Note: FilmArray Instrument should be powered on and ready for use prior to pouch preparation.

**STEP 1: Prepare Pouch**
- Insert pouch into Pouch Loading Station.
  - **Note:** Use only materials provided with or validated for the BioThreat Panel kit when testing air, powder or surface swab samples
- Place Sample Injection Vial into red well.
- Place Hydration Injection Vial into blue well.

**STEP 2: Hydrate Pouch**
- Twist off Hydration Injection Vial, leaving blue plastic cover in Pouch Loading Station, and insert into pouch hydration port.
- Forcefully push down to puncture seal and verify that Hydration Solution has been drawn into pouch.

**STEP 3: Prepare Sample Mix**
- Add Sample Buffer:
  - Hold the Sample Buffer Tube so that the tip is facing up.
  - Gently pinch the textured plastic tab on the side of the tube until the seal snaps.
  - **Note:** Do not touch the tip of the tube.
  - Invert the tube over the Sample Injection Vial.
  - Dispense Sample Buffer by squeezing the liquid-filled end using a slow, forceful squeeze, followed by a 2nd squeeze.
    - **Note:** Squeezing the tube additional times will generate excessive bubbles, which should be avoided.
- For Air Samples (Air-into-PBS):
  - **Note:** If using dry air filter, follow all steps below. If using liquid air sample, skip to 4th bullet point below.
  - Place dry air filter into a 50mL conical tube with collection side facing away from the side of the tube.
  - Add 10mL PBS with 0.1% Triton X-100 to the conical tube.
  - Cap tube and shake by hand for 2 minutes.
  - Using Transfer Pipette, draw filter wash or liquid air sample to the 3rd line (0.3mL) and add to Sample Injection Vial.
STEP 3: Prepare Sample Mix (cont.)
- For Powder Samples:
  - Wet the tip of the swab by dipping into the sample buffer in Sample Injection Vial.
  - Touch the tip of sample swab to powder sample. Place swab into the Sample Injection Vial and swirl for 5 seconds.
  - Break the swab handle at the molded break point, leaving the tip of the swab in the Sample Injection Vial. Discard the handle in an appropriate waste container.
- For Surface Swab Samples:
  - After collecting sample with swab, place the tip of the swab into the Sample Injection Vial and swirl for 5 seconds.
  - Break the swab handle at the molded break point, leaving the tip of the swab in the Sample Injection Vial. 
    **Note:** If the swab handle does not have a molded break point, cut the tip of the swab with clean scissors, leaving the tip of the swab in the Sample Injection Vial.
  - Discard the handle in an appropriate waste container.
- Tightly close lid of Sample Injection Vial and mix sample by gently inverting 3 times.
- Return Sample Injection Vial to red well of Pouch Loading Station.

STEP 4: Load Sample Mix
- With Sample Injection Vial in the Pouch Loading Station, twist to loosen it from the red plastic cover and pause for 5 seconds.
- Lift Sample Injection Vial from red plastic cover and insert into Sample port.
- Forcefully push down to puncture seal and verify that Sample Mix has been drawn into pouch.

STEP 5: Run Pouch
- Screw vials back into plastic covers in Pouch Loading Station before disposing of them in a biohazard container.
- Remove pouch from Pouch Loading Station. **Optional added operator protection:** Before removal from biosafety cabinet, run a bleach wipe, a paper towel with 10% bleach (one part household bleach to nine parts water), across the top of the pouch from the Hydration port to the Sample port, and follow with a water wipe. This reduces the potential for contact with small amounts of sample mixed with sample buffer that may be retained at the sample injection port.
- Follow instructions on computer for inserting pouch in the FilmArray.
- Scan or enter pouch information and select the correct BioThreat protocol.
- Start run.

**Note:** If repeated ‘error’ messages are obtained, contact BioFire Defense Technical Support.