Document Title: Ouchterlony Plates QC
Controlled: Yes, with red stamp present
Controlled By: Quality Manager

Prepared By: _____ Date: _____

Approved By: _____ Date: _____

A. PURPOSE:

To prepare Ouchterlony plates for species determination and to perform quality control on prepared reagents or purchased antisera/sera.

B. RESPONSIBILITY:

Forensic Science Examiners 1 and 2 in the Forensic Biology Section. Ordering information is maintained in a log book in the Forensic Biology Section.

C. SAFETY:

Use appropriate measures for the proper handling of Trypan Blue and sodium azide according to SOP-GL-2 (Safety Manual) and the Material Safety Data Sheets.

D. DEFINITIONS:

PBS: Phosphate Buffered Saline

E. PROCEDURE:

1. Materials (1% Agarose, 0.25% Trypan Blue):
   a. Phosphate buffered saline 100mL
   b. Type I agarose 1g
   c. Trypan blue 0.0125g
   d. Sodium azide ~0.025g
   e. Sterile petri dishes (50x9mm)
   f. Serological pipets (10ml)
   g. Controls: Known positive control(s) and negative reagent blank(s)

2. Procedure:
   a. Heat 0.0125g of Trypan blue in 50mL PBS to dissolve (**do not boil**).

   b. In a separate container, heat 1g of agarose in 50mL PBS until dissolved. Re-measure and add distilled water to volume as needed.

   c. Add Trypan blue solution to agarose solution. Heat to boiling.

   d. Add approximately 0.025g of sodium azide.

E. 2. e. Swirl to mix thoroughly.

f. Using a serological pipet, add 4mL of agarose media to each sterile petri dish and swirl gently so agarose covers the bottom of the plate. Avoid the formation of bubbles.

g. Allow to cool, then cover with lids and place in the refrigerator in a zip lock bag, inverted to prevent condensation.

3. Test each new lot with the appropriate antisera and corresponding controls as needed before use according to SOP-FB-09 (Species Double Diffusion Test), Ouchterlony Quality Record Worksheet (FBQR-08) and the Ouchterlony Reagent Log Sheet. Record the required information.

4. If the appropriate results are not obtained, review the procedure and repeat the test with a 2nd plate.

5. If the appropriate results are still not obtained, discard and make new plates.

6. If the plates are suitable for use, store inverted in the refrigerator in a zip lock bag labeled with the lot # (date of preparation), control date and examiner's initials.

7. Discard any unused plates after six (6) months or sooner if any bacterial growth or dehydration of the gel occurs.

F. REFERENCES:

1. Ouchterlony, 0., 1948a, “Antigen-antibody reactions in gels," Acta. Pathol. Microbiol. Scand. 26 (1949), 507.

2. Ouchterlony, 0. “Antigen-antibody reaction in gels”, Ark. Kemi. Mineral Geol. 26B (14).

3. Ouchterlony, 0. 1949b. Antigen-antibody reactions in gels II. Factors determining the site of the precipitate. Ark. Kemi. 1:43-48.

4. Ouchterlony, 0. 1949c. Antigen-antibody reactions in gels III. The time factor. Ark. Kemi. 1:55-59.

5. Ouchterlony,O. 1968. Handbook of lmmunodiffusion and Immuno-electrophoresis, Ann Arbor Science Publishers, Inc., Ann Arbor, Michigan.

6. SOP-GL-2 (Safety Manual).

7. Material Safety Data Sheets.