

# The effects of rocket travel and near-space environment on dried blood and blood plasma

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## SUMMARY

A crew of astronauts embarking on an extended space mission will typically experience at least one medical emergency, such as traumatic injury or complications from space anemia, both further aggravated by lack of gravitational forces acting on the circulatory system. Treatment for these conditions requires blood transfusions; however, liquid blood has a short shelf life. Spray-dried and freeze-dried blood storage solutions are being investigated for their extended shelf life, with research into dried blood applications on the battlefield, in austere terrestrial environments, with reduced-gravity environments, and under extreme forces all showing promise. Advancements could significantly improve health outcomes for astronauts. We sought to determine if dried red blood cells and blood plasma can survive a sounding rocket launch into sub-orbital space and return with intact red blood cells and blood plasma. We hypothesized that dried blood stored in a shielded, protected container will survive rocket launch and a near-space environment because the solid state of matter will protect it from acceleration relative to Earth's gravity, vibrations, and temperature. Results showed that blood plasma survived the launch with minimal impact, but the red blood cells experienced a significant loss of viable cells. While the results were disappointing for the red blood cell samples, the dried blood plasma shows promise as a treatment option for traumatic space injury. Future experiments should include a ground-based control group, improved preservation techniques, and samples vacuum-sealed in blood bags to better protect against extreme forces and environmental damage.

## INTRODUCTION

Given the inherent risks faced by astronauts on manned space missions, the National Aeronautics and Space Administration (NASA) conducts regular research-based risk assessments on the impacts of space travel on human systems across five hazardous domains found in the space environment including altered gravity (1). For our study, we focused on the effects of altered gravity on the cardiovascular system.

A recent study investigating the impact of space flight on the human body has shown the long-term physiological impacts of zero gravity (2). Due to the absence of gravity, the hydrostatic pressure in the body is decreased and body fluids shift towards the head and upper body (2). When coupled with reduced physical activity, the cardiovascular system

experiences physical and biochemical changes, which result in a 10–15% reduction in blood plasma volume throughout the body (2, 3). While red blood cell (RBC) mass initially increases with a decrease in blood plasma volume, new RBCs produced through erythropoiesis are destroyed by hemolysis as the body attempts to maintain homeostasis. This results in a net loss of approximately 1% of RBC mass per day, or 54% increased loss per mission, as compared to on Earth, a condition called space anemia (2–5). This condition persists even after the return to Earth, leaving these astronauts with a 30% higher hemolysis rate more than a year after landing (5).

Traumatic injury is another cause of blood loss in space. While injuries have not been common aboard spacecraft, the risk has been estimated to be approximately 0.06% per person-year of flight, which would mean the crew should expect one medical emergency in a 900-day mission (6). Since hemorrhage can occur internally or externally and the cardiovascular system is under increased stress complicated by zero gravity, isolating and treating the injury can be extremely difficult, especially with limited medical facilities and equipment aboard the spacecraft (7). Research into battlefield data has demonstrated that blood transfusions performed within 30 minutes of traumatic injury improved outcomes over 24-hour and 30-day windows making a fresh, ready supply of transfusable blood crucial to survival rates (8).

The remedy for space anemia and traumatic injury typically involves blood transfusions; however, liquid blood only remains viable for 24 hours at room temperature before degradation begins (9). Blood can be stored for longer under refrigeration (35 days for whole blood and 42 days for RBCs), making transport to and storage at inhospitable locations difficult (10). During World War II, medics began administering dried blood plasma, and in particular the protein albumin, to patients for battlefield resuscitation to treat hemorrhage because it was shelf-stable, transportable, and simple to reconstitute and administer (11). It provided an effective treatment for military men undergoing shock because it controlled bleeding and restored blood volume (11). Today, countries such as the United Kingdom, which uses the "Blood Far Forward" program, continue to invest in research to refine distribution of dried blood plasma and whole blood products to austere, remote, and harsh environments, such as war zones (12, 13). The goal is to deploy dried blood products for use during remote damage control resuscitation (RDCR) to improve health outcomes for patients by increasing oxygen delivery, reducing shock, and decreasing coagulopathy (12, 13).

Lyophilizing (freeze-drying) blood plasma is the next-

generation advancement in dried blood preservation using a three-step process of supercooling, sublimation, and diffusion to remove the solvent water (11). Freeze-dried blood plasma maintained an improved coagulation profile and factor activity and demonstrated anti-inflammatory effects in studies of treated animals (11). Furthermore, triple concentration spray-dried blood plasma, created by atomization and superheating, increased the available clotting factor and improved clotting performance as opposed to fresh frozen blood plasma, which displayed significant degradation of clotting factor and strength after thawing (11). While freeze-dried blood plasma is more alkaline, studies have shown this higher pH to be tolerated in humans; buffering with ascorbic acid mitigates these pH changes while preserving 84% of coagulation factor activity (11). Results from this study demonstrated that freeze-dried or spray-dried blood plasma may be suitable for packaging and transporting to austere environments such as space (11).

As NASA continues planning for the Artemis campaign to send the first long-term, manned mission to the Moon, research is underway to bolster the shelf life and durability of blood products to be carried aboard a spacecraft (14). One area of research has focused on freeze-dried RBC preservation by trehalose loading using techniques such as electroporation and ultrasonic sonoporation, resulting in an RBC recovery rate of 70.9% and 95%, respectively (15, 16). Trehalose is a disaccharide sugar, which has demonstrated ability to stabilize cellular structures when water has been removed (15). Additionally, trehalose preservation of freeze-dried blood and blood plasma has been shown to be best suited for room temperature storage and provide protection against the effects of increased temperatures, both of which are considerations with space travel (17). Another study with trehalose-loaded RBCs explored the feasibility of rehydrating freeze-dried blood in a reduced gravity environment similar to space (18). Scientists were able to successfully reconstitute freeze-dried RBCs in 0 G with less hyperosmotic shock as demonstrated by a recovery rate approximately 30% more than that of bulk freeze-dried RBCs (18). The next advancement in the research would be to test the effects of launching dried blood into space to outfit the emergency medical supply cache aboard spacecraft with sustainable blood products.

Provisioning a manned space vehicle with a ready supply of dried blood products requires consideration for the launch environment and the extreme vibrations and forces involved. In prior research, RBCs exposed to sustained extreme vibrations of up to 300 km/s<sup>2</sup>, or over 30,000 Gs, for 30 minutes while in a laboratory vortex mixer hemolyzed up to 76.6% of all cells (19). The Terrier-Improved Orion sounding rocket can fly 73 miles (116.7 kilometers) above sea level for an estimated 15 minutes reaching a peak acceleration of 1,330 m/s, exceeding 25 Gs, before parachuting into the Atlantic Ocean (20, 21). Although these forces represent 0.1% of the forces necessary to destroy three-quarters of the RBCs in the samples in the research study, we expect that the launch forces may be sufficient to hemolyze a percentage of our RBC samples (19). Hemolyzed RBCs compromise the blood supply by reducing its capacity to carry oxygen and increasing the risk of contamination from toxic intermediates

such as reactive free hemoglobin and elevated potassium, which can have adverse effects on human health (22, 23). The samples from the vortex mixer experiments experienced hemolysis that was well above the U.S. Food and Drug Administration's (FDA) recommendation of 1%, rendering the blood potentially harmful to recipients (19, 24).

In this study, we sought to determine if dried blood and blood plasma would survive a rocket launch experiencing upwards of 25 Gs for eight minutes on a Terrier-Improved Orion sounding rocket and return within transfusable limits. The findings would help find a workable solution for two medical challenges facing astronauts in space – space anemia and traumatic injury (2, 6, 25). We hypothesized that dried blood stored in a shielded, protected container would survive rocket travel and a near-space environment because the container's design and the solid state of matter would protect it from acceleration relative to Earth's gravity, vibrations, and temperature. Our results indicated that spray-dried and freeze-dried blood samples, as currently prepared, experience significant degradation rendering them potentially unsafe for transfusion; however, blood plasma concentrations remain strong, making this a promising treatment option for traumatic injury in space. Future research into long-term dried blood storage for extended space missions would need to improve preservation techniques and protective measures to shield RBCs against the extreme forces of rocket launch.

## RESULTS

Since all space-bound medical supplies are launched into space aboard a rocket, our experiment served to test the impact of a rocket launch on both dried blood and blood plasma samples with the expectation that the RBCs and blood proteins would remain viable post-launch. Space limitations in the rocket allowed for only four 1.5 mL microcentrifuge tubes, consisting of one untreated control sample of spray-dried porcine RBCs, two trehalose-treated replicates of freeze-dried porcine RBCs, and one untreated spray-dried porcine blood plasma sample. We chose porcine blood for this experiment as it most closely matches human blood in structure and anatomy (26). A treated group in this experiment was preserved by way of trehalose loading using sonoporation; however, we did not include treated samples of blood plasma in this experiment. The dried blood samples were tested pre-launch for complete blood counts (CBCs) and dried blood plasma for total protein concentrations before being placed in a plastic cube lined with lead foil to weigh the cube down to the required weight (**Figure 1**). The cube was launched aboard a Terrier-Improved Orion sounding rocket, flying over seventy miles above sea level up to the edge of space in the transitional zone between the upper atmosphere and outer space. The rocket flew approximately 15 minutes at upwards of 25 Gs before parachuting into the Atlantic Ocean (20, 21). During the sub-orbital flight, the dried blood and blood plasma samples experienced intense acceleration relative to Earth's gravity, vibrations, radiation, and extreme temperatures. When the cube returned to Earth, we sent the samples to a laboratory to have the dried blood and blood plasma tested again for CBCs and total protein concentrations, respectively, to determine if the samples survived the extreme conditions they were exposed to (**Table**



**Figure 1: Packed cube experiment.** A sample packet containing blood samples was placed in a plastic cube, packed with foam to protect against vibrations, and lined with lead foil to combat radiation.

1).

In analyzing the pre- and post-launch hematology results, we made the decision to discard the results from one treated sample as the data was incomplete with most parameters returning null values, making it impossible to use those results for comparison and analysis. The raw data from the viable sample has been reported in the accompanying table and figures and was used for the statistical analysis with results summarized here as percentages only to assist with interpretation of the findings. When comparing the control with the remaining treated sample, it was observed that the RBCs, RBC ghosts (RBCs devoid of intracellular material and hemoglobin), the hemoglobin (HGB), platelets (PLT), and white blood cells (WBC) were severely depleted for both the control and treated blood samples (**Table 1**). When comparing the control with the treatment group post-launch, there was a statistically significant change in RBC concentrations based on a Chi-square test indicating more dead RBCs in the treatment group, contrary to our hypothesis ( $\chi^2 = 780361.69$ ,  $p < 0.001$ ) (**Figure 2**). Specifically, nearly 100% of RBCs in the treatment group were destroyed, while closer to 60% died in the control group (**Figure 2**). The effect size was moderate (0.51), as indicated by Cramer's V test. When comparing other data, we observed that the control group's mean corpuscular volume (MCV) dropped 23% and red cell distribution width (RDW) increased 92%, while the treated blood saw increases of 1% and 59%, respectively, for these same measures (**Table 1**). The percentage of the sample composed of RBCs as measured by hematocrit (HCT) fell to 0.1% for the control group, while the treated sample experienced a total decline to 0% (**Table 1**).

RBC ghost cells experienced similar losses as RBCs. We performed a Chi-square test to determine if there was

a statistically significant change in RBC ghost cell counts for the control and treatment groups post-launch. In this instance, the number of lysed RBCs measured as RBC ghosts decreased 43% for the control group and 87% for the treated group ( $\chi^2 = 106296.70$ ,  $p < 0.001$ ) with a moderate effect size of 0.22 as indicated by Cramer's V test (**Figure 3**). When determining the disposition of HGB present within and without the remaining RBCs, HGB levels in the control and treatment groups decreased by 50% and 66%, respectively (**Table 1**). However, mean corpuscular hemoglobin (MCH) increased by 10% for the control group and over 2,100% for the treated blood. There were higher increases of 43% and nearly 22,000%, respectively, for mean corpuscular hemoglobin concentration (MCHC) (**Table 1**). Meanwhile, the PLT and mean platelet volume (MPV) in untreated blood showed a 47% decrease and 6% increase, respectively, and a larger decrease of 91% and 61%, respectively, for the treated blood (**Table 1**). These PLT counts resulted in a statistically significant decline in concentrations for the treated group compared to the control group, as indicated by Chi-square test ( $\chi^2 = 159517.96$ ,  $p < 0.001$ ), and a moderate effect size of 0.48, as indicated by Cramer's V test (**Figure 4**). Finally, the number of WBCs decreased by 38% for the control group and by 50% for the treated group (**Table 1**). However, unlike with the treated and untreated dried blood samples, the untreated blood plasma protein counts saw an insignificant loss of less than 3% (**Figure 5**).

## DISCUSSION

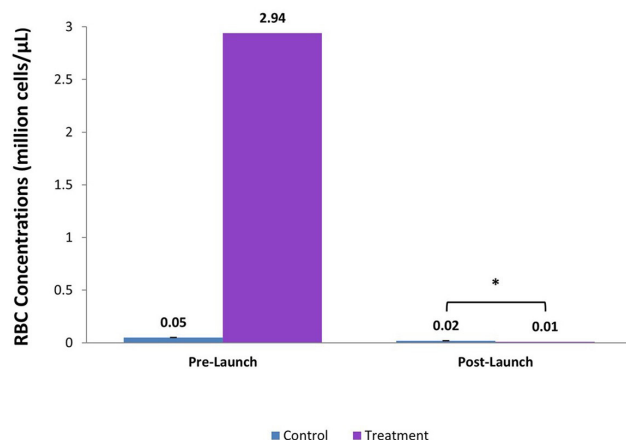
In this experiment, we sought to determine if dried blood and blood plasma would be able to withstand the extreme forces of a rocket launch to supply manned space missions with shelf-stable blood products during a medical emergency. We deployed untreated and preserved blood samples loaded with trehalose to determine if treatment would have an impact on survivability. The hematology results from post-launch testing showed significant degradation of the RBCs for both the untreated and treated dried blood samples when

**Table 1: Hematology Results for Control and Treatment Groups of Dried Blood Samples**

Measure	Units	Reference Range	Control		Treatment	
			Pre-Launch	Post-Launch	Pre-Launch	Post-Launch
RBC	million cells/ $\mu$ L	5-8	0.05	0.02	2.94	0.01
MCV	fL	50-68	78.80	60.60	63.70	64.50
RDW	%	<15	34.20	65.70	17.90	28.50
RBC Ghosts	million cells/ $\mu$ L		0.07	0.04	2.07	0.27
PLT	thousand cells/ $\mu$ L	200-500	179	95	526	48
MPV	fL	39-44	11.60	12.30	47.90	18.50
HCT	%	28-43	0.40	0.10	18.70	0.00
HGB	g/dL	10-16	3.20	1.60	13.10	4.50
MCHC	g/dL	30-34	815.20	1166.80	69.80	15424.30
MCH	pg	17-21	642.70	707.20	44.50	9948.70
CHCM	g/dL		34.80	33.20	25.20	21.10
CH	pg		27.70	21.60	15.90	13.00
WBC	thousand cells/ $\mu$ L	11-22	0.29	0.18	11.73	5.87

**Table 1: Hematology results for control and treatment groups of dried blood samples.** Complete blood count (CBC) testing performed on dried blood samples using Siemens ADVIA 2120i hematology system in pre- and post-launch for one untreated sample of dried blood cells and two treated replicates with the results of one replicate discarded due to nulls in the data. Porcine reference ranges extracted from Merck Veterinary Manual (27).





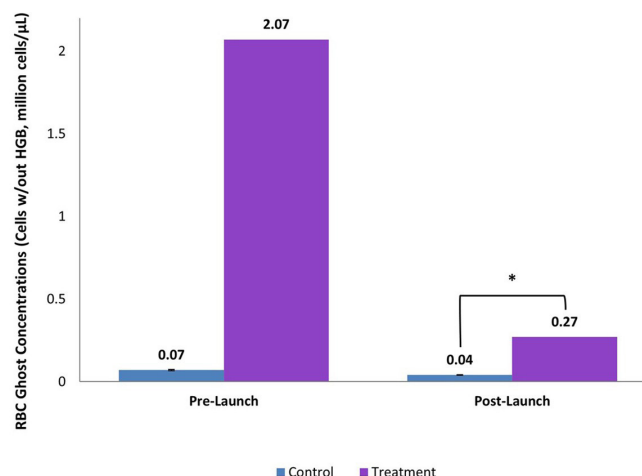
**Figure 2: RBC concentrations for control and treatment groups.** Red blood cell (RBC) concentrations measured by Siemens ADVIA 2120i hematology system in pre- and post-launch for one untreated sample and one treated sample of dried blood cells. RBC concentrations declined 60% and nearly 100%, respectively, with statistical significance of change based on Chi-Square test of  $*p < 0.001$  with 1 degree of freedom and effect size of 0.51, as indicated by Cramer's V test.

compared to pre-launch data; however, a greater number of RBC ghosts were hemolyzed in the treated samples. These cellular shells release HGB and other intracellular material during hemolysis (28). HGB levels also declined for both untreated and treated dried blood samples due to the loss of viable RBCs. Conversely, the dried blood plasma showed only a slight decline in concentration. Our other results were less conclusive. Studies have shown that MCH, MCHC, and MCV will be artificially elevated and HCT will be artificially reduced, as seen in our results, when the RBC count is abnormally low due to in vitro hemolysis of blood samples (27, 28). These conflicting results may occur because of the mathematical relationship between the values; MCH results are derived from dividing HGB by RBC count and HCT results are determined by multiplying MCV by RBC count (28, 29). Any extreme changes to RBC and HGB counts will impact values such as MCH and HCT (29). Additionally, an increase in intracellular material caused by hemolysis can interfere with optical and spectrophotometric readings, which will artificially increase cellular hemoglobin concentration mean (CHCM) and corpuscular hemoglobin (CH) (28). Only RBCs, RBC ghosts, and HGB, were directly measured by the hematology analyzer, therefore the only values evaluated for this study.

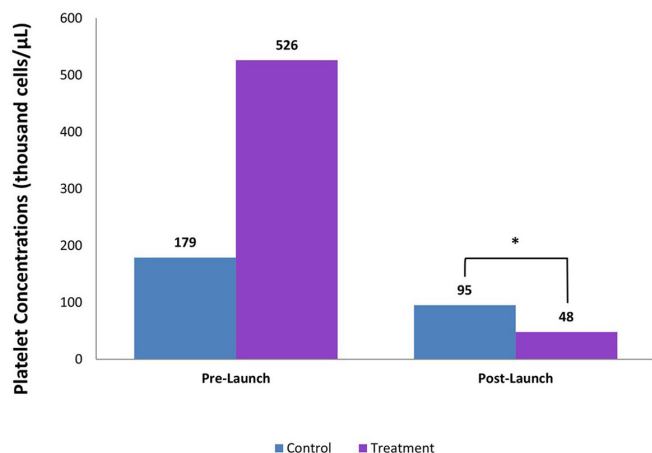
Comparable pre-launch data, consistent laboratory procedures, and prior research describing the effects of extreme acceleration on RBCs would suggest that the extreme forces of a rocket launch as well as experimental limitations may have been contributing factors in our results. Based on our research and findings, we concluded that these blood samples would not be suitable for transfusion based on FDA guidelines (30). Patients treated with hemolytic blood risk a hemolytic transfusion reaction (HTR), the presentation of which may include burning at the infusion site, diffuse pain, shortness of breath, and flu-like symptoms (31). Less common symptoms may include low blood pressure, accelerated

heart rate, changes in the skin's overall appearance, red discoloration to the urine, and other more severe symptoms (31). In some cases, delayed onset transfusion reactions may occur approximately 24–30 days after transfusion with symptoms indicative of anemia, and in rare cases, may lead to death; hospital stays are lengthy with delayed transfusion reactions (31). Replication of the experiment with modifications to the treatment would be necessary to attempt to mitigate these outcomes.

Experimental limitations which may have also impacted our findings include laboratory constraints, number of trials, absence of a ground-based control group, and confounding variables of transportation, storage conditions, temperature, and radiation. Our efforts to find a laboratory willing to test porcine blood products proved difficult; human laboratories would not test animal biologics and animal laboratories required coordination through a veterinarian. While one research laboratory agreed to test the samples, future research should attempt to coordinate hematology testing through multiple independent research laboratories to rule out any laboratory constraints. Due to space limitations aboard the rocket, the experiment was confined to a small plastic cube. The space limits allowed for only four microcentrifuge tubes, thereby limiting the number of trials for each blood type from which to obtain conclusive quantitative results. We recommend that future research includes a more comprehensive set of trials to test treated and untreated samples of freeze-dried and spray-dried RBCs and blood plasma. Furthermore, weight restrictions limited the amount of lead foil shielding used to reduce the effects of radiation; additional layers may have improved protections. The rocket launch was delayed for over a month due to unfavorable launch conditions, shipping of the samples back to the laboratory was hampered by funding challenges, and the laboratory was backlogged causing the samples to be subjected to variable storage and transportation conditions for an additional eight months. A second control



**Figure 3: RBC ghost concentrations for control and treatment groups.** Red blood cell (RBC) ghost concentrations measured by Siemens ADVIA 2120i hematology system pre- and post-launch for one untreated sample and one treated sample of dried blood cells. RBC ghosts declined 43% and 87%, respectively, with statistical significance of change based on Chi-Square test of  $*p < 0.001$  with 1 degree of freedom and effect size of 0.22, as indicated by Cramer's V test.



**Figure 4: Platelet concentrations for control and treatment groups.** Platelet concentrations measured by Siemens ADVIA 2120i hematology system in pre- and post-launch for one untreated sample and one treated sample of dried blood cells. Platelets declined 47% and 91%, respectively, with statistical significance of change based on Chi-Square test of  $*p < 0.001$  with 1 degree of freedom and effect size of 0.48, as indicated by Cramer's V test.

group could have been stored in a temperature-controlled, ground-based environment for the duration of the experiment and tested pre- and post-launch for comparative analysis, to account for confounding effects of variable transportation and storage.

With consideration for these challenges, our results suggest that the current treatment may not be sufficient to protect dried blood products from extreme forces and atmospheric conditions of rocket launch, including radiation exposure, temperature variations, and high-pressure, rendering it potentially unsafe for intravenous administration. More research and testing are necessary to isolate and eliminate factors that may have contributed to the high degree of hemolysis observed in all our dried blood samples to perfect future preservation techniques to combat these effects. While we recommend additional research and testing, our results suggest that the dried blood plasma may be suitable for delivery by rocket travel to a manned spacecraft. In addition to improving the storage and transportation environment, future experiments might want to buffer against forceful mixing of the sample within the empty space of the microcentrifuge tubes, temperature variations during the launch, and radiation exposure.

Our experiment was conducted using a Terrier-Improved Orion sounding rocket generating an average of 20 Gs of force, while the NASA Space Launch System cargo rockets that would transport the dried blood and blood plasma for medical use would have a maximum acceleration of 5 Gs (32). Future research studies might consider additional trials launched simultaneously aboard one or more types of sounding rockets to subject samples to various accelerations relative to Earth's gravity with all samples concurrently sent for pre- and post-launch testing at multiple laboratories. We recommend that future experiments also explore improved preservation methods.

In a prior study, one group of mesenchymal stem cells was air-dried and vacuum sealed for several days and was the only

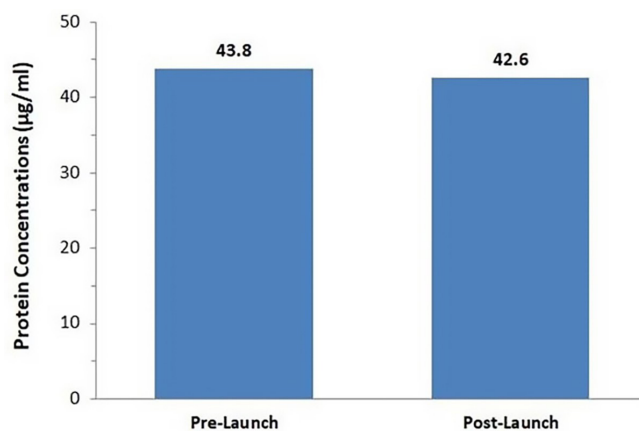
group of cells in the study to survive the reconstitution process (33). The supposition made was that improved survival may have been attributed to oxygen deprivation preventing free radicals from forming (33). Preserving these cells with trehalose and glycerol prior to dehydration improved recovery rates, suggesting that preserving dried blood products with a combination of trehalose and glycerol and/or vacuum sealing the material in blood bags may prevent moisture contamination, provide a buffer against launch vibration and forces, and improve reconstitution, RBC recovery rates, and delivery of transfusable blood products (33).

In summary, we concluded that current preservation methods are not sufficient to protect against the destruction of RBCs exposed to the impacts of rocket launch, transportation, and storage. Current preparations for dried blood products, when subjected to these conditions, would not be safe for transfusion. Future research may improve upon the preservation process in such ways as by loading RBCs with a combination of trehalose and glycerol, securing dried blood products with vacuum sealing technologies, and taking extra measures to ensure that confounding variables are isolated or removed in subsequent experiments. Traumatic injury and space anemia are inherent risks for astronauts during space flight, which are complicated by the effects of altered gravity, but the advancements in space travel and the increasing need for sustainable life support solutions for astronauts embarking on deep space missions demand that the research continue into viable, shelf-stable dried blood and blood plasma products.

## MATERIALS AND METHODS

### Selecting laboratory environment

A nationwide search for a suitable laboratory was conducted with consideration for the following criteria: ability to test porcine blood without the possibility of contamination, ability to handle novel preservation techniques, and ability to reconstitute dried blood and blood plasma without damaging the RBCs. Several concerns were the cross-contamination of samples or unsterilized/insufficient laboratory equipment, airborne pollutants or unclean laboratory setting, and



**Figure 5: Total protein concentrations for untreated blood plasma.** Total protein concentrations as measured by bicinchoninic acid (BCA) assay in pre- and post-launch for one sample of untreated blood plasma. The sample experienced a less than 3% decline post-launch.

transportation or storage barriers. A research laboratory startup was chosen due to their expertise in dried blood products and their proprietary freeze-drying process of RBCs loaded with trehalose by way of ultrasonic technology. The laboratory donated two treated porcine blood samples to this experiment. We also obtained samples of untreated spray-dried porcine blood and untreated spray-dried porcine blood plasma from an agricultural company.

### Pre-Launch hematology analysis

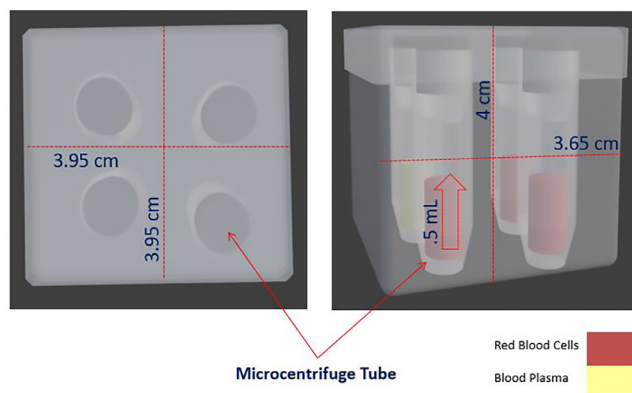
The spray-dried blood and blood plasma samples were sent to the research laboratory for pre-launch hematology testing. The research laboratory reconstituted the untreated spray-dried and treated freeze-dried blood samples, containing 25% volume of dry components, by adding 0.5 mL of saline to each 1.5 mL microcentrifuge tube and placing them into a Siemens ADVIA 2120i hematology system for analysis. CBC tests were performed to measure blood sample characteristics such as RBC count, HGB levels, hematocrit, RBC size, the amount of HGB per cell, and HGB concentration per cell. A bicinchoninic acid (BCA) assay was used to measure total protein concentrations of the dried blood plasma sample. Results were digitally charted and graphed before being emailed to our team. The research laboratory mailed a prepared sample packet that contained four 1.5 mL microcentrifuge tubes consisting of two treated freeze-dried porcine blood samples, one untreated spray-dried porcine blood sample, and one untreated spray-dried porcine blood plasma sample. These combined with desiccant pack were all wrapped in a sealed plastic bag with a total weight of 3.5 g (Figure 6).

### Cube preparation

The sample packet was placed in a small plastic cube with an inside length of 3.65 cm, outside length of 3.95 cm, rim depth of 0.15 cm, and height of 4 cm including the lid (Figure 7). The total weight of the sample packet, containing the four 1.5 mL microcentrifuge tubes with dried blood and blood plasma, desiccant pack, and plastic bag, was 3.5 g with only 25% volume of dry components to allow for rehydration using 0.5 mL of saline per tube during testing. Lead foil was



**Figure 6: Sample packet contents and wrapping technique.** Packet contained four microcentrifuge tubes filled with dried blood and blood plasma and one desiccant pack, all sealed in a bag tied with an elastic band to conserve space in the cube.



**Figure 7: Top and side views of cube depicting microcentrifuge tubes containing dried blood and plasma samples.** Depiction of the internal space and relative positioning for the contents of the cube, which contained four 1.5 mL microcentrifuge tubes with two replicates of treated dried blood sample, one untreated dried blood sample, and one untreated blood plasma sample. Cube had inside length of 3.65 cm, outside length of 3.95 cm, rim depth of 0.15 cm, and height of 4 cm including the lid. Diagram created using Blender.

used to line the cube to protect against the effects of radiation (Figure 1). Foam packing materials were placed around the sample packet to function as a cushion to reduce vibrations and movement (Figure 1). The inside and outside of the cube were wrapped with tape to prevent the cube contents from being contaminated by the lead foil and spilling out (Figure 1). The total cube weight with consideration for the samples, packing materials, and cube itself was 64 g. Space limitations allowed for only four microcentrifuge tubes.

### Launch environment

Our experimental tubes were launched aboard a Terrier-Improved Orion sounding rocket at NASA's Wallops Flight Facility in Wallops Island, Virginia. The rocket endured acceleration forces of between 20 and 25 Gs and extreme vibrations during flight. The rocket reached an altitude of 73 miles (116.7 km) above sea level reaching the edge of space. The payload remained at constant atmospheric pressure of 101.325 kPa. The rocket was exposed to temperatures of 20–55°C. The highest temperatures were those during descent, caused by friction from the atmosphere. The rocket's descent was slowed by a parachute until it landed in the Atlantic Ocean where it was taken aboard by a recovery boat and shipped back to the research laboratory.

### Post-Launch hematology analysis

The samples were delivered to the research laboratory two months after launch. All four samples, each housed in 1.5 mL microcentrifuge tubes, which included two treated freeze-dried porcine blood samples, one untreated spray-dried porcine blood sample, and one untreated spray-dried porcine blood plasma sample, were reconstituted in 0.5 mL saline and inserted into the hematology analyzer. CBC tests were performed to measure blood sample characteristics such as RBC count, HGB levels, HCT, RBC size, the amount of HGB per cell, and HGB concentration per cell. BCA assay



was used to measure total protein concentrations for the dried blood plasma sample. Results were digitally charted and graphed before being emailed to our team for analysis.

### Statistical analysis

Statistical significance of the change in RBC, RBC ghost, and PLT concentrations was determined by a Chi-square test performed using a Python script with a  $p$  value < 0.001 and 1 degree of freedom. The strength of the effect size was calculated by Python script using the Cramer's V test.

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