IDAHO DEPARTMENT OF

LAW ENFORCEMENT

BUREAU OF

FORENSIC SERVICES

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SEROLOGY MANUAL

MAKING EXTRACTS

1. From Swabs

Cut a portion, use no more than half of the swab. Avoid taking lots of unstained underlayer.

2. From Clothes or Sheets

A. Locate stain using any of the following: U.V., visual clues, alternate light source.

B. May use brentamine for screening.

C. If stain is large, cut area about ½ cm square (more if dilute), but vary shape to encompass edge and center of stain.

D. If stain is small, take no more than half, unless the appropriate authority is consulted.

E. If the circumstances warrant, take a cutting of similar size to questioned sample from adjacent unstained area.

F. If the garment is expensive, try to use scrapings instead of making a cutting, if possible.

G. Mark the location of each cutting taken with the appropriate number or use photos and/or detailed diagrams. Use whatever method records the location in the most appropriate way.

3. How to Soak and Extract

A. *"Hole-in-the-lid" Method*

1. Place cutting in labeled 1.5 ml plastic centrifuge tube.

2. Add 100 μl of water or physiological saline.

3. Agitate using wooden stick, toothpick, or washed tweezers, if needed to assure sample is moistened.

4. Soak at least half an hour (refrigerated).

5. Agitate again.

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6. Puncture lid with hot needle in two places in inner depression.

7. Cram wet cutting into lid (use clear tape to hold in "springy" cuttings).

8. Spin in microfuge 3-4 minutes.

9. The cutting should not be discarded. It can be retained or returned with the evidence.

10. Replace "holey" lid with new, intact lid.

B. *"Piggy-back" Method*

1. Label a 1.5 ml plastic centrifuge tube as above. Place the cutting in a 0.4 ml centrifuge tube which has a hole in the bottom, sitting piggy-back in the 1.5 ml tube.

2. Add 100 μl of water.

3. Agitate using wooden stick, toothpick, or washed tweezers, if needed to assure sample is moistened.

4. Soak at least half an hour (refrigerated).

5. Agitate again.

6. Spin 3-4 minutes in microfuge while tubes are still "piggy-back." Removing the Eppendorf tube lid may help in fitting the stack into the tube holder.

7. Replace the Eppendorf lid if it was removed. Return the cutting to the evidence container, properly labeled, or retain.

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MAKING EXTRACTS

1. From Swabs

Cut ~ 1/4 of the swab with a pair of scizzors. Use wooden end of same swab to poke swab cutting into the tube. Can use either up to 1/2 of the swab in the first step or can later add additional swab if 1/4 is not enough.

2. From Clothes or Sheets

A. Locate stain either visibly or with the ALS.

B. Circle the stain with a marking pen.

C. Number the circled areas.

D. Screen area with the Brentamine Test.
   a) moisten a sterile swab with nano water
   b) press the end of the swab against the area to be tested, then rub firmly for 3-5 seconds
   c) place one drop of Brentamine solution on the swab
   d) swab should change color within 30 seconds if strong and four minutes if weak
   e) test area even if it is weak

E. Cut out an ~ 3 X 3 mm piece of the material to be tested.

F. Place a 0.4 ml centrifuge tube, which has a hole in the bottom, into a labeled 1.5 ml tube. Put the cutting into the inner tube.

3. How to Soak and Extract

A. Add 100 ul of nano water or PBS to each tube.
   a) Use blue-topped pipetteman-set at 100 ul
   b) PBS is in a squirt bottle on top of the counter

B. Agitate cutting against the wall of the tube using a sterile wooden stick or toothpick. (try not to push an excessive amount of fluid through the hole in the bottom).

C. Let incubate at room temperature for 30 minutes to one hour.
   NOTE: Procedure can be continued the next day, place samples in freezer.

D. Spin in a microcentrifuge for 3-5 minutes.

E. Remove and discard internal tube.

4. How to Prepare and Stain the Slide

A. Pipette 5 µl of extract onto a microscope slide. Place slide on warmer to dry. Heat fix by passing briefly through a bunsen burner flame.

B. Cover the dry sediment spot with Kernechtrot (Nuclear Fast Red) solution. Let stand for 15 minutes.

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C. Gently wash off the stain with water. Take care to leave the sediment on the slide.

D. Cover the sediment with Picroindigocarmine (Green) solution.

E. Let stand 15 seconds, then rinse with methanol.

F. Blot dry with paper towels, examine under a microscope.

5. Interpretation

A. Spermatozoa will be stained with red heads (frequently two-toned) and green tails.

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ABSORPTION ELUTION TESTING

1. General

Blood group antigens on a substrate are allowed to bind either ∝-A, ∝-B, or ∝-H lectin antibodies. Excessive antibody is washed away. The piece of material with absorbed antibodies is covered with a drop of saline containing BSA. Raising the temperature to 60°C causes the absorbed antibody to be released into the saline/BSA. A drop of indicator cells is added and agglutination is observed after rotation.

2. Sample Preparation

Most frequently, stains are tested on threads. These can be threads teased from a cloth stain or threads which have been moistened and dragged through a dried deposit. Liquid samples may be tested by dipping a thread into the liquid then drying the thread. (For very dilute liquids, the dipping and drying can be repeated). If the threads are very fine or lightly stained, more than one may be used. Occasionally, a non-thread substrate may be used, such as a cutting of leather or strong paper. In every case, a non-stained substrate control should also be tested.

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3. Method

Attach threads to a hydrophobic surface, such as a silicated 3x5 glass slide (SERI S231). Fingernail polish has been used as an adhesive with good success. The surface should be ruled and/or marked to identify the threads. An example is below:

	1	2	3	4	5	6
∝-A						
∝-B						
∝-H Lectin						

Group A, B, and O known blood stain threads should be used and are usually put in columns 1, 2, and 3. Column 4 usually contains thread blanks (negative control).

After the threads are attached, a drop of anti-A antisera (Polyclonal only) SERI is placed individually over each thread in the ∝-A row, and ∝-B, and ∝-H lectin are added in their rows. The slide is covered and placed in a refrigerator overnight, or for at least 4 hours.

After absorption, the antisera is washed off the samples. Any rinsing technique is acceptable which produces correct results with the positive and negative controls. One such method is rinsing the slide with cold water or saline and blotting the threads about six times, then washing the slide in a bath of cold saline with agitation for about an hour. A final series of rinse-and-blot is then performed.

After rinsing, a drop of 0.3% BSA in physiological saline is added to cover each thread. Both 10 μl and 25 μl have been used with success, with the larger amount for longer or thicker threads. The slide is covered and placed in a 60° oven for 20 minutes.

After elution, the appropriate 0.3% suspension of triple-washed red cells in 0.3% BSA/Saline is added in an amount equal to the quantity of BSA/Saline used for the elution step (either 10 μl or 25 μl). "A" cells are added across the ∝-A row, B cells across the ∝-B row, etc.

The slides are kept in a moisture chamber and then rotated. Before reading the agglutination microscopically, the drops are individually stirred to promote agglutination and free the cells from adhering to the slide. Extended rotation may be needed for weak reactions to develop. The controls and blanks should be rotated, agitated, and read at whatever intervals the questioned samples and their substrate controls are read.

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Reference: This procedure is adapted from "Absorption Elution Testing", SERI Procedure Manual.

Adopted Date: March 20, 1997

AMYLASE DIFFUSION

1. General

Amylase diffusion is a test for an elevated amylase level that could indicate the presence of saliva.

2. Buffer (pH 6.9)

2.7 g NaH_2PO_4, Anhydrous (0.045 M).
3.9 g Na_2HPO_4, Anhydrous (0.055 M).
0.2 g NaCl (7 mM).
500 ml Deionized H_20.

Or use a commercial buffer preparation, following the instructions provided.

3. Gel: 1% Agarose/0.1% Soluble Starch

0.1 g Agarose.
0.01 g Soluble Starch.
10 ml of above buffer (pH 6.9).

Heat to melt agarose, then pour into a 15 x 100 mm petri dish and allow to gel.

4. Application

Punch holes about 2 mm in diameter in gel, remove plug. A Pasteur pipette is a convenient punch.
Leave at least 1.5 cm between holes.
Label holes, fill with controls and samples.
Cover and incubate at 37°C overnight.

5. Controls and Samples

Use liquid saliva diluted 1/100 and 1/500.
Extracts of known semen and saliva stains may be run.
Samples and their substrate controls should be extracted in the same way (same size cutting and same amount of elutant for unknown and substrate control).

6. Development

Pour a 1:100 dilution of a saturated iodine solution (use GLO I iodine). Clear circles around holes indicate amylase. Measure and record diameter of clear circles. Photographs may be taken.

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7. Interpretation

The presence of amylase is indicated by clearing zones around individual wells. This is the result of the hydrolytic action of the enzyme on the starch in the gel. The result is a lack of starch in these zones with which iodine can react. The blue areas of the gel are negative for the presence of the enzyme, but provide a control to establish that the chemistry of the system is performing correctly. The procedure may be used semi-quantitatively with the size of the clearing zones around the wells being directly proportional to the concentration of enzyme in the extract. Controls which should be considered (liquid and/or dry) include normal human serum, semen, saliva, blood, urine, and perspiration.

References

Schill, W.B. and Schumachar, G.F.B., "Radial Diffusion in Gel for Microdetermination of Enzymes," Anal. Biochem. Vol. 46, 1972, pp.502-503.

Kipps, A.E. and Whitehead, P.H., "The Significance of Amylase in Forensic Investigations of Body Fluids," For. Sci. Vol. 6, 1975, pp. 136-144.

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BRENTAMINE TEST FOR ACID PHOSPHATASE

General

This is a screening test for the presence of acid phosphatase.

1. Moisten a swab with water and touch it firmly to the questioned stain. Or deposit 5-10 μl of a questioned stain extract on the swab.

2. Use two separate swabs to prepare positive and negative controls (using a known semen stain and plain water, respectively).

3. Apply a drop of the brentamine reagent to each swab.

4. Observe the swabs for a rapidly developing purple color. The negative control may show a faint purple reagent color. Weak or faint reactions on negative controls must be taken into account when interpreting reactions from other swabs.

Brentamine Reagent: (To make 5 ml of each Solution A and B)

(If you use a commercial source, use their directions.)

Solution A: Freezer Door (Ser) — 50 mg o-dianisidine tetrazotized (Fast Blue B Salt) in 5 ml of pH 5 10x buffer. (.25 grams)(.25 mL)

Solution B: Freezer Door (Ser) — 50 mg Na α-naphthyl phosphate in 5ml of H_20. (.25 grams)(.25 mL)

pH 5 10x Buffer: Fridge Door (Ser) — 1.2 gm anhydrous sodium acetate, 400 μl acetic acid (approximate amount as needed to adjust to pH 5), and 10 ml H_20. NOTE: Can be microwaved on low to dissolve

Spot Test:

Mix 1 drop of Solution A and 1 drop of Solution B with 8 drops of water.

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Mapping:

Whole garments, bed sheets, panty crotch panels, etc. may be assayed for the possible presence of seminal stains by using this technique. It should be done as follows:

a. Cut Whatman #1 filter paper to appropriate size.

b. Saturate the filter paper with deionized water using a spray bottle and blot.

c. Lay the moist filter paper over the area of interest and press onto the item for 30-60 seconds. A glass or plastic plate and weight (e.g.--books or bricks) placed on the paper may be useful at this step. Large areas may require multiple filter paper overlays, laid side by side, to map efficiently. If multiple sheets are used be sure to mark them in a manner that facilitates their orientation to one another so as to relate positive reaction areas back to specific areas of the evidence item.

d. After pressing, hang filter paper in a chemical hood and spray with brentamine test reagent.

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Interpretation:

The positive control should be a rapidly developing dark purple color. The negative control (blank) should be colorless (it may sometimes show a faint hint of purple color).

Read samples as positive, negative, or weak reaction.

Reference:

Gaensslen: Sourcebook in Forensic Serology, Immunology, and Biochemistry has a good discussion of the interpretation of acid phosphatase tests.

SERI Methods Manual, Serological Research Institute, Richmond, CA.

Adopted March 20, 1997

LONG TERM STORAGE OF LIQUID BLOOD

Recommended Protocol

1. Log data into "Freezer Log Book" and assign sample a number.

2. Make a stain on clean cloth (at least 3 quarter-sized spots, if sample allows) and label.

3. Let stain dry. Store in freezer.

4. Place in envelope so no contamination can occur with other bloodstains in your storage area.

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LATTES CRUST TEST

1. General

This technique relies on the presence (in a blood crust or stain) of the antibodies common to the particular type of blood of which the crust or stain is composed. If known indicator cells are allowed to come in contact with antibodies diffusing into solution from such a crust or stain, agglutination will occur between the antibodies and cells bearing the homologous antigens. The greatest difficulties with this procedure are encountered because of the labile nature of the antibodies involved.

2. Reagent Preparation

Known cell suspensions (approximately .3%) of A, B, and O cells in saline.

3. Procedure

A. Split the sample of questioned blood (stain or crust) into three portions and place on microscopic slides labeled for each cell type.

B. Prepare a known stain or crust of Group O blood as a control.

C. Add cover slip and allow the indicator cells to flow under the cover slip.

D. Place slides in a moist chamber for two hours or more; refrigeration may promote agglutination.

E. Read results microscopically.

4. Interpretation

A Cells	B Cells	O Cells	Group of Sample
0	+	0	A
+	0	0	B
+	+	0	0
0	0	0	AB

+ = Agglutination
0 = No Agglutination

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The known stain or crust of group O blood should give the reactions shown for Group O.

If any unknown sample shows agglutination when O cells are added, no conclusion should be drawn.

No Lattes crust result should be reported without a result for A/E on the same sample.

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FORWARD AND REVERSE TYPING-LIQUID BLOOD

The following instructions are compatible with the reagents currently in use. If the manufacturer's insert conflicts with this procedure, then use the reagents according to the package insert.

1. Label 12 x 75 mm test tubes as follows:

anti-A	A_1
anti-B	B
anti-A,B	O

2. Centrifuge whole blood three minutes in Serofuge. Remove serum (or plasma) to clean tube.

3. Place two drops of serum in each tube labeled A_1, B, and O (reverse typing).

4. Add one drop of 3-5% A_1, B, and O cells to the appropriately labeled tube and mix.

5. Centrifuge 15 seconds in Serofuge at "high" speed. Read, as per step 13.

6. Add physiological saline to tube of red cells left from step 2. Mix.

7. Centrifuge long enough to separate cells from saline. Decant or remove saline layer.

8. Repeat steps 6 and 7 twice more.

9. Using washed cells, make 2-5% solution of cells with physiological saline.

10. Add one drop of anti-A, anti-B, and anti-A,B antiserum into the appropriately labeled tubes.

11. Add one drop of 3-5% washed cell suspension from step 9 to anti-A, anti-B, and anti-A,B and gently mix each tube.

12. Spin tubes for 15 seconds in Serofuge (high speed).

13. Read agglutination by gently rotating button and observing with agglutination viewer.

For interpretation, see the package insert supplied by the reagent manufacturer.

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Interoffice Memo

IDAHO STATE POLICE

TO: Ann Bradley and Jennifer Taylor

FROM: Carla J. Finis

SUBJECT: p30 Testing

DATE: March 21, 2000

Effective today, we will employ the Abacus Diagnostics OneStep ABAcard® system for the detection of the seminal antigen, p30. In validation testing, this system demonstrated specificity and sensitivity equal to or greater than our current system that employs rocket immunoelectrophoresis. Please follow the draft protocol that has been distributed.

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BI-0XX	

IDENTIFICATION OF SEMEN BY p30 DETECTION

Abacus Diagnostics OneStep ABAcard® Procedure

1.0 BACKGROUND:

P-30 is a seminal-fluid-specific protein. Its presence in semen is independent of the presence of spermatozoa. Immunological detection of p30 is commonly used as a confirmatory test for the presence of semen.

Sensabaugh, G.F. Isolation and Characterization of a Semen-Specific Protein from Human Seminal Plasma: A Potential New Marker for Semen Identification. (1978) *Journal of Forensic Sciences*, 23(1): 106-115.

Spear, T.F. and Khoskebari, N. The Evaluation of the ABAcard® p30 Test for the Identification of Semen. (2000) *Crime Scene*, 26(1): 9-12.

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2.0 SCOPE:

This procedure is to be used as a confirmatory test for human semen, in instances where a positive presumptive result has been obtained but no spermatozoa are seen upon microscopic examination.

3.0 EQUIPMENT/REAGENTS:

OneStep ABAcard® p30 Test Kit

4.0 PROCEDURE:

4.1 All samples must be at room temperature prior to testing.

4.2 Add 10µl of each sample and control extract (see **BI-0XX** on making stain extracts) to approximately 190µl (4 drops) of saline and mix thoroughly. **Note: Either an extract of a known semen stain or Seri™ Semen standard (add only ~0.5µl [~50ng p30] of this standard**

to saline) may be used as a positive control. Use approximately 200µl of saline as a negative control.

4.3 Label an ABAcard® p30 test device for each of the samples, including positive and negative controls.

4.4 Transfer each extract to the 'S' well of the appropriately labeled test device and incubate at RT for 10 minutes.

4.5 A positive result is recorded when two pink lines are visible, one in the test 'T' area and one in the control 'C' area. A negative result is recorded when no pink line is visible in the 'T' area after ten minutes but a pink line is visible in the 'C' area. Results are inconclusive if there is no pink line visible in the 'C' area.

DRAFT

5.0 COMMENTS:

5.1 The time it takes for positive reaction to occur is a function of the p30 concentration as well as other sample-specific characteristics. Therefore, it is necessary to wait the full ten minutes before recording a negative result.

5.2 Incubation times should not exceed 10 minutes as nonspecific reactions leading to a false positive may occur.

5.3 As with any antigen/antibody interaction, excess antigen may lead to 'high dose' effect resulting in false negatives when the p30 concentration is very high. This effect should be considered when examination and presumptive tests have indicated the likelihood of the presence of semen. In those instances, the sample should be diluted and the test repeated.

5.4 Other biological or DNA compatible buffers may be substituted for saline in the extraction process.

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P^{30} ROCKET ELECTROPHORESIS

1. General

P^{30} rocket electrophoresis is a method to demonstrate the presence of the prostate specific antigen P^{30} and to estimate its titer.

2. Buffers - Tank and Gel

25.2 g Trizma base (0.208M).
2.5 g EDTA, free acid (8.55 mM).
1.9 g boric acid (30.7 mM).
1.0 liter distilled/deionized H_20.
Bring to pH 9.1 with concentrated sodium hydroxide.

Or use a commercial buffer preparation and follow the manufacturer's directions.

3. Gel

1% Agarose Type V (EEO 0.1 or less).
Add anti-P^{30} to the gel solution at 60°C just before pouring.
Gels may be poured on glass or FMC Gel Bond® film.
Use the following amounts:

3" x 2" Plate	7 cm x 11 cm Plate
0.08 g agarose	0.20 g agarose
8.0 mls buffer	20 mls buffer
120 μl anti-P^{30}	300 μl anti-P^{30}

4. Origin

1.5 to 2 mm diameter sample wells punched 0.8 - 1.0 cm from the cathodic edge of the plate and approximately 0.5 cm apart. 2 μls of sample extract are carefully pipetted into the wells. The following sample dilutions are recommended to start.

5. Samples

Stains: Neat extract and 1:10 dilution.
Liquid Semen: 1:50, 1:100, and 1:200 dilutions.

6. Controls

50, 25, 12.5, and 6.25 μg/ml P^{30} (diluted from SERI Semen Standard - See product insert instructions).

7. Electrophoresis Conditions

Gel is run at room temperature; samples run toward anode.
In some chambers, gel will be upside down, resting directly on sponge wicks.
Run at 40V (measured at power supply) overnight.

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8. Staining

Remove from tank.
Place moist filter paper on gel side with layers of paper towels over filter paper.
Press under heavy books (approximately ½ hour).
Place in 60° oven until the gel is dry.

Stain in 0.2% Coomassie Blue made in the following destain solution: Alcohol/Acetic Acid/H_20 (50/10/50) for 15-30 minutes.

Clear the background by soaking the stained gel in destain solution.

9. Rocket Measurement

Peak height is measured with a millimeter ruler from the center of the sample well to the tip of the rocket peak.

10. Calculation of P^{30} Concentration

Measurements of the four control samples are used to plot a calibration line (peak height in mm vs. antigen concentration in μg/ml). At P^{30} concentrations below 50 μg/ml this plot approximates a straight line which can be used to determine P^{30} amounts in the unknown samples. P^{30} rocket height to antigen concentration is a logarithmic function. The difference relative to a straight line plot at levels below 50 μg/ml is negligible relative to the inherent experimental error, therefore it is treated as a direct relationship for convenience. The peak height of an unknown sample is compared to the control line and the corresponding P^{30} concentration is found.

Once an estimation of the P^{30} amount in μg/ml has been determined, this value can be used to calculate the semen dilution of the extract. SERI studies have indicated a range of 100 to 5900 μg/ml of P^{30} in individual semen samples studied. It is therefore assumed that the original concentration of P^{30} in the semen in the test stain is 5000 μg/ml. The μg/ml value of each unknown is thus divided into 5000 to give an estimation of semen dilution, which provides a basis for interpreting genetic markers present in the sample.

10. Calculation of P^{30} Concentration (Cont.)

Example: An extract is made with a portion of the stain or swab in approximately 100 μls saline. A 2 μl sample is run by the rocket technique and the peak heights are measured:

Semen Standard Controls:	50 μg/ml	=	34 mm
	25 μg/ml	=	19 mm
	12.5 μg/ml	=	10 mm
	6.25 μg/ml	=	8 mm

Stain Extract = 28 mm which corresponds to 39 μg/ml. Therefore, the semen dilution = 5000/39 = 1/128.

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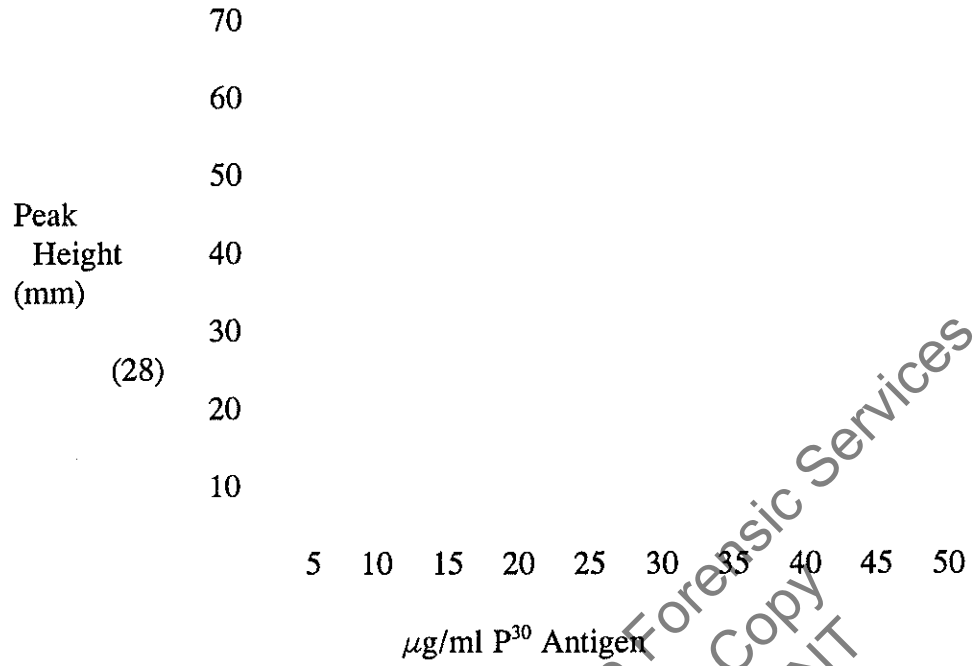
Example Calibration Plot:



Reference: SERI Methods Manual.

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Adopted March 20, 1997

PHENOLPHTHALEIN PRESUMPTIVE TEST FOR BLOOD

(Kastle-Meyer Test)

The test is performed as a two-step test.

1. Reagents should be prepared as directed in QC binder resulting in a working solution of phenolphthalein and a separate solution of H_20_2.

2. The suspected stain is rubbed with either a water moistened cotton-tipped applicator (e.g. Q-tip) or a folded filter paper moistened at the point. An unstained substrate sample may be tested as well. A known blood stain should always be tested as a positive control; H_20 can serve as a negative control.

3. The moistened area is watched while one drop of the phenolphthalein reagent is added. Any color change should be noted.

4. One drop of the peroxide solution is added directly on top of the previous phenolphthalein drop and, again, the color noted.

5. Occasionally, an old stain may appear very fixed to fabric and not be extracted by damp rubbing. In this case, a short section of thread can be cut and tested in the well of a white spot plate. (It may even be helpful to observe the test under a stereoscope).

6. Interpretation of Results

   Bloodstains will give a pink-purple color within a few seconds after the peroxide solution is added. This color should not appear after the first solution is applied. If it does, then the stain is catalyzing the change without relying on any hemoglobin being present. No conclusion can be drawn. The positive control should be pink-purple very fast. The negative control should be colorless.

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References:

Lee, Henry C., PhD. "Identification and Grouping of Bloodstains" Forensic Science Handbook. Ed. Richard Saferstein, PhD, p. 271-276. Prentice Hall, 1982.

Gaensslen, R.E. "Identification of Blood; Catalytic Tests" in Sourcebook in Forensic Serology, Immunology, and Biochemistry, National Institute of Justice, U.S. Government Printing Office, 1983.

Adopted March 20, 1997

HEMOCHROMOGEN (TAKAYAMA) TEST

(Used for the Identification of Blood)

1. Place the sample on a slide, cover with a cover slip, and allow reagent to flow under the slip (See QC book for reagent recipe).

2. Warm the slide for 10-30 seconds. Observe the orange crystals (stellate or rhomboid-shaped) microscopically.

3. A known blood control should be run using the same procedure.

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Interpretation

A positive reaction for the presence of blood occurs when the stellate or rhomboid-shaped crystals are observed. A pink or red coloration appears around the specimen, and the crystals are deep red in color.

A known blood sample and reagent blank are run concurrently with any unknowns to ensure that the reagent is reacting normally.

Reference

Spalding, Robert and Cronin, William. Technical and Legal Aspects of Forensic Serology: A Laboratory Manual. FBI, 1984.

Adopted March 20, 1997

IMMUNODIFFUSION TEST FOR SPECIES

(Ouchterlony)

1. Principle

A commercially available anti-human anti-sera is allowed to diffuse through agar where it meets the extract of a questioned stain. If the questioned stain extract contains proteins against which the anti-sera was produced, a precipitin line will form where the two meet.

2. Specimen Preparation

A cutting of the questioned stain is moistened with a minimum amount of physiological saline or water. This may be done in the well of a spot plate or in a 1.5 ml centrifuge tube (or the "piggy-back" insert for such a tube). A substrate control is similarly cut and soaked. Very old or heat-fixed stains may require extended soaking periods. Some investigators have used 0.05 M dithiothreitol on denatured stains for 3-4 days. The liquid, after soaking, is drawn off by pipette or centrifugation and used below.

3. Plate Preparation

Boil 1 g of agarose in 90 ml of water. Let cool to 50-60° and add 10 µl of 1:1000 aq. (Thimerosal) merthiolate solution. Pour into petri dishes using about 15 ml/plate. Allow agar to solidify in plate (plates can be stored in the refrigerator at this point). Just before use, punch holes in the agar so that a center hole is surrounded by a ring of holes. Commercial punches are available. Typical dimensions are: hole diameter of 2-4 mm and distance (edge-to-edge) from center hole to outer hole of 3 mm.

4. Procedure

Add the commercial anti-human anti-serum to the center well. To the separate outer wells, add the questioned stain extract, the substrate control, a known human bloodstain extract or liquid blood as a positive control, and any other controls deemed appropriate for the circumstances (inclusion of animal serum of the species used in the antiserum preparation as a control may also be useful). Cover and allow to stand at room temperature for at least four hours (usually overnight).

Optional staining for Ouchterlony on GelBond

Place moist filter paper on the gel side of GelBond, with a layer of paper towels over the filter paper. Press under a heavy weight (several books or a brick) for approximately 30 minutes. Place gel in 60° C oven until the gel is dry. Stain using 0.2% Coomassie Blue in destain solution for 15-30 minutes. Clear the background by soaking the stained gel in the destaining solution until precipitin interaction lines are visible.

Destaining solution: Methanol/Acetic acid/Water (50:10:50)

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5. Interpretation

The observation of a visible precipitin band (or bands) between the anti-human serum well and the stain extract well indicates the presence of human proteins if a similar band (or bands) is observed for the known human blood (positive control) and no such band is observed for the negative or substrate controls. The absence of observable precipitin bands for the questioned stain extract constitutes a negative reaction for human protein if the control samples exhibit expected results. Additional information may be obtained by placing known human blood in a well adjacent to one or two questioned stain extracts and observing the continuation of precipitin banding for specific proteins.

Reference

Lee, Henry C., "Determination of Species of Origin" in Forensic Science Handbook, Ed., Richard Saferstein, Prentice Hall, 1982.

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STAINING OF EXTRACTS FOR MICROSCOPIC IDENTIFICATION OF SPERM

1. Make an extract of a suspected semen stain (See "Making Extracts").

2. From the centrifuged extract, take 5 μl or less of the pelleted material and place on a labeled microscopic slide.

3. Dry the sediment spot (may use a slide warmer to speed the drying), then fix the sediment to the glass by heating the slide in a Bunsen burner flame.

4. Cover the dry sediment spot with Kernechtrot (Nuclear Fast Red) solution. Let stand 15 minutes.

5. Gently wash off the stain with water. Take care to leave the sediment on the slide.

6. Cover the sediment with Picroindigocarmine solution.

7. Let stand 15 seconds, then rinse with methanol.

8. Air dry, examine under the microscope.

9. Return the slide with the evidence.

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Interpretation

Spermatozoa will be stained with red heads (frequently two-toned) and green tails.

Reagents

Nuclear Fast Red:

5 gm of aluminum sulfate/100 ml of hot distilled H_20. Immediately add 0.1 gm of Nuclear Fast Red and stir with glass rod. Allow to cool.

Picro-indigo carmine:

4 g of picric acid + 300 ml of water. Cover and allow to stand to produce a saturated solution. Dissolve 1 gm of indigo carmine dye in the solution. Filter.

Reference: Oppitz, E. Arkive fur Krimin 144, pg. 145 (1969).

Adopted March 20, 1997