HHS Public Access

Author manuscript

Artif Organs. Author manuscript; available in PMC 2018 February 01.

Published in final edited form as:

Artif Organs. 2017 February ; 41(2): 175–178. doi:10.1111/aor.12724.

A Reusable, Compliant, Small Volume Blood Reservoir For *In Vitro* Hemolysis Testing

Salim E. Olia[*,†,‡], **Luke H. Herbertson**[§], **Richard A. Malinauskas**[§], and **Marina V. Kameneva**[*,†,¶]

[*]McGowan Institute for Regenerative Medicine, University of Pittsburgh, Pittsburgh, PA, USA

[†]Department of Bioengineering, University of Pittsburgh, Pittsburgh, PA, USA

[‡]Artificial Heart Program. University of Pittsburgh Medical Center, Pittsburgh, PA, USA

[§]Center for Devices and Radiological Health, U. S. Food and Drug Administration, Silver Spring, MD, USA

[¶]Department of Surgery, University of Pittsburgh, Pittsburgh, PA, USA

Abstract

Bench-top *in vitro* hemolysis testing is a fundamental tool during the design and regulatory safety evaluation of blood-contacting medical devices. While multiple published experimental protocols exist, descriptions of the test loop reservoir remain ambiguous. A critical fixture within the circuit, there is no readily available blood reservoir that ensures thorough mixing and complete air evacuation: two major factors which can affect results. As part of the Food and Drug Administration (FDA) Critical Path Initiative, we developed a three-piece reservoir consisting of a 3D-printed base, a plastic clamp set, and a medical-grade blood bag. This simple, reusable, and cost-effective design was used successfully in the hemolysis assessment of FDA benchmark nozzles and prototype rotary blood pumps, and may be useful as an integral component to any *in vitro* blood circulation loop.

Keywords

blood reservoir; in vitro hemolysis testing; mechanical circulatory support devices; cardiopulmonary bypass

Introduction

The characterization of blood damage is essential for the development and safety evaluation of many blood-contacting medical devices including ventricular assist devices,

Corresponding Author: Dr. Marina V, Kameneva, McGowan Institute for Regenerative Medicine, 450 Technology Drive, Suite 300, Pittsburgh, Pa 15219, kamenevamv@upmc.edu, Phone: 412-624-5281, Fax: 412-624-5363.

Conflicts of Interest:

The authors declare that they have no conflicts of interest.

Disclaimer: The mention of commercial products, their sources, or their use in connection with material reported herein is not to be construed as either an actual or implied endorsement of such products by the US Department of Health and Human Services.

cardiopulmonary bypass (CPB) components, and hemodialysis catheters.[1,2] In addition to pre-clinical *in vivo* animal studies,[3] *in vitro* hemolysis testing is an important tool for assessing the hemocompatibility of mechanical circulatory support devices.[4] Although multiple publications describe variations of the typical experimental loop (Figure 1-A), they lack details regarding the blood reservoir [5–7]: a vital component towards ensuring accuracy and reproducibility.

Most commercial blood reservoirs cannot 1) accommodate a wide range of blood volumes, 2) provide thorough blood mixing, 3) eliminate the air-blood interface, while 4) remaining reusable or inexpensive. Hard-shell CPB or cardiotomy reservoirs, unable to be de-aired, retain a large air interface. Single-use, soft-shell CPB reservoirs or clinical blood bags often contain extraneous or undersized ports leading to stagnation, pressure loss, and potential collapse. In support of the Food and Drug Administration (FDA) Critical Path Initiative Computation Fluid Dynamics (CFD)/Blood Damage Project, a cost-effective, small volume blood reservoir consisting of a disposable bag clamped to a reusable base (Figure 1-B) was developed to enhance washout while allowing for the complete elimination of air during *in vitro* testing.[8]

Materials and Methods

½”-Port Reusable Base

The rigid base was designed in SolidWorks® (Dassault Systemes®, Velizy, France) with two barb fittings to accept ½” ID tubing, a flow separator between the inlet and outlet ports, and a 0.10” external circumferential lip (Figure 2-A). The flow separator was shelled to remove excess material while maintaining a minimum thickness of 3/64”. All corners and edges were smoothed on the model before manufacturing. Fabrication was accomplished with a stereolithography additive (SLA) printer (Viper™ SLA® System, 3D Systems® Inc, Valencia, CA, USA) using the Somos® Watershed XC11122 (DSM®, Elgin, IL, USA) photopolymer resin. Following light sanding to remove the printer-generated structural supports, a liquid coat of the same photopolymer resin was brushed onto the blood-contacting surfaces before a 30 minute UV cure and a final isopropanol rinse.

Disposable Blood Bag

A medical-grade, three-port, 500-mL compliant polyvinyl chloride (PVC) blood bag (Qosina®, Edgwood, NY, USA) was diagonally heat-sealed and trimmed along the bottom to remove the existing orifices. An optional Luer fitting (Qosina®, Edgwood, NY, USA) was fixated into the apex with cyanoacrylate glue before fitting the bag over the base.

Clamp Set

The clamp set consisted of two plastic halves designed to secure the compliant bag against the rigid base (Figure 2-B). Milled from rigid PVC stock, the clamps have a 0.10” wide groove to accept the reservoir lip and two throughput holes for assembly using 10–32 machine bolts and wing-nuts.

CFD Analysis

A computational fluid dynamics (CFD) analysis to assess reservoir performance at low flow rates was conducted using SolidWorks® Flow Simulation and compared with a previously employed three-port (two ¼" ID, one ½" ID fenestrated), 1-L commercial CPB venous reservoir bag using a non-Newtonian, power-law blood model (asymptotic viscosity: 3.0 cP).[9,10] The reservoirs were assumed to be rigid with inlet flow set to 0.5 L/min to assess their stagnation potential, and an outlet boundary condition of atmospheric pressure at room temperature (20°C).

Results

The single-piece design of the 3D-printed, optically clear, reusable base, devoid of seams or adhesives, reduced the possibility of cracking and contamination. Sufficient wall thicknesses and fillets ensured rigidity and fluid washout of the reservoir base, while lowering printing time and resin cost. With similar material properties as clinically used ABS plastic, the Watershed XC11122 resin meets ISO 10993 standard specifications for cytotoxicity, sensitization, and irritation, along with USP Class VI standards. As the SLA printing process leaves an inherently porous exterior, the integrated liquid resin finish reduced surface roughness to an Ra<0.3 μm (Contour GT-KI®, Bruker AXS, Madison, WI, USA), avoiding concerns of delamination and wear associated with topical sealants. Compressing the PVC bag between the circumferential lip and clamp created a labyrinth face seal and a leak-proof assembly (Figure 2-C). Stain-resistant and easy to clean, the rigid bases have been washed and sanitized with degreasers, enzymatic detergents, 70% ethanol, and 10% bleach without issue before reuse with a new blood bag.

The cross-sectional velocity profiles from CFD-analysis of the commercial venous reservoir bag and proposed reservoir at 0.5 L/min are presented in Figure 3. Regions below 0.1 cm/s shown in black designate possible areas of stagnation. After equally distributing blood flow through the two smaller ports to minimize dead space, the three-port commercial bag was examined in two flow configurations. Defining the fenestrated port as the reservoir inlet created large areas of stasis below the inlet sleeve and above the bag outlets (Figure 3-A). Reversing the reservoir fluid flow direction decreased those regions by the development of two distinct circulation patterns from the overlapping flows between the shared inlet ports (Figure 3-B). In both conditions, peak velocity of 10.8 cm/s was located inside the sleeve of the fenestrated port. In contrast, the designed reservoir assembly had a maximum velocity of 16.5 cm/s and maintained a well-developed, counter-clockwise flow regime (Figure 3-C). The flow fields looked similar at higher flow rates (5 L/min, not shown) with reduced stagnation areas due to jetting, though with more pronounced secondary flow fields in the commercial reservoir.

While vertical operation is suggested to ensure adequate preload and allow hanging using the existing reinforced opening (Figure 4), the reservoir has also been utilized horizontally allowing for possible water bath submersion.[7] Priming and air removal was straightforward with the Luer fitting deairing port. The angled heat seal reduces blood volume and, in conjunction with the flow separator within the base, directs flow to minimize stagnant regions within the reservoir. As assembled, the ½"–port reservoirs have a fluid capacity of

250 mL and are customizable by adjusting the bag size, heat-seal angle, and insertion depth of the base before clamping.

Summary

This cost-effective assembly provides a useful *in vitro*, bench-top reservoir for hemolysis testing of blood-contacting devices. The reusable, single-piece base enables attachment to low cost, disposable blood bags. The rigid, large bore ports and flow separator minimize resistance, promote mixing, and prevent bag collapse, while the angled compliant bag allows for complete de-airing. The versatility of the reservoir makes it an effective component for any flow loop utilizing blood or other fluids, with an added heat exchanger if temperature control is required during vertical operation. Aside from possible natural yellowing of the reusable base due to ambient UV-light exposure, performance remains unchanged after four years of consistent use in a multi-laboratory study.[8] This novel reservoir has been used successfully during the hemolysis assessment in the FDA benchmark nozzles, rotary blood pump prototypes, and for flow visualization experiments.[8]

Acknowledgments

This work was supported by the U.S. Food and Drug Administration's Critical Path Initiative and in part by the National Institute of Health (Training Grant T32-HL076124 Cardiovascular Bioengineering Training Program) for Mr. Olia. We would like to thank J. Andrew Holmes from the Swanson Center for Product Innovation at the University of Pittsburgh, Swanson School of Engineering for sharing his technical and fabrication expertise.

References

1. Food and Drug Administration Center for Devices and Radiological Health: Guidance for Cardiopulmonary Bypass Oxygenators 510(k) Submissions; Final Guidance for Industry and FDA Staff. Silver Spring, MD: Food and Drug Administration; 2000.
2. Food and Drug Administration Center for Devices and Radiological Health: Implanted Blood Access Devices for Hemodialysis; Guidance for Industry and Food and Drug Administration Staff. Silver Spring, MD: Food and Drug Administration; 2016.
3. Food and Drug Administration Center for Devices and Radiological Health: Guidance for Industry and FDA Staff: General Considerations for Animal Studies for Cardiovascular Devices. Silver Spring, MD: Food and Drug Administration; 2010.
4. Zhang J, Gellman B, Koert A, et al. Computational and Experimental Evaluation of the Fluid Dynamics and Hemocompatibility of the CentriMag Blood Pump. Artificial Organs. 2006; 30:168–177. [PubMed: 16480391]
5. Naito K, Suenaga E, Cao ZL, et al. Comparative Hemolysis Study of Clinically Available Centrifugal Pumps. Artificial Organs. 1996; 20:560–563. [PubMed: 8817955]
6. Giridharan GA, Sobieski MA, Ising M, Slaughter MS, Koenig SC. Blood Trauma Testing for Mechanical Circulatory Support Devices. Biomed Instrum Technol. 2011; 45:334–339. [PubMed: 21812617]
7. ASTM International. ASTM Standard F1841-97 (2013): Standard Practice for Assessment of Hemolysis in Continuous Flow Blood Pumps. West Conshohocken, PA: ASTM International; 2013.
8. Herbertson LH, Olia SE, Daly AR, et al. Multi-Laboratory Study of Flow-Induced Hemolysis Using the FDA Benchmark Nozzle Model. Artificial Organs. 2015; 39:237–248. [PubMed: 25180887]
9. Johnson CA, Vandenberghe S, Daly AR, et al. Biocompatibility assessment of the first generation PediaFlow pediatric ventricular assist device. Artificial Organs. 2011; 35:9–21. [PubMed: 20626737]

10. Maul TM, Kocyildirim E, Johnson CA, et al. In Vitro and In Vivo Performance Evaluation of the Second Developmental Version of the PediaFlow Pediatric Ventricular Assist Device. Cardiovasc Engr and Technol. 2011; 2:253–262.

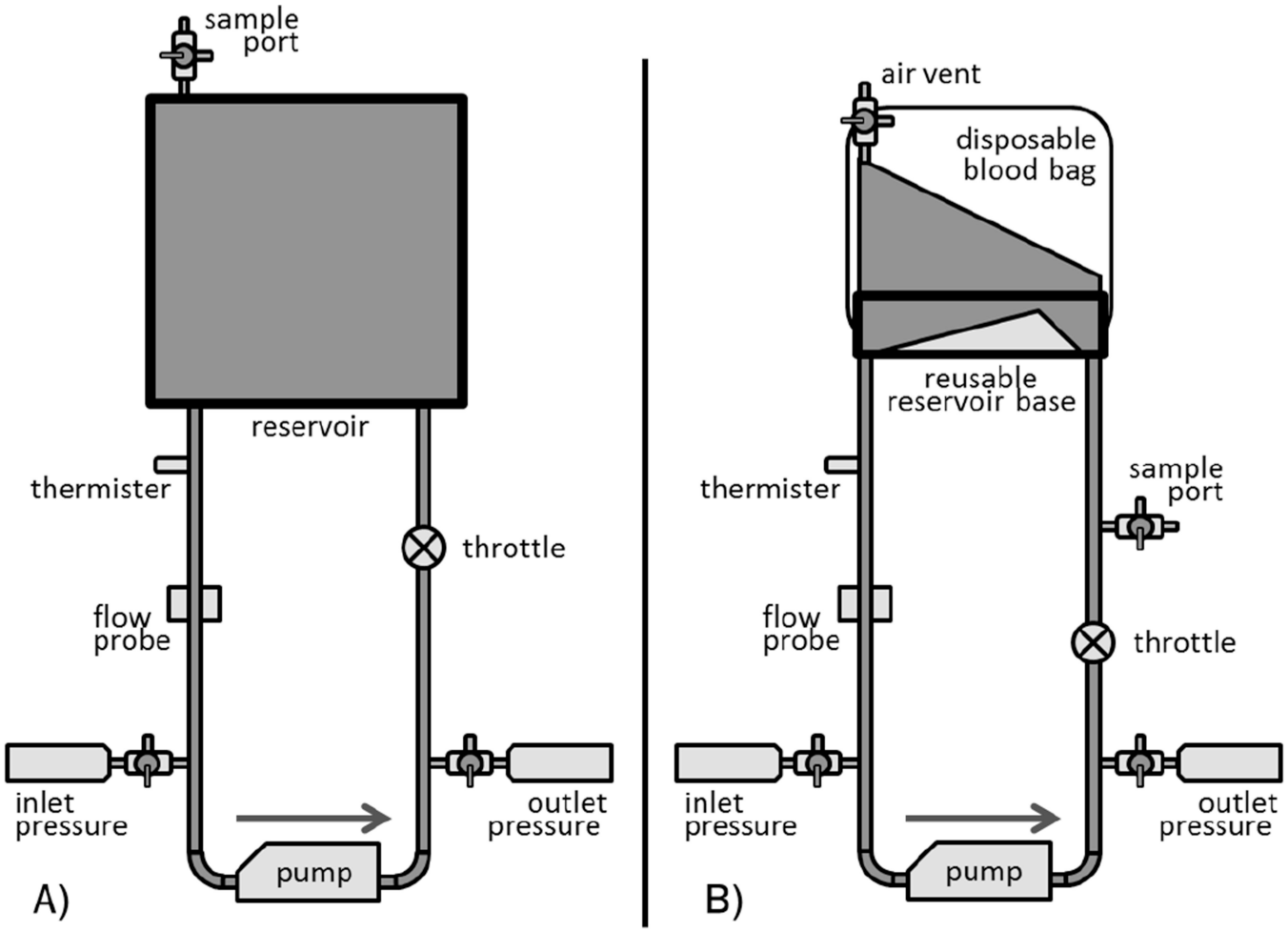

Figure 1.

Example mock loops used for *in vitro* hemolysis testing of blood pumps with **A)** the typical generic reservoir representation and **B)** the proposed reservoir design.

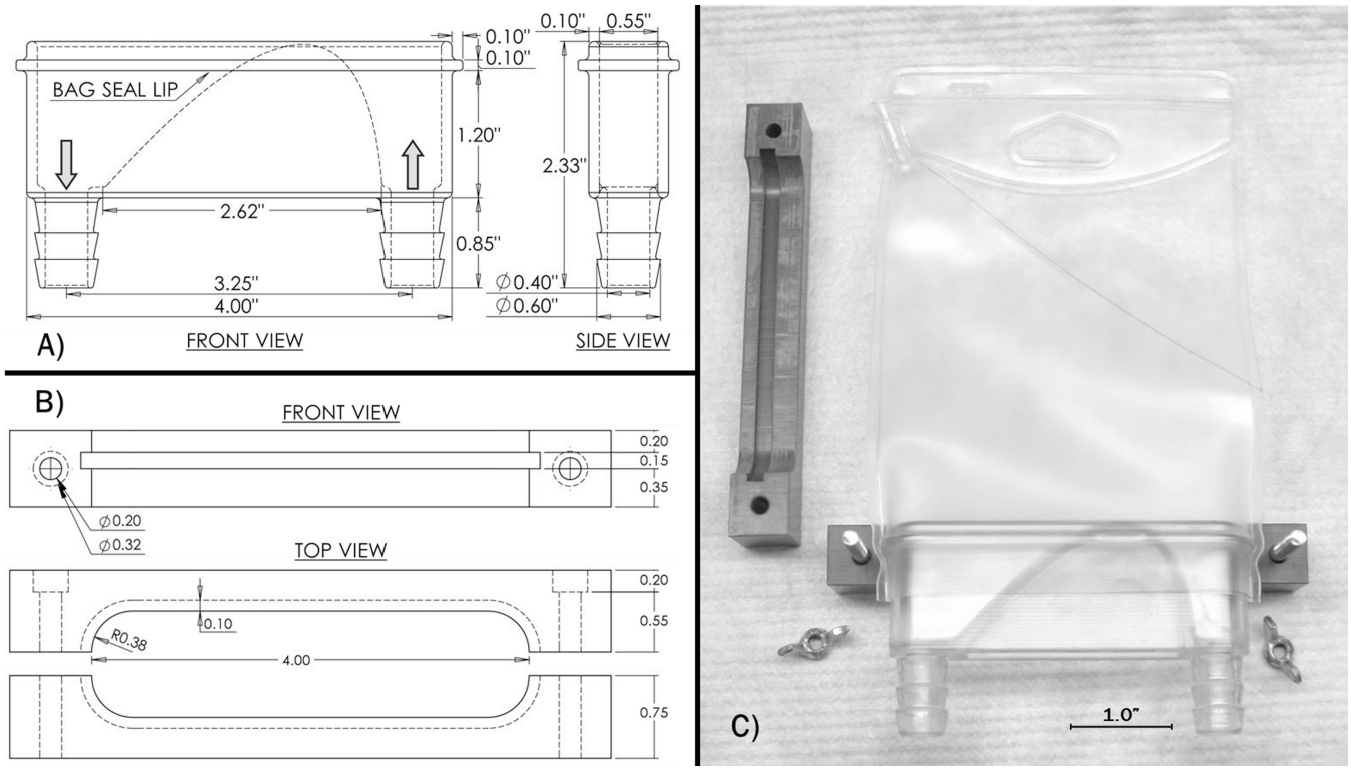

Figure 2.

Schematic of the reusable ½"–port **A)** printed base, **B)** machined clamp set, and **C)** the manufactured reservoir components with the modified blood bag before assembly.

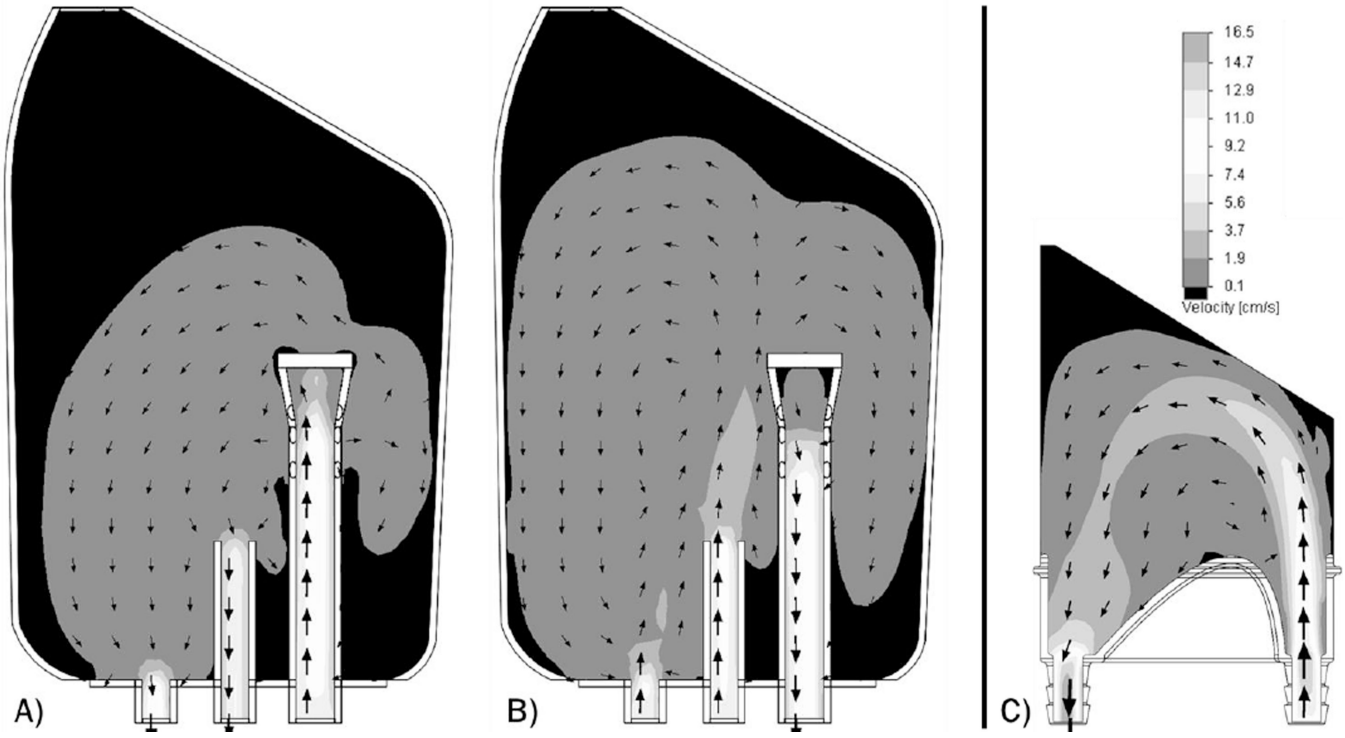

Figure 3.

CFD predicted velocity contours at 0.5 L/min for the commercial venous blood bag with the fenestrated port defined as the reservoir **A)** inlet or **B)** outlet, and **C)** the developed ½”-port reservoir assembly.

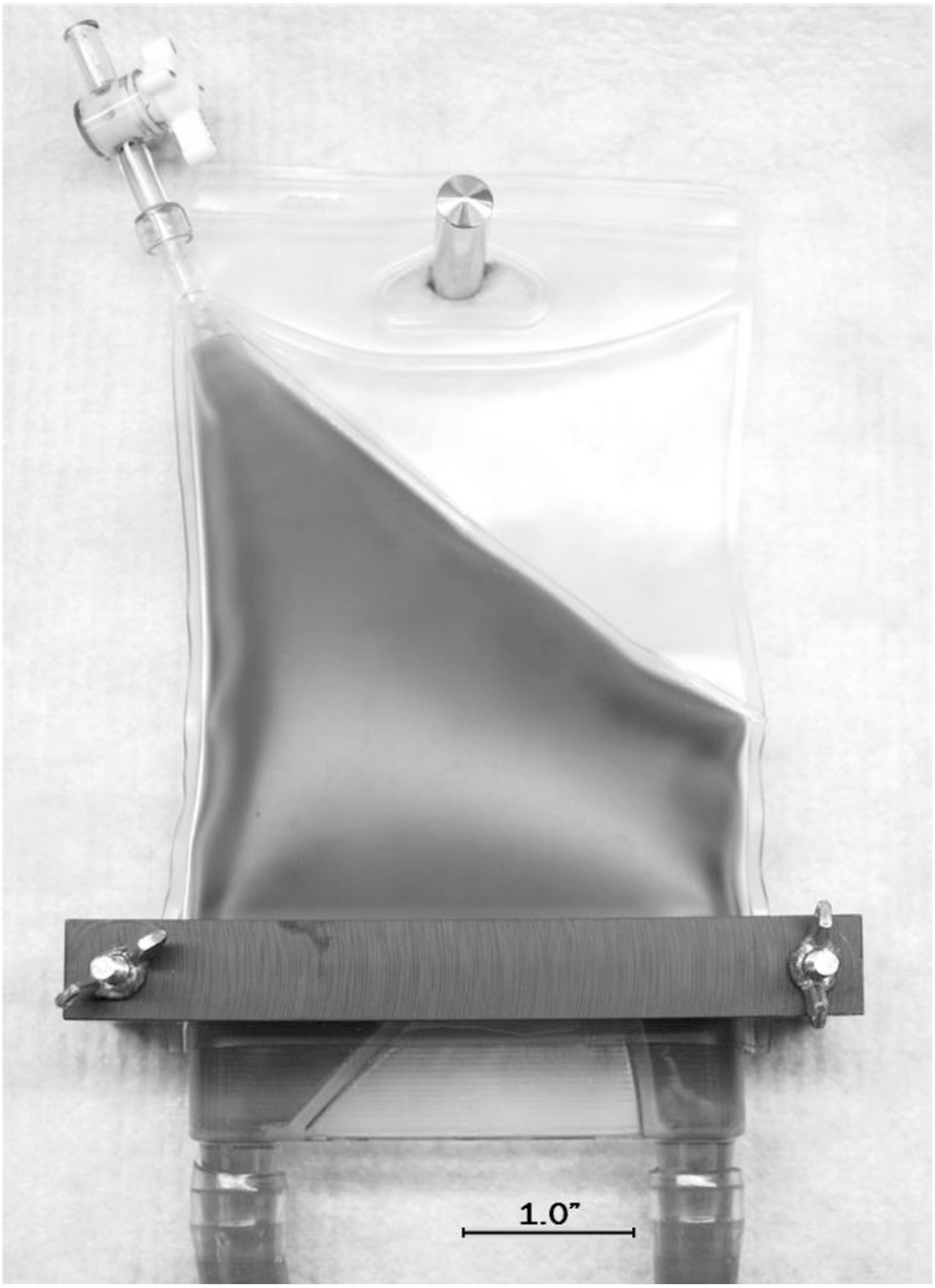

Figure 4.
The vertically hung, ½"–port blood reservoir assembly after clamping, filling and de-airing.