**PROTOCOL:**

**1. Rig Assembly**

1.1) Assemble prototypecomponents(fluidics, optical, electronic system control/ data acquisition) for a simple flow cytometry system to be used in reduced gravity conditions **[lunar g + micro g –** to note the differences in conditions**]..**

**1.1.2) A fluid source container that can be loaded without trapping air (See Step 3.3)**

**1.1.3) A fluid waste container to collect waste without building a backpressure that will compromise flow**

**1.1.4) A sample loader for use in reduced gravity**

**1.1.5) A micromixer that does not rely on powered mechanical subcomponents to function**

**1.1.6) A palm-sized miniature optical block to detect individual flowing particles. [opt block]**

**1.1.7) Electronics and software for device control and data acquisition**

1.1.7.2) A program custom software (example in Fig. 2E) for the laptop to operate rig devices and synchronize all data.

**1.3) Electrical power scheme**

1.3.1) A mechanism for quick and complete electronics shutdown (required for safety reasons on reduced-gravity flights)

1.3.1.1) Connect a single power strip (with single I/O button) to the aircraft power distribution panel (120 VAC 60 Hz).

**1.4) Flight-ready rig layout**

1.4.1) Considerations for successful in-flight performance

1.4.1.1) Total space available is limited to a smaller area than provided for a similar demonstration on the ground (**Figure 3A**) ) **[cabin view]**. Consider total space available and how that space will be divided between experimental rig space (including for components beyond those formally part of the prototype) and user space surrounding the rig **[test op]**.

1.4.1.2) Determine which components are more appropriately accessed at a standing, kneeling, or floor height, as well as considering which components will benefit most from the protection attained within a structure support structure.

1.4.2) Rig support structure

1.4.2.1) Obtain or construct a vertical equipment rack that meets considered layout needs, contains all components, enables different vertical levels for organization, withstands flight accelerations, and securely attaches to the intended aircraft cabin floor.

**1.6) In-flight demonstration implementation**

1.6.1) Simple interventions to proceed through demonstrations

1.6.1.1) Incorporate additional components that eliminate required manual tubing adjustments in-flight or other actions that require significant dexterity or could risk leaking fluids into the cabin environment.

1.6.1.2) Program software to proceed through demonstrations (**Figure 6**) using single-button interventions (e.g., single click on the laptop).

**1.7) Flight disturbance readiness:** Ready system for possible sudden jolting forces, vibration, or passenger collision in flight.

1.7.2) Physical disturbance testing

1.7.2.1) Shake rig support structure with all components in place. **[motion test]**

1.7.2.2) Check individual component functionality after subjecting the rig to the disturbance, particularly aligned optical components.

**2. Demonstration Preparation and Logistics**

**2.1) In-flight and ground team role assignments**

2.3.5) Train for unexpected in-flight occurrences including sudden forces hitting the rig or the plane suddenly leveling out in the middle of an experiment.

2.3.7) Train multiple individuals as primary operators **[alt user]** to expertly operate the device in-flight. It is unpredictable who will get sick during the parabolas, and a given user may be unaffected on one flight and become sick on another.

**2.6) Pre-flight testing --** Perform pre-flight testing at the flight location to check functionality of all components several days before the flights **[ground prep]**.

**3. In-flight Demonstrations**

**3.4) Load fluid source vials**

3.4.1) Apply fresh, powder-free latex diaphragm to vial (cut finger from glove acceptable). Make sure the diaphragm is long enough to extend from the vial floor and fold over the top outer rim. Slide the vial ring over the folded portion.

3.4.3) Before filling the vial, negatively pressurize the vial with a syringe to expand the diaphragm. Pour fluid to top of vial and insert the cap at an angle such that no air is trapped under the cap during cap placement (some fluid will spill out). Briefly remove slide clamp to prime the outlet tubing and release collapsing pressure exerted by the diaphragm.

3.7) Position rig operators once in-flight, nearing dedicated parabola airspace. **[op pos]** Provide enough space to allow rig operators to lie down during high-gravitation intervals and enable access to leg straps. Once parabolas begin, do not apply strong forces on body during reduced gravity as this may send the body up too quickly and somewhat dangerously **[head crash]**.

**3.8) Perform microfluidic mixer demonstration (Day A only)**

3.8.2) Mix blood and saline in a 1:1 ratio at 1.5, 2, 3, 4, 5, and 6 psi, for at least 2 parabolas each, recording video data synchronized to other readings. **[lunar g blood mix]**

3.8.3) Inject air into saline inlet to test whether channel architecture will trap a bubble that could prevent optimal mixing. **[air bubble]**

3.8.4) Mix blue and yellow food dyes at 1.5, 2, 3, 4, 5, and 6 psi for at least 2 parabolas each, again recording synchronized data. **[lunar g dye mix]**

**3.9) Perform optical block and sample loader demonstrations (Day B only)**

3.9.6) When the plane enters reduced gravity, use a sample syringe to place a drop of the counting bead dye mixture on a fingertip to simulate a finger prick sample. **[samp load]** Use an unrealistically large drop (**Fig. 1B**) to test the limits of keeping a finger prick sample on a finger during reduced gravity.

3.9.7) Use capillary consumable to pick up sample (about 10 uL) off finger and load into capillary loader. **[samp load]**

3.9.9) Drive sample into optical system for detection. **[samp load]**