Stomacher® sample preparation:

We have developed this protocol to prepare a large number of tissue samples (salamander tail clips, body wall, liver) for inoculation onto cell culture monolayers (for virus screening, plaque assays. etc.). We generally use small bits of tissue (0.5-4cm^2), but whole animals can be used[1]. The Stomacher is made by Seward Ltd. (London: phone +44 (0) 181 365 4105), but can be purchased through Brinkmann (catalogue # 030-01-001-9). Samples are placed in disposable bags with some liquid, put in the machine, and then the Stomacher uses reciprocating paddles to pummel the crap out of them. Fast, neat, clean, although it is a bit loud.

We have also figured out that spinning these samples at 9000 x g for 10 minutes removes most of the bacteria and only lowers the titer of the virus by at most an order of magnitude, no worse than 0.45µ filters and a lot cheaper.

<div align="right">

Jesse Brunner
Danna Schock
Arizona State University
brunner@asu.edu
2000

</div>

1) Put tissue sample (tail, body wall, liver[2], gill) in a stomacher bag with 3ml media (we use 2% fetal bovine serum-minimum essential medium Eagles for EPC cell lines; use whatever you like) with pen-strep-neomycin (1:100) and a pinch of diatomaceous earth.

2) Fold the bag to make a 'pocket' with the sample and the media in it, and tape the fold.
3) Stomach on 'high' for 60 seconds on one side and 30 seconds on the other. Two bags at once, slightly offset works best.
4) Pour ~1.5ml of the slurry into a centrifuge tube and centrifuge at 9,000 x g for ten minutes (preferably at 4ºC).
5) In a laminar flow hood carefully pipette 1ml of the supernatant into a sterile centrifuge tube or cryotube and freeze in -80C.

---

[1] When using bony, pointy, or otherwise sharp samples, such as whole salamanders, it is generally a good idea to use two bags to avoid rips and tears. Larger samples, of course, also require more liquid.

[2] Liver tends to leave a layer of fat or grease on the surface of the supernatant, so plunge the pipette tip well below the surface and watch for the fat layer to descend