Forensic Chemistry Section

Method for Preparing Ortho-Tolidine Spray

1. Scope

This document outlines the method for preparing ortho-tolidine spray.

2. Safety

2.1 Ortho-tolidine is carcinogenic. Precautions must be taken to minimize exposure. Disposable laboratory coats and gloves will be worn while preparing the reagent.

2.2 Reagent preparation will occur in a total exhaust fume hood with the sash as low as practicable.

3. Reagent Preparation

3.1 Acetate Buffer will be prepared as follows:

- 25.0 g sodium acetate will be dissolved in 215 ml glacial acetic acid.
- 250 ml reagent water will be added to the solution.
- The above constituent amounts may be varied as long as the final concentrations remain the same (final volume of approximately 50 ml achieved by using 2.5 g sodium acetate, 21.5 ml acetic acid, and 25 ml water).
- The bottle will be labeled, dated, and stored in the refrigerator.

3.2 The ortho-tolidine spray is prepared as follows:

3.2.1 0.4 g ortho-tolidine dihydrochloride will be dissolved into 10 ml of acetate buffer in a small erlenmeyer flask using a magnetic stirrer.

3.2.2 The solution will be stirred until it appears creamy.

3.2.3 0.5 g sodium perborate will be added to the solution and mixed until most of the solids appear dissolved. The solution will be removed from the stirring plate.

3.2.4 35 ml collodion will be mixed with 15 ml ethanol/reagent alcohol in a large erlenmeyer flask with a stirring bar on the stirring plate for 3-4 minutes. **DO NOT HEAT.**

3.2.5 120 ml ethyl ether will be **SLOWLY** added to the collodion solution with constant stirring to avoid precipitation.

3.2.6 The ortho-tolidine/sodium perborate solution will be added to the collodion solution and stirred for another 3-4 minutes. The resulting solution should appear golden.

3.2.7 The solution will be tested against a swabbing of known blood. A blue color should begin to develop within 20 - 30 seconds.

3.2.8 The solution will be poured into an aerosol spray bottle and labeled with the name of the solution, date made, initials of preparer, and expiration date (one day).

3.3 The remaining unused solution is poured into the appropriate waste container.