OVERVIEW

The ULTRASITE® Valve is designed to reduce needlestick injuries and reduce healthcare worker exposure to pathogenic organisms resulting from exposure to infected blood. The ULTRASITE Valve prevents microbial access through the valve by providing a closed system when not activated and can be accessed with a standard Luer lock connection. The bi-directional valve allows both the infusion of fluids and the aspiration of blood through the same device. As the ULTRASITE system does not require a cap, the use of a sterile 70% Isopropanol (IPS) wipe is recommended prior to each access.

A study was undertaken to evaluate the effect of the ULTRASITE Valve on the cellular components of blood when in direct contact. The study utilized in vitro hemolysis and selected hematological parameters of human blood as indices to determine adverse effects. Both the ULTRASITE Valve and a control, an 18 gauge hypodermic needle in combination with a B. Braun IN-3000 intermittent injection port, were utilized in a simulated transfusion of citrated whole human blood.

Both the test and control articles were administered in vitro, directly to the test system. This was the optimal route of administration available in this test system. The use of human blood as a test system is recommended by ISO guidelines for the determination of activity of a test article on the cellular components of blood.

The study utilized percentage hemolysis and the hematological parameters of Complete Blood Count, including Hemoglobin, Hematocrit, Platelets, and Erythrocyte indices to determine the effect on human blood. The system for determination of hemolytic activity of a test article, when in direct contact, was based on a modification of the DHEW Publication, Evaluation of Hemodialyzers and Dialysis Membranes. The guidelines have no alternate methods.
PROTOCOL

Toxikon Corporation of Bedford, Massachusetts, was commissioned to perform an independent *in vitro* study to simulate the effect of the ULTRASITE Valve and the control article, an 18 gauge hypodermic needle/B. Braun IN-3000 intermittent injection port combination, during transfusion.

Approximately 500 mL of citrated whole blood was transferred gravimetrically through both the test and control articles connected to individual blood bags via intravenous delivery at a rate of 10 drops per minute.

At 100cc, 200cc, 300cc, 400cc, and just before 500cc of the lapsed mock transfusion, 2 aliquots (1 mL each) from each transfusion set were taken. Two samples (1 mL each) from the blood bag were concurrently taken.

To determine percent (%) Hemolysis, the sampled blood from the blood bag (i.e. before transfusion) was diluted sufficiently in Sodium chloride until 0.2 mL was hemolyzed in 10 mL Sterile Water for injection (WPI) and the spectrophotometric reading at 545 nm was approximately 1.0 Absorbance unit (A).

Transfused human blood (0.2 mL) was sampled from each transfusion set up and added to tubes (10 mL) each of saline and water for injection (positive control), in duplicate; the tubes were centrifuged for 5 minutes at approximately 500 x g. The absorbance of the supernatant was determined against a Sodium chloride blank at 545 nm.

The average absorbance values were used to determine the percent (%) Hemolysis of both the test and control articles in comparison to the Hemolysis exhibited by pretransfused blood samples.

To determine the system’s effect on various hematological parameters, a Coulter 4C Plus Cell Control Counter was utilized to analyze the blood aspirated through both systems for Complete Blood Counts (CBC), Hemoglobin, Hematocrit, Erythrocyte indices and Platelet count.

The mean and standard deviation for the test sample and comparison control article results were calculated. The test means were compared to the control means. Data analysis was performed by statistical methods such as Analysis of Variance (ANOVA) of P.J. Tallarida and R. B. Murray (Manual of Pharmacologic Calculations with Computer Programs, Springer-Verlag, New York, 1986) and the student’s t-test. Differences between the test and control values were considered statistically significant only if the probability of the differences being due to chance was equal to or less than 5% (p<=0.05). For each of the hematological parameters, the test article was considered to have failed if the differences were statistically significant from the control. Biological significance was considered in the evaluation.

The average absorbance values were used to determine the percent (%) Hemolysis of each of the test and control articles in comparison to the Hemolysis exhibited by the pretransfused blood.
RESULTS

Hemolysis experienced with the use of the ULTRASITE Valve did not exceed 1.79%; the mean hemolysis exhibited by the ULTRASITE Valve was determined to be –0.85 ± 3.8%. Under the conditions of the test, 5.00% hemolysis is considered hemolytic.

Hemolysis experienced with the use of the 18 gauge hypodermic needle/IN-3000 intermittent injection port ranged as high as 26.11%; the mean hemolysis exhibited by the 18 gauge hypodermic needle/IN-3000 intermittent injection port was determined to be 7.6% ± 10.6%.

Both the test and control articles statistically decreased Red Blood Cell Count, Hemoglobin content, and Hematocrit in comparison with pretransfused blood. Neither device statistically affected White Blood Cell Count, Platelet Content, mean Corpuscular Volume, mean Corpuscular Hemoglobin, or mean Corpuscular Concentration as indicated in Table 1.

Table 1: Hematological Parameters

<table>
<thead>
<tr>
<th>Mean</th>
<th>Normal Range</th>
<th>Pretransfused Blood</th>
<th>ULTRASITE® Valve</th>
<th>18 gauge needle/IN-3000</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC (103/1)</td>
<td>9.3 ± 0.6</td>
<td>6.34</td>
<td>6.66</td>
<td>6.46</td>
</tr>
<tr>
<td>RBC (106/1)</td>
<td>4.21 ± 0.22</td>
<td>3.87</td>
<td>3.59</td>
<td>3.67</td>
</tr>
<tr>
<td>HgB (g/dl)</td>
<td>13.3 ± 0.6</td>
<td>12.00</td>
<td>11.16</td>
<td>11.37</td>
</tr>
<tr>
<td>Hct (%)</td>
<td>36.8 ± 2.0</td>
<td>34.53</td>
<td>32.15</td>
<td>32.57</td>
</tr>
<tr>
<td>MCV (fl)</td>
<td>87.5 ± 3.0</td>
<td>89.30</td>
<td>89.42</td>
<td>89.37</td>
</tr>
<tr>
<td>MCH (pg)</td>
<td>31.4 ± 2.5</td>
<td>31.04</td>
<td>31.08</td>
<td>31.02</td>
</tr>
<tr>
<td>MCHC (g/dl)</td>
<td>35.9 ± 2.8</td>
<td>34.75</td>
<td>34.75</td>
<td>34.72</td>
</tr>
<tr>
<td>PLT (103/1)</td>
<td>211 ± 40</td>
<td>125.05</td>
<td>154.15</td>
<td>140.15</td>
</tr>
</tbody>
</table>

WBC (White Blood Cell Count)  MCV (Mean Corpuscular Volume)
RBC (Red Blood Cell Count)    MCH (Mean Corpuscular Hemoglobin)
HgB (Hemoglobin)              MCHC (Mean Corpuscular Hemoglobin Concentration)
Hct (Hematocrit)              Plt (Platelet Count)
CONCLUSION

The study determined that the use of the ULTRASITE Valve during transfusion does not cause hemolysis at any stage of the transfusion procedure. The study determined that the traditional practice of utilizing an 18 gauge hypodermic needle in combination with an IN-3000 intermittent injection port during transfusion does not cause hemolysis.

The study determined that the ULTRASITE Valve had no effect on White Blood Cell Count, mean Corpuscular Volume, mean Corpuscular Hemoglobin, or mean Corpuscular Concentration.

Analysis of the hematologic parameters determined that both the ULTRASITE Valve and the 18 gauge hypodermic needle/IN-3000 intermittent injection port exhibited a significant adverse decrease in Red Blood Cell Count, Hemoglobin and Hematocrit. The values were similarly decreased for both systems. It can be concluded that use of the ULTRASITE Valve does not have any greater effect on hematological parameters of whole human blood than the use of an 18 gauge hypodermic needle and IN-3000 intermittent injection port.

SUMMARY

The study was undertaken to evaluate the effect of the ULTRASITE Valve during transfusion and/or aspiration of blood. Use of the ULTRASITE Valve results in no hemolysis of human Red Blood Cells. The use of the ULTRASITE Valve has no greater effect on hematological parameters of human blood than the traditional system of 18 gauge hypodermic needle and an intermittent injection system.