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SEALING WHIRL-PAK®-TYPE BAGS CONTAINING WET SAMPLES

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ABSTRACT—Whirl-Pak® and similar "fold-and-twist" blended polyethylene bags (FATs) are excellent containers for many types of biological samples because they are shatter-proof, lightweight, flat and thin when empty, less expensive than glass containers, nearly transparent, and available in a variety of sizes. Nevertheless, experience suggests that few know how to properly seal FATs, and this matter seems exacerbated by incorrect manufacturer instructions regarding the sealing of FATs containing wet samples. We provide instructions to securely seal FATs containing wet samples which require: a sample without sharp structures that may puncture the FAT, proper FAT loading, proper FAT compression, and proper locking of the FAT seal. Executing the steps necessary to meet these requirements will produce a sealed FAT that can undergo subsequent changes in pressure and remain well-sealed for months to years and even if dropped.

Science and industry have used Whirl-Pak® (Nasco, Fort Atkinson, WI), Twirl'em® (Labplas Inc., Ste-Julie, QB, Canada), and similar "fold-and-twist" blended polyethylene bags (FATs) for over three decades. These inexpensive bags are useful containers for many types of biological samples in that they are durable and versatile while also being ideal for field collections and shipment because they are lightweight, flexible, and flat and thin when empty. Three of us (HWP, GWB, GBS) have each used FATs extensively over the past 25 years, most often to contain wet biological samples (tissue biopsies or entire organisms) for periods of days to years. On the extreme end of that scale, GWB has kept some samples in FATs containing 10% formalin for 7 years.

Although we prefer FATs for storing many types of wet samples, we periodically receive leaking, improperly sealed FATs from other collectors, some of whom consequently claim that FATs are unreliable and inadequate for securing wet samples. Such feedback has confused us because of our favorable use of FATs as well as the fact that we have easily taught others to successfully use them. Interestingly, further consideration of the matter prompted GWB, GBS, and SAB to realize that all whom they knew who were capable of properly using FATs could trace their skill back to HWP (who in turn learned to seal FATs from John Sieburth, University of Rhode Island). The problem of leaking FATs could be confounded by the fact that manufacturers do not ship directions with FATs, perhaps incorrectly presuming that the procedure for sealing a FAT is intuitive to all. Sealing directions can, however, be obtained from manufacturer websites or by contacting customer service staff. Even so, four sets of instructions we obtained from two FAT manufacturers were all different and defective in various ways regarding sealing FATs containing wet samples without leaks. Consideration of the common sealing mistakes that resulted in leaking FATs sent to GWB made it obvious that at least some sealing failures were the product of people following defective manufacturer directions (such as twirling or whirling FATs before sealing them, folding the sealing wires across the FAT rather than twisting them together, and not pressurizing FATs before sealing; see below). With this in mind, we herein provide a quick and reliable method for sealing FATs containing wet samples to retain their vapors and liquids.

METHODS AND DISCUSSION

The effective sealing of wet samples in FATs requires: a sample without sharp structures that may puncture the FAT, proper FAT loading, proper FAT compression, and proper locking of the FAT seal. These requirements can easily be achieved via the following six steps (Fig. 1).

1) After tearing off the perforated FAT mouth seal, open the FAT by pulling the FAT-mouth finger tabs apart so the wire-supported mouth band can later be closed without kinking (Fig. 1, Panel A).
2) Fill the FAT ≤ 60% full (Fig. 1, Panel B). This is important to consider when fixing samples requiring at least a 10:1 formalin to sample volume ratio because overfilled FATs are difficult or impossible to properly seal. While sharp samples should not be wet-stored in FATs, the modification of some samples before bagging, e.g., by removing an unessential sharp structure or by wrapping them in cheesecloth, might eliminate puncture risk. It also should be noted that seawater will corrode FAT sealing wires to the point of failure over many months of contact. However, we have routinely kept

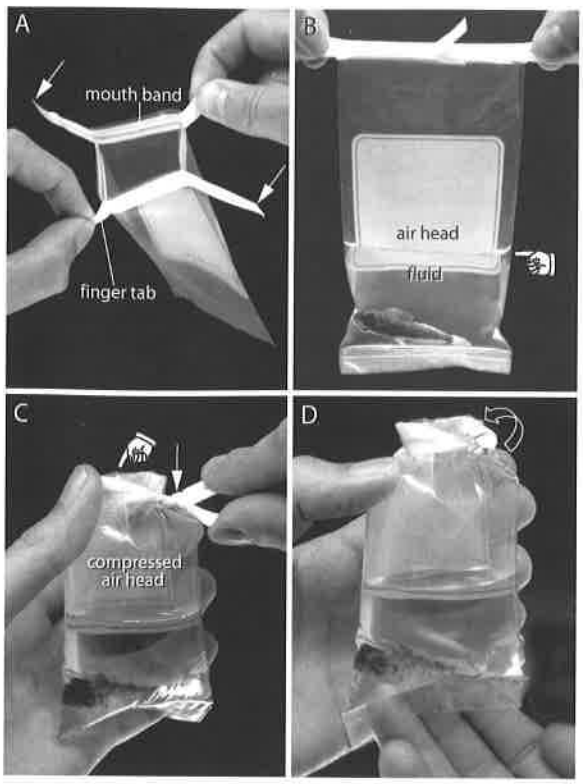

FIG. 1. Method for sealing wet samples in Whirl-Pak® or similar "fold-and-twist" bags (FATs) to prevent leaking (see text for details). A. FAT opening and "anatomy." The mouth seal has been removed and the FAT mouth band is being spread open by pulling the finger tabs. Arrows indicate ends of sealing wires. B. FAT loading, mouth band closing, air head capture; note fluid level (finger). C. Proper bag compression through mouth band folding and sealing by wire twisting (arrow); note smooth arc of mouth band (finger). D. Properly sealed FAT; note sealing wires folded back halfway along their lengths (arrow).

samples comprised partially of seawater in FATs for several years when sealing wires were kept dry.

3) Close the FAT by applying the inner-facing surfaces of the mouth band flat against each other while capturing a head of air within the FAT (Fig. 1, Panel B).
4) Compress the FAT contents by neatly and tightly folding the mouth band over itself at least four times (folds should equal band width) such that concomitant shortening of the FAT compresses the head of air in the FAT (Fig. 1, Panels B and C). When properly executed, this procedure transforms the FAT into a turgid container (Fig. 1, Panel C). The amount of air captured before folding will dictate the number of folds needed to achieve the requisite rigid, balloon-like condition. FATs sealed by less than four folds or FATs that are flaccid will likely leak. In instances when FAT compression results in less than four folds (due to overfilling) or when insufficient bag pressure is developed, one can carefully unfold the FAT and readjust matters (add or remove sample or liquid, capture a larger or smaller air head) before resuming the sealing process.
5) Once the FAT is well-folded and pressurized, bend the sealing wires toward the open side of the folded mouth band, cross one wire over the other near to where the wires extend beyond the FAT sides, and evenly twist the sealing wires neatly about one another (Fig. 1, Panel C) all the way to their tips. Properly sealed FATs will lack acute bends in the mouth band along the FAT mouth. Rather, the mouth band should be bent in a smooth arc that creates a bow-like tension between the twisted sealing wires (Fig. 1, Panel C). Ideally, when twisting the sealing wires, the mouth band should be slightly inclined toward the open side of the FAT fold to place added sealing pressure on the open side of the folded mouth band.
6) To complete the sealing process, fold the tips of the sealing wires back halfway along their twisted lengths (Fig. 1, Panel D). This reduces the likelihood they will puncture the FAT or a neighboring FAT during transport or storage.

The trade names used for some FATs imply that bag whirling or twirling is required to achieve an effective seal. However, these names are unfortunate because they are linked to unreliable product directions which often cause FAT leakage. In light of the directions provided above, "whirling" could be thought of as the neat folding of the mouth band to achieve bag compression and "twirling" could be thought of as the neat twisting of the sealing wires. While the aforementioned directions may seem lengthy, they are easily mastered such that it takes but a few seconds to seal a FAT so that it will remain well-sealed for many months to years and even if dropped.