TOOLKIT – STANDARD OPERATING PROCEDURE FOR SPATT BAGS ASSEMBLAGE AND EXTRACTION OF HAB TOXINS

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Introduction:

Solid Phase Adsorption Toxin Tracking (SPATT) is "a simple and sensitive *in situ* (monitoring) method that involves the passive adsorption of biotoxins onto porous synthetic resin filled sachets (SPATT bags; Figure #1) and their subsequent extraction and analysis"[1].

In countries with indigenous shellfish industries, regular monitoring of algae blooms and biotoxin levels in marine waters are essential public health measures; warnings of imminent or actual contamination events may minimize consumer exposure to potentially harmful shellfish products. Occurrences of harmful algae blooms have dramatically increased in recent decades due to increasing eutrophication and other factors such as the spread of toxins in ballast water discharges, climate change, ocean acidification, and global shellfish product trading[2]. SPATT technology, in common with other continuous monitoring techniques, can be directly deployed in the marine and freshwater environment, an advantage over traditional lab-based methods such as shellfish tissue testing or phytoplankton microscopic cell counting.

Figure #1: SPATT bags ready for deployment

In addition, SPATT technology is a simple, cost-effective technique for recovering targeted analytes from a aqueous environment. However, SPATT will only detect dissolved biotoxins (extracellular biotoxins) which is one of its limitations. Furthermore, the SPATT technique is not able to detect at the ng/g of toxin concentration required by health advisory authorities. However, it is still a useful predictive and preventative tool for biotoxin monitoring when used in conjunction with analytical instrumentation (e.g., LC-MS/MS) for the screening of seafood[2].

Procedure for SPATT Bag Construction

1. Turn on a heat-sealer and warm up according to manufacturer's instructions.
2. Cut two 60 mm x 12 mm pieces of mesh that will form SPATT bag sides (use mesh size in the range 80 to 100 µm). When marking out to cut by hand, ensure no pen or Sharpie ink stains the mesh as the will contaminate resin once immersed in MeOH. Mesh can also be cut with a laser cutter/engraver.
3. Overlay mesh pieces carefully then, using the heat sealer, seal three edges of the mesh by applying heat twice to each edge. Use a heat setting to create seams without melting holes in the weld. Without disturbing the mesh allow the weld to cool for between five and ten seconds, then apply heat again to re-weld the seam. This step ensures the weld fully penetrates both layers of mesh (Figure #2).
4. Check visually for gaps in welds and confirm weld strength by gently trying to pull seams apart. Weaknesses are most common at corners, where welds cross. Ensure heat-sealed sides of the mesh do not have any gaps which result in resin loss during deployment.

Figure #2: Redesigned SPATT bags with integrated 'numbered' laser-cut plastic label, with all four sides heat sealed.

5. Place a weighing boat on a scale (tare to zero) and weigh out 3 g of dry equivalent resin using a clean spatula. Resin with 42.5% moisture: 5.22 g wet weight = 3 g dry weight.

$$MC = (w - d) / w * 100$$

Where MC is the moisture content (%)
w is the weight while wet
d is the weight while dry

6. Pour the resin into the SPATT bag (3 g dry weight) using a miniature funnel (Figure #3).
7. Push the resin toward the bottom of the SPATT bag.
8. Heat-seal the fourth open edge, 15 mm from the top of the bag (repeating the double heat application technique described in step 3) to trap the resin. Check the integrity of this weld carefully (see step 4.)
9. Insert a characteristically numbered laser-cut plastic label, size 6 mm x 42 mm, into the pocket at the top of the bag. The label should be engraved with a unique ID and have a 3 mm diameter hole near one end (hole centre 3 mm from the end).
10. Heat-seal the fourth edge 4 mm from the top of the bag (repeat technique in step #3). This final weld traps the label. The bag should look like Figure #2.
11. With a soldering iron tip positioned over the hole in the plastic label, melt through both sides of the bag. Heat from the soldering iron will melt mesh around the circumference of the hole, reinforcing it (Figure #4).
12. Prepare a weighted deployment line, inserting plastic zip ties at required depth intervals.
13. During deployment of SPATT bags in the field, secure activated SPATT bag to the deployment line with the pre-positioned zip ties (Figure #5).

Figure #3: Filling SPATT bags with adsorbent phase, using funnel.

Figure #4: Application of a soldering iron to generate hole in label for zip tie attachment.

Resin Activation and Deployment:

1. Submerge SPATT bag with resin in methanol for approximately 24 hours.
2. After 24 hours, rinse off methanol with ultrapure water.
    a. Fill a 500 ml beaker with ultrapure water and dip the SPATT bag in the water, lightly agitate the ring to ensure all the resin contacts the water.
    b. The methanol will react with the water and increase the temperature of the water.
    c. Pour the water out, refill the beaker, and repeat rinses until the temperature of the water does not increase when the hoop is placed in the beaker.
3. Place the SPATT bags in a Ziplock bag with ultrapure water covering the resin to ensure the resin does not dry out.
4. Store in a refrigerator until deployment (resin is stable for months in a refrigerator).
5. Use zip tie to attach SPATT to a structure or a weighted line (Figure #5).

Figure #5: Redesigned SPATT bags with integrated 'numbered' laser-cut plastic label connected to deployment line.

Retrieving SPATT Samplers from the Field:

1. Upon collection rinse as much silt and debris from the SPATT bag as possible using field water.
2. Put SPATT bag into a labelled Ziplock bag (does not need to be in water). Writing with sharpie pens directly onto the Ziplock bag is recommended.
3. Freeze immediately at < -20 °C until extraction of toxins in the lab.

Figure #5: SPATT bags (n=4) deployed at a specific depth, retrieved after a 2-week deployment.

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References:

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