Document Title: Evaluation of Percipitating Antisera QC
Controlled: Yes, with red stamp present
Controlled By: Quality Manager

Prepared By: _____ Date: _____

Approved By: _____ Date: _____

A. PURPOSE:

To determine the titer (sensitivity) of a precipitating antiserum and to determine if the antiserum cross-reacts with other body fluids and/or other species of bloodstains.

B. RESPONSIBILITY:

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

C. SAFETY:

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

D. DEFINITIONS:

PBS: Phosphate Buffered Saline

E. PROCEDURE:

1. Materials:
   a. Ouchterlony plates
   b. Antisera:
      aa. Animal antisera (anti-dog, -cat, -deer, etc.)
      bb. Anti-human serum
   c. Known bloodstain extracts or thawed sera controls: human and/or animal.
   e. PBS
   f. 0.05% ammonia
   g. Distilled water (dH_2O)
   h. Disposable pipets or micropipet and tips
   i. Microcentrifuge tubes

2. Procedures:
   a. If the antiserum to be tested is lyophilized, reconstitute according to manufacturer's specifications.

E. 2. b. Prepare samples for titer determination of human antisera:

aa. Extract a 1cm^2 portion of a human bloodstain in 100µl of PBS and extract overnight at 4°C.

bb. Make dilutions from this neat extract with PBS according to the table below.

c. Prepare samples for the detection of cross reactivity with human and animal antisera according to SOP-FB-09 (Species Double Diffusion Test) and step 2.d. below.

d. Typical samples and suggested dilutions for testing precipitating antisera:

Anti-human serum	Bloodstain extract: Neat; 1:10, 1:100, 1:500, 1:1T, 1:10T, 1:20T
	Semen stain extract
	Seminal-vaginal mixture swab extract
	Saliva stain extract
	Urine stain extract
	Vaginal swab extract
	Breast milk stain extract
	Fecal swab extract
	Animal bloodstain extracts
Animal antisera	Human bloodstain extract
	Corresponding animal control bloodstain &/or thawed serum
	All available animal bloodstain extracts & thawed sera

e. Test the antisera according to SOP-FB-09 (Species Double Diffusion Test). Record the results and other appropriate information on the Ouchterlony Quality Record Worksheet (FBQR-08), see examples of sample placement on page 4, below.

3. Evaluation:

a. The highest dilution to yield a positive result is the titer of the antiserum. Consideration will be taken to determine if the antiserum has a sufficient titer suitable for use.

b. An antiserum that yields a positive result with a sample other than its corresponding control, exhibits cross reactivity. If this occurs, consideration will be taken to determine if the anti-serum is suitable for use.

c. If the appropriate results are not obtained and the antiserum is determined to be unsuitable for use, review the procedure and replace the antiserum as needed.

E. 4. If the antiserum is determined to be suitable for use, store as follows:

a. Antiserum received in a lyophilized state:

aa. Aliquot 50µl volumes of the reconstituted antiserum into microcentrifuge tubes labeled with the antiserum type and lot #. Store in the freezer in a zip lock bag labeled with the antiserum, lot #, date received, date reconstituted, titer and examiner's initials.

bb. Store additional bottles with lyophilized antiserum in the freezer. Label each bottle with the date received and examiner's initials. Re-titrate lyophilized antiserum after thawing and reconstituting as above.

b. Antiserum received in a liquid state:

Aliquot 50µl volumes into microcentrifuge tubes labeled with the antiserum type. Store in the freezer in a zip lock bag labeled with the antiserum, lot #, date received and examiner's initials.

c. Thawed antiserum may be stored in the refrigerator until consumed. Discard if a decrease in activity or bacterial growth is observed.

d. Avoid repeated freezing and thawing of the antiserum.

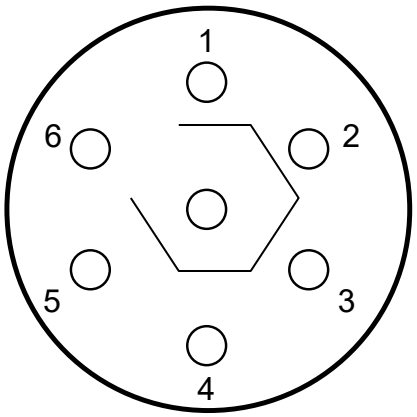
F. REFERENCES:

1. SOP-GL-2 (Safety Manual).
2. Material Safety Data Sheets.

OUCHTERLONY WORKSHEET

I.

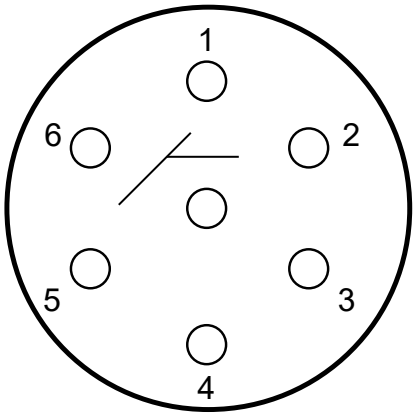
C - anti-HuSer
1 -1:10 human blood
2 -1:100
3 -1:200
4 -1:300
5 -1:400
6 -1:500



Examiner: K J L
Date: 11/15/99
αSerum
Lot #: 19H4816
Titer: 1:400
Ouchterlony
Date: 11/01/99

II.

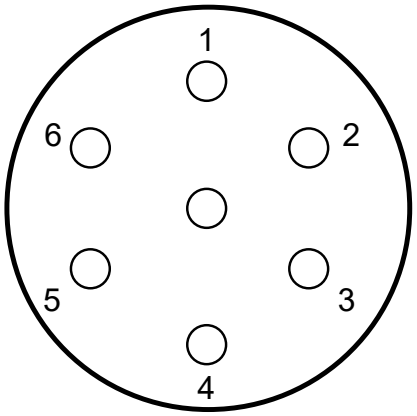
C - anti-Dog
1 -dog serum #55983
2 -cat
3 -chicken
4 -cow
5 -deer
6 -1:10 human blood



Examiner: KJL
Date: 11/15/99
αSerum
Lot #: 02727
Titer: N/A
Ouchterlony
Date: 11/01/99

III.

C
1
2
3
4
5
6



Examiner:
Date:
αSerum
Lot #:
Titer:
Ouchterlony
Date: