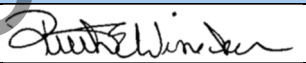
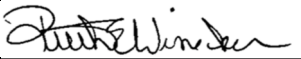
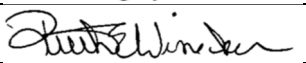
SOP 005 - Preparation of Tissue Homogenates

Table of Contents

SOP Name: Preparation of Tissue Homogenates		SOP #: 005
North Carolina Office of the Chief Medical Examiner Toxicology Laboratory	**Revision:**	**Revision Date/Initials:**
Approving Authority Name	**Approving Authority Signature**	**Approval Date**
Ruth E. Winecker, Ph.D.		04/07/2015
Ruth E. Winecker, Ph.D.		06/06/2016
Ruth E. Winecker, Ph.D.		08/21/2017

SOP 005 - Preparation of Tissue Homogenates

1. **Principle**

    1.1. procedure describes the method for preparing 1:4 tissue homogenates for determining drug concentrations in the tissues. Tissues obtained from the pathologist or medical examiner are diluted with water and completely homogenized.

2. **Specimens**

    2.1. This procedure is applicable to liver, brain, spleen, kidney, muscle, or other tissue specimens.

3. **Reagents and Materials**

    3.1. Deionized water

    3.2. Tissue

    3.3. Disposable scalpel

    3.4. 50mL Falcon Tubes

    3.5. 10% Bleach Solution

4. **Standards, Controls, and Solutions**

    4.1. N/A

5. **Equipment and Special Supplies**

    5.1. Top-loading balance

    5.2. Silent Crusher M homogenizer

6. **Procedure**

    6.1. Note: When preparing an hd2 (second homogenate prepared from parent liver specimen) clean homogenizer generator thoroughly (6.10) prior to next step.

    6.2. In a 50mL Falcon Tube, weigh approximately 4g of tissue on the top-loading balance using a scalpel to cut it into small enough pieces to fit in the generator (Approx. 1/8 inch).

    6.3. Without taring the balance, multiply the exact amount of tissue weighed by four.

6.3.1. Add deionized water to the Falcon tube containing the tissue until the final mass reaches the calculated mass.

6.3.2. **E.G. 4.0g tissue (4g x 4 = 16g) → add water to a final mass of 16g.**

6.4. Place the Falcon tube under the generator of the Silent Crusher M homogenizer.

6.5. Lower the generator into the Falcon tube so that it rests approximately ¼ inch off the bottom and tighten the adjustment knob to hold it in place.

6.6. Turn the Silent Crusher M on, press the start button, and increase the speed to 10000 RPM.

6.7. Blend the tissue until completely homogenized. (1-2 min)

6.8. Pour the contents of the bottle into a 20 mL scintillation vial labeled with T#, S#, contents and dilution factor (e.g.1:4 hd1 liver), date prepared, and initials of preparer.

6.9. Store in the refrigerator at about 4° C.

6.10. Clean the homogenizer by disassembling the generator and cleaning all parts thoroughly with bleach solution and clean running DI water.

7. **References**

7.1. Levine, Barry. "Postmortem Forensic Toxicology." *Principles of Forensic Toxicology*. 2nd ed. Washington, DC: AACC, 2006. 3-13. Print.

7.2. Heildolph Instruments, comp. *SilentCrusher M*. 14 Oct. 2011. Instruction manual. Room 2606 OCME Toxicology, Raleigh, NC.