Protocol for counting oly larvae using the Z2 Coulter Counter at Taylor Shellfish Hatchery

Pre-work up

* Be sure to have plenty of 95% ethanol with you
* Have clear packing tape to protect labels
* Apply Packing Tape
* Visually inspect sample for gray larvae or large white larvae (possibly too large for counter)
* Have clean sample beakers ready
* Build up dual screen larvae catchers with 120 um screen on top and 48 um screen on bottom.
* Have lots of paper towels ready

Sample Prep

1. After writing down label information, pour sample over the 120 um screen. Larvae should pass through leaving only large gunk on the 120 um screen. The 48 um screen will catch larvae. Save the 120 screen to inspect for larger larvae.
2. From below, dry 48 um screen thoroughly but gently with paper towel to remove excess ethanol.
3. When EtOH is fully removed, wash screen off into sample beaker with filtered sea water (FSW)
4. Fill sample beaker to 50 ml with FSW. NO MORE, NO LESS.
5. Place empty sample falcon tube in rack next to counter operator in order from back to front. No more than 5 samples at a time.

Counting Larvae

1. Ensure that aperture is submerged in clean isotonic solution before starting.
2. Pull down sample stand, remove isotonic solution from aperture, and replace with sample beaker.
3. Slide stand back up, open sample door, and align the beaker in such a way as to have the aperture close to, but not touching, the back wall of the beaker while the sample propeller is partially submerged. **Do not submerge propeller fully**, it will either stop spinning or will interfere with the aperture. Be careful not to spin too vigorously.
4. If everything is aligned correctly, press start on digital display next to counter.
5. Counter will begin to whirr and count sample. It will beep to note count completion. Write down first count and restart count again. Repeat twice more.
6. When final count completed, wait until counter stops whirring then remove sample. Ignoring this step WILL damage machine.
7. Replace aperture in isotonic solution and press unblock button to clear aperture of previous sample.
8. Move on to next sample.

Unblock Aperture

1. Occasionally counting will stall and not complete. This is most likely due to blockage in the aperture.
2. Use view screen in top right corner of machine to determine if aperture is blocked. If center clear or light brown, then aperture is clear. If center has a black or dark brown spot, aperture is clogged.
3. Press unblock button and allow machine to eject material stuck in aperture.
4. Count will restart immediately following the unblock maneuver with no extra prompting.

Recollecting sample

1. After sample has been counted thoroughly, move labeled empty falcon tube to a separate stand. Pour the FSW sample solution through a clean 48 um sieve.
2. Use a squirt bottle of FSW to collect sample along an edge of the screen.
3. Remove excess FSW by gently running finger over back of the sieve.
4. Use a squirt bottle of EtOH to rinse and collect sample into original falcon tube using only 10-15 ml 95% EtOH.
5. Mark cap with an X to show that sample has been counted.
6. Return tube to rack making sure lid is tightly secured.