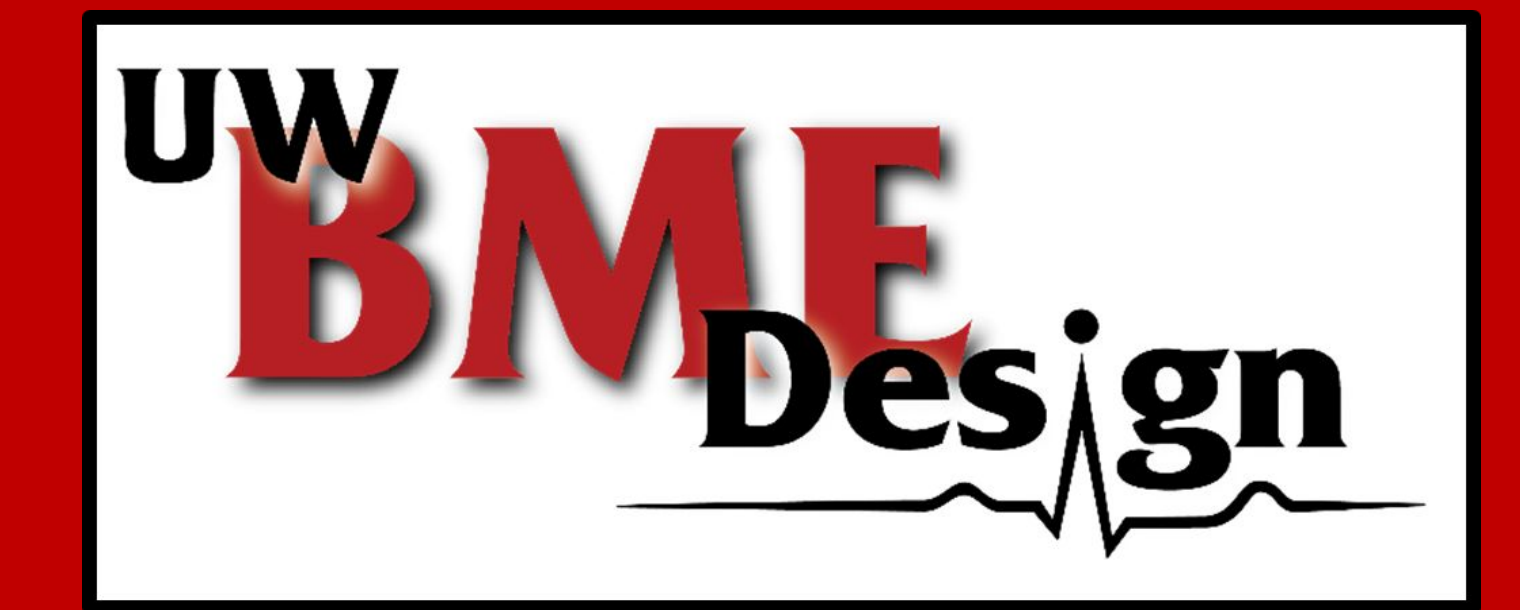




LARGE ANIMAL PLATELETPHERESIS



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Abstract

The COBE Spectra Apheresis System by Terumo performs plateletpheresis on horses. Our clients would like to use this method to efficiently extract equine platelets for their research. They have developed a novel equine platelet therapy that has been shown to be effective in mice. However, a one time use tubing kit from Terumo can cost up to \$2600. The team has been tasked with bringing this cost down to \$100 per use. This can be done in one of three ways: create a simplified tubing design, create a new sterilization protocol for current tubing, or to remake current tubing with new material. The create a simplified tubing design won our design matrix, so this option was carried out. A basic tubing skeleton (\$56.03) was made of sterilizable materials (PVC, polypropylene connectors), harvesting pressure sensors, air traps, and centrifuge component from the original tubing set. This was demonstrated to interface with the COBE Spectra Apheresis System without leaking. An additional safety component to remove air was partially manufactured but could not be completed due to late material shipments.

Motivation & Background

Platelets are therapeutically useful blood components

- Blood clotting and wider healing functions
- Can be injected into patients to help them heal faster [1]

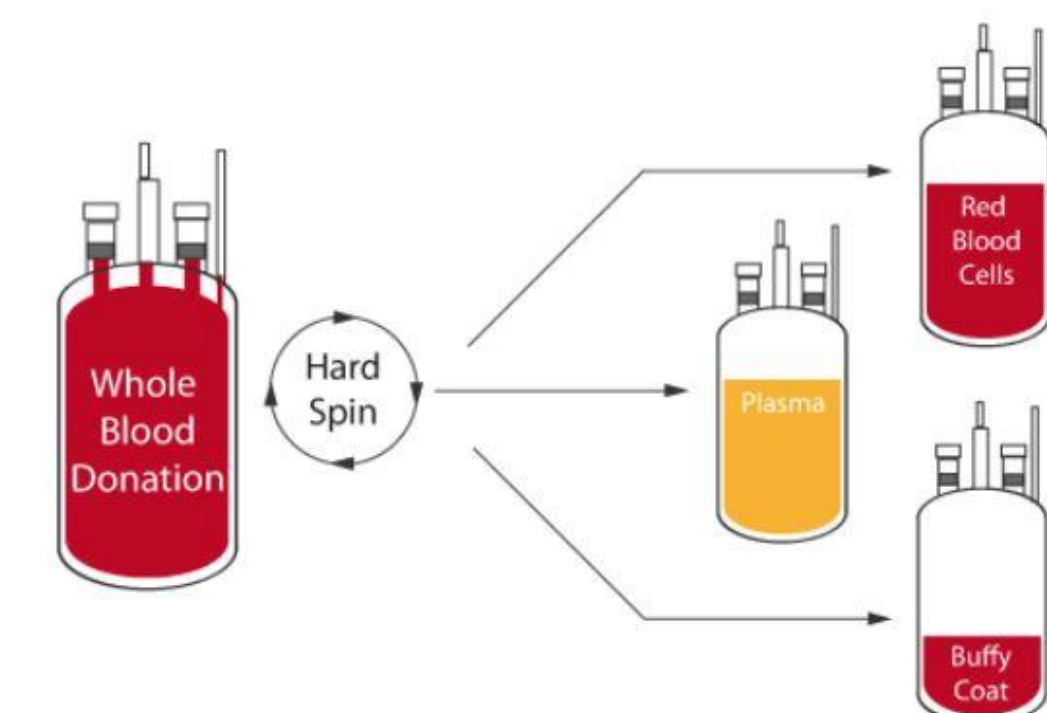
Why horse platelets?

- Washed Equine Platelet Extract (WEPLEX) can be used cross-species [2]
- Anti-inflammatory effects shown in mice with colitis

Problem 1: Whole blood extraction is inefficient

- Requires two centrifuge steps
- Yields lower platelet concentration

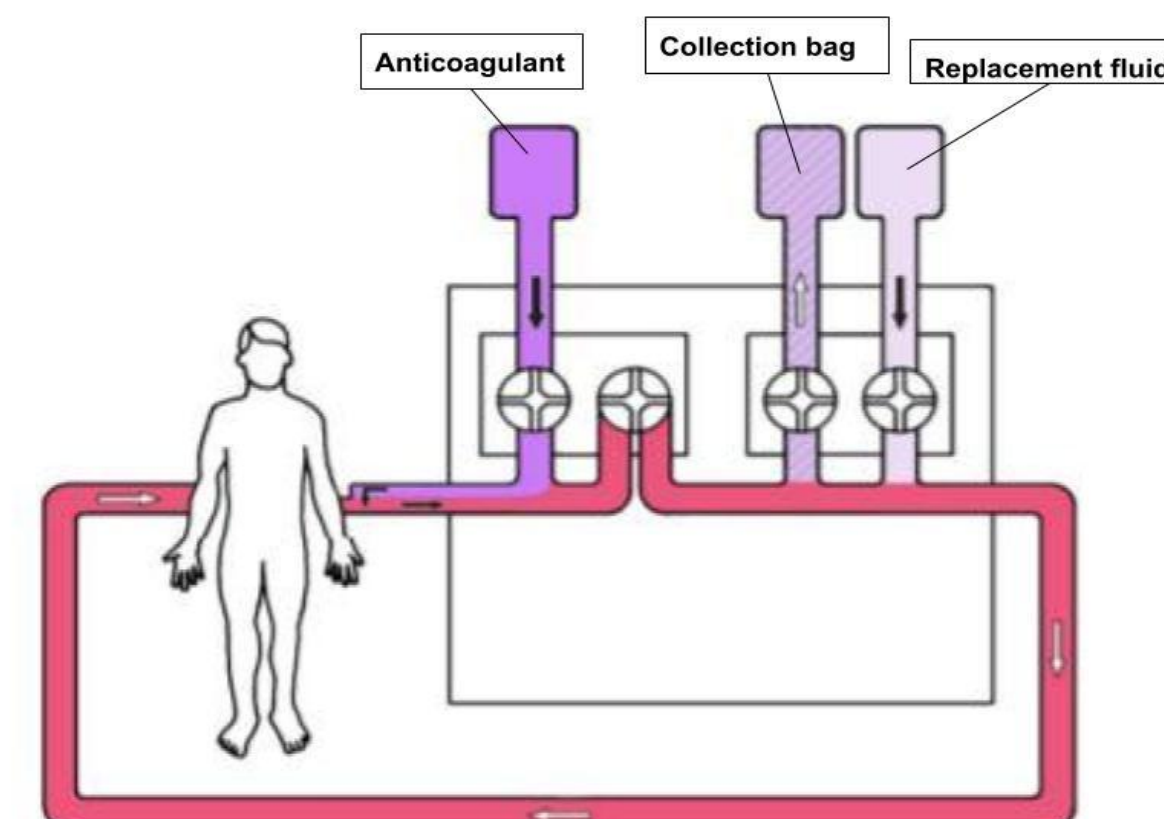
Figure 1: Whole blood extraction schematic [3]



Problem 2: Automated extraction (plateletpheresis) is efficient but expensive

- Continuously circulates blood
- Centrifuges out platelets
- \$2000-2600 per use tubing sets [4]

Figure 2: Plateletpheresis schematic [5]. Red whole blood continuously circulates with anticoagulant and replacement fluid, platelets collected.



COBE Spectra Apheresis System

- Terumo BCT Extended-Life Platelet Disposable Tubing Set with Leukoreduction Chamber
- Separation and collection of different blood components

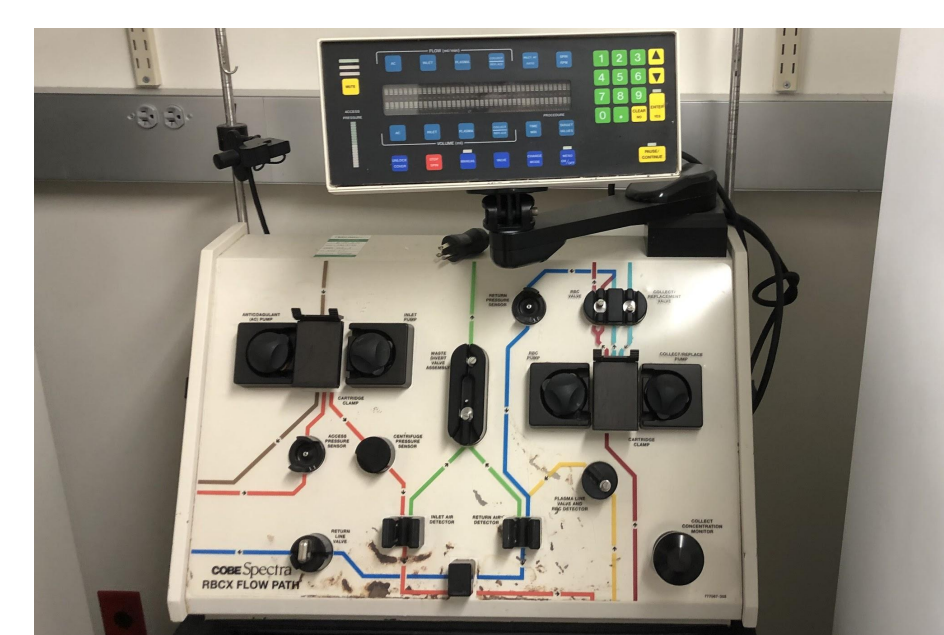


Figure 3: COBE Spectra Apheresis System without a tubing set loaded into it.

Project Goals

- Fabricate a new tubing set that is cheaper than the current \$2600 cost
- Create a cost estimate of the tubing
- Increase the flow rate of the machine

Design Specifications

- Low cost tubing with sterilization abilities
- Ability to handle repeated uses without losing structural integrity
- Tubes should be able to be easily assembled with airtight and leak proof joints
- Malleable enough to curve around the peristaltic pumps
- Material and size properties of original tubing
 - Outer Diameter: (.157 in to .163 in)
 - Inside Diameter: (Maximized for highest output)

Methods and Testing

- Create a simplified version of the current tubing set, reusing critical components that cannot be fabricated
- Sterilize tubing and other components via gas sterilization for reuse
 - Essential components that cannot be fabricated from scratch readily (Figure 4)
 - Centrifuge Loop (28, 27, 10)
 - Pressure Sensors (7, 19)
 - Air Filters (9, 20)
 - Access Pump Cartridge (8)
 - Components to be purchased and attached to tubing set separately after each use
 - Waste Collection Bag (22)
 - Specimen Collection Bag (14)
 - Access and Return Needle (1, 24)
 - Saline and Anticoagulant Access Lines (3, 4)

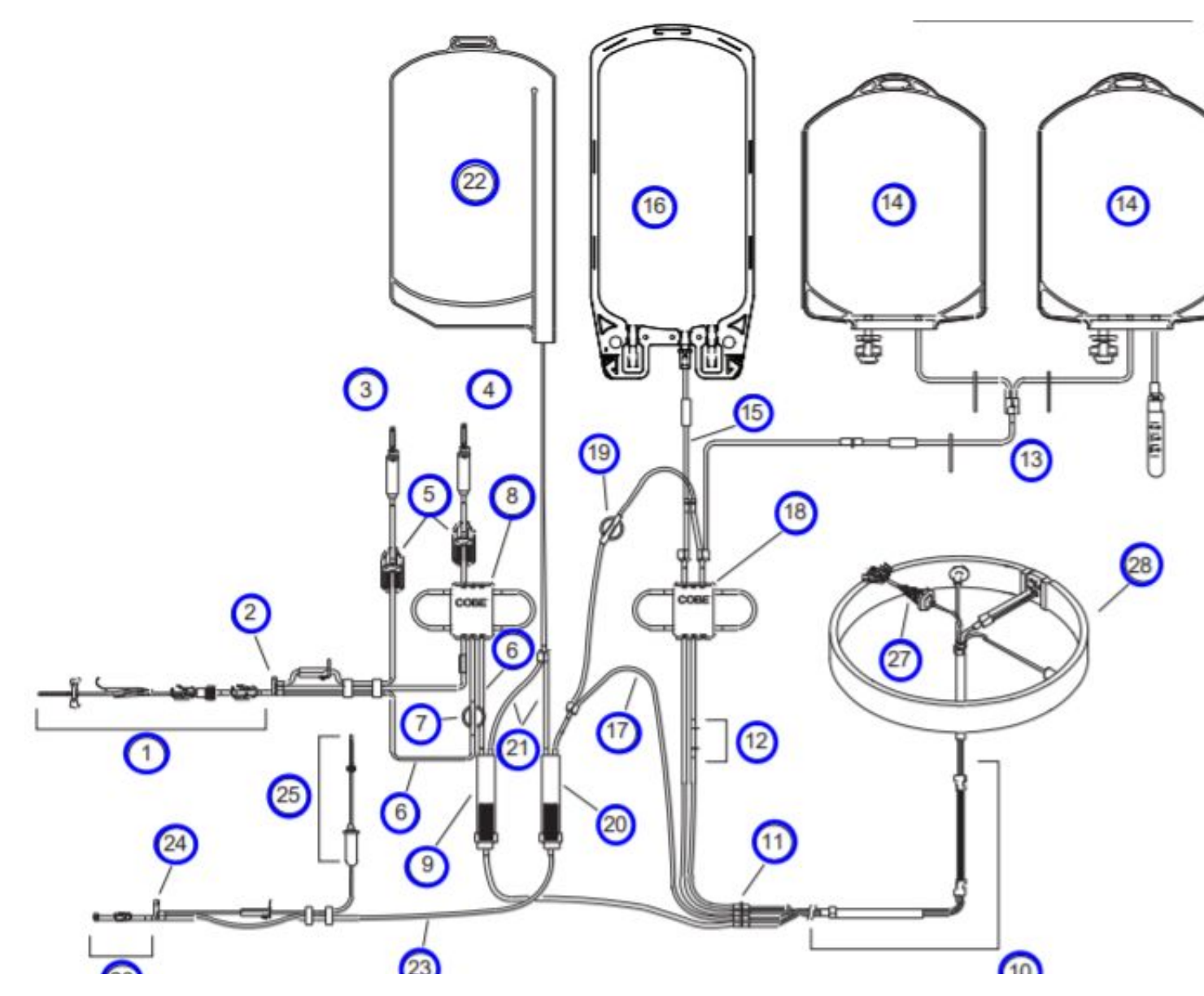


Figure 4: Schematic for Extended-Life Plateletpheresis Tubing Set

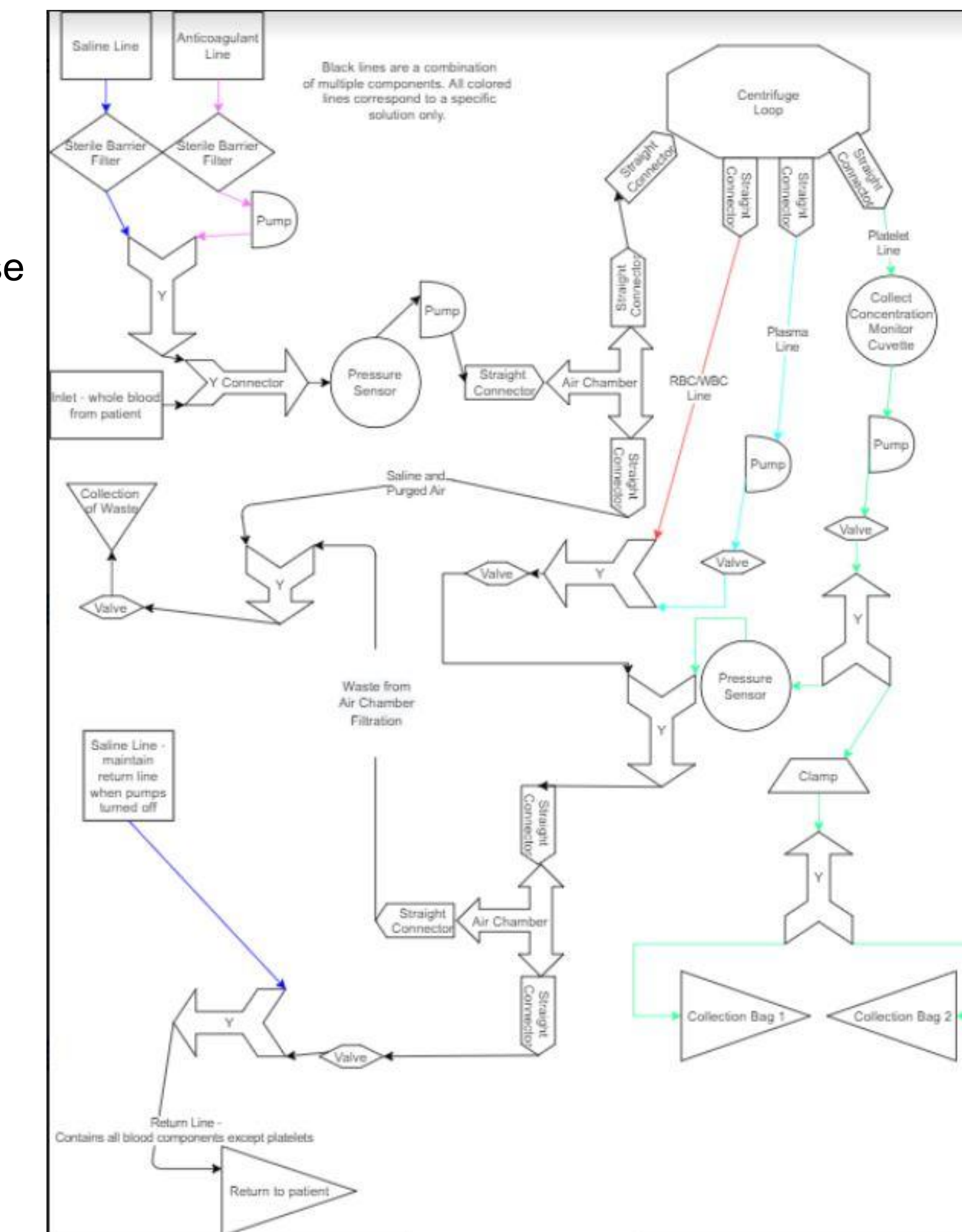


Figure 5: Final Schematic for Simplified Tubing

Prototype Test with Machine

- Prototype created from clear PVC tubing and straight connectors replacing sections of the existing set
- Ran machine with prototype to test for airtight seals at connectors
- Successful test with no visible leaks or pressure issues

Fabrication of Bubble Trap

- Bubble trap was constructed to ensure parts fit together correctly
- Could not be tested due to undelivered membrane filter

Comparing Connectors

- Two sets of tubing were fabricated to compare connectors, one with 1/16" connectors and one with 1/8" connectors
- Water was ran through both sets to determine water retention and flow rate



Figure 6: A close image of a connector and tubing section in the prototype.

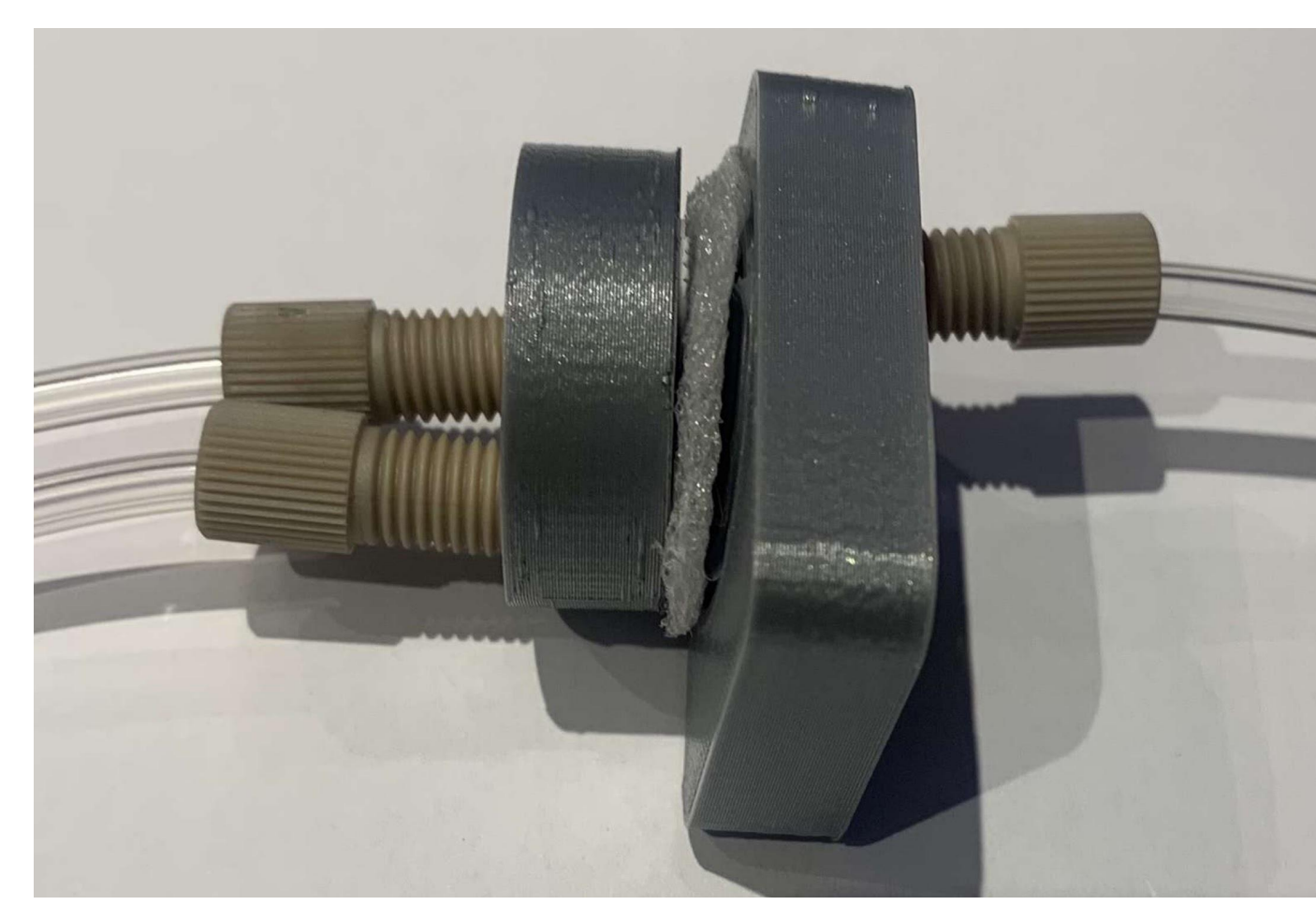


Figure 7: The fabricated bubble trap.

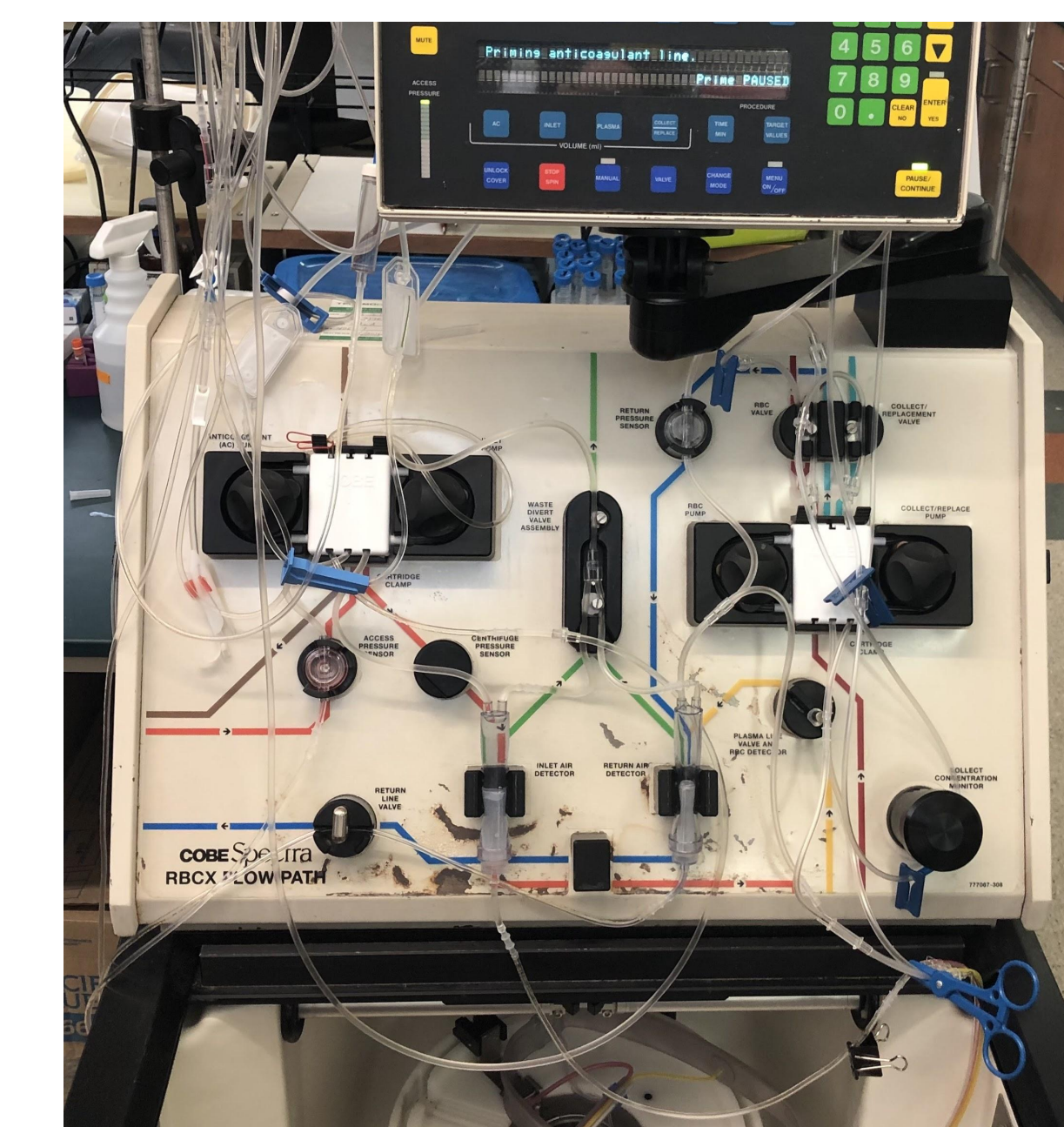


Figure 8: The prototype tubing set being used with the plateletpheresis machine.

Results

Comparing Connectors

- 1/8" connectors are preferred over the 1/16" connectors
- Fabrication for the 1/8" is more difficult and requires heat, but creates a stronger seal
- Flow rate for the 1/8" connectors is 16.57 mL/min and the flow rate for the 1/16" connectors is 16.53 mL/min
- Standard deviations for the path times for the 1/8" and 1/16" connectors are 0.0058 and 0.0100 respectively, the standard error values are 0.0033 and 0.0058 respectively
- No tubing set, including control, had full retention of water
- Loss of water was caused by the droplets that accumulate on the inside of the tubing that are difficult to remove (results in Figure 9)

Water Regained with Error Bars

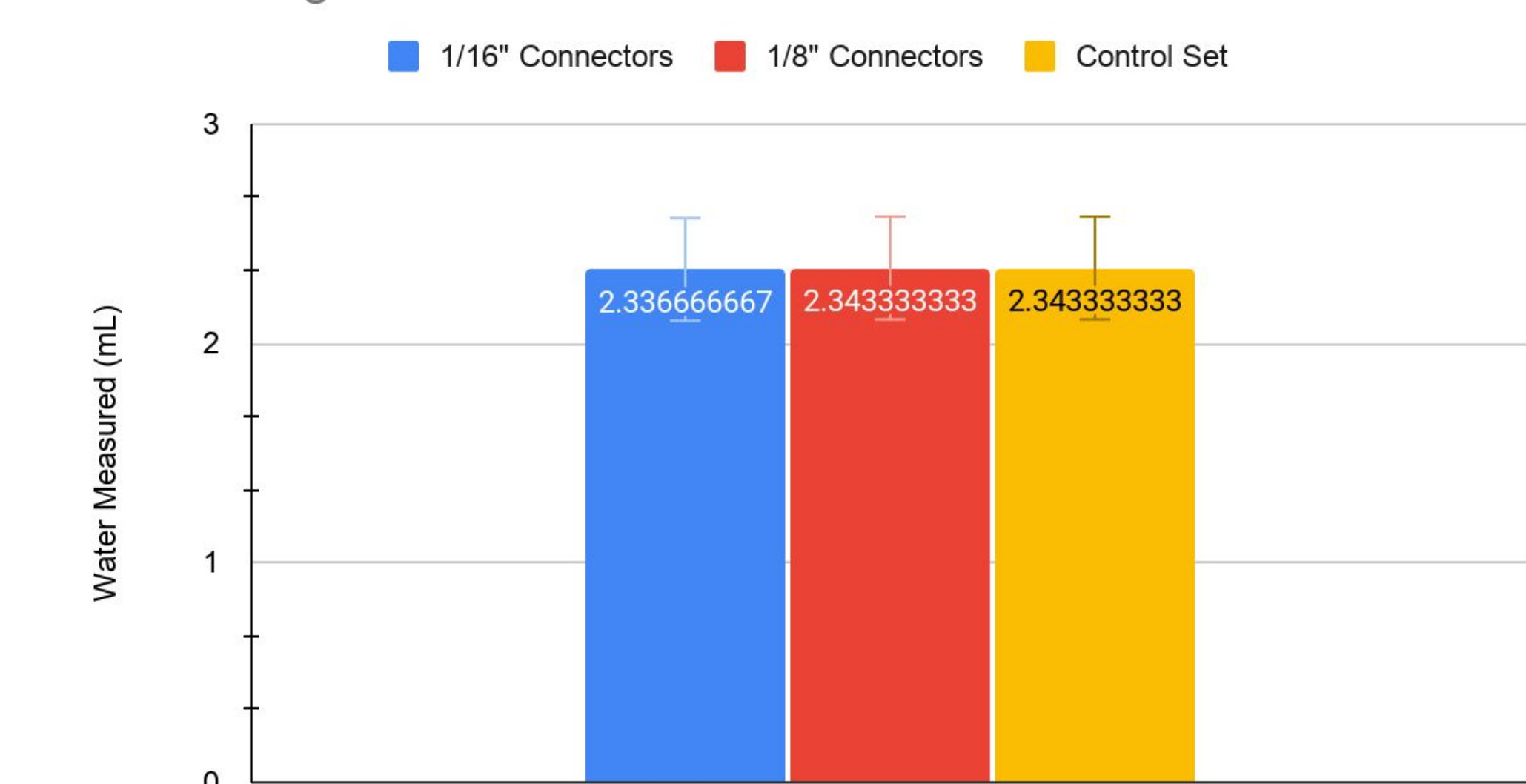


Figure 9: Graph of water recovered from tubing (loaded with 2.5mL). Results were not significantly different (ANOVA p = 0.88)

Discussion

- Platelets extracted from our clients' novel method have 266x[2] the growth factors as platelets extracted from normal platelet lysate
- Global implications since project will improve use of platelet lysate in anti-inflammatory and regenerative therapies
- Main ethical consideration is that horses cannot consent to donating their blood. However, since this project is for the furthering of science and will improve human quality of life we understand why it is needed.
- Largest difficulty we faced was getting our tubing to interface with the COBE Spectra Apheresis Machine. This was accomplished by reading in depth about the apheresis machine.

Future Work

- Compare the material properties of the tubing before and after sterilization
- Use a blood substitute (or actual blood) to test the centrifuge loop
- Fabricate pressure sensors, air sensors, and centrifuge loop
- Optimize the flow rate of the tubing
- Test the efficiency of the bubble trap

Estimated Costs (Not Including Separate Attachments)

| Component | Amount Needed | Unit Cost | Cost per Use |
|--|------------------------|------------------|---|
| PVC Tubing 2mm ID, 4mm OD | 64.56ft | \$0.48/ft | \$31.00 |
| Polypropylene Y-tubing connector; 0.125– 0.156in.; 4mm | 6 Y connectors | \$1.52/connector | \$9.09 |
| Kynar Straight Coupler 1/8 in ID | 23 straight connectors | \$0.69/connector | \$15.94 |
| | | | Total Cost per Tubing Set: \$56.03 |

Acknowledgements

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References

- Townsend, Mary, and Marissa Li. "Chapter 6 - Apheresis Blood Component Collections." In *Transfusion Medicine and Hemostasis* (Third Edition), edited by Beth H. Shaz, Christopher D. Hillyer, and Morayma Reyes Gil, 39–41. Elsevier, 2019. <https://doi.org/10.1016/B978-0-12-813726-0.00006-4>.
- Pennati A, Apfelbeck TM, Brounts SH, Galipeau J. Washed equine platelet extract (WEPLEX) as an anti-inflammatory biologic pharmaceutical [published online ahead of print, 2020 Aug 28]. *Tissue Eng Part A*. 2020;10.1089/ten.TEA.2020.0160. doi:10.1089/ten.TEA.2020.0160
- Terumo BCT. "OPERATIONAL PRINCIPLES OF THERAPEUTIC PLASMA EXCHANGE (TPE) PROCEDURES EXCHANGE (TPE) PROCEDURES." Accessed October 13, 2020. <https://www.terumobct.com/Public/306670728.pdf>
- S Brounts. VETMED: CONVERSION OF HUMAN COBE PLATELETPHERESIS MACHINE FOR LARGE ANIMAL USE. UW Madison BME Department. 2020. Accessed Sept. 7, 2020. Available: <https://bmedesign.engr.wisc.edu/selection/projects/25de148e-1405-47a7-9894-c76d2366793d>.
- PluriSelect USA. "Buffy Coat - Sample Material - Knowledge Base." Accessed October 13, 2020. <https://www.pluriselect.com/us/knowledge-base/sample-material/buffy-coat.html>.