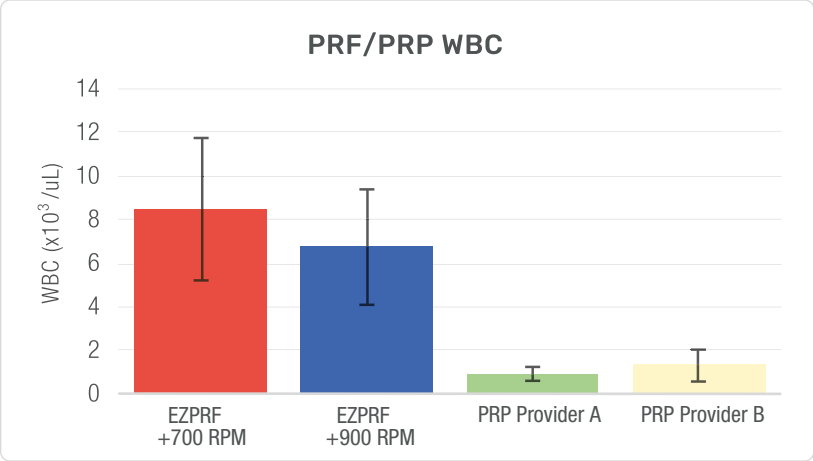

Figure 5. The WBC concentrations in the PRP/PRF (MEAN±SD)

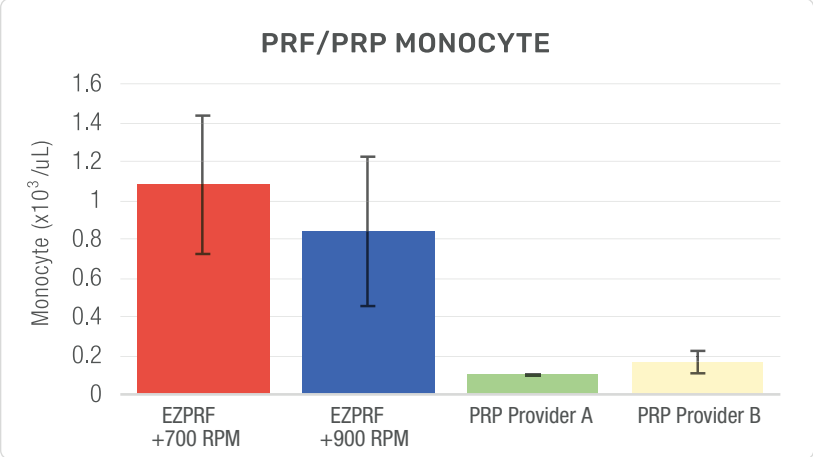

Figure 6. The Monocyte counts in the PRP/PRF (MEAN±SD)

5. RBC in PRP/PRF

There are more RBC retained in the EZPRF tubes, although they are neglectable (less than 4% of normal RBC count) when compared to normal RBC count, as in Fig. 7. The standard deviations of RBC count are also higher for EZPRF tubes, for the same reason as the larger variation of the WBC count.

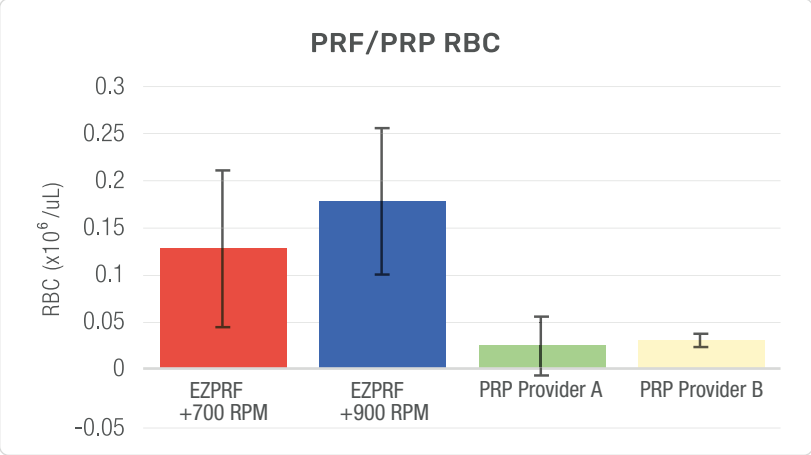

Figure 7. The RBC counts in the PRP/PRF (MEAN±SD)

6. Volume of Obtained PRP/PRF

With the assistance of gel and higher centrifugation force, Provider A kit capture more volume than other PRP kits, although at a lower platelet count. Both EZPRF tubes and Provider B tubes have smaller volume but higher platelet count. Their variations are higher as well, due to subjectivity of extracting the PRP/PRF after centrifugation.

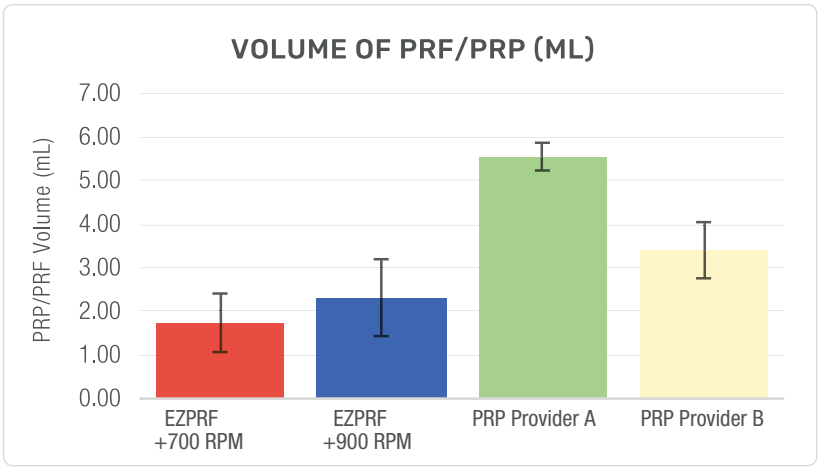

Figure 8. The volumes of PRP/PRF extracted (MEAN±SD)

CONCLUSION AND DISCUSSION

Because of the lack of well-recognized corrections between different metrics of PRP/PRF with the therapeutic outcomes, doctors are free to choose what parameters are more important for their own practice.

This study has observed consistently higher platelet concentration and ratio of harvest in the EZPRF tubes than Provider A and Provider B. The EZPRF tubes also capture more WBC and Fibrinogen. Provider A PRP kit captures the least amount of WBC and RBC, but also the lowest in this study regarding the platelet.

1. Gel or no gel

In the PRP/PRF kits tested, both Provider A and Provider B use gel as the primary means of separation, while EZPRF doesn't use gel. The gel does make the extracting step much easier because the PRP can be poured out of the gel tube without the need for pipetting. It is also more consistent and less subjective, although this also reduced the flexibility for some expert users. There was small, but visible, amount of gel coming into the PRP/PRF samples. The safety concern is unknown because the gels are mostly proprietary.

2. Red Cell Content in PRP/PRF

The red blood cell counts are higher in EZPRF tubes than Provider A or Provider B tubes. This is again due to the lack of more solid gel barrier. If compared with RBC count in normal human, the EZPRF tube had 2%-3% of the normal RBC concentration, and less than 1% for Provider A and Provider B tubes. The benefit or risk has not been assessed thoroughly, although most surgeons may prefer less RBC in their PRP/PRF samples.

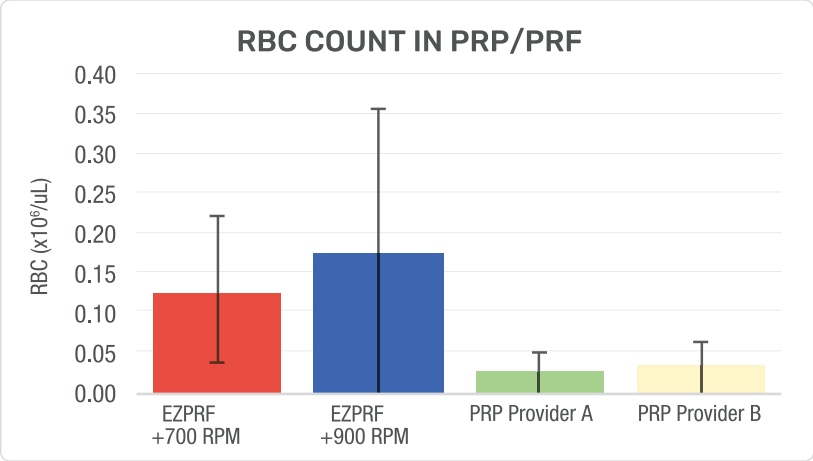

Figure 9. The red cell count in PRP/PRF (MEAN±SD)

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