Staining Protocol using the Regressive Acetocarmine approach

Before starting, wipe down dishes with water and a chem wipe (not paper towels) to remove fibers/hairs so that they don't get stuck to your specimens.

Staining:

1) Place specimen in dish with 2 squirts of acetocarmine stain. Leave for 15-30 mins (depending on the thickness of the worm) **(**always transfer from liquid to liquid!)**
2) Using a paintbrush, gently transfer worm to dish containing 70% ethanol (add approx 2 pipette squirts of ETOH into the dish)
3) De-stain using HCl (2-8 drops) until desired effect
   - Swirl dish as you add HCl **slowly**
   - Look for faint white outline around exterior
4) Transfer worm to new 70% ETOH

Dehydration:

1) Begin in 70% ETOH for 1 hour
2) Transfer to 85, 90, 95, and 100% each for 1 hour
3) Place in 100% a second time, again for 1 hour

Clearing:

1) Transfer worm to ¼ xylene + ¾ 100% EtOH, leave for 30 min
2) Transfer to ½ xylene + ½ 100% EtOH, then ¾ xylene +1/4 100% EtOH, and then full xylene, each for 30 min.

Mounting:

1) Combine Permount/Canada balsam and xylene to desired consistency in a vial (not runny, but easily distributed).
2) Clean glass slides and cover slip in 70% EtOH.
3) Label slides (with pencil) indicate the specimen ID, date, etc
4) Place 1-2 drops or Permount/xylene onto center of the slide.
5) Using a paint brush place the worm, suckers facing up, onto the slides. Use brush to adjust worm.
6) Carefully place the cover slip onto the worm.
7) Slowly pipette more Permount/xylene underneath the coverslip, add slowly to avoid bubbles
8) Place slide on the slide warmer.
9) If bubbles have formed, leave the slide on the warmer for a few days to allow the bubbles to move to the edges of the coverslip, then bubbles can be removed using xylene on a paintbrush and filled in with more Permount.