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# The Use of Hydroxy-Ethyl Starch Sedimentation for Autologous Buffy Coat Preparation

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**Abstract:** The use of hydroxy-ethyl starch (HES) has been used in the cord blood banking industry for the separation of blood into its individual components. The focus of this study is to examine whether the use of HES is feasible in obtaining a buffy coat for the use in the construction of platelet gels. Blood was collected from seven canines into anticoagulant citrate dextrose at a ratio of 1:8. A sample of whole blood was used to obtain initial cell counts and a base line Thrombelastograph® (TEG®). 6% Hespan was added to the whole blood and allowed to sediment for 60 minutes. Cell counts and TEG®s were performed on the resulting layers: red blood cells (RBCs), buffy coat (BC), and plasma. The results of this study (see Table 1) suggest that the gravity sedimentation of whole blood with HES can significantly reduce the RBCs in the BC and plasma layers while increasing the platelet count in the plasma layer. These data also suggest that HES does not affect the clotting capabilities of the BC and plasma layers. **Keywords:** buffy coat, platelet gel, hetastarch, Thrombelastograph® *JECT. 2005;37:311–314*

The growing market for platelet gels in recent years has given rise to many different mechanisms for of producing platelet-rich plasma (PRP) that is used in the construction of the platelet gel. These gels are used in the aid of wound healing after different types of surgeries, for example, face lifts, reduction mammaplasties, abdominoplasties, skin grafts, bone grafts, and dressing of diabetic ulcers (1,2).

Hydroxy-ethyl starch (HES) is readily used in the blood banking industry for its red blood cell sedimentation properties (1). HES coats and helps in the agglutination of red blood cells (RBCs). The agglutination and the viscosity of the HES create a density gradient between the whole blood components (e.g., RBCs, white blood cells [WBC], platelets [Plts], plasma) (3,4). Over time, the heavier RBCs are pulled downward by gravitational forces, thereby separating the RBCs from the lighter-weight components, such as WBCs and Plts. Typically, blood banking procedures require the sample/collected product to be spun in a centrifuge to enhance the separation process, but current studies have been done that dispute the need to spin the product (5).

This study examines whether the HES sedimentation procedure is capable of producing a buffy coat (BC) to use in the construction of platelet gels and create a simple procedure to obtain PRP for use when there is no access to machines to separate blood.

## MATERIALS AND METHODS

The following procedure was conducted at The Ohio State University Circulation Technology Department canine laboratory. All animals used in this study received humane care in compliance with the "Guide for the Care and Use of Laboratory Animals," published by the National Institutes of Health. This experiment was conducted on seven canine during the fall of 2003. Before the collection of blood, a 20-mL syringe was filled with anticoagulant citrate dextrose (ACD, Baxter, Deerfield, IL) to coat the inside of the syringe. The ACD was then aspirated out of the syringe until 2 mL of ACD remained in the syringe. Sixteen cubic centimeters (mL) of blood was collected, resulting in a ratio of 8:1 blood to ACD, from the arterial line of the canine, before chest incision. The blood was then mixed with the ACD by drawing up 1 mL of air into the syringe. The syringe was then inverted six times to ensure good mixing. The air was then aspirated from the syringe along with 3 mL of the ACD/whole blood mixture. This sample was used to obtain an initial hematocrit (Hct),

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WBC count, platelet count, and a Thrombelastograph® (TEG, Haemoscope Corporation, Niles, IL). Next, 3 mL of 6% Hespan™ (HES, Baxter, Deerfield, IL) were added to the 15 mL of ACD/whole blood resulting in a 1.2% HES in the ACD/whole blood/HES solution. This solution was mixed by inverting the syringe six times. The syringe was then allowed to sit motionless, needle port down, for 60 minutes to allow the blood solution to separate by gravity.

After the blood was allowed to separate by gravity the resulting layers—RBC, BC, and plasma —were aliquoted out into separate test tubes and sent for cell counts on RBCs, WBCs, Plts, and TEG analysis. The amount removed for each sample was calculated with the following calculations. The RBC volume to be removed for analysis was calculated by taking the canine's initial hematocrit and multiplying by the remaining 15 mL of blood; this is the amount of whole blood left in the syringe after removing the baseline sample.

$$\text{Fraction baseline Hct.} \times 15\text{ mL whole blood} = \text{estimated RBC volume to be removed}$$

The BC volume to be removed for analysis was calculated by adding the baseline hematocrit to 55%, average plasma water volume present in humans (6), and subtracting this sum from 100%. This gave us an estimated percentage of what the BC would be of the final volume. This percentage was then multiplied by the 15 mL of the initial whole blood solution to give us the amount of volume we should remove for the BC. The calculation for the BC is as follows:

$$100\% - (\text{fraction baseline Hct.} + 55\%) = \text{estimated BC\%}$$

$$\text{estimated BC\%} \times 15\text{cc whole blood} = \text{estimated BC volume to be removed}$$

The remaining volume in the syringe was then aliquoted into a test tube to be used as the plasma layer. Next, TEG analysis was performed on the whole blood sample, RBC layer, BC layer, and plasma layer according to the Thrombelastograph® 5000 Hemostasis Analyzer protocol. The 340 μL of the whole blood sample and the RBC sediment sample were placed in the cuvette, and then 20 μL of 0.2 M $CaCl_2$ were added to initiate the clotting of the samples. After the $CaCl_2$ was added, the TEG® 5000 Hemostasis Analyzer was started. For the BC and plasma layer samples, 330 μL of sample was placed in the cuvette and 30 μL of 0.2 M $CaCl_2$ solution was added to the sample to initiate the clotting of the samples. After the $CaCl_2$ was added, the TEG® 5000 Hemostasis Analyzer was started. The remainder of each sample was sent for cell counts on the Hct, WBCs, and Plts. These counts were preformed on the COULTER™ Ac.T DIFF™ cell counter (Beckman-Coulter, Miami, FL) located in the Central Processing Lab at The Ohio State University Medical Center. Statistical analysis (one-way analysis of variance) was conducted by using Minitab™ Release Statistical Software Release 13.30 (State College, PA).

## RESULTS

Tables 1 through 4 compare the hematocrit, cell counts and TEG® parameter comparison between the three HES sedimentation layers.

## DISCUSSION

The data in Table 1 suggests that there is a significant difference between the whole blood and the resulting layers of the sedimentation process for all parameters. Further, analysis shows that there is a significant increase in Hct ($p = .004$) in the RBC sediment after the 60-minute sedimentation. This can be seen in Table 2. Also, Table 2 data suggest that there was a significant decrease in the

**Table 1.** Sedimentation of RBCs with hespan: separation results by layer.

| Parameter | Whole Blood | RBC Sediment | Buffy Coat | Plasma Layer | ANOVA *p* Value |
|---|---|---|---|---|---|
| Hematocrit (%) | 30 | 49 | 17 | 6 | <.001 |
| WBC count ($\times10^{-3}/\mu L$) | 6.7 | 2.4 | 8.1 | 8.2 | .004 |
| Platelet count ($\times10^{-6}/\mu L$) | 121.4 | 70 | 173.1 | 236.1 | <.001 |
| TEG MA (mm) | 58 | 57 | 47 | 26 | <.001 |
| TEG alpha angle (degree) | 56 | 67 | 56 | 23 | <.001 |
| TEG Coag Index | 1.7 | −1.3 | 1 | 1.8 | .005 |

The mean values of seven observations are listed for the initial whole blood and the sedimentation layers. *p* values are the result of ANOVA. TEG Coag Index is The Thrombelastograph® Coagulation Index; TEG MA, Thrombelastograph® maximum amplitude; TEG AA, Thrombelastograph® alpha angle.

**Table 2.** Comparison of the whole blood and the RBC sediment.

| Parameter | Whole Blood | RBC Sediment | ANOVA *p* Value |
|---|---|---|---|
| Hematocrit (%) | 30 | 49 | .004 |
| WBC count ($\times10^{-3}/\mu L$) | 6.7 | 2.4 | .014 |
| Platelet count ($\times10^{-6}/\mu L$) | 121 | 70 | NS |
| TEG MA (mm) | 58 | 26 | <.001 |
| TEG AA (degree) | 56 | 23 | .003 |
| TEG Coag Index | 1.7 | −1.3 | .006 |

The mean values of seven observations are listed for the initial whole blood and the sedimentation layers. *p* values are the result of ANOVA. TEG Coag Index is The Thrombelastograph® Coagulation Index; TEG MA, Thrombelastograph® maximum amplitude; TEG AA, Thrombelastograph® alpha angle.

**Table 3.** Comparison of the whole blood and the buffy coat layer.

| Parameter | Whole Blood | Buffy Coat | ANOVA *p* Value |
|---|---|---|---|
| Hematocrit (%) | 30 | 17 | NS |
| WBC count ($\times 10^{-3}/\mu L$) | 6.7 | 8.1 | NS |
| Platelet count ($\times 10^{-6}/\mu L$) | 121 | 173 | NS |
| TEG MA (mm) | 58 | 47 | NS |
| TEG AA (degree) | 56 | 56 | NS |
| TEG Coag Index | 1.7 | 1 | NS |

**Table 4.** Comparison of the whole blood and the plasma layer.

| Parameter | Whole Blood | Buffy Coat | ANOVA *p* Value |
|---|---|---|---|
| Hematocrit (%) | 30 | 6 | <.001 |
| WBC count ($\times 10^{-3}/\mu L$) | 6.7 | 8.3 | NS |
| Platelet count ($\times 10^{-6}/\mu L$) | 121 | 236 | .002 |
| TEG MA (mm) | 58 | 57 | NS |
| TEG AA (degree) | 56 | 67 | NS |
| TEG Coag Index | 1.7 | 1.8 | NS |

WBC values, TEG MA, TEG AA, and the TEG CI values, ($p = .014$, $p < .001$, $p = .003$, $p = .006$). This decrease in the TEG values show a decrease in the platelet activity (TEG AA), clot strength and max viscoelasticity (TEG® MA) and the strength of the clot formed (TEG CI).

There was no significant difference between the Plt values for the whole blood and RBC sediment. This suggests that the RBC layer of the whole blood sample can be concentrated with the use of HES sedimentation.

Table 3 shows no significant differences in all parameters between the whole blood and the BC layer. From this, one may interpret that the sedimentation procedure was capable of producing a BC layer comparable, in values, to the whole blood. Table 4 data suggests that there is a significant decrease in the Hct, $p < .001$, between the whole blood and the plasma layer, whereas there is a significant increase in the Plt count ($p = .002$). There was no significant difference in the WBC values. Also, we see there are no significant differences between the TEG values of the whole blood and the plasma layer.

When we compare Tables 2 through 4 with Table 1, we can see where the one-way analysis of variance (ANOVA) values in Table 1 originate. For example, the significant difference ($p < .001$) in the Hct is seen in comparing the whole blood to the plasma layer. Also, we see in Table 2 the increase in Hct in the RBC Sediment, ($p = .004$). These data show that the use of HES sedimentation can significantly reduce the number of RBCs in the plasma layer. Next we see that the ANOVA value for the WBC ($p = .014$) is caused by the reduction of WBCs in the RBC sediment. This along with the no significant difference between the whole blood and the BC and plasma layers suggests that the WBCs are retained after the HES sedimentation. The value for the Plt ANOVA ($p = .002$) in Table 1 is caused by the difference between the whole blood and the plasma layer. These data suggest that most platelets sequestered remain in the plasma layer, thereby creating PRP.

Next we see in Table 1 that the values for the TEG MA, TEG AA, and TEG CI all originate from the comparison of the whole blood and the RBC sediment. These values show a significant reduction in the coagulation properties of the RBC sediment, which is what one would expect with a reduction in the number of platelets, although there is no significant difference in the number between the whole blood and the RBC sediment. Also, this reduction may be caused by the majority of the HES being located in the RBC layer along with a majority of the clotting factors remaining in the plasma and BC layers.

In comparing the TEG values of both the BC and plasma layers to the whole blood, we did not see a significant difference between the coagulation properties, which suggests that the resulting BC and plasma layers of the sedimentation are not hindered by the use of HES. This, we believe, is the result of a low percentage of HES, 1.2%, being in the final solution of whole blood. Also, a majority of the HES remained in the RBC sediment. Finally, in Table 5 we compared the differences in values between the BC and plasma layer and found that there was no significant difference between any of the parameters, suggesting that the resulting layers are the same and can be used together to increase the volume of the final product of the HES sedimentation.

The results suggest that the gravity sedimentation of whole blood with HES can significantly increase the platelet count in the plasma and BC layers while reducing the amount of RBCs. The majority of the platelets are contained in the plasma layer. The data from TEG analysis suggests that the use of HES does not affect the clotting capabilities of the BC and plasma layers. Finally, it may be beneficial to remove the RBC layer after separation and combine the BC and plasma layer to recover a majority of the platelets.

Further experimentation will be needed to elucidate whether the final product of the HES sedimentation will form a platelet gel comparable with those formed from PRP from mechanical devices.

**Table 5.** Comparison of the buffy coat and plasma layers.

| Parameter | Whole Blood | Plasma Layer | ANOVA *p* Value |
|---|---|---|---|
| Hematocrit (%) | 17 | 6 | NS |
| WBC count ($\times 10^{-3}/\mu L$) | 8.1 | 8.3 | NS |
| Platelet count ($\times 10^{-6}/\mu L$) | 173 | 236 | NS |
| TEG MA (mm) | 47 | 57 | NS |
| TEG AA (degree) | 56 | 67 | NS |
| TEG Coag Index | 1 | 1.8 | NS |

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