1.0 PURPOSE:

To replace the use of real blood or red blood cell suspensions, use a known Blood-mimicking fluid (BMF). BMF is an ultrasound compatible fluid that mimics the haemodynamics and ultrasound properties of blood.

2.0 SCOPE:

The procedure involves the mixing of specific materials. The mixed solution is later filtered, degassed, viscosity tested and, if necessary, altered to refine the quality of the BMF.

3.0 RESPONSIBILITIES:

A WHMIS trained individual should perform the mixing and degassing procedure in a properly functioning class B fumehood. Filtering and viscosity testing can take place in any laboratory area.

4.0 DEFINITIONS:

BMF – Blood-mimicking fluid
Clean – Free of any possible contaminants

5.0 REFERENCES:

Ramnarine, K. V., P. R. Hoskins, et al. (1999). "Doppler backscatter properties of a blood-mimicking fluid for Doppler performance assessment." Ultrasound in Medicine & Biology **25**(1): 105-10.

Ramnarine, K. V., D. K. Nassiri, et al. (1998). "Validation of a new blood-mimicking fluid for use in Doppler flow test objects." Ultrasound in Medicine & Biology **24**(3): 451-9.

6.0 MATERIALS and EQUIPMENT:

6.1 MATERIALS

- Glycerol
- Jet dry
- Orgasol
- Dextran
- CA24
- Water (distilled)

6.2 EQUIPMENT

- Fumehood
- Digital weight scale
- Measurement container
- Funnel
- Graduated cylinder

- Magnetic stir plate
- Peristaltic pump
- Tygone R-36 tubing
- 0.25” PVC tubing
- Vacuum chamber
- Retort stand
- Spatula
- Spoon
- Cannon-Fenske Routine Viscometer
- Digital timer

7.0 PROCEDURES:

7.0.1 PREPARATION

1. Refer to *section 20: Appendix 10* of the *Robarts Laboratory Health and Safety Manual* for information about the use and maintenance of the fumehood.
2. Clean a large *square-bottomed* container with soap and rinse thoroughly with water. Leave to dry.
3. Clean any plastic bowls that will be used to move substances from the digital scale to the large container.
4. Obtain the materials: glycerol, jet dry, orgasol and dextran. Orgasol can be found in a container in the wet lab room B-07.1 and must be transported in a sealed plastic container to the fumehood.
   IMPORTANT: DO NOT use dextran with lot number 88HO744 because it is defective.

△**WARNING:** Use a NIOSH approved respirator when handling orgasol. It is harmful to the lungs.

7.0.2 MIX MATERIALS

Make all measurements with the digital scale. Remember to tare the digital scale each time a clean plastic bowl is used for measuring a substance. Use a funnel to pour all measured substances into the large container. Measured material should be accurate within 0.3% of the desired value.

△**CAUTION:** Mixing and measurements must take place inside the fumehood to prevent the inhalation of any harmful airborne particles.

1. Measure and gently pour each substance into the large *square-bottomed* container using a funnel in the following order:

	Substance	Percent by Weight [%]
1	Glycerol	10.06
2	Jet dry	0.90
3	Orgasol	1.82
4	Dextran	3.36
5	CA24 (preservative)	0.3

6	Water (distilled)	83.86

Table 7.1 Mixing proportions.

Densities and sample measurements can be found in ATTACHMENT A.

2. Apply a WHMIS workplace label to the large container as well as a list of the mixed contents (Flammability – 0, Reactivity – 0, Health – 0, Personal Protection - 0).
3. Use a magnetic stir plate to mix the contents in the large container. Adjust the setting until the mixing is silent.
4. Leave on stir plate until consistency is uniform. This may take a few hours.

7.0.3 FILTER

To turn OFF the peristaltic pump in case of an emergency, set the gauge to 0 or flick the onboard switch.

A. SETUP

1. Clean all tubing to be used during the filter process. This is a very sensitive procedure and must be free of any contaminants.
2. In order, layer an O-ring, 400 mesh (38 μm sieve opening) screen, and a plastic placement screen between the two filter pieces.
3. Clamp the two filter pieces tightly together. Check that O-ring is sealed.
4. Place Tygone R-36 tubing into the peristaltic pump.
5. Connect two 0.25” PVC hoses at each end of the Tygone R-36 hose. A click sound will indicate a secure connection.
6. Note the direction in which the fluid will flow through the peristaltic pump. Put the 0.25” PVC hose end (receiving unfiltered BMF) into the BMF container.
7. Connect the 0.25” PVC hose end (where BMF will exit) to the filter.
8. Connect 0.25” PVC tubing to the other end of the filter. Lead this tubing into a clean and empty 2L container. The setup is shown in Figure 1.
   IMPORTANT: *The vacuum chamber must accommodate the size of the 2L container.*
9. Secure both tubes leading into the containers with clamps. Avoid causing any obstructions to flow.
10. Apply a WHMIS label on the 2L container.

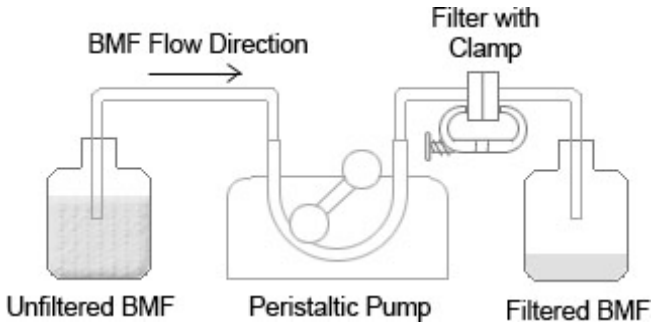

Figure 1: Filtering Setup

B. START THE PERISTALTIC PUMP

1. Set the gauge to 0.
2. Turn ON the pump with the onboard switch.
3. Slowly turn the gauge up to 30.

C. AGITATE FILTER PIECE

The fluid over the sieve must be constantly moving so that there is no accumulation of solid deposits in the filter.

1. Shake the clamped filter piece manually with a free hand.

△**CAUTION:** Make sure that the tubing is not bent during the shaking. It may cause a pressure buildup that could result in a disconnection and spill.

D. STOP THE PERISTALTIC PUMP

1. Set the gauge to 0 or flick the onboard switch OFF.

7.0.4 DEGAS

1. Place an **unsealed** 2L container of filtered BMF into the vacuum chamber.
2. Turn both blue valves OPEN (Both *vent valve* and *pump vacuum valve* levers point parallel to the tube)
3. Turn ON the pump. The switch is located on the power cable.
4. Close the *pump vacuum valve* FIRST.

△**CAUTION:** DO NOT make the mistake of closing the *vent valve* first. Doing so will cause the contents of the container to explode because of the large internal pressure. Adjusting the pressure over 50mmHg will have a similar result. Refer to *7.5.1.B BMF SPILL CLEANUP* for a cleanup procedure in case of a spill.

5. Close the *vent valve.*
6. Use both valves to carefully adjust for the desired pressure. Opening the *pump vacuum valve* will increase the pressure. Opening the *vent valve* will decrease the pressure.
7. Set the pressure to 10 mmHg.
8. Turn OFF pump. Let it degas for 10 minutes.
9. Turn ON pump. Set the pressure to 15 mmHg. Turn OFF pump. Let it degas for 10 minutes.
10. Turn ON pump. Set the pressure to 20 mmHg. Turn OFF pump. Let it degas for 5-10 minutes.
11. Open the *vent valve*.
12. Open the *pump vacuum valve*.
13. Seal the 2L container of the degassed BMF.
14. Label the container with the date BMF was made.

7.0.5 VISCOSITY TEST

1. Insert a suction syringe over the larger opening of the viscometer.
2. Invert the viscometer and apply suction to the larger opening, immersing the smaller opening of the viscometer in a sample of the BMF.
3. Use the suction syringe to draw BMF into the viscometer.
4. Remove the suction syringe. Clamp the viscometer, vertically aligned, to a retort stand.
5. Apply suction to the smaller opening to draw the BMF above the two indication lines on the viscometer.
6. Remove the suction syringe.
7. Record the time it takes (in seconds) for the meniscus of the BMF to fall between the two indication lines on the viscometer. Use a digital timer.
8. Perform additional trials to improve accuracy.
9. Record the temperature of the room, as it will affect the viscosity constant.

7.0.6 VISCOSITY ALTERATION

[*Needs to be revised and checked by Hristo. The "calculated amounts" perhaps need to be outlined.]*

A. VISCOSITY IS TOO LOW

If the viscosity happens to be too low, add a calculated amount of dextran.

B. VISCOSITY IS TOO HIGH

If the viscosity happens to be too high, you will typically want to make a fresh batch of BMF. However, you can attempt to add a calculated amount glycerol and water in their respective proportions found in Table 7.1.

7.0.7 CLEANUP

- Use soap and water to wash any spare containers that were exposed to any of the materials.
- Apply a WHMIS label on any containers storing BMF.
- Wipe down used countertops with dish soap and water.
- Clean all the tubing that was used.
- Put all equipment back where it was found.

7.1 STORAGE AND HOLDING TIMES:

BMF is stored at room temperature in a labeled container that has the date it was mixed. There is no maximum holding time for BMF, but if there is evidence of contamination, inconsistency, or of a change in viscosity, it should no longer be used.

7.2 RISKS TO PERSONNEL AND PRECAUTIONS FOR RISK REDUCTION:

7.2.1 Refer to *section 7: Basic Safety* of the *Robarts Research Laboratory Health and Safety Manual* for standard lab safety practices.

7.2.2 Wear impervious (preferably non-latex) gloves and safety glasses during the mixing procedure. If orgasol or dextran contact the eyes, flush for 15 minutes with water at an eye wash station.

7.2.3 Wear a NIOSH approved respirator when handling the powder forms of orgasol and dextran. If inhaled, person must be moved to fresh air immediately. If breathing becomes difficult, seek medical attention by contacting security at x34041.

7.3 CALCULATIONS:

The viscosity constant, C_o, is linearly dependant on the ambient room temperature, T, by the following formula:

$$C_o = 0.01507 – (10^{-6})•T \qquad [cS/s]$$

The viscosity, C, can be found using the following formula:

$$C = C_o•t \qquad [cS]$$

Where t is the time it took for the BMF to pass between the two indication lines on the viscometer in seconds.

NOTE: You can assume the viscosity constant to be 0.01503 cS/s to get a reasonably accurate estimate of the viscosity if you are operating within room temperature.

7.4 ACCEPTANCE RANGES:

The viscosity value of the BMF must be in the range of 4.02±0.10 cS, which is the viscosity value of real blood.

7.5 CONTINGENCIES:

7.5.1 SPILLS

The hazardous material user is responsible for cleaning up a spill. If the spill is beyond the resources or abilities of the users to cleanup, contact security at x34041 who will then notify the Robarts Emergency Response Team. During off-hours and weekends call the Emergency Pager at 14111 or 9-911 if the situation warrants immediate attention. This duty must not be delegated to other staff such as caretakers.

Follow the general spill procedure outlined in "section 10.6: Chemical Spills" of the Robarts Research Health and Safety Manual.

A. DEXTRAN AND ORGASOL CLEANUP

Sweep or scoop up waste and place in a bag, avoid raising dust. Hold the bag for waste disposal. Ventilate area and wash spill site after material pickup is complete.

B. BMF SPILL CLEANUP

Clean up with a mop. Then clean the surface with soap and water.

Awaiting for a response about proper disposal of BMF from the Health and Safety Officer.

8.0 DOCUMENTATION:

Record all measurements and calculations in a book for future reference.

9.0 REVIEWS AND REVISIONS:

This procedure shall be reviewed for compliance and effectiveness and revised as necessary.

10.0 ATTACHMENTS

ATTACHMENT A. BMF-5μm

ATTACHMENT B. BMF-10μm