Document Title: Species Double Diffusion Test (Ouchterlony)
Controlled: Yes, with red stamp present
Controlled By: Quality Manager

Prepared By: _____ Date: _____

Approved By: _____ Date: _____

A. PURPOSE:

To determine the species of origin in cases when animal blood is suspected, to test for the presence of human semen or to determine human origin of other body fluid samples.

B. RESPONSIBILITY:

Forensic Science Examiners from the Connecticut State Forensic Science Laboratory who have been trained in the discipline of the species double diffusion test procedure according to SOP-FB-31 (Training Manual).

C. SAFETY:

Use appropriate measures for the proper handling of the Ouchterlony plates according to SOP-GL-2 (Safety Manual).

D. DEFINITIONS:

1. PBS: Phosphate Buffered Saline
2. RSID: Rapid Stain Identification

E. PROCEDURE:

This procedure will be performed at the discretion of the examiner based on the submitting agency requests, case information and the condition of the evidence.

1. Materials:
   a. Ouchterlony plates
   b. Anti-sera:
      aa. Animal anti-sera (anti-dog, -cat, -deer, etc.)
      bb. Anti-human serum
   c. Known bloodstain extracts or thawed sera controls: human and/or animal.
   e. PBS
   f. RSID™-Semen Extraction Buffer
   g. 0.5% ammonia
   h. Disposable pipets or micropipet and tips
   i. Microcentrifuge tubes

E. 2. Procedure:

a. Preparing extracts:

Blood (animal anti-sera):

aa. Extract a portion of the sample in a microcentrifuge tube with 30-50µl of PBS. If necessary, sample can be extracted for a longer period of time on a shaker at room temperature or overnight at 4°C.

bb. 0.5% ammonia may be used in place of PBS to extract older samples or samples that are difficult to extract.

Other body fluids (anti-human serum):

Extract a portion of the sample in a microcentrifuge tube with 30-50µl PBS, overnight at 4°C.

b. Each plate must contain a positive control and negative control.

aa. Known blood stain extracts for animal anti-sera or anti-human serum should be made up fresh for each use. Store remaining extract(s) in the refrigerator and discard after six (6) months or sooner if necessary.

bb. Frozen aliquots of animal sera may be thawed and used as positive controls for animal anti-sera. Store thawed, remaining animal sera in the refrigerator and discard after six (6) months or sooner if necessary.

cc. PBS, RSID™-Semen Extraction Buffer or 0.5% NH_4 may be used as a negative reagent blank control according to the extraction buffer used.

c. Show placement of the controls, samples and anti-serum/anti-sera on the schematic diagram of the Ouchterlony Quality Record Worksheet (FBQR-08).

d. Fill the wells in the Ouchterlony plate with a pipet according to the diagram. Avoid air bubbles or over flowing the wells.

e. Allow plate to sit upright at room temperature until all samples have diffused into the plate.

f. Turn the plate upside down and allow the plate to stand overnight (12-16 hrs) at 4°C.

g. Examine the plate for precipitin lines using back lighting and record the results on the schematic diagram of the Ouchterlony Quality Record Worksheet (FBQR-08).

Include a copy of this worksheet in the case jacket, file the original in the designated notebook.

E. 2. h. If no precipitin line is observed, re-examine the plate for up to 48 hours.

i. If the controls do not give the appropriate results, the test is considered inconclusive and the sample(s) should be re-run.

3. Results:

a. *Positive.* A white precipitin line between a sample well and the anti-serum well indicates a positive result.

b. *Negative.* No line will appear between a sample well and the anti-serum well, indicating a negative result.

c. *Inconclusive.* No discernible precipitin line and/or insufficient extraction of sample.

F. REFERENCES:

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

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

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

4. Ouchterlony, O. 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).