Department of Forensic Biology

Forensic Biochemistry & Hematology Laboratory

Methods Manual

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I. Introduction

The goal of the Department of Forensic Biology is to develop information through the identification and individualization of physiological fluids such as blood and semen. Among other benefits, this information can aid in the investigation of a crime or suspected crime, help tie a victim to a crime scene, connect a suspect to a crime, or eliminate a suspect.

This manual contains policies and methods available for use in the Biochemistry and Hematology Laboratory. The genetic markers analyzed were chosen, in part, to yield the most information out what is often limited sample. This results in a choice of genetic markers that have a high discrimination potential, are stable, or both.

The best use of limited evidence requires that information about the case be available. Such information as whether suspects were injured or victims transfused can help guide the analysis of evidence. Often, this may require the submittal of additional evidence such as a blood standard from the suspect or an injured, living, victim.

Depending on the case, required analysis can range from determining the species of blood present on an item to full genetic marker analysis of stained items for comparison to victims and/or suspects. The decision of what analyses are to be performed is made by a supervising Forensic Scientist after evaluation of the evidence through discussions with detectives or assistant district attorneys.

Analysis follows a logical progression. Items are first examined, most often looking for human blood and staining patterns. If human blood is present, and genetic marker analysis is required, the blood standard of the victim, and any other possibly bleeding person, is typed. It is generally preferable to type the standards first for a variety of reasons:

by discovering the types, the most informative system or systems (i.e. discrimination potential) can be used for the evidence

if during the preliminary typing, problems arise with the typing of the standards, they can be worked out before the evidence is analyzed; for example, obtaining the victim's clothing to use as a source of victim blood

if more than one person was bleeding, the preliminary typing can determine where their blood types differ and the evidence can be analyzed accordingly

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Once the blood standards are typed, decisions can be made by the supervisor and analysts regarding the potential of typing the evidence items.

Occasionally, investigative information desired or the particulars of a specific case may require that genetic typing of evidence be performed before all standards are submitted and typed.

II. Documentation

A. Casefiles

There is one case file per incident, which usually means one case file per victim; a double-homicide is still one incident, so there would be a file with <u>two</u> victims. Each has a unique FB (Forensic Biology) number, and all evidence associated with the victim(s) will use the same FB number.

The left-hand side of each file contains the:

chain of custody forms, documenting the flow of evidence received and released

evidence vouchers, the police department documentation of evidence collected

laboratory request forms listing the analyses desired

miscellaneous correspondence, such as memos to outside laboratories

case contact/control forms, documenting:

basic information on the victim (and suspect, if applicable)

discussions with detectives, attorneys, or others

what items are to be analyzed, and in what manner

assignment date, due date, and report date

The right-hand side of each file contains the:

handwritten notes documenting the evidence examinations

worksheets documenting the analyses performed

reports generated at the conclusion of the analyses

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All information regarding a case, including records of phone conversations, **must** be kept in the case file, in a neat and organized manner.

B. Notetaking

Notetaking is one of the most important aspect of casework. The notes are used to document the condition of the evidence, describe any stains that may be found, present the results of analytical tests, support the conclusions of the report, and refresh the analyst's memory when required to testify in court.

Each page of notes, both handwritten and worksheets, **must** have on it the following information:

case number
date
analyst's initials
page number (at the bottom)

Place page numbers at the bottom of pages. Page numbering is easiest if the bottom page in a file, usually the "Blood Processing" worksheet, is page 1. As more notes are generated, they are placed on top of existing notes and numbered. The last page, the "Case Summary" worksheet, will have the highest number and be on the top.

Notes should be legible and organized. If a mistake is made, draw a single line through the error and initial and date the correction. NEVER obliterate any notes or entry in a worksheet.

Notetaking starts with a description of the evidence, beginning with the packaging:

type of package - paper bag, manila envelope, zip-loc bag, etc.
condition of package - wet, bloody, etc.
type of seal - stapled, taped, unsealed
identifying marks - labels, tags, handwritten notations

Each package **must** be marked by the analyst with the case number, item number, date, and analyst's initials. Finding the marks in court is easier if the analyst always chooses the same location to put his or her marks.

Next is a description of the contents, the evidence itself. Note the following:

color or pattern of clothes
condition of evidence - torn, dirty, wet, moldy, etc.
dimensions of holes or tears in clothing or other items
any trace evidence (hairs, fibers, etc.) seen or collected
dimensions of knifes, sticks, etc.

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Each item **must** be marked with identifying case information, either by affixing a tag with the information or by writing directly on the item.

Any stains **must** be documented by diagrams, and occasionally photography, also note of the condition of the stains:

location of stains
size of stains
heaviness of stain - soaked into fabric, on surface, etc.
directionality of stain, if evident

Standardized worksheets are available with diagrams of pants, shirt, shoes, etc., to aid in documenting staining patterns. If a diagram must be hand-drawn, make sure it is large enough to allow room to document all of the stains present. Each stain **must** be given a unique, identifying number, and the evidence marked accordingly by affixing a tag with the information or by writing directly on the item. If tags are used, color-coding can be useful: red for blood, yellow for semen, and a third color to identify the unstained control area.

A representative unstained control should be selected for analysis. Its location should be documented in the same way as stained areas. The unstained controls are used to ascertain the effects of substrates on the test procedure and should be collected as close to the stained area as possible. It may be necessary to analyze more than one unstained area in order to obtain a negative result when used in sensitive tests, i.e., PCR.

Further analyses, such as species determination and genetic marker analyses, make use of worksheets. Make sure all worksheets are filled out completely and legibly, and that the appropriate gels or photographs are attached. If more than one case is analyzed on a worksheet, put original worksheet in one file and photocopies in the others. On the photocopied pages, note where the original is to be found.

C. Case Summary Sheet

The case summary sheet, which will be the top page of the analyst's notes, has two functions:

to summarize the examinations, presumptive testing, confirmatory testing, and typing results

to document the total number of examinations and/or tests performed for laboratory statistical purposes

For every piece of evidence examined there **must** be an entry in the summary sheet, even if no tests were performed (for example, a shoe with no stains).

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For every test performed on an item of evidence, a result **must** be entered in the summary sheet.

The quality control (QC) numbers associated with each test **must** be noted in the appropriate place.

The number of standards and controls also needs to be counted. This can be most easily done by counting the total number, not worrying about what the results were. The following should be counted in this group:

positive and negative controls for presumptive tests
positive and negative controls for confirmatory tests
normal rabbit controls for crossover electrophoresis
unstained/substrate controls from evidence
standards used in electrophoresis

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III. Screening Tests

A. Kastle Meyer (KM) Presumptive Test For Blood

ALL REAGENTS ARE AVAILABLE PRE-MADE AND QUALITY CONTROL CHECKED. DO NOT MAKE YOUR OWN OR USE SUPPLIES THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

stock solution:

10 g phenophthalin
50 g KOH

Dissolve in 1 l distilled water.

working solution:

200 ml stock solution
800 ml ethanol

Mix together; store in a dark tightly closed bottle over zinc dust to prevent oxidation.

OR

4 g phenolphthalein
40 g NaOH
20 g zinc dust
1 l water

Reflux this mixture until colorless. Cool and bring volume to 1200 ml with absolute ethanol. Store in a dark tightly closed bottle over zinc dust to prevent oxidation.

standards: blood and saline or water

Method:

1. A test may be performed directly on a cut out portion of a stain, an extract of a stain, or a "wipe" of the stained material. A wipe may be a piece of filter paper, thread, or swab; wet wipe with ethanol, then rub over the stained area while still wet.

2. Apply a drop of ethanol.

3. Apply a drop of KM reagent and observe any color change.

   A PINK COLOR HERE IS DUE TO THE PRESENCE OF AN OXIDIZING AGENT (e.g., vegetable peroxidase), NOT BLOOD

4. Add a drop of 3% hydrogen peroxide. An immediate pink color is a positive result.

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5. Test the postive and negative controls at the same time.

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III. Screening Tests

A. Kastle Meyer (KM) Presumptive Test For Blood

ALL REAGENTS ARE AVAILABLE PRE-MADE AND QUALITY CONTROL CHECKED. DO NOT MAKE YOUR OWN OR USE SUPPLIES THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

stock solution:

10 g phenophthalin
50 g KOH

Dissolve in 1 l distilled water.

working solution:

200 ml stock solution
800 ml ethanol

Mix together; store in a dark tightly closed bottle over zinc dust to prevent oxidation.

OR

4 g phenolphthalein
40 g NaOH
20 g zinc dust
1 l water

Reflux this mixture until colorless. Cool and bring volume to 1200 ml with absolute ethanol. Store in a dark tightly closed bottle over zinc dust to prevent oxidation.

standards: blood and saline or water

Method:

1. A test may be performed directly on a cut out portion of a stain, an extract of a stain, or a "wipe" of the stained material. A wipe may be a piece of filter paper, thread, or swab; wet wipe with ethanol, then rub over the stained area while still wet.

2. Apply a drop of ethanol.

3. Apply a drop of KM reagent and observe any color change.

   A PINK COLOR HERE IS DUE TO THE PRESENCE OF AN OXIDIZING AGENT (e.g., vegetable peroxidase), NOT BLOOD

4. Add a drop of 3% hydrogen peroxide. An immediate pink color is a positive result.

5. Test the postive and negative controls at the same time.

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A1. LEUCOMALACHITE GREEN PRESUMPTIVE TEST FOR BLOOD

ALL REAGENTS ARE AVAILABLE PRE-MADE AND QUALITY CONTROL CHECKED. DO NOT MAKE YOUR OWN OR USE SUPPLIES THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

solution:

1 g leucomalachite green
100 ml glacial acetic acid
150 ml distilled water
5 g zinc dust

Mix together. Simmer until the solution is almost colorless (pale yellow); this will take a few hours. Allow to cool, then filter. Add more zinc dust and store in a dark tightly closed bottle to prevent oxidation.

standards: blood and saline or water

1. A test may be performed directly on a cut out portion of a stain, an extract of a stain, or a "wipe" of the stained material. A wipe may be a piece of filter paper, thread, or swab; wet wipe with ethanol, then rub over the stained area while still wet.

2. Apply a drop of ethanol.

3. Apply a drop of LMG reagent and observe any color change.

   A BLUE-GREEN COLOR HERE IS DUE TO THE PRESENCE OF AN OXIDIZING AGENT (e.g., vegetable peroxidase), NOT BLOOD

4. Add a drop of 3% hydrogen peroxide. An immediate blue-green color is a positive result.

5. Test the positive and negative controls at the same time.

References:

Saferstein, Forensic Science Handbook

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B. Acid Phosphatase Presumptive Test For Semen

ALL BUFFERS AND REAGENTS ARE AVAILABLE PRE-MADE AND QUALITY CONTROL CHECKED. DO NOT MAKE YOUR OWN OR USE SUPPLIES THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Buffer: 8.21 g anhydrous sodium acetate

Dissolve in 1 l distilled water; adjust to pH 5.5 with acetic acid.

Reagents: 5 mg sodium alpha-naphthyl phosphate
5 mg Fast Blue B salt

In two separate test tubes, dissolve each in 5 ml buffer.

OR

1.58 g SERI spot test reagent

Dissolve in 5 ml distilled water.

The SERI spot test reagent contains alpha-naphthyl phosphate, Fast Blue B salt, and acetate buffer.

For either method, prepare the reagents fresh each time.

Standards: semen and water or saline

Two-step method:

1. A test may be performed directly on a portion of a stain, an extract, or a "wipe" of the stained material.
2. Apply a drop of the alpha-naphthyl phosphate reagent; wait 60 seconds.
3. Apply a drop of the Fast Blue B reagent. An immediate purple color is a positive reaction.
4. Test the positive and negative controls at the same time.

One-step method:

1. A test may be performed directly on a portion of a stain, an extract, or a "wipe" of the stained material.
2. Apply a drop of AP reagent and observe any color changes. A purple color developing within 60 seconds is a positive.

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3. Test the positive and negative controls at the same time.

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C. Presumptive Test for Saliva: Amylase Difusion

Gel buffer:

5.40 g anhydrous NaH_2PO_4
7.80 g anhydrous Na_2HPO_4
0.40 g NaCl

Dilute to 1 l with distilled water; adjust to pH 6.9.

Gel prep:

0.80 g Sigma Type I agarose
0.08 g starch (cornstarch)
80 ml gel buffer

OR

0.80 g SERI EA agarose (agarose plus starch)

Dissolve the agarose and starch in the buffer by heating in a flask. Pour onto a 20x20 cm glass plate and allow to solidify.

If a different size plate is used, adjust reagents accordingly.

A 10x10 cm disposable petri dish can also be used; adjust reagents accordingly.

I_3 solution:

16.5 g KI
25.4 g I_2

Dilute to 1 l in warm distilled water. Stir for 5 minutes, then filter. Store in brown bottle in the refrigerator.

Sample prep:

Extract an approximately 5 x 5 mm stain or a portion of a swab in 100 ul water for 30 minutes at room temperature.

Standards:

α-amylase standard: 20, 2, 0.2, 0.02, and 0.002 units or a comparable ten-fold dilution series.

The standard can be purchased amylase or dilutions of saliva standardized against the purchashed amylase.

1. Punch wells in the gel, leaving at least 1.5 cm between wells.
2. Fill wells (~8 ul) with standards, blank, samples, and sample controls (unstained areas).
3. Incubate for 3-8 hours at 37 °C or overnight at room

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temperature; keep the plate in a humid chamber to avoid drying.

4. Pour a 1:100 dilution (200 ul stock iodine solution added to 20 ml water) of a saturated iodine solution onto the gel; clear areas indicate regions of amylase activity.

5. Measure the diameter of the clear areas and record on the worksheet.

Entire items (blood spatter patterns, etc.) can be tested for amylase. Prepare a plate and allow to solidify; bring item (or area of item) into contact with the gel for 5 minutes. Follow steps 3-5 above to visualize any amylase pattern.

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Amylase$_1$ and Amylase$_2$ Differentiation by Differential Inhibition (Gel Diffusion Method)

Materials:

Kidney Bean Extract (KBE) - See Solutions Manual.

Wheat Lectin (WL) - Purchased commercially from Sigma (A-1520). Isolated from Triticum aestivum. Reconstitute 5mg of solid with 1ml distilled water.

Crude Pancreatic Extract - See Solutions Manual.

Crude Salivary Extract - See Solutions Manual.

Diffusion Gels - See Amylase Diffusion Method.

Lugol's Iodine - See Amylase Diffusion Method.

Preparation of Standardized Controls:

Determine the amylase activity of an equal amount of crude salivary extract and crude pancreatic extract using the amylase diffusion quality control test. Dilute extracts appropriately with 1mM calcium chloride to produce extracts with equal amylase activities.

Preparation of Standards:

Using standardized controls, prepare 3:1 v/v, 2:2 v/v, and 1:3 v/v mixtures of salivary and pancreatic extracts. Prepare dried stains on cotton swatches of the 3 mixtures and of the crude salivary and crude pancreatic extracts.

Assay:

Cut three 2-4mm^2 pieces of each of the samples to be tested (including the five standards) and incubate for 30 minutes at room temperature (20-25°C) in a minimal amount (approximately 50ul) of distilled water, KBE, and WL, respectively.

Place 8ul of a distilled water blank and each of the samples into punched wells (3mm in diameter) in the diffusion gels. Allow the samples to diffuse in the gel for 16 hours at room temperature under moist conditions. Pour Lugol's Iodine over the gel after the incubation period to develop the plate.

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Immediately measure the amylase activity by determining the area ($A=\pi r^2$) of the clear circles around each well. Measurements of radii should be taken under a stereoscope using a ruler with 0.5mm graduations if possible. The radius of each diffusion circle is calculated by averaging four readings measured at 90° from one another. Each reading is measured from the edge of the sample well to the outer border of the diffusion circle.

Calculate the percent inhibition of amylase activity for each sample or standard by using the equation:

$$1 - \frac{\text{(Area KBE or WL diluted sample)}}{\text{(Area water diluted sample)}} \times 100$$

Plot the inhibition ratio for each of the standards against the salivary:pancreatic ratio (4:0 v/v, 3:1 v/v, 2:2 v/v, 1:3 v/v). Similarly, determine the inhibition ratio of the test samples and plot on the standard curve.

Interpretation of Results:

In general, samples giving an inhibition ratio which approximates the value for unmixed salivary extract can be considered Amy_1 in origin. Conversely, samples giving an inhibition ratio which approximates the value for unmixed pancreatic extract can be considered Amy_2 in origin. Values in between those for the unmixed extracts should be interpreted with caution. The origin should not be considered until after consultation with a supervisor.

Reference:

JFSS, Vol. 33, No. 2 (1993), pp. 87-94.

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D. Presumptive Test for Urine: Urea Diffusion

Reagents

Bromothymol Blue (BTB) - Dissolve 1.5g of BTB in 100ml distilled water. Add one drop of phosphoric acid diluted 1:10 with distilled water. Adjust pH below 6.0 with 0.1N sodium hydroxide if necessary.

Urease Solution - Dissolve enough urease in 100ml distilled water to produce a solution containing 2500-300 units of urease activity.

Agarose Solution - Prepare a 1% solution (w/V) by dissolving an appropriate amount of agarose (Sigma type I or equivalent) in boiling water. Cool to 56°C.

Blank Plate - Prepare a 1% (v/v) solution of BTB in agarose solution. Pour approximately 30ml of this solution in 10cm² square petri dishes and allow to solidify.

Test Plates - to 1% (v/v) solution BTB in agarose add an appropriate amount of urease so that 30ml of test solution will contain approximately 10 units of urease activity. Aliquot 30ml fractions of test solution in 10cm² square petri dishes and allow to solidify.

	15 Blank Plates	15 Test Plates	20 Blank Plates	20 Test Plates
BTB	4.5ml	4.5ml	6.0ml	6.0ml
Urease	--	5.0ml	--	6.6ml
Agarose (1g/100ml)	445.5ml	445.5ml	594ml	594ml

Sample Preparation:

Extraction a 1cm² stain or equivalent portion of swab or other substrate in a pipette tip with 200ul distilled water. Extend the lower portion of the pipette tip into a test tube and slowly rotate for 30 minutes. After this period, centrifuge the sample allowing the extract to descend to the bottom of the tube.

Standards Preparation:

Prepare standard solutions containing 5g urea/100ml distilled water, 0.5g Urea/100ml distilled water, 0.05g urea/100ml distilled water, and 0.0005g urea/100ml distilled water. Also prepare a 1cm² urine stain on either

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filter paper or cotton fabric. Extraction the stain in 200ul water and prepare a 1:2 dilution of the extract in distilled water.

Assay:

Punch wells in both blank plates and test plates (for 10cm^2 plates, 9 wells can easily be punched). For the distilled water blank, each urea standard, both extracts of know urine stain (neat and 1:2 dilution), and sample extracts place 9ul aliquots in both blank plate and test plate wells. After a diffusion period of 20 minutes, measure the mean radius of the ;diffusion circle for each sample in the two corresponding plates. If the diffusion area of the blank plate is of significantly lower intensity than the test plate diffusion area, the measurement should be recorded as zero. Record results on worksheet.

Standard Curve:

For each urea standard, plot the mean diffusion radius (determined by subtracting the mean diffusion radius of standard blank plate from mean diffusion radius of standard test plate) vs. log of urea concentration (g/10ml). Measurement of radius should be taken in at least 3 points of the diffusion circle.

Plot the mean diffusion radius for neat and 1:2 diluted extracts of known urine stain curve.

Results:

Values which exceed or bracket the values obtained with he neat and 1:2 diluted extracts from the known urine stain should be considered positive for urine. All test samples falling below the value obtained for the 0.005g urea/100ml should be considered inconclusive. If results are in doubt consult a supervisor.

Photography:

All plates must be photographed and

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kept in case folders. Due the blue and green background of the plates an orange filter is recommended when photographing plates. Recommended photographic parameters are listed below.

Test plates; f stop - 22
shutter speed - 1/8

transmitted and oblique lighting

Blank Plates f stop - 22
shutter speed - 1/30 oblique lighting only

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IV. Confirmatory Tests

A. Takayama Hemoglobin Test

PERFORM THE TEST IN THE HOOD BECAUSE OF THE PRESENCE OF PYRIDINE.

Reagent: 5 ml 10% dextrose (glucose)
10 ml 10% NaOH
20 ml pyridine

Dilute to 100 ml with distilled water. Store in the refrigerator.

For crusts or scrapings:

1. Place a blood crust or flake on a glass slide.
2. Add a drop of reagent to the slide and cover with a cover slip.
3. Heat the slide very gently over a flame or on a hot plate for 1-3 minutes.
4. Allow slide to cool slowly.
5. View under the microscope (about 100X), looking for characteristic salmon red/pink crystals.
6. Test positive and negative controls at the same time.

For clothing:

1. Extract stain with 1-3 drops water.
2. Place extract on slide and allow to evaporate. Scrape off the dried substance and gather in the center of the slide.
3. Add a drop of reagent to the slide and cover with a cover slip.
4. Heat the slide **very gently** over a flame or on a hot plate for 1-3 minutes.
5. Allow slide to cool slowly.
6. View under the microscope (about 100X), looking for salmon red/pink crystals.
7. Test positive and negative controls at the same time.

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B. Ouchterlony Radial Diffusion

ALL BUFFERS, GELS, AND ANTISERA ARE AVAILABLE PRE-MADE AND QUALITY CONTROL CHECKED. DO NOT MAKE YOUR OWN OR USE SUPPLIES THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Gel buffer: 1:1 species Cross-Over buffer: distilled water

Mix 100 ml tank buffer with 100 ml distilled water.

Gels: Prepare tubes of 1% agarose gel in advance by dissolving 2 g of Sigma type I agarose in 200 ml gel buffer, then dispensing 7 ml aliquots into 20x150 mm test tubes. Allow to solidify, then cover with Parafilm and store in the refrigerator.

Sample prep: Prepare extracts using gel buffer, water, or saline. If you don't want to make extracts, a small, wet thread or small piece of swab can be inserted directly into the well.

Plate prep: Have a supply of 2.5 x 3.5 inch pieces of GelBond

Standards: positive control, negative control, sample controls

1. Obtain a premade test tube of gel; heat in a boiling water bath until it is liquified.
2. Pour onto the hydrophilic side of the GelBond and let solidify.
3. Punch an array of wells consisting of a central well surrounded by four wells; use the template on the worksheet.
4. Apply anti-sera to the central well.
5. Apply the positive control to one of the surrounding wells.
6. Apply the sample(s) so that a stain is always next to a positive control.
7. Apply negative control and stain (substrate controls) to the remaining wells.
8. Place the plate in a moisture chamber and incubate at 37 °C overnight.
9. A positive result is when the precipitin bands for the positive controls and the samples meet in a smooth curve. No spur formation should be seen.

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10. Rinse the plate in saline overnight, then do two 10 minute rinses in distilled water.

11. Press the gel between paper towels with a weight on top for 30 minutes, then dry in the oven for about 30 minutes.

12. Stain (see "Coomassie Blue Staining" Section C.3.)

13. Dry either at room temperature or in the oven.

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C. Species Crossover Electrophoresis

ALL BUFFERS, GELS, AND ANTISERA ARE AVAILABLE PRE-MADE AND QUALITY CONTROL CHECKED. DO NOT MAKE YOUR OWN OR USE SUPPLIES THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Tank buffer: 8.76 g sodium barbiturate
1.38 g diethyl barbituric acid (Barbital)
0.38 g calcium lactate

Dilute to 1 l with distilled water; adjust to pH 8.6

Gel buffer: 1:1 tank buffer:distilled water

Mix 100 ml tank buffer with 100 ml distilled water.

Gels: Prepare tubes of 1% agarose gel in advance by dissolving 2 g of Sigma type I agarose in 200 ml gel buffer, then dispensing 7 ml aliquots into 20x150 mm test tubes. Allow to solidify, then cover with parafilm and store in the refrigerator.

Sample prep: Prepare extracts using gel buffer, water, or saline. If you don't want to make extracts, a small, wet thread or small piece of swab can be inserted directly into the well.

Plate prep: Have a supply of 2.3x3.5 inch pieces of GelBond

Standards: positive control, negative control, sample controls

Method:

1. Obtain a premade test tube of gel; heat in a boiling water bath until it is liquified.
2. Pour onto the hydrophilic side of the Gelbond and let solidify
3. Punch small wells (about 1-2 mm) in rows using the template on the worksheet. Punch enough wells for controls, samples, and sample controls.
4. Apply anti-sera in the left-hand wells using capillary tubes
5. Apply samples in the right-hand wells
6. Place plate in electrophoresis tank with the antibodies toward the cathode (black)
7. Electrophoresis is at 120V for 20 minutes.
8. A sharp, white, precipitin band (not a cloud) is a positive

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9. Rinse the plate in saline overnight, then do two 10 minute rinses in water.

10. Press the gel between paper towels with a weight on top for 30 minutes, then dry in the oven for about 30 minutes.

11. Stain (see "Coomassie Blue Staining", Section C.3)

12. Dry after staining either at room temperature or in the oven.

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D. Christmas Tree Stain For Spermatazoa

The nuclear material within the cell is stained red by the nuclear fast red stain. Sperm heads are usually well differentiated with the acrosome staining significantly less densely than the distal region of the head. Epithelial membranes and sperm tails are stained green by the PIC stain; nuclei inside epithelial cells appear purple. Yeast cells also stain red, however the stain is uniform throughout the cell and extends into polyp-like structures which are occasionally seen in yeast.

Reagents: Nuclear Fast Red

2.5 g aluminum sulfate
50 mg nuclear fast red (C.I. 60760)

Dissolve the aluminum sulfate in 100 ml of warm distilled water and add the nuclear fast red. Stir and allow to cool; filter. The solution is stable for many months in the refrigerator.

Picro Indigo Carmine (PIC)

1.30 g picric acid
0.33 g indigo carmine (C.I. 73015)

Dissolve the picric acid in 100 ml of warm distilled water; add the indigo carmine and stir overnight. The solution is stable for many months in the refrigerator.

Method:

1. Fix cells to the slide by heating
2. Cover cell debris with a few drops of nuclear fast red and allow to sit for at least 10 minutes (if longer, a humid chamber may be necessary)
3. Wash away the nuclear fast red with distilled water
4. Add one drop of the PIC stain to the still-wet slide; allow to sit for no more than 30 seconds
5. Wash away the PIC stain with ethanol
6. Let slide dry; examine the slide at 100X or 400X (don't use immersion oil)

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E. P30 Crossover Electrophoresis

ALL GELS, BUFFER, AND ANTISERA ARE AVAILABLE PRE-MADE AND QUALITY CONTROL CHECKED. DO NOT MAKE YOUR OWN OR USE SUPPLIES THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Tank buffer: 25.2 g Tris base
2.5 g EDTA, free acid
1.9 g boric acid

Dilute to 1 l with distilled water; adjust to pH 9.1

OR

use SERI pre-made tank buffer

Gel buffer: same as tank buffer

Gels: Prepare tubes of 1% agarose gel in advance by dissolving 2 g of Sigma type III agarose in 200 ml gel buffer, then dispensing 7 ml aliquots into 20x150 mm test tubes. Allow to solidify, then cover with parafilm and store in the refrigerator.

Sample prep: Prepare extracts using gel buffer, water, or saline. If you don't want to make extracts, a small, wet thread or small piece of swab can be inserted directly into the well.

Plate prep: Have a supply of 2.3x3.5 inch pieces of Gelbond

Standards: human semen stain, saline, and sample (substrate) controls

Method:

1. Obtain a premade test tube of gel; heat in a boiling water bath until it is liquified.
2. Pour onto the hydrophilic side of the Gelbond and let solidify.
3. Punch small wells (about 1-2 mm) in rows using the template on the worksheet. Punch enough wells for controls, samples, and sample controls.
4. Apply anti-sera (~ 5 ul) in the left-hand wells using capillary tubes or pipettes; do not fill wells to over flowing.
5. Apply samples (~ 5 ul) in the right-hand wells using capillary tubes or pipettes; do not fill wells to over flowing.

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6. Place plate in electrophoresis tank with the antibodies toward the cathode (black).

7. Electrophoresis is at 120V for 30 minutes.

8. A sharp, white, precipitin band (not a cloud) is a positive.

9. Rinse the plate in saline overnight, then do two 10 minute rinses in water.

10. Press the gel between paper towels or thick blotting paper with a weight on top for 30 minutes, then dry in the oven for about 30 minutes.

11. Stain (see "Coomassie Blue Staining", Section C.3).

12. Dry after staining either at room temperature or in the oven.

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F. P30 ELISA

ALL BUFFERS, STOCK SOLUTIONS, AND NEAT ANTISERA ARE AVAILABLE PRE-MADE AND QUALITY CONTROL CHECKED. DO NOT MAKE YOUR OWN OR USE SUPPLIES THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Prostate specific antigen (PSA) is another name for P30 antigen.

Phosphate buffered saline (PBS)

To prepare 200 mL, dissolve 1 tablet in 200 mL of distilled water. This can be stored at 4°C for up to 2 weeks.

To prepare 1 L, dissolve 5 tablets in 1 L of distilled water. This can be stored at 4°C for up to 2 weeks.

Casein stock solution

Thoroughly dissolve 10 g Hammerstein casein in 500 mL distilled water; adjust to pH 8.0 with 3M NaOH. Add 500 mL PBS and 0.1 g sodium azide. Freeze in 40 mL aliquots.

PBS with 0.02% w/v Hammerstein casein (PBS-casein)

To prepare 1 L, add 20 mL of casein stock solution to 1 L of PBS.

One plate uses approximately 800 mL.

PBS with 0.1% bovine serum albumin (PBS-BSA)

To prepare 100 mL, add 100 uL bovine serum albumin to 100 mL PBS.

Alkaline substrate buffer

Add 97 mL diethanolamine, 0.2 g sodium azide, and 0.1 g $MgCl_2$ to 800 mL of distilled water; adjust to pH 9.8 with concentrated HCl. Make up to 1 L with distilled water.

Standards

P30 antigen

When P30 antigen arrives, divide into 100 uL aliquots and freeze.

Prepare 1ug/mL solution of P30 antigen by diluting 100 uL antigen with 100 mL PBS-BSA. Divide into 500 uL aliquots and freeze at -40°C.

Plates

Immulon II microELISA plates

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Antibodies

Mouse monoclonal anti-human PSA (prostate specific antigen, P30)

When needed, reconstitute with 100 uL of distilled water. Store at 4°C.

Rabbit polyclonal anti-human PSA

Store at 4°C.

Swine anti-rabbit IgG alkaline phosphatase conjugate

Store at 4°C.

Mouse IgG1, Kappa chain (MOPC 21, mouse myeloma protein)

When needed, reconstitute with 100 uL of distilled water. Store at 4°C.

Initials: [illegible] Date: 4/23/93

PLATE PREPARATION:

COATING THE PLATES

1. Prepare a 1:8000 dilution of mouse monoclonal anti-human PSA by adding 10 uL antiserum to 80 mL PBS. **Always make dilutions in glass, not in plastic.** This is enough for 16 plates.

2. Prepare a 1:8000 dilution of MOPC by adding 10 uL MOPC to 80 mL PBS. **Always make dilutions in glass, not in plastic.** This is enough for 16 plates.

3. Coat the plate as shown in the diagram. Use 100 uL of the appropriate solution per well.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	M	M	M	M	M	M	M	M	M	M	M
B	PBS	M	M	M	M	M	M	M	M	M	M	M
C	PBS	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA
D	PBS	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA
E	PBS	M	M	M	M	M	M	M	M	M	M	M
F	PBS	M	M	M	M	M	M	M	M	M	M	M
G	PBS	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA
H	PBS	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA	PSA

PBS = phosphate buffered saline
PSA = mouse monoclonal anti-human PSA
M = MOPC

4. Cover plates with Parafilm and incubate overnight at 4°C.

BLOCKING THE PLATES:

1. Aspirate contents of wells. Wash each plate twice with wash buffer (PBS-casein), then fill the wells. Let the filled plate sit at room temperature for 15 minutes.

2. Aspirate the final wash and blot dry. Plates which are not used immediately should be wrapped in plastic and stored at 4°C; they can be stored like this for up to 2 weeks.

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SAMPLE PREPARATION:

Prepare samples and standards on the day of use.

1. **Stains/swabs** Using the pipet tip/test tube method, extract 2.5 x 2.5 mm samples in 100 uL of PBS for 30 minutes at room temperature. Centrifuge, then prepare a 1:25 dilution by adding 20 uL sample extract to 500 uL wash buffer (PBS-casein).

   Retain the remainder of the sample extract until the ELISA is complete.

2. **Standards** 10 ng/mL: 10 uL of 1 ug/mL P30 + 990 uL wash buffer (PBS-casein)

   6 ng/mL: 6 uL of 1 ug/mL P30 + 994 uL wash buffer (PBS-casein)

   2 ng/mL: 2 uL of 1 ug/mL P30 + 998 uL wash buffer (PBS-casein)

SAMPLE AND STANDARD APPLICATION:

1. Apply PBS, wash buffer, standards and samples as shown in the diagram. Use 100 uL of the appropriate solution per well.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	W	2ng	10ng	S2	S4	S6	S8	S10	S12	S14	S16
B	PBS	W	2ng	10ng	S2	S4	S6	S8	S10	S12	S14	S16
C	PBS	W	2ng	10ng	S2	S4	S6	S8	S10	S12	S14	S16
D	PBS	W	2ng	10ng	S2	S4	S6	S8	S10	S12	S14	S16
E	PBS	W	6ng	S1	S3	S5	S7	S9	S11	S13	S15	S17
F	PBS	W	6ng	S1	S3	S5	S7	S9	S11	S13	S15	S17
G	PBS	W	6ng	S1	S3	S5	S7	S9	S11	S13	S15	S17
H	PBS	W	6ng	S1	S3	S5	S7	S9	S11	S13	S15	S17

PBS = phosphate buffered saline
W = wash buffer (PBS-casein)
S = samples

2. Cover the plates with Parafilm and incubate at room temperature for 1 hour.

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3. Aspirate contents of wells. Wash the plate three times with wash buffer (PBS-casein). Make sure that there is no excess buffer remaining in the wells.

4. Prepare a 1:1000 dilution of rabbit polyclonal anti-PSA by adding 10 uL antiserum to 10 mL PBS.

5. Apply 100 uL of the 1:1000 dilution of rabbit anti human polyclonal anti-PSA to each well of columns 2-12. Apply 100 uL of PBS to each well of column 1.

6. Cover the plates with Parafilm and incubate the plates at room temperature for 1 hour.

7. Aspirate contents of wells. Wash the plate three times with wash buffer (PBS-casein). Make sure that there is no excess buffer remaining in the wells.

8. Prepare a 1:1000 dilution of swine anti-rabbit IgG alkaline phosphatase conjugate by adding 10 uL antiserum to 10 mL of PBS.

9. Apply 100 uL of the 1:1000 dilution of swine anti-rabbit alkaline phosphatase conjugate to each well of columns 2-12. Apply 100 uL of PBS to each well of column 1.

10. Cover the plates with Parafilm and incubate at room temperature for 1 hour.

11. During the incubation, allow alkaline substrate buffer (ASB) to come to room temperature, about 30 minutes. For each two plates, dissolve one 20 mg p-nitrophenyl phosphate tablet (PNPP) in 20 mL of ASB.

12. Aspirate contents of wells. Wash the plate three times with wash buffer (PBS-casein). Make sure that there is no excess buffer remaining in the wells.

13. Apply 100 uL of PNNP substrate solution to each well of columns 1-12.

14. Cover the plates with Parafilm and incubate for 1 hour at 37°C. Uncover and read at 405nm.

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CALCULATION OF P30 ELISA RESULTS

The background readings from column 1 (reagent blank) are automatically subtracted by the microtiter plate reader.

1. Calculate the plate threshold value (PT) of the plate:

   Determine the average (AVE) and standard deviation (SD) of the values in column 2.

   PT value = 2(AVE) + 3(SD)

2. Subtract the PT value from the values in columns 3-12.

3. For all standards and samples, calculate the average of the duplicate samples for both the MOPC and monoclonal PSA coated wells.

4. Subtract the MOPC averages from their corresponding monoclonal PSA averages. The remaining value is the P30 ELISA result.

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EXAMPLE:

The 2 ng standard gave a final ELISA value of 0.055.

Sample 1 is in wells A3–D3; sample 2 is in wells E3–H3

	Value		Value	
A2	0.114	A3	0.081	} MOPC
B2	0.091	B3	0.063	} MOPC
C2	0.123	C3	0.356	} monoclonal PSA
D2	0.063	D3	0.325	} monoclonal PSA
E2	0.081	E3	0.266	} MOPC
F2	0.085	F3	0.272	} MOPC
G2	0.085	G3	0.711	} monoclonal PSA
H2	0.070	H3	0.847	} monoclonal PSA

average of column 2 values: 0.089
standard deviation of column 2 values: 0.020

plate threshold = 2(0.089) + 3(0.020)
= 0.238

Subtract the PT value from each value in column 3; if less than zero, enter zero.

A3	0.000	
B3	0.000	average of A3 and B3 = 0.000
C3	0.118	
D3	0.087	average of C3 and D3 = 0.103
E3	0.011	
F3	0.009	average of E3 and F3 = 0.010
G3	0.473	
H3	0.609	average of G3 and H3 = 0.506

P30 ELISA results:

sample 1 = (AVE C3/D3) – (AVE A3/B3)
= (0.103) – (0.000)
= 0.103

sample 2 = (AVE G3/H3) – (AVE E3/F3)
= (0.506) – (0.010)
= 0.496

Any sample with a P30 ELISA result greater than the value obtained for the 2 ng standard is positive for the presence of semen.

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SOURCES FOR P30 ELISA SUPPLIES:

Sigma Chemicals

P4417	PBS tablets
N2770	PNNP tablets
M9269	mouse IgG, Kappa chain (MOPC 21)

SERVA Biochemicals

48005	Hammerstein casein

DAKO Corporation
6392 Via Real
Carpenteria, CA 93013
1 800 235 5743

A562	rabbit polyclonal anti-human PSA
D306	swine anti-rabbit IgG alkaline phosphatase conjugate

INKSTAR
Stillwater, MN 55082

22516	mouse monoclonal anti-human PSA

Dynatech Laboratories Inc.
14340 Sullyfield Circle
Chantilly, VA 22021

0110103450	Immulon II microELISA plates

SCRIPPS
San Diego, CA

P0714	P30 antigen

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V. Red Cell Antigen Systems

A. Blood Processing Policy

The majority of blood samples need only to be processed for storage and possible enzyme, protein, or DNA analysis.

1. Find the case files; the blood processing forms and chain of custody forms should have been filled out by a member of the Evidence Unit. If not, alert a supervisor.

2. Prepare cloth for dried stains by affixing colored tape tags. Label the tags and coin envelopes or zip-loc bags with the following information:

   case number
   date
   initials of person preparing stain
   subjects name

3. Make stains on the cloth using whole blood; set aside to dry.

4. Label 12 x 75 mm test tubes and 1.5 ml microfuge tubes (two per sample) with FB numbers.

5. Fill 12 x 75 mm test tubes about 2/3 full with whole blood and centrifuge in the Serofuge for three minutes to spin down the cells.

6. Place the serum into one of the 1.5 ml microfuge tubes.

7. Place the packed red blood cells into the other microfuge tube.

8. Package dried stains into their envelopes. Freeze the stains, cells, and sera.

9. Indicate on the "Liquid Blood Processing" forms that stains were made and that cells and sera were obtained (where applicable).

THE REMAINDER OF THE LIQUID BLOOD WILL BE DISCARDED IN APPROXIMATELY TWO MONTHS BY MEMBERS OF THE EVIDENCE UNIT.

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B. ABO TYPING

ABO (autopsy blood) typing needs to be performed only on blood samples in cases where an ABO inhibition test might need to be done. This includes cases which have vaginal, oral, or rectal swabs or evidence such as cigarette butts where saliva stains may be present. The blood samples are identified by the RED DOT sticker on the lids.

Reagents:
anti-A serum
anti-B serum
anti-H lectin
A indicator cells
B indicator cells

Method:

1. Find the case files; the blood processing form and chain of custody form should have been filled out by a member of the Evidence Unit. If not, alert a supervisor.

2. Label test tubes (three for each sample), 1.5 ml Eppendorf tubes (two for each sample), blood typing slides, cloth for dried stains, and coin envelopes or small zip-loc bags. The cloth and coin envelopes or zip-loc bags should have on them the following information:

   case number
   date
   initials of person preparing stain
   subjects name

   The cloth is easiest labeled by affixing a tape "tag" onto which the information can be written.

   The other items should be labeled with the case number.

3. Make stains on the cloth using whole blood; set aside to dry.

Forward (DIRECT) Typing:

a. Add one drop of whole blood to each of three wells

b. Add one drop of anti-A to one well
   Add one drop of anti-B to another well
   Add one drop of anti-H to a third well

c. Rotate briefly (on rotator or by hand) until agglutination can be seen

d. Interpretation of Results:

   If anti-A agglutinates, the blood is type A. The

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anti-H may or may not agglutinate.

If anti-B agglutinates, the blood is type B. The anti-H may or may not agglutinate.

If anti-A and anti-B agglutinates, the blood is type AB. The anti-H may or may not agglutinate.

If anti-H agglutinates, the blood is type O.

If there's no agglutination, the ABO type is inconclusive.

5. Fill a 12 x 75 mm test tube about 2/3 full with whole blood and centrifuge in the Serofuge for three minutes to spin down cells.

Reverse Typing:

a. Put two drops of the supernatant serum into each of two test tubes or microscope slides labeled with the case number and "A" and "B".

b. Add one drop of the A1 indicator cells (undiluted) into the "A" tube or slide.

c. Add one drop of the B indicator cells (undiluted) into the "B" tube or slide.

d. Centrifuge tubes for one minute, forming a "button" of cells, or gently rock the slide.

e. Incubate at room temperature for 10 minutes.

f. Shake tubes gently and read macroscopically for agglutination.

g. Interpretation of Results

Agglutination with B cells - type A

Agglutination with A cells - type B

Agglutination with both A and B cells - type O

Agglutination with neither A or B cells - type AB or inconclusive (depending on forward typing results).

7. Place the remainder of the serum into one of the 1.5 ml Eppendorf tubes.

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8. Place the packed red blood cells into the other Eppendorf tube. If Lewis typing is to be done, take a few drops of the cells and place in a 12 x 75 mm test tube. Fill about 2/3 full with saline (see "Lewis Typing").

9. Package dried stain into its envelope. Freeze the stain, cells, and serum.

10. Indicate on the "Liquid Blood Processing" form the results of the forward and reverse typing. If it is the second typing, add the date and initials of the analyst.

11. Use the "Comments" section to document any unusual results, condition of sample, etc.

THE REMAINDER OF THE LIQUID BLOOD WILL BE DISCARDED IN APPROXIMATELY TWO MONTHS BY MEMBERS OF THE EVIDENCE UNIT.

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C. Lewis Typing

Lewis typing is to be performed on whole blood (autopsy blood) samples in cases where an ABO inhibition test might need to be performed. This includes cases which have vaginal, oral, or rectal swabs or evidence such as cigarette butts. The blood samples are identified by the RED DOT sticker on the lids.

Alsevier's buffer:

4.00 g trisodium citrate*2 H_2O
0.25 g anhydrous citric acid
10.25 g dextrose
2.09 g NaCl

Dilute to 500 ml with distilled water; adjust to pH 6.0 if necessary. Store in refrigerator.

Phosphate-buffered Saline (PBS):

5.38 g NaH_2PO_4*1 H_2O
16.35 g Na_2HPO_4*7 H_2O
9.00 g NaCl

Dilute to 1 l with distilled water; adjust to pH 7.0 if necessary. Store in refrigerator.

4% ficin:

1.00 g ficin
25.00 ml Alsever's solution

Mix on a magnetic stirrer until the ficin has dissolved as completely as possible. Filter through Whatman #1 filter paper using a vacuum and freeze in 0.2 ml aliquots.

Lewis antisera:

Dilute antisera 1:5 with PBS (200 ul antisera, 800 ul PBS). Store in refrigerator.

Capillary tubes:

Chown type capillary tubes
0.4 mm I.D. x 90 mm, catalog #BB-100
Diagnostic Technology Inc.
240 Vanderbilt Motor Parkway
Hauppage NY 11788 800-645-6288

Standards:

Le a+b- and Le a-b+

Method:

1. Wash red blood cells (RBC) three times with saline by adding

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saline to RBC's, mixing, and centrifuging 3 minutes in the Serofuge.

2. Using the washed RBC, prepare a solution of 50 ul RBC, 50 ul Alsever's, and 25 ul ficin solution. Mix gently.

3. By capillary action, draw diluted Lewis antisera into the capillary tube to a distance of 2.5 cm.

4. Wipe the tip of the capillary tube with a Kimwipe.

5. By capillary action, draw the treated RBC into the capillary tube to a distance of 1.0 cm. Keep the capillary tube in a vertical position to prevent air bubbles from being trapped between the RBC and the Lewis antisera. If air gets trapped, discard the tube and repeat the process.

6. Wipe the tip of the capillary tube with a Kimwipe.

7. Seal that end of the capillary tube used to pick up the antisera and RBC by gently pushing the capillary tip into Critoseal (replace cover on the Critoseal when finished to prevent drying).

8. Invert the sealed capillary tube, sealed end up, and place in a stand set at a 60 degree angle.

9. Results may be read after 10 minutes. You may wish to use a magnified light source to aid in reading the results.

Interpretation of Results:

a positive result is indicated by a clumped, broken column of RBC along the length of the capillary tube

a negative result is indicated by a unbroken, needle-like column of RBC along the length of the capillary tube

A Le a-b- sample should have the RBC tipped through the antisera several times; clumping may eventually occur.

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D. Absorption-Elution Thread Method

ANTISERA MUST BE QUALITY CONTROL CHECKED. DO NOT USE REAGENTS THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Reagents:
polyclonal anti-A anti-sera (MUST be polyclonal)
polyclonal anti-B anti-sera (MUST be polyclonal)
anti-H anti-sera

A1, B, and O test cells
30% bovine serum albumin (BSA)

saline

Standards:
A bloodstain
B bloodstain
O bloodstain
unstained cloth

Sample prep: If the bloodstain is on an absorbent thread material (such as clothing), the threads may be used as is. If not (such as a knife), swab off the blood onto threads. For scrapings, dissolve in water and apply onto threads.

Always prepare an unstained area in a similar fashion as a control.

Method:

1. Label enough Petri dishes for the standards, samples, and sample controls. Make wells on the bottom of the Petri dish with a hot test tube.

2. Using a waterproof adhesive such as nail polish, affix threads (3-10 mm) to the appropriate wells.

3. After the adhesive is dry, add one drop anti-A to the wells in the "A" columns, one drop anti-B to the wells in the "B" columns, and one drop anti-H to the wells in the "O" columns; make sure each thread is submerged.

4. Place the lids on the Petri dishes and allow to absorb for a minimum of two hours at 4°C. Absorption can be as long as 48 hours; place Petri dishes in a humid chamber to prevent evaporation of the anti-sera.

5. Prepare 0.05 - 0.1 % suspensions of A1, B, and O test cells in the following manner:

   Label three 12 x 75 test tubes "A", "B", and "O".

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Fill each tube with saline to about 2 cm from the top (about 4.5 ml); add 1 drop of 30% BSA to each tube.

To the A tube add 2 drops of 2-4% A2 test cells; to the B tube add 2 drops B cells and to the O tube add 2 drops O cells.

6. Prepare a dilute solution of BSA by mixing 100 ul 30% BSA to 10 ml saline.

7. Using cold saline (4 °C), rinse the anti-sera off the Petri dishes.

8. Rinse the Petri dishes in cold saline (4 °C) on a rotator for two hours, changing the saline every 30 minutes.

9. Gently blot dry with tissue.

10. Add a drop of dilute BSA to each well, covering each thread. Place the lids on the Petri dishes and incubate at 55 °C for approximately 25 minutes.

11. Add a drop of A1 test cell suspension to each well in the "A" columns, a drop of B test cell suspension to each well in the "B" columns, and a drop of O test cell suspension to each well in the "O" columns.

12. Place the lids on the Petri dishes and rotate at room temperature for 30 minutes.

13. Read agglutination microscopically at 100X. Score the agglutination as below:

- \- cells are free; no clumps are seen
- 1 small clumps, a few cells each, are seen; the majority of the cells are free
- 2 more and larger clumps are seen; not many free cells
- 3 large clumps are seen; few free cells are seen
- 4 very large, solid, clumps are seen; essentially no free cells

14. The A, B, and O bloodstain standards should give results similar to below:

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	A	B	O
A bloodstain	3-4	-	-, 1-4
B bloodstain	-	3-4	-, 1-4
O bloodstain	-	-	3-4
unstained	-	-	-

If the agglutination is weaker than above, rotate for an additional 15 minutes.

15. Substrate controls should have no agglutination; if any agglutination is seen in the substrate control, the stain results MUST be called inconclusive.

Initials: RCJ Date: 3/31/92

E. Absorption - Elution Slide Method

ANTISERA MUST BE QUALITY CONTROL CHECKED. DO NOT USE REAGENTS THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Reagents:
polyclonal anti-A anti-sera (MUST be polyclonal)
polyclonal anti-B anti-sera (MUST be polyclonal)
anti-H anti-sera

A1, B, and O test cells
30% bovine serum albumin (BSA)

saline
5% aqueous NH_4OH

Standards:
A bloodstain
B bloodstain
O bloodstain

Sample prep: Extract an approximately 5 x 5 mm stain in an appropriate amount of 5% NaOH. The volume used should give a translucent red extract; for a weak stain, a volume of 150 ul is usually sufficient.

Method:

1. Label enough Kline slides or blood typing slides for the standards, samples, and sample controls.

2. Pipette one drop of extract into each of the A, B, and O labelled wells and allow to dry (overnight is convenient).

3. The next day, prepare 0.05 - 0.1 % suspensions of A2, B, and O test cells in the following manner:

   Label three 12 x 75 test tubes "A", "B", and "O".

   Fill each tube with saline to about 2 cm from the top (about 4.5 ml); add 1 drop of 30% BSA to each tube.

   To the A tube add 2 drops of 2-4% A1 test cells; to the B tube add 2 drops B cells and to the O tube add 2 drops O cells.

4. Add anti-A to the wells in the "A" columns, anti-B to the wells in the "B" columns, and anti-H to the wells in the "O" columns; use enough to cover the dried extract.

5. Allow to absorb in a humid chamber for 45 minutes at room temperature.

6. Using cold saline (4 °C), rinse the anti-sera off the slides.

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7. Rinse the slides in cold saline (4 °C) on a rotator for three hours, changing the saline every 30 minutes.

8. Gently blot dry with tissue.

9. Add a drop of A1 test cell suspension to each well in the "A" columns, a drop of B test cell suspension to each well in the "B" columns, and a drop of O test cell suspension to each well in the "O" columns. Incubate in a humid chamber at 55 °C for 25 minutes.

10. Rotate in a humid chamber for 25 minutes.

11. Read agglutination microscopically at 100X. Score the agglutination as below:

    - cells are free; no clumps are seen

    1 small clumps, a few cells each, are seen; the majority of the cells are free

    2 more and larger clumps are seen; not many free cells

    3 large clumps are seen; few free cells are seen

    4 very large, solid, clumps are seen; essentially no free cells

11. The A, B, and O bloodstain standards should give results similar to below:

	A	B	O
A bloodstain	3-4	-	-, 1-4
B bloodstain	-	3-4	-, 1-4
O bloodstain	-	-	3-4

If the agglutination is weaker than above, repeat the incubation and rotation steps.

12. Substrate controls should have no agglutination; if any agglutination is seen in the substrate control, the stain results MUST be called inconclusive.

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F. Absorption - Inhibition Titer Determination

ANTISERA MUST BE QUALITY CONTROL CHECKED. DO NOT USE REAGENTS THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Reagents:

polyclonal anti-A anti-sera (MUST be polyclonal)
polyclonal anti-B anti-sera (MUST be polyclonal)
anti-H anti-sera

A2, B, and O test cells

30% bovine serum albumin (BSA)

Method:

1. Prepare doubling dilutions of anti-A, anti-B, and anti-H sera in the following manner:

   label column wells on a microtiter plate 1/2 - 1/512

   label row wells A, B, and H

   pipet 100 ul saline into each well (a total of 27 wells)

   add 100 ul anti-A to the well labeled A and 1/2; this creates the 1/2 dilution of anti-A

   take 100 ul of the 1/2 and add it to the saline in the well labeled A and 1/4 and mix thoroughly. Continue doing this all the way to the well labeled A and 1/512.

   OR

   prepare dilutions of anti-sera as described in "Absorption -Inhibition, Section G.1.

   prepare the dilutions of anti-B and anti-H the same way

2. Prepare 0.05 - 0.1 % suspensions of A2, B, and O test cells in the following manner:

   Label three 12 x 75 test tubes "A", "B", and "O".

   Fill each tube with saline to about 2 cm from the top (about 4.5 ml); add 1 drop of 30% BSA to each tube.

   To the A tube add 2 drops of 2-4% A2 test cells; to the B tube add 2 drops B cells and to the O tube add 2 drops O cells.

3. Label three 100 x 100 mm square Petri dishes, one each for anti-A, anti-B, and anti-H. Each plate will have nine wells, one for each dilution of anti-sera. Either the lids or the

bottoms can be used; some dishes have convenient markings on the bottom.

1/2	1/4	1/8
1/16	1/32	1/64
1/128	1/256	1/512

4. Add 10 ul of saline to each well.

5. Add 10 ul of 1/2 dilution of anti-A to the 1/2 well, 10 ul of 1/4 dilution of anti-A to 1/4 well, etc.; do the same with the dilutions of anti-B and anti-H.

6. Place the lids on the Petri dishes and allow to absorb for at least 30 minutes.

7. Add 10 ul of A2 test cell suspension to each well of the "A" plate, 10 ul of B test cell suspension to each well of the "B" plate, and 10 ul of O test cell suspension to each well of the "O" plate.

8. Cover and rotate for 30 - 45 minutes.

10. Read agglutination microscopically at 100X. (It may be easier to read if the other objectives are removed). Score the agglutination as below:

- \- cells are free; no clumps are seen
- 1 small clumps, a few cells each, are seen; the majority of the cells are free
- 2 more and larger clumps are seen; not many free cells
- 3 large clumps are seen; few free cells are seen
- 4 very large, solid, clumps are seen; essentially no free cells

The weakest dilutions of anti-sera which give scores of 4 are the working dilutions to be used for absorption-inhibition.

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G. Absorption - Inhibition

ANTISERA MUST BE QUALITY CONTROL CHECKED AND TITERED. DO NOT USE REAGENTS THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Reagents:
polyclonal anti-A anti-sera (MUST be polyclonal)
polyclonal anti-B anti-sera (MUST be polyclonal)
anti-H anti-sera

A2, B, and O test cells

30% bovine serum albumin (BSA)

Standards:
A secretor saliva
B secretor saliva
O secretor saliva
non-secretor saliva

Sample prep: Extract an approximately 1 x 1 cm stain or half of a swab in 200 ul saline for 30 minutes at room temperature. Centrifuge at high speed to separate extract from sample.

Any cell pellet formed should be retained and stored frozen for possible DNA analysis.

Method:

1. Check the QC files for the current titer of anti-sera being used. Prepare the appropriate dilutions of anti-sera using the amounts below:

dilution	anti-sera	saline
1/8	200 ul	1.4 ml
1/16	100 ul	1.5 ml
1/32	100 ul	3.1 ml
1/64	100 ul	6.3 ml
1/128	50 ul	6.3 ml
1/256	25 ul	6.3 ml

Any other necessary dilutions would be prepared similarly.

2. Prepare 0.05 - 0.1 % suspensions of A2, B, and O test cells in the following manner:

Label three 12 x 75 test tubes "A", "B", and "O".

Fill each tube with saline to about 2 cm from the top (about 4.5 ml); add 1 drop of 30% BSA to each tube.

To the A tube add 2 drops of 2-4% A2 test cells; to the B tube add 2 drops B cells and to the O tube add 2 drops

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O cells.

3. Label enough 100 x 100 mm square Petri dishes for the standards, samples, and sample controls. Either the lid or the bottom can be used; some dishes have convenient markings on the bottom.

4. Transfer the extracts to a microtiter plate. Prepare 1/10 dilutions by diluting 20 ul of the extracts in 180 ul saline.

5. To the appropriate spaces across the labeled Petri dishes, add three 10 ul aliquots of the neat samples and three 10 ul aliquots of the 1/10 diluted samples in the rows labeled "N" and "1/10" (see diagram).

6. Add 10 ul of anti-A to each space in the "A" columns, 10 ul of anti-B to each space in the "B" columns, and 10 ul of anti-H to each space in the "O" columns (see diagram).

7. Cover and allow to absorb for at least 30 minutes at room temperature.

8. Add 10 ul of A2 test cell suspension to the "A" columns, 10 ul of B test cell suspension to the "B" columns, and 10 ul of O test cell suspension to the "O" columns.

9. Cover and rotate for 30 - 45 minutes.

10. Read agglutination microscopically at 100X. (It may be easier to read if the other objectives are removed). Score the agglutination as below:

    - cells are free; no clumps are seen

    1 small clumps, a few cells each, are seen; the majority of the cells are free

    2 more and larger clumps are seen; not many free cells

    3 large clumps are seen; few free cells are seen

    4 very large, solid, clumps are seen; essentially no free cells

11. The A, B, and O secretor standards and the non-secretor standard should give results similar to below:

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	A	B	O
A secretor, N	-	4	-
, 1/10	-	4	-, 1-4
B secretor, N	4	-	-
, 1/10	4	-	-, 1-4
O secretor, N	4	4	-
, 1/10	4	4	-, 1-4
non-secretor, N	4	4	3, 4
, 1/10	4	4	3, 4

If the agglutination in the standards is too weak, cover and rotate for an additional 15 minutes. Read as before.

12. Substrate controls should give results similar to the non-secretor standard. If any inhibition or weak agglutination (1, 2) is seen in the substrate control, the stain results MUST be called inconclusive.

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VI. SEMEN ANALYSIS - POST MORTEM SAMPLES

A. Semen Analysis Policy

Once a week (Wednesday), an assigned analyst analyzes the accumulated swabs, sexual offense evidence collection kits, and any other applicable autopsy items for the presence of blood and semen.

1. Remove the swabs and kits from the freezer and coldroom.

2. Find the case files associated with the swabs and kits; they may be in the file cabinet, already assigned to an analyst, or in the "waiting to be assigned" box.

3. Fill out the "Swab Processing" worksheet or the "Kit Inventory" worksheet, indicating what samples are present; note the number of swabs.

5. Perform Kastle-Meyer spot tests on any swabs that appear to be bloody (see "Kastle-Meyer Presumptive Test for Blood").

6. Cut an approximately 2.5 x 2.5 mm piece out of each set of swabs (e.g., only test one vaginal swab out of four). Place the samples in clearly labeled blue pipet tip/test tubes and give to the person assigned to perform the P30 ELISA.

7. When the raw data is returned, fill out a P30 ELISA worksheet for each case. Put the P30 ELISA worksheet, a P30 ELISA plate worksheet, and the raw P30 data in each casefile. For the P30 ELISA plate worksheet and raw data, note where the original can be found.

8. Discard any vaginal or rectal swabs that are negative for semen.

9. For swabs that are negative for semen, discard thier corresponding slides.

10. For swabs that are positive for semen, examine their corresponding slides for sperm (see "Christmas Tree Stain for Spermatozoa".) If there are two slides, stain only one.

11. Discard any vaginal, oral, or rectal slides that are negative for sperm.

12. Check the Blood Processing worksheet for the ABO and Lewis typing results of the deceased.

    If the ABO type was inconclusive AND the oral swab is negative for semen, discard the oral swab.

    If there is an ABO type, the Lewis type is a-b+ or a+b-, AND the oral swab is negative for semen, discard the oral swab.

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13. If there is an ABO type, the Lewis type of the deceased is a-b-, AND the oral swab is negative for semen, an absorption inhibition and an amylase test (see "Absorption Inhibition" and "Amylase Diffusion") must be done on the oral swab.

    If the oral swab is KM positive, follow the procedure in "Processing of KM Positive Oral Swabs."

    If the case has physical evidence associated with it, the A/I and amylase are the responsibility of the assigned analyst.

    If there is no physical evidence, the A/I and amylase are the responsibility of the analyst processing the swabs.

14. Repackage all positive swabs and/or slides in small envelopes and label with:

    case number
    date
    analyst's initials
    type of swab

    If the swabs and/or slides came from a kit, they stay in their original envelopes.

15. If an envelope of cuttings from a case already exists (ask assigned analyst), place the swabs and slides in that envelope. If not, prepare an envelope for them and place in the freezer along with other retained samples.

16. Once all the items in a kit have been processed, the kit box itself can be discarded.

17. If a case doesn't have an analyst assigned, give the file to the clerical for typing of a report.

NOTE: If P30 ELISA is not available, analyze the swabs using the acid phosphastase presumtive test, followed by the Christmas Tree stains and/or P30 crossover electrophoresis.

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VI. Post-Mortem Semen Analysis

A. Semen Analysis Policy

Once a week (Wednesday), an assigned analyst analyzes the accumulated swabs, sexual offense evidence collection kits, and any other applicable autopsy items for the presence of blood and semen.

Method:

1. Remove the swabs and kits from the freezer.

2. Find the case files associated with the swabs and kits; they may be in the file cabinet, already assigned to an analyst, or in the "waiting to be assigned" box. As the tests are performed, transfer results to the "Swab Processing" worksheet in the case file.

3. Examine any slides for sperm (see "Christmas Tree Stain for Spermatozoa", Section: IV.D.). If there are two slides, stain only one.

4. Perform seminal acid phosphatase (SAP) spot tests on all swabs (see "Acid Phosphatase Presumptive Test for Semen", Section: III.B.).

5. Perform Kastle-Meyer spot tests on any swabs that appear to be bloody (see "Kastle-Meyer Presumptive Test for Blood", Section: III.A.).

6. For all swabs that are SAP positive, make a slide for a sperm search (see "Christmas Tree Stain for Spermatozoa", Section: IV.D.).

7. For all swabs that are sperm negative, do a P30 crossover (see "P30 Crossover Electrophoresis").

   Prepare the crossover plate so that swabs from each case have their own individual row with the appropriate standards.

   After rinsing, drying, and staining, cut the GelBond and affix to the corresponding crossover worksheets; place in the appropriate file.

8. Discard any vaginal or rectal swabs that are negative for semen.

9. Discard any vaginal, oral, or rectal slides that are negative for sperm.

10. Check the Blood Processing worksheet for the ABO and Lewis typing results of the deceased.

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If the ABO type was inconclusive AND the oral swab is negative for semen, discard the oral swab.

If there is an ABO type, the Lewis type is a-b+ or a+b-, AND the oral swab is negative for semen, discard the oral swab.

11. If there is an ABO type, the Lewis type of the deceased is a-b-, AND the oral swab is negative for semen, an absorption inhibition and an amylase test (see "Absorption Inhibition" and "Amylase Diffusion") must be done on the oral swab.

    If the oral swab is KM positive, follow the procedure in "Processing of KM Positive Oral Swabs."

    If the case has physical evidence associated with it, the A/I and amylase are the responsibility of the assigned analyst.

    If there is no physical evidence, the A/I and amylase are the responsibility of the analyst processing the swabs.

12. Repackage all positive swabs in small envelopes and label with:

    case number
    date
    analyst's initials
    type of swab

    If the swabs came from a kit, they stay in their original envelopes.

13. Package all positive slides in small envelopes and label with:

    case number
    date
    analyst's initials
    type of slide

    If the slides came from a kit, they stay in their original envelopes and slide holders.

14. If an envelope of cuttings from a case already exists (ask assigned analyst), place the swabs and slides in that envelope; if not, place the swabs and/or slides in the freezer in the storage box designated for swabs.

15. Once all the items in a kit have been processed, the kit box itself can be discarded.

16. If a case doesn't have an analyst assigned, give the file to the clerical for typing of a report.

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B. Processing of KM Positive Oral Swabs

When determining the secretor status of a decedent from an oral swab (contingent on obtaining an ABO type), care must be taken to remove any possible interference from a KM-positive sample. To remove blood ABO antigens from KM-positive oral swabs, the following method is suggested.

Method:

1. Extract a swab as per the absorption-inhibition method (Section: V.G.).
2. Centrifuge sample test tubes (at high speed) for 5 minutes.
3. Decant supernatant into another test tube and discard debris.
4. Take sample test tubes, place in a water bath, and boil for 5 minutes.
5. Centrifuge the sample (at high speed) test tubes.
6. Proceed with the amylase and absorption-inhibition methods using the supernatant.

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VII. Electrophoresis

A. Guidelines

1. Agarose Electrophoresis

Buffers: **Gel, tank, and reaction buffers are prepared by lab personnel and quality control checked before use. Do not make your own or use reagents that have not been quality control checked.**

Reagents: **Miscellaneous reagents and reaction mixtures are quality control checked before use. Do not make your own or use reagents that have not been quality control checked.**

Equipment: The following equipment is needed:

balance
glass plates, various sizes
leveling stand
cooling bath and platens
power supplies capable of 500V
electrophoresis tanks
incubator
photography equipment
miscellaneous glassware

Tank prep: The buffer reservoirs must be filled with tank buffer; the amount depends on the type of tanks being used.

Discard tank buffer after each use.

Gel prep: The amounts of gel buffer and agarose depend on the size of the gel:

gel size	g agarose	ml gel buffer
10 x 20 cm	0.40	40
15 x 20 cm	0.60	60
20 x 20 cm	0.80	80

The type of agarose (amount of electro-endo-osmosis, EEO) is specified by the method.

The agarose is dissolved in the gel buffer by heating. Make sure the agarose is completely dissolved by checking the solution for visible agarose granules.

Pour gel solution onto a leveled glass plate, making sure the gel is evenly distributed. Allow

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gel to solidify at room temperature or on a cooling platen.

Sample prep: Stains on thread (clothes, or stains swabbed off items) can be used directly; alternatively, an extract can be made and applied to cotton threads.

Soak threads in a minimal amount (just enough to wet the sample) of 0.05 M DTT. Do not allow threads to dry out.

Scrapings need to be dissolved in a minimal amount of 0.05 M DTT and applied to cotton threads.

Lysates need to be treated with 0.05 M DTT and applied to cotton threads.

Sample slots: Using a metal comb, make slots in the gel at the origin location specified by the method. Place sample threads in slots.

Unless otherwise specified, it is generally preferable to use 1 cm wide sample slots.

Wicks: Moisten filter paper wicks, 12 cm wide and as long as the gel, with tank buffer. Use 2-6 wicks, depending on the thickness of the wicks.

Gel assembly: Place gel on platen at 4 °C. All genetic marker electrophoresis methods used in the laboratory run at this temperature.

Make sure there is a THIN layer of water between the plate and the platen; no air bubbles and not so much water that the plate is "skating" around. The layer of water ensures even cooling of the gel. Any bubbles will result in a "hot spot" on the gel which can affect migration and may cause unreadable results.

Place wicks on either side of the gel to give a wick distance of 12-17 cm. To make sure the wicks stay in contact with the gel, place a border stick on each wick and cover with a glass plate.

Plug in electrodes (if applicable) and close the tank lid. Connect the power supply, making sure the positive on the tank is connected to the positive on the power supply.

Parameters: Conduct electrophoresis at the voltage and for the time specified by the method.

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Agarose electrophoresis is run at constant voltage; the amperage and watts can be set at the maximum for the power supply being used.

Overlays:

If water has condensed on a gel, remove excess moisture with filter paper or Kimwipes.

Filter paper overlays should be wet, but not dripping, with the reaction mixture. Remove filter paper before viewing or photographing.

Cellulose acetate membrane (CAM) overlays should be wet, but not dripping, with the reaction mixture.

To pour an agarose overlay, make a "corral" of black border sticks around the area where the overlay goes. Use melted 1% agarose to plug the corners where the sticks meet. Pour on the overlay, making sure the overlay is evenly distributed.

An agarose overlay can be removed upon solidifying and a new one poured if necessary.

Incubation:

Incubate the gel with overlays at 37 or 55 °C. Use a humid chamber or a glass plate lid to keep the gel from drying out.

Results:

For a phenotype to be called, the banding pattern and band separation must fulfill the requirements specified by the method.

Results are recorded by noting the phenotype on the worksheet. If the sample can't be called, indicate with the following symbols why:

-,NR	no visible activity
inc	activity, but no clear bands
()	possible type, not clear enough to call

If the required standards don't work, the entire plate **must** be called inconclusive, UNLESS there are secondary standards that did work on the plate. For example, a secondary standard could be a victim blood sample that was previously typed.

Double-reading:

All electrophoresis results **must** be called **INDEPENDENTLY** by a second reader. The initials of the second reader **must** be on the bottom of the

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electrophoresis worksheet.

If there is a disagreement on a call, a Forensic Scientist supervisor should be consulted. If there is no agreement, the sample must be called inconclusive.

Photography: All electrophoresis results should be documented by photographs. The quality of a photo is determined by film speed, f-stop, and shutter speed.

The f-stop controls the size of the aperture (shutter opening); a small f-stop number means a large aperture letting in lots of light.

The shutter speed controls how long the aperture is open. The "faster" the shutter speed, the less is the amount of light reaching the film. On the camera, the speeds are in sec^{-1}; therefore, 1=1 sec, 2=1/2 sec, all the way to 60=1/60 sec. The B setting holds the aperture open until the lever is released.

The lab generally uses two types of film - fast (3000 ASA, Polaroid type 107) and slow (80 ASA, Polaroid types 665 and 667). A slow film requires more light; therefore, a larger aperture and/or a longer exposure time.

Suggested settings are:

1. Type 667

ASA 3000	UV photo	non-UV photo
f-stop	11	22 or 32
shutter	1/8	1/60

2. Type 665

ASA 80	UV photo	non-UV photo
f-stop	5.6	8
shutter	B (10 sec)	1/30

A filter must be used with U.V. light. The exposure settings given are for the orange filter.

If a photo is too dark (under exposed), increase the amount of light reaching the film by either increasing the aperture (moving the f-stop to a smaller number) or decreasing the shutter speed

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(moving it to a smaller number). The reverse is true for a photo that is too light (over exposed).

For UV photos, it may be helpful to turn the plate over and photograph the underside of the gel.

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2. Isoelectric Focusing (IEF)

Buffers: **Reaction buffers and IEF gels are prepared by lab personnel and quality control checked before use. Do not make your own or use reagents that have not been quality control checked.**

Reagents: **Miscellaneous reagents and reaction mixtures are quality control checked before use. Do not make your own or use reagents that have not been quality control checked.**

Equipment:
balance
glass plates, various sizes
cooling baths and platens
power supplies capable of 2000 V
isoelectric focusing tanks
isoelectric focusing electrodes
incubator
photography equipment
miscellaneous glassware

Plate prep: Described in detail in "Preparation of ultrathin polyacrylamide gels".

Gel prep: Described in detail in "Preparation of ultrathin polyacrylamide gels".

The amount of gel solution prepared depends on the size and numbers of the gels being prepared:

Gel Size	ml Solution
10 x 20 cm	3.5
15 x 20 cm	6-7
12 x 20 cm	4-5
12 x 25 cm	6-7

The acrylamide and other reagents are dissolved in distilled water. Make sure the acrylamide and sucrose is completely dissolved by checking the solution for visible granules.

Sample prep: Use the pipet tip and test tube method for the strongest extracts.

For stains, extract an approximately 3x3 mm stain in 50 ul of the appropriate extractant for 30 minutes at room temperature.

If a stain is small or light, use less; if it is heavy, it may need to be diluted. Experience will tell you if sample size or volume of extractant

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need to be adjusted.

For lysates, prepare a solution of 5 ul of red blood cells with 100 ul of the appropriate extractant.

If a sample needs to be analyzed in both PGM and ACP or ESD, there are two approaches:

extract in DTT and use that extract for all analyses; there will be a blue discoloration at the PGM origin

OR

extract in water and run the PGM first. Take the remaining ~40 ul extract and add 10 ul of a 0.25 M DTT (5X) solution

Plate loading: Use a silicone applicator strip to apply samples at the origin specified by the method. Comparison of the strips with applicator tabs showed a clear improvement in results with the applicator strip.

It is generally preferable to use the 1x7 mm sample slots.

Generally, 10 ul of extract is used. If a sample is very light, up to 15 ml can be used in the 1x7 mm slots. For very weak samples, use up to 15 ul in the 2x3.5 mm slots.

If a sample has been run before and was too intense, use less extract or dilute it the next time.

If a sample has been run before and gave distorted results, it may be due to salts in the sample (especially in semen samples or samples contaminated with sweat or dirt). Try running a volume reduction series of the sample (10, 5, and 2.5 ul) or a dilution series (neat, 1/2, 1/4, etc).

It may be helpful to place standards right next to samples whose type was ambiguous on a prior run.

It may be helpful to leave blank spaces around samples that have caused distortion during a prior run.

Wicks: Moisten filter paper wicks, 6 mm wide and as long as the gel (not the plate), with the appropriate electrolyte. The wicks should be uniformly wet,

but not dripping or too dry. The wicks **must** be parallel.

Place wicks on gel to give the wick distance specified by the method.

Gel assembly: Place gel on platen at 4 °C. All genetic marker electrophoresis methods used in the laboratory run at this temperature.

Make sure there is a THIN layer of water between the plate and the platen; no air bubbles and not so much water that the plate is "skating" around. The layer of water ensures even cooling of the gel. Any bubbles will result in a "hot spot" on the gel which may affect migration and may cause unreadable samples.

Adjust electrodes to give the electrode gap specified by the method. Make sure the electrodes are parallel by measuring at each end. Lower electrodes onto wicks, making sure there is contact all the way along the wicks.

Plug in electrodes and close the tank lid. Connect the power supply, making sure the positive on the tank is connected to the positive on the power supply.

Parameters: Conduct electrophoresis at the voltage and for the time or volt-hours specified by the method.

Isoelectric focusing is run with a power (10 watts) maximum to limit heat build-up; the voltage and amperage are set at 2000V and 15mA.

Two gels can be run simultaneously from one power supply. Change the Watt setting to 20W.

Overlays: Cellulose acetate membrane (CAM) overlays should be wet, but not dripping, with the reaction mixture. Leave CAM on gel when viewing or photographing; trying to remove the overlay will tear the gel.

To pour an agarose overlay, make a "corral" of black border sticks around the area where the overlay goes. Use melted 1% agarose to plug the corners where the sticks meet. Pour on the overlay, making sure the overlay is evenly distributed.

An agarose overlay can be carefully removed upon

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solidifying and a new one poured if necessary.

Incubation: Incubate the gel with overlays at 37 or 55 °C. Use a humid chamber or a glass plate lid to keep the gel from drying out.

Results: For a phenotype to be called, the banding pattern and band separation must fulfill the requirements specified by the method.

Results are recorded by noting the phenotype on the worksheet. If the sample can't be called, indicate with the following symbols why:

-, NR	no visible activity
inc	activity, but no clear bands
()	possible type, not clear enough to call

If the required standards don't work, the entire plate **must** be called inconclusive, UNLESS there are secondary standards that did work on the plate. For example, a secondary standard could be a victim blood sample that was previously typed.

Double-reading:

All electrophoresis results **must** be called by a second reader. The initials of the second reader **must** be on the bottom of the electrophoresis worksheet.

If there is a disagreement on a call, a Forensic Scientist supervisor should be consulted. If there is no agreement, the sample must be called inconclusive.

Photography: See Agarose Electrophoresis, Section VII.A.1.

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a. Preparation of Ultrathin Polyacrylamide Gels

Method:

1. Prepare the ultra-thin mold by sticking strips of smooth PVC adhesive tape, approximately 1 cm wide, 0.15 mm thick, to the edges of clean 15 x 20 cm or 12 x 25 cm glass plates. Avoid over-stretching the tape and avoid gaps and overlaps at the corners since this can hinder polymerization. Use different colored tape for each system to allow clear and rapid identification of the plates:

PGM	blue
ACP	green
ESD	red
Hb	red/yellow

2. Prepare enough plates and plate "lids" (plain glass) by cleaning their surfaces thouroughly with alcohol; this removes any oils left on the plate and helps prevent bubbles and tearing. Place the mold up on a petri dish or other support. **DO NOT TOUCH THE SURFACE OF THE PLATES WITH YOUR FINGERS ONCE THEY ARE CLEAN**; handle by the edges.

3. Prepare the acrylamide solution using the amount of reagents shown in Table 1. Ensure that the solids are completely dissolved (let sit for about 10 minutes) and avoid too vigorous of mixing.

4. Add the appropriate amount of the correct ampholyte as shown in Table 2. Note that each system has a different ampholyte.

5. To initiate polymerization, two different methods are available. These are chemical polymerization using ammonium persulfate and photopolymerization using riboflavin (**RIBOFLAVIN GIVES BETTER RESULTS**). Use one or the other:

   add the appropriate volume of riboflavin solution (1.0 mg per 10 ml water, stored in the refrigerator) to the gel solution; mix well

   OR

   add the appropriate volume of freshly prepared 0.2M ammonium persulfate solution (0.228 g per 10 ml water); mix well

6. Pour 6-7 ml of the solution onto the upper edge of a glass mold. Carefully and slowly lower the plain glass "lid" down, allowing the solution to spread over the mold. Take care not to trap any air bubbles; if this happens, raise the top plate and lower again. **DO NOT PRESS THE PLATES TOGETHER OR USE A WEIGHT** - once the force is removed, the solution will pull

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away from the edges and ruin the plate. Any excess solution will flow out and can be removed with tissues.

WITH PRACTICE, 6 ml WILL COVER ONE LARGE PLATE. AT FIRST, USE 7-8 ml.

7. Allow the plates to polymerize:

   For riboflavin plates, expose them to UV light overnight. They may be stacked, but no more than three in one pile and each plate must be separated by strips placed along the edges to prevent them from sticking to each other.

   For ammonium persulfate plates, leave the plates at room temperature for 12 hours.

8. The plates are then placed in the refrigerator, even if they are to be used that day; cooling aids in separating the plates. The plates can be stored in the refrigerator for up to 2 months, but do not use them if they are dried out, discolored, or have obvious damage.

9. To use the plates, separate the plates immediately before sample application by placing the whole plate on a firm horizontal surface and inserting a spatula between the plates. Twist gently and slowly and carefully lift the top plate; once you start, don't stop.

10. If the gel has torn or ragged edges, trim them so they are straight. If there is a bubble or gap in the gel, do not apply a sample in that position.

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B. Red Cell Isoenzyme Determination

1. Esterase D and Phosphoglucomutase

ALL BUFFERS AND PGM REACTION MIXTURE ARE PRE-MADE AND QUALITY CONTROL CHECKED. DO NOT MAKE YOUR OWN OR USE REAGENTS THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Tank buffer:

12.11 g Tris base
11.62 g maleic acid
2.92 g EDTA free acid
2.03 g $MgCl_2*6H_2O$
5.00 g NaOH

Dissolve in 1 l distilled water; adjust to pH 7.4 with NaOH.

Gel buffer: 1:14 dilution of tank buffer

Dilute 60 ml tank buffer with 840 ml distilled water; adjust to pH 7.4 if necessary.

ESD reaction buffer:

4.1 g sodium acetate, anhydrous

Dissolve in 1 l distilled water; adjust to pH 6.5 with 1% acetic acid.

PGM reaction buffer:

12.0 g Tris base
4.0 g $MgCl_2*6H_2O$

Dissolve in 1 l distilled water and adjust to pH 8.0 with HCl.

Gel prep:

0.8 g Sigma type I agarose
80 ml gel buffer

Dissolve agarose in gel buffer by heating until clear. Pour gel onto a leveled 20x20 cm plate making sure that the gel is evenly distributed; allow gel to solidify.

If a different plate size is used, adjust reagents accordingly.

Standards: ESD 1 and 2-1, PGM 1 and 2-1

Sample prep: Stains are soaked in a minimal amount (just enough to wet the sample) of 0.05 M DTT (Cleland's

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reagent) in a spot plate.

Stains can also be extracted in a minimal amount (just enough to wet the sample) of 0.05 M DTT using the pipet tip/test tube method; apply the extract to cotton threads.

For most stains, 2-4 threads should be sufficient. Up to 6 threads can be used for weak stains.

Origin: 3 cm from cathode

Parameters: Place gel on platen at 4 °C.

Conduct electrophoresis at 400 V for 3 hours.

Development for ESD isoenzymes:

Reagents: 6 mg methumbelliferyl acetate
15 ml reaction buffer

Method: Dissolve the MUA in a small amount of acetone and mix in the reaction buffer. Soak onto a strip of 20x8 cm filter paper, allowing excess reaction mixture to drip off, and apply the paper to the gel from the origin forward.

Incubate in a humid chamber for 15 minutes at 37 °C and read under UV light (it may be helpful to remove the filter paper).

Photograph the plate as bands develop.

Mark the position of the ESD 2 band.

After ESD development, rinse plate in water for about 30 seconds to remove any remaining ESD reaction mixture.

Development for PGM Isozymes:

Reagents: 47 mg glucose-1-phosphate with 1% glu-1,6-diphosphate
3 mg NADP sodium salt
4 mg MTT
15 ml reaction buffer
75 ul glucose-6-phosphate dehydrogenase
200 ul meldola blue (1 mg per ml)
15 ml overlay agarose, 1% Sigma type I in distilled water

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Method: Dissolve dry ingredients in reaction buffer; add meldola blue and enzyme. Mix in agarose and pour onto plate between the origin and the ESD 2 band.

Incubate in a humid chamber at 37 or 55 °C until bands are clear.

Photograph the plate as bands develop.

Interpretation of Results:

Compare the banding pattern to the ESD and PGM schematics.

The allowable separation between bands is:

ESD 1-2	> 4 mm
ESD 2-3	> 4 mm
PGM a-b	> 3 mm
PGM a-c	> 7 mm
PGM b-d	> 7 mm
PGM a-d	> 14 mm

The separations are based on experimental data. If the actual band separation is less than that allowed, all of the results must be called inconclusive.

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2. Acid Phosphatase by Isoelectric Focusing

ALL BUFFERS, GELS, AND REAGENTS ARE PRE-MADE AND QUALITY CONTROL CHECKED. DO NOT MAKE YOUR OWN OR USE REAGENTS THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Gel preparation: Prepare the gels as described, using pH 4-6 and 6-8 ampholytes from LKB; SERVA ampholytes are not acceptable.

ACP typing gels can be identified by the green tape.

Standards: B, BA and A

If standards are placed in lanes 2, 5, 8, 11, etc., a sample will always be next to a standard. THIS IS NOT MANDATORY, but can assist with typing problem samples.

Sample preparation: For lysates, mix 5 ul of washed packed red blood cells with 100 ul DTT; store frozen if the sample may be typed again.

For stains, extract an approximately 3x3 mm stain in 50 ul DTT for 30 minutes at room temperature. Experience will tell you if the samples need to be further diluted.

For the strongest extracts, use the pipet tip/test tube method; let the tubes lie horizontally during the 30 minutes so the water stays in contact with the sample. Centrifuge at high speed to separate extract from sample.

Plate loading: Use an application mask (slots 1x7 mm) to apply the samples 1 cm from the anode wick. The application strip stays on the gel during the entire run.

Use 10 ul of extract; if a sample is very light, up to 15 ul can be used in the 1x7 mm slots. For very weak samples, use up to 15 ul in the 2x3.5 mm applicator strip.

Electrolytes: Prepare electrode wicks as long as your gel; they should not be touching the plastic tape. Saturate the anode wick with acid (1% acetic acid) and the cathode wick with base (1% ethanolamine). The wicks should be uniformly wet, but not dripping.

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Apply the wicks to give an electrode gap of 10.5 cm.

Use the LKB template for the proper placement of the electrode wicks.

Run conditions: Place gel on platen at 4 °C. Apply the electrodes and cover. Conduct electrophoresis at 2000V for a total of 3000 volt-hours; the Hb should be focused.

Power Supply Settings

Volts	**Amps**	**Watts**
2000V	15mA	10W

This method is **POWER LIMITED**; the wattage will never be allowed to be greater than 10 W, even if the voltage if forced to be lower. This means that the voltage may not be 2000V at the beginning.

Reaction buffer:

1.92 g citric acid
0.8 g NaOH

Dissolve the citric acid in 200 ml distilled water and adjust to pH 5.0 with the NaOH.

Reaction mixture: Have the reaction mixture already dissolved and the cellulose acetate membrane (CAM) cut before your plate is finished.

ACP reaction mixture:

3 mg methumbilliferyl phosphate

Dissolve in 3 ml reaction buffer.

1. Absorb reaction mixture onto a single piece of 5 cm wide CAM as long as your gel.

2. Apply the CAM to the gel surface beginning 4 cm from the origin and towards the anode.

3. Place a glass plate over the gel or place in a humid chamber.

4. Do not have the CAM extending from under the glass plate lid. This can cause distortion of the samples on the edges.

5. Incubate for 10 minutes at 37 or 55 °C and view the gel (with CAM) under UV light; leave up to 30 minutes if bands are weak.

Interpretation of Results:

Compare the banding pattern to the ACP schematic, using the "class" as the type.

In order for a sample to be called, at least one secondary band (a_3, a_4, b_3, b_4, c_3, c_4) must be visible.

The allowable separation between bands is:

B1/B2 bands	> 8 mm
B/A bands	> 10 mm
A/Hb bands	> 1 mm

The separations are based on experimental data. If the actual band separation is less than that allowed, all of the results **must** be called inconclusive.

initials: RC Date: 3/31/92

3. Esterase D by Isoelectric Focusing

ALL BUFFERS, GELS, AND REAGENTS ARE PRE-MADE AND QUALITY CONTROL CHECKED. DO NOT MAKE YOUR OWN OR USE REAGENTS THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Gel preparation: Prepare the gels as described, using pH 4-6.5 ampholytes.

ESD typing gels can be identified by the red tape.

Standards: 2-1 and 5-1

If standards are placed in lanes 2, 5, 8, 11, etc., a sample will always be next to a standard. THIS IS NOT MANDATORY, but can assist with typing problem samples.

Sample preparation:

For lysates, mix 5 ul of washed packed red blood cells with 100 ul DTT.

For stains, extract an approximately 3x3 mm stain in 50 ul DTT for 30 minutes at room temperature. Experience will tell you if the extract needs to be further diluted.

For the strongest extracts, use the pipet tip/test tube method; let the tubes lie horizontally during the 30 minutes so the DTT stays in contact with the sample. Centrifuge at high speed to separate extract from sample.

Plate loading: Use an application strip (slots 1x7 mm) to apply the samples 4 cm from the cathode wick. The application mask stays on the gel during the entire run.

Use 10 ul of extract; if a sample is very light, up to 15 ul can be used in the 1x7 mm slots. For very weak samples, use up to 15 ul in the 2x3.5 mm applicator strip.

Electrolytes: Prepare electrode wicks as long as your gel; they should not be touching the plastic tape. Saturate the anode wick with acid (1% acetic acid) and the cathode wick with base (1% ethanolamine). The wicks should be uniformly wet, but not dripping.

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Electrode wicks should have a gap of 18 cm.

Run conditions: Place gel on platen at 4 °C. Apply the electrodes and cover. Conduct electrophoresis at 2000V for a total of 4000 volt-hours.

Power Supply Settings

Volts	**Amperage**	**Watts**
2000V	15mA	10W

This method is **POWER LIMITED**; the wattage will never be allowed to be greater than 10 W, even if the voltage if forced to be lower. This means that the voltage may not be 2000V at the beginning.

Reaction buffer: 4.1 g anhydrous sodium acetate

Dissolve the sodium acetate in 1 l distilled water; adjust to pH 6.5 with 1% acetic acid.

Reaction mixture: Have the reagents measured and the cellulose acetate membrane (CAM) cut before your plate is done.

ESD reaction mixture:

3 mg methumbilliferyl acetate (MUA)

Dissolve the MUA in MINIMAL (a few drops) acetone; add 3 ml reaction buffer.

1. Absorb reaction mixture onto a single piece of 5 cm wide CAM as long as your gel.

2. Apply the CAM to the gel surface beginning 4 cm from the origin and towards the anode.

3. Place a glass plate over the gel or place in a humid chamber.

4. Do not have the CAM extending from under the glass plate lid. This can cause distortion of the samples on the edges.

5. Incubate for 10 minutes at 37 or 55 °C and view the gel (with CAM) under UV light; leave up to 30 minutes if bands are weak.

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Photograph the plate as bands develop.

Interpretation of Results:

Compare the banding pattern to the ESD IEF schematic.

The allowable separation between bands is:

type 1	> 3	mm
type 2-1	> 1	mm
	> 1	mm
type 5-1	> 3	mm

The separations are based on experimental data. If the actual band separation is less than that allowed, all of the results **must** be called inconclusive.

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4. Phosphoglucomutase by Isoelectric Focusing

BUFFERS, GELS, AND REAGENTS ARE PRE-MADE AND QUALITY CONTROL CHECKED. DO NOT PREPARE YOUR OWN OR USE REAGENTS THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Gel preparation: Prepare the gels as described, using pH 5-7 ampholytes and N-2-hydroxyethylpiperazine-N-2-propanesulfonic acid (abbreviated EPPS or HEPPS) as a separator.

PGM subtyping gels can be identified by the blue tape.

Standards: 2+2-1+1- in any combination (i.e., 2-1+ and 2+1-) or use a 4-band standard (available from SERI or prepared in the laboratory).

If standards are placed in lanes 2, 5, 8, 11, etc., a sample will always be next to a standard. THIS IS NOT MANDATORY, but can assist with typing problem samples.

For samples whose PGM type is known, an appropriate PGM sub standard can be placed next to it: e.g., a PGM sub 1+1- next to samples that are PGM 1.

Sample preparation: For lysates, mix 5 ul of washed packed red blood cells with 100 ul distilled water.

For stains, extract an approximately 3x3 mm stain in 50 ul distilled water for 30 minutes at room temperature.

For the strongest extracts, use the pipet tip/test tube method; let the tubes lie horizontally during the 30 minutes so the water stays in contact with the sample. Centrifuge at high speed to separate extract from sample.

A reducing agent (DTT) is not necessary for PGM typing and will cause a blue discoloration near the origin. If DTT is used (for example, if the same extract is to be used for ESD), trim away the blue discoloration on the agarose overlay. An alternative method is to use water to extract the sample and apply part of the extract to the PGM plate. Treat the remainder of the sample (approximately 40 ul) with 10 ul 0.25 M DTT, bringing the concentration of DTT in the sample to 0.05 M.

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Plate loading:

Use a silicone application strip (slots 1x7 mm) to apply the samples 2 cm from the anode edge. The application strip stays on the gel during the entire run.

Use 10 ul of extract for stains and living persons; use 5 ul of extract for post-mortem samples. If a sample is very light, up to 15 ul can be used in the 1x7 mm slots. For very weak samples, use up to 15 ul in the 2x3.5 mm applicator strip.

Electrolytes:

Prepare electrode wicks as long as your gel; they should not be touching the plastic tape. Saturate the anode wick with acid (1% acetic acid) and the cathode wick with base (1% ethanolamine). The wicks should be uniformly wet, but not dripping.

Apply the wicks to give an electrode gap of 10.5 cm. Use the LKB template for the proper placement of the electrode wicks.

Run conditions:

Place gel on platen at 4 °C. Apply the electrodes and cover. Conduct electrophoresis at 2000V for approximately 2 hours, for a total of 4000 volt-hours.

The settings on the power supply are:

2000V 15mA 10W

This method is POWER LIMITED; the wattage will never be allowed to be greater than 10 W, even if the voltage if forced to be lower. This means that the voltage may not be 2000V at the beginning.

Reaction buffer:

12.0 g Tris base
4.0 g $MgCl_2*6H_2O$

Dissolve in 1 l distilled water and adjust to pH 8.0 with HCl.

Reaction mixture:

Have the reaction mixture already dissolved and the agarose melted before your plate is done.

PGM reaction mixture:

47 mg glucose-1-phosphate with
1% glu-1,6-diphosphate

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3 mg NADP sodium salt
4 mg MTT
15 ml reaction buffer
75 ul glucose-6-phosphate dehydrogenase
200 ul meldola blue (1 mg per ml)
15 ml overlay agarose, 1% Sigma type I in distilled water, melted

Dissolve dry ingredients (available pre-measured in the freezer) in reaction buffer; add meldola blue and enzyme. Mix in agarose and pour onto plate between the origin and the hemoglobin. Incubate at 37 or 55 °C or until bands are clear.

Post-mortem samples can have enhanced PGM activity and may develop quickly; check the plate often.

Photograph the plate as bands develop.

Interpretation of Results:

Compare the banding pattern to a known PGM subtype.

The allowable separation between bands is:

type 2+2-	> 4	mm
type 2-1+	> 6	mm
type 1+1-	> 2	mm

The separations are based on experimental data. If the actual band separation is less than that allowed, all of the results must be called inconclusive.

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C. Protein Polymorphism Determination

1. Group Specific Component (GC) - Conventional

ALL BUFFERS AND ANTISERA ARE PRE-MADE AND QUALITY CONTROL CHECKED. DO NOT MAKE YOUR OWN OR USE REAGENTS THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Tank buffer: 41.2 g anhydrous sodium phosphate, dibasic
19.2 g anhydrous citric acid

Dissolve in 1 l distilled water; adjust to pH 5.5

Gel buffer: 0.81 g anhydrous sodium phosphate, dibasic
0.48 g anhydrous citric acid

Dissolve in 1 l distilled water; adjust to pH 5.5

Plate prep: 0.8 g Sigma Type I agarose
80 ml gel buffer

Dissolve agarose in gel buffer by heating until clear. Pour gel onto a leveled 20x20 cm plate, making sure that the gel is evenly distributed; allow gel to solidify.

If a different plate size is used, adjust reagents accordingly.

Standards: Gc 2-1

Sample prep: Stains are soaked in a minimal amount (just enough to wet the sample) of distilled water.

For most stains, 5-6 1 cm long threads should be sufficient. Up to 8 threads can be used for weak stains.

Origin: Midpoint of gel

Parameters: Place gel on cooling platen at 4 °C.

Conduct electrophoresis at 400 V for 2.5 hours.

Development of GC:

5 x 20 cm cellulose acetate membrane (CAM)

anti-Gc, diluted with distilled water to the concentration specified by the QC file. For a 20 cm long CAM, a total volume of 600-750 ul is sufficient.

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Soak the CAM in diluted anti-sera. Apply to the anodic end of the gel from the edge of the wick towards the origin.

Incubate the plate in a moisture chamber at room temperature for 2 hours.

Processing: Following incubation, remove the overlays and submerge the gel in saline and wash overnight in the refrigerator (2-5 °C).

Rinse by submerging the gel in distilled water for 30 minutes.

Remove the gel from the glass plate and place it on the hydrophilic side of a piece of GelBond. Put a sheet of filter paper on top of the gel, then several layers of paper towels; press the gel for 30-60 minutes.

Dry the gel in the oven at 55-60 °C for approximately 60 minutes. Do not overdry; the gel may separate from the Gel Bond.

Once the gel is dry (the gel should be clear, flat, and tightly adhered to the GelBond), stain in Coomassie Blue for approximately 10 minutes then destain (see "Coomassie Blue Staining").

Results: Compare the banding pattern to a known Gc pattern.

The allowable separation between bands is:

all bands	> 2 mm

The separations are based on experimental data. If the actual band separation is less than that allowed, all of the results must be called inconclusive.

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2. Hemoglobin by Isoelectric Focusing

GELS ARE PREMADE AND QUALITY CONTROL CHECKED. DO NOT MAKE YOUR OWN OR USE REAGENTS THAT HAVE NOT BEEN QUALITY CONTROL CHECKED.

Gel preparation: Prepare the gels as described, using pH 6-8, 7-9, and 3-10 ampholytes (use ammonium persulfate and TEMED).

Hb typing gels can be identified by the yellow/red tape.

Standards: AFSC in any combination

If standards are placed in lanes 2, 5, 8, 11, etc., a sample will always be next to a standard. THIS IS NOT MANDATORY, but can assist with typing problem samples.

Sample preparation: For whole blood, lysates, or AFSC standard, mix 10 ul with 100 ul 0.05% KCN.

For stains, extract an approximately 1x3 mm stain in 50 ul 0.05% KCN for 30 minutes at room temperature.

All samples should be slightly lighter than the diluted AFSC standard; dilute if necessary.

For the strongest extracts, use the pipet tip/test tube method; let the tubes lie horizontally during the 30 minutes so the water stays in contact with the sample. Centrifuge at high speed to separate extract from sample.

Plate loading: Use an application strip (slots 1x7 mm) to apply the samples 1 cm from the anode wick. The application strip stays on the gel during the entire run.

Use 10 ul of extract; if a sample is very light, up to 15 ul can be used in the 1x7 mm slots. For very weak samples, use up to 15 ul in the 2x3.5 mm applicator strip.

The application mask with 2x3.5 mm slots also works well; use 5 ul of extract.

Electrolytes: Prepare electrode wicks as long as your gel; they should not be touching the plastic tape. Saturate the anode wick with acid (1% acetic

acid) and the cathode wick with base (1% ethanolamine). The wicks should be uniformly wet, but not dripping.

Apply the wicks to give an electrode gap of 10.5 cm.

Run conditions:

Place gel on platen at 4 °C. Apply the electrodes and cover. Conduct electrophoresis at 2000V for approximately 3000 volt-hours, or until the bands are focused and the separation meets the allowed separation.

Power Supply Settings

Voltage	**Amperage**	**Watts**
2000V	15mA	10W

This method is **POWER LIMITED**; the wattage will never be allowed to be greater than 10 W, even if the voltage if forced to be lower. This means that the voltage may not be 2000V at the beginning.

Photograph the bands.

Interpretation of Results:

Compare the banding pattern to a known Hb pattern.

The allowable separation between bands is:

A - F	> 2 mm
F - S	> 3 mm
S - C	> 6 mm

The separations are based on experimental data. If the actual band separation is less than that allowed, all of the results **must** be called inconclusive.

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D. Related Techniques

1. Coomassie Blue Staining of Gels

Destain solution:
500 ml water
500 ml methanol
100 ml glacial acetic acid

Stain solution:
0.2 g coomassie blue

Dissolve in 200 ml destain solution; filter if necessary.

Have three jars labeled STAIN, DESTAIN 1, and DESTAIN 2. Put the stain solution into the STAIN jar, and about 200 ml of destain into the other two jars.

Two or three gels can be stained and destained simultaneously; more than that can make destaining difficult or result in scratches to the gel surface.

Method:

1. Place pressed, dried GelBond plate into the STAIN jar; rotate for 3-5 minutes.
2. Place into the DESTAIN 1 jar and rotate for 5 minutes, remove and
3. Place into the DESTAIN 2 jar and rotate for 5 minutes.
4. Remove and dry at room temperature or in the oven.

The stain is good for at least 2-3 months. As DESTAIN 1 gets too dark, discard **(ACCORDING TO OCME SAFETY STANDARDS)** and replace with DESTAIN 2. Put new destain into DESTAIN 2.

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VIII. Report Writing

A report is the last step in a case. It brings together all of the analytical results and conclusions found in the case notes, in an easily readable style. Overly technical terminology or misleading statements must be avoided.

Each report will have certain identifying information at the top:

name of deceased, FB number, and ME number
doctor that did autopsy, date, and borough
precinct submitting evidence and their complaint number

With this information, both the ME and the detective who get the reports will know where to file them.

The bottom of a handwritten report should have your name or initials and the date you wrote the report.

The body of a report will have three or four sections, depending on the complexity of the case.

For examples, see completed case files for 1990 and later.

SUMMARY:

THE SUMMARY SHOULD ANSWER QUESTIONS!

The summary should be a brief synopsis of the analytical results; it should **answer the questions** that were posed by the submission of the physical evidence. Is there blood? Could it be the victim's? Are there blood types foreign to the victim? Is there semen? Could the suspect be the semen donor?

Before you write your summary, ask yourself **"WHAT DOES READER OF THE REPORT NEED TO KNOW?** Then write a short, clear summary answering those questions! The summary should give all of the answers in a simple manner; save all technical explantions for the examinations section.

For the majority of cases, the following type of summary is sufficient:

Human blood was found on the knife, but the amount was insufficient for further analysis.

Human blood was found on the shirt (S) "from suspect". Typing results show that it could have come from the victim and could not have come from the suspect (see examinations).

Amylase, a component of saliva, was found on all three cigarette butts found in the "living room." Analysis showed types foreign to the victim (see examinations).

No blood was found on the pants or shoes.

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For cases where there are similar items, but they can be differentiated by color or other descriptions:

Human blood was found on the blue shirt. No blood was found on the green shirt.

Human blood was found on the samples from the "doorway" and "hall."

It is always clearest to avoid the use of item or voucher numbers in the summary. If you refer to items only by their numbers, that forces the reader to look elsewhere to find out what you are referring to.

If this is not possible, the items need to be identified by item and/or voucher numbers:

Human blood was found on the shirt (item 1). No blood was found on the shirt (item 2).

Human blood was found on the shirt (item 1, voucher E111111). No blood was found on the other shirt (item 1, voucher E111112).

If there are samples taken off of objects or locations (swabs, threads, or blood flakes), it is helpful to put that information into the report. Since you don't have personal knowledge of where the sample came from, use quotes to describe the item:

Human blood was found on the sample taken from the "bedroom door."

Human blood was found on the shirt taken from "the defendant."

If when examining evidence, you collect trace evidence (hairs, fibers, etc.), they should be mentioned in the summary:

Hairs and/or fibers were collected from the shirt.

Glass fragments were found on the sneakers.

All items submitted must be mentioned in the report. If nothing of evidentiary interest was found on an item:

No blood was found on the shirt or pants.

No semen was found on the vaginal, oral, or rectal swabs from (V).

If items were not examined:

The clothes from (V), (V) head hair, and (V) fingernails were not examined.

The hairs taken from "deceased's hand" were not examined due to lack of serological value.

After you write a summary, review it carefully. Does it answer all of the

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questions? Is it clear? Are all submitted items accounted for?

EXAMINATIONS:

This section is used when there are analytical results, such as enzyme or antigen typing, that need a more detailed presentation than is used in the summary. In most cases, this will take the form of a table comparing typing results with the blood of the victim and/or suspect.

A standard explanatory statement regarding genetic markers has been prepared and should be used for all reports where these results are to be reported.

However, the standard statement can be modified to reflect the analyses performed in a specific case.

The standard statement follows:

Blood and other physiological fluids and tissues contain polymorphic ("many forms") genetic markers which can differ from person to person. These genetic markers are inherited, that is, pass from generation to generation and can be used to compare biological samples from different sources.

Genetic markers occur because of changes (mutations) that occur in a person's hereditary material, DNA (Deoxyribonucleic Acid). These genetic markers can be identified through the analysis of enzymes, such as esterase D (ESD), phosphoglucomutase (PGM), and erythrocyte acid phosphatase (ACP) and red blood cell antigens such as ABO (blood group) and Lewis, or by direct analysis of the DNA itself, i.e., HLA-DQα.

Alternative forms of DNA are called alleles; they are found at the same location of the DNA (locus) on homologous (matching) chromosomes. An individual can have a maximum of two different alleles at a particular locus, one on each homologous chromosome.

With HLA-DQα there are 8 alleles which can be identified. Six of the alleles are distinguishable and give 21 types. The HLA-DQα alleles are typed using a technique known as the polymerase chain reaction (PCR).

After the standard statement, an introduction to the table of results can be inserted such as:

Typing was done with the following results:

Genetic marker analysis was done with the following results:

Stains from the shirt were compared to blood from the victim with the following results:

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Then comes the table itself.

1. **For a simple case:**

ITEM	ABO	ESD	PGM
(S) Smith blood	B	1	2-1
(V) Jones blood	O	2-1	2-1
shirt from "suspect"	*	2-1	-

* = typing not done
\- = typing done with inconclusive results

2. **For many different items and multiple stains on one item:**

ITEM	ABO	ESD	PGM
(S) Smith blood	B	1	2-1
(V) Jones blood	O	2-1	2-1
shirt from "suspect"			
right sleeve, stain 1A	*	2-1	2-1
left chest, stain 1B	*	1	2-1
pants from "suspect"			
right leg, stain 2A	*	1	2-1

* = typing not done

In a case such as above where there are stains of different types, the table could also be arranged so that items of the same type are together:

ITEM	ABO	ESD	PGM
(V) Jones blood	O	2-1	2-1
shirt from "suspect"			
right sleeve, stain 1A	*	2-1	2-1
(S) Smith blood	B	1	2-1
shirt from "suspect"			
left chest, stain 1B	*	1	2-1
pants from "suspect"			
right leg, stain 1A	*	1	2-1

* = typing not done

Appendix A

Forensic Biochemistry & Hematology Laboratory

Methods Manual

Initials: RCO Date: 3/31/92

TABLE 1

REAGENTS	NUMBER OF PLATES REQUIRED				
	5-6	10-12	15-18	20-24	**
distilled water	20	40	60	80	mL
sucrose	2.5	5.0	7.5	10.0	g
3% acrylamide premix	1.00	2.00	3.00	4.00	g
OR					
5% acrylamide premix acrylamide	0.60 0.40	1.20 0.80	1.80 1.20	2.40 1.60	g g
OR					
acrylamide bisacrylamide	0.97 0.03	1.94 0.06	2.91 0.09	3.88 0.12	g g
riboflavin	150	300	450	600	uL
OR					
ammonium persulfate	150	300	450	600	uL
TOTAL VOLUME (approx)	24	48	72	96	mL

FOR POLYMERIZATION:

riboflavin — 1.0 mg per 10 ml water

OR

ammonium persulfate — 0.228 g per 5 ml water, made up fresh

** The number of plates is approximate and is based on the medium sized plates. With the given amounts of reagents, there is an plenty to make the lesser number of plates; with careful pouring, more plates can be poured. If different sized plates are being poured, calculate how many using the following:

SIZE	DIMENSION	mL REQUIRED
S	10 x 20 cm	3 - 3.5
M	13 x 20 cm	4 - 4.5
L	15 x 20 cm	6 - 6.5
L	13 x 27 cm	6 - 6.5

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TABLE 2

SYSTEM	AMPHOLYTE	NUMBER OF PLATES REQUIRED				
		5-6	10-12	15-18	20-24	**
ACP	pH 4-8	1.0	2.0	3.0	4.0	mL
	OR					
	pH 4-6	0.5	1.0	1.5	2.0	mL
	pH 6-8	0.5	1.0	1.5	2.0	mL
ESD	pH 4-6.5	1.0	2.0	3.0	4.0	mL
PGM	pH 5-7	1.0	2.0	3.0	4.0	mL
	PLUS					
	EPPS/HEPPS	0.25	0.50	0.75	1.00	g
Hb	pH 6-8	0.5	1.0	1.5	2.0	mL
	pH 7-9	0.5	1.0	1.5	2.0	mL
	pH 3-10	0.2	0.4	1.5	2.0	mL
	TEMED	10	10	10	10	mL

** number of plates is approximate

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DISPOSITION:

This section tells what has happened to the evidence. Always keep a dried stain of (V) blood:

> A dried stain prepared from (V) blood will be retained in the laboratory.

If there are hair standards, fingernails, etc., retain them as well.

If semen is found on vaginal, oral, or rectal swabs, they are retained. If they are negative, they are discarded:

> The vaginal, oral, and rectal swabs were discarded after analysis.

Stains from clothing, stains taken off of knives, etc., may be retained for possible further analysis. This is determined on a case-by-case basis.

> Stains and unstained controls from the pants and shirt will be retained in the laboratory.
>
> The blood swabbed off the knife will be retained in the laboratory.

Sometimes the entire item is retained:

> Item 1, sample from "bedroom door", will be retained in the laboratory.

If numerous items are being kept, it is easier to write it in this way:

> The following items are being retained in the laboratory:
>
> dried stain prepared from (V) blood
> (V) head and pubic hairs
> stains and unstained controls from shirt and pants
> item 1, sample from "bedroom door"

If an item has left the lab, NOT through our evidence unit:

> The gun was returned to Det. Smith on 5-7-90.
>
> The vaginal swabs from (V) have been submitted to the FBI for further analysis.

For everything else:

> The remainder of the evidence is being held pending release to the submitting agency.

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IX. References

GENERAL REFERENCES

Boorman, K.E. and B.E. Dodd, **"An introduction to blood group serology"**, Little, Brown, & Co., Boston (1961).

Gaensslen, R.E., **"Sourcebook in forensic serology, immunology, and biochemistry"**, (1983).

Issitt, P.D. and C.H. Issit, **"Applied blood group serology"**, Spectra Biologicals, Oxnard CA (1979).

"Isoelectric focusing: principles and methods", Pharmacia Fine Chemicals, Uppsalla, Sweden (1982).

Righetti, P.G., **"IEF: theory, methodology, and applications"**, Elsevier Biomedicals Press, New York (1983).

Stites, et al., **"Basic and clinical immunology"**, 4th ed., Lange Medical Publishing, Los Altos, CA (1982).

PRESUMPTIVE AND CONFIRMATORY TEST REFERENCES

Kastle-Meyer and other presumptive tests for blood

Burdett, P.E., **"Presumptive tests for blood - a comparative survey"**, HOCRE, report 201: 1-10 (1983).

Gaensslen, R.E., **"Catalytic Tests"** in "Sourcebook in forensic serology, immunology, and biochemistry", section 6: 101-116 (1983).

Garner, D.D., et al., **"An evaluation of tetramethylbenzidine as a presumptive test for blood"**, J. For. Sci. 21(4): 816-821 (1976).

Higake, R.S. and W.M.S. Philp, **"A study of the sensitivity, stability and specificity of phenolphthalein as an indicator test for blood"**, Can. Soc. Foren. Sci. J. 9(3): 97-102 (1976).

Saferstein, R., **"Forensic characterization of bloodstains"** in "Forensic Science Handbook", 3rd edition, chapter 12: 319-324 (1987).

Sheehan, F.X., and L. Kobilinsky, **"Human blood identification: a forensic science approach"**, J. Chem. Ed. 61(6): 542-546 (1984).

Species determination

Gaensslen, R.E., **"Determination of species of origin"** in "Sourcebook in forensic serology, immunology, and biochemistry", sections 15-17: 215-256 (1983).

Wilson, M.W. and B.H. Pringle, **"interpretation of the Ouchterlony precipitin test"**, J. Immunol., 75: 460-469 (1955).

Initials: RCS Date: 3/31/92

Acid phosphatase presumptive test for semen

Gaensslen, R.E., "Identification of semen" in "Sourcebook in forensic serology, immunology, and biochemistry", section 10: 149-182 (1983).

Spermatozoa identification

Chapman, R.L. et al., "The siolation of spermatozoa from sexual assault swabs using Proteinase K", J. For. Sci. Soc. 23(4): 207-212 (1989).

Cortner, G.V. and A.J. Boudreau, **"Phase contrast microscopy versus differential interference contrast microscopy as applicable to the observation of spermatozoa"**, J. For. Sci. 23(4): 830-832 (1978).

Ellis, H.D., **"Recovery of spermatozoa from semen stains"**, Amer. J. Clin. Path. 34(1): 95-98 (1960).

Gaensslen, R.E., **"Identification of semen"** in "Sourcebook in forensic serology, immunology, and biochemistry", section 10: 149-1822 (1983).

Hueske, E.E., **"Techniques for extraction of spermatozoa from stained clothing: a critical review"**, J. For. Sci. 22(3): 597-598 (1977).

Keating, S.M., **"The laboratory's approach to sexual assault cases: sources of information and acts of intercourse"**, J. For. Sci. Soc. 28(1): 35-48 (1988).

Keating, S.M., **"The laboratory's approach to sexual assault cases: demonstration of the possible offender"**, J. For. Sci. Soc. 28(2): 99-110 (1988).

Wilcott, G.M. and M.A. Crosse, **"Detection of spermatozoa in the mouth"**, J. For. Sci. Soc. 26(2): 125-128 (1986).

P30 identification

Gaensslen, R.E., **"Identification of semen"** in "Sourcebook in forensic serology, immunology, and biochemistry", section 10: 149-182 (1983).

Amylase

Gaensslen, R.E., **"Identification of saliva"** in "Sourcebook in forensic serology, immunology, and biochemistry", section 11: 457-462 (1983).

Kipps, A.E. and P.H. Whitehead, **"A method for quantitating amylase and its use in the investigation of various body fluids"**, Ann. Clin. Biochem. 11: 219-223 (1974)

Kipps, A.E. and P.H. Whitehead, **"The significance of amylase in forensic investigations of body fluids"**, For. Sci. 6: 137-144 (1975)

Initials: RCJ Date: 3/31/92

GENETIC MARKER REFERENCES

ABO typing

Gaensslen, R.E., **"The ABO and secretor systems"** in "Sourcebook in forensic serology, immunology, and biochemistry", section 19: 261-328 (1983).

Le Pendu, J., **"A hypothesis on the dual significance of ABH, Lewis, and related antigens"**, J. of Immunogenetics 16: 53-61 (1989).

Roy, R., **"Concentration of urine samples by three different procedures: ABO typing from concentrated urine samples"**, JFS 35(5): 1133-1137 (1990).

Zhou, B., et al., **"The rapid determination of the ABO blood group from body fluids (or stains) by dot enzyme-linked immunosorbent assay (Dot-ELISA) using enzyme-labeled monoclomal antibodies"**, JFS 35(5): 1125-1132 (1990).

Acid phosphatase

Blake, E.T. and G.F. Sensabaugh, **"Genetic markers in human semen: a review"**, JFS 21: 784-796 (1976).

Burdett, P.E. and P.H. Whitehead, **"The separation of the phenotypes of phosphoglucomutase, erythrocyte acid phosphatase, and some hemoglobin variants by isoelectric focusing"**, Anal. Bioch. 77: 419-428 (1977).

Gaensslen, R.E., **"Erythrocyte acid phosphatase"** in "Sourcebook in forensic serology, immunology, and biochemistry", section 29: 445-456 (1983).

Giblett, E.R. and N.M. Scott, **"Red cell acid phosphatase: racial distribution and report of a new phenotype"**, Am. J. Hum. Genet. 17: 425-432 (1965).

Hopkinson, D.A. et al., **"Red cell acid phosphatase variants: a new human polymorphism"**, Nature, 199: 969-971 (1963).

Karp, G.W. and H.E. Sutton, **"Some new phenotypes of human red cell acid phosphatase"**, Am. J. Hum. Genet. 19: 54-62 (1967).

Luffman, J.E. and H. Harris, **"A comparison of some properties of human red cell acid phosphatase in different phenotypes"**, Ann. Hum. Genet. 30: 387-400 (1967).

Nelson, M. et al., **"Three new phenotypes of red cell acid phosphatase"**, Human Genetics 67:369-371 (1984).

Randall, T. et al., "A method of phenotyping erythrocyte acid phosphatase by iso-electric focusing", Med. Sci. Law 20(1): 43-47 (1980).

Rogers, P.A. et al., **"An examination of the age-related patterns of decay of acid phosphatase (ACP 1) in human red cells from individuals of different phenotypes"**, Bioch. Genet. 16: 727-738 (1978).

Initials: RCS Date: 3/31/92

Sorenson, S.A., **"Report and characterization of a new variant, EB, of human red cell acid phosphatase"**, Am. J. Hum. Genet. 37: 31-38 (1975).

Tahir, M.A. and J.V. Welch, **"Simultaneous typing of EAP, AK and ADA in human hair root sheaths"**, J. For. Sci. Soc. 26(5): 335-340 (1986).

Wraxall, B.G. and E.G. Emes, **"Erythrocyte acid phosphatase in bloodstains"**, J. For. Sci. Soc. 16: 127-132 (1976).

Wraxall, B.G. and M.D. Stolorow, **"Recent advances in electrophoretic techniques of bloodstain analysis"**, presented at the 30th annual meeting of the American Academy of Forensic Science, St. Louis, MO (1978).

Yeshion, T.E., **"Thermal degradation of erythrocyte acid phosphate isozymes in a case sample"**, J. For. Sci. 25: 695-698 (1980).

Zamir, A., **"The simultaneous phenotyping of erythrocyte acid phosphatase, esterase D, phosphoglucomutase and adenosine deaminase by isoelectric focusing"**, J. For. Sci. Soc. 28(4): 219-226 (1988).

Esterase D

Blake, E.T. and G.F. Sensabaugh, **"Genetic markers in human semen:a review"**, J. For. Sci. 21: 784-796 (1976).

Gaensslen, R.E., **"Esterase D"** in "Sourcebook in forensic serology, immunology, and biochemistry", section 31: 463-476 (1983).

Hopkinson, D.A. et al., **"Esterase D: a new human polymorphism"**, Ann. Hum. Genet. 37: 19-137 (1973).

Hayward, J.W. and A.L. Bosworth, **"Esterase D types in human bloodstains"**, J. For. Sci. Soc. 15: 289-291 (1975).

Parkin, B.H. and E.G. Adams, **"The typing of Esterase D in human bloodstains"**, Med. Sci. Law 15: 102-105 (1975).

Wraxall, B.G. and M.D. Stolorow, **"Recent advances in electrophoretic techniques of bloodstain analysis"**, presented at the 30th annual meeting of the American Academy of Forensic Science, St. Louis, MO (1978).

Zamir, A., **"The simultaneous phenotyping of erythrocyte acid phosphatase, esterase D, phosphoglucomutase and adenosine deaminase by isoelectric focusing"**, J. For. Sci. Soc. 28(4): 219-226 (1988).

Group specific component

Gaensslen, R.E., **"Group specific component"** in "Sourcebook in forensic serology, immunology, and biochemistry", section 41: 581-588 (1983).

Grunbaum, B.W. and P.L. Zajac, **"Rapid phenotyping of the Group Specific Component by immunofixation on cellulose acetate"**, J. For. Sci. 22(3): 586-589 (1977).

Initials: RCJ Date: 3/31/92

Horscroft, G.A. and S.A. Westwood, **"Group-specific component content in bloodstains. An aging and distribution study"**, J. For. Sci. Soc. 26(4): 275-280 (1986).

Westwood, S.A. and K. Fryer, **"The typing of group-specific component in case bloodstains using narrow pH interval isoelectric focusing"**, J. For. Sci. Soc. 26(4): 267-274 (1986).

Westwood, S.A. and D.J. Werrett, **"The collaborative study on typing group-specific component by eith forensic laboratories"**, J. For. Sci. Soc. 29(3): 173-184 (1989).

Thomas, A.S. et al., **"The application of immunoblotting to the phenotyping of goup-specific component"**, J. For. Sci. Soc. 29(3): 197-206 (1989).

Zajac, P.L. and B.W. Grunbaum, **"Determination of Group Specific component phenotypes in dried bloodstains by immunofixation of cellulose acetate"**, J. For. Sci. 23(2): 353-355 (1978).

Hemoglobin

Bunn, H. and J. Drysdale, Biochem. Biophys. Acta, 229: 51 (1971).

Gaensslen, R.E., **"Hemoglobin"** in "Sourcebook in forensic serology, immunology, and biochemistry", section 38: 545-562 (1983).

Schmidt, R.M., et al., **"Laboratory diagnosis of hemoglobinopathies"**, JAMA 224: 1276-1280 (1973).

Schneider, R.G. et al., **"Abnormal hemoglobins in a quarter million people"**, Blood 48(5): 629-637 (1976).

Wiggins, K.G., **"The identification of five hemoglobin variants in bloodstains"**, J. For. Sci. Soc. 18: 57-60 (1978).

Lewis typing

Gaensslen, R.E., **"The Lewis system"** in "Sourcebook in forensic serology, immunology, and biochemistry", section 20: 329-334 (1983).

Phosphoglucomutase

Bark, J.E., et al., **"Typing of common PGM variants using IEF - a new interpretation of the PGM system"**, J. For. Sci. Soc. 16: 115-120 (1976).

Blake, E.T. and G.F. Sensabaugh, **"Genetic markers in human semen: a review"**, J. For. Sci. 21: 784-796 (1976).

Blake, N.M., and K. Omoto, **"Phosphoglucomutase types in the Asian-Pacific area: a critical review including new phenotypes"**, Ann. Hum. Genet. 38:251-273 (1975)

Initials: RCS Date: 3/31/92

Budowle, B., **"Phosphoglucomutase-1 subtyping of human bloodstains on ultrathin-layer polyacrylamide gels"**, Electrophoresis 5: 165-167 (1984).

Burdett, P.E. and P.H. Whitehead, **"The separation of the phenotypes of phosphoglucomutase, erythrocyte acid phosphatase and some hemoglobin variants by isoelectric focusing"**, Anal. Bioch. 77: 419-428 (1977).

Burdett, P.E., **"Isoelectric focusing in agarose: phosphoglucomutase (PGM locus 1) typing"**, J. For. Sci. 26: 405-409 (1981).

Burgess, R.M. et al., **"An improved means of enzyme typing of hair roots using isoelectric focusing"**, J. For. Sci. 24: 392-396 (1979).

Culliford, B.J., **"The determination of phosphoglucomutase (PGM) types in bloodstains"**, J. For. Sci. Soc. 7: 131-133 (1967).

Eastwood, M.E., **"Phosphoglucomutase typing of vaginal swabs"**, J. For. Sci. 22:771-773 (1977).

Gaensslen, R.E., **"Phosphoglucomutase"** in "Sourcebook in forensic serology, immunology, and biochemistry", section 27: 425-438 (1983).

Gill, P. and J.G. Sutton, **"Enhanced resolution of phosphoglucomutase (PGM 1) by the addition of a separator to ultrathin isoelectric focusing gels"**, Electrophoresis 6: 23-26 (1985).

Hopkinson, D.A. and H. Harris, **"Rare phosphoglucomutase phenotypes"**, Ann. Hum. Genet. London 30: 167 (1966).

Kuhnl, P. et al., **"Evidence for two additional common alleles at the PGM 1 locus: a comparison by three different techniques"**, Hum. Genet. 35: 219-223 (1977).

Sensabaugh, G.F., et al., **"Genetic markers in semen III: alteration of phosphoglucomutase isozyme patters in semen contaminated with saliva"**, J. For. Sci. 25: 470-478 (1980).

Spencer, N. et al., **"Phosphoglucomutase polymorphism in man"**, Nature 204: 742 (1964).

Sutton, J.G., **"Further alleles of phosphoglucomutase in human semen detected by isoelectric focusing"**, J. For. Sci. 24:189-192 (1979).

Sutton, J.G. and R. Burgess, **"Genetic evidence for four common alleles at the phosphoglucomutase-1 locus (PGM 1) detectable by isoelectric focusing"**, Vox Sang. 34: 97-103 (1978).

Wraxall, B.G. and B.J. Culliford, **"A thin layer starch gel method for enzyme typing of bloodstains"**, J. For. Sci. Soc. 8: 81-82.

Wraxall, B.G. and M.D. Stolorow, **"Recent advances in electrophoretic techniques of bloodstain analysis"**, presented at the 30th annual meeting of the American Academy of Forensic Science, St. Louis, MO (1978).

Initials: RCS Date: 3/31/92

Zamir, A., **"The simultaneous phenotyping of erythrocyte acid phosphatase, esterase D, phosphoglucomutase and adenosine deaminase by isoelectric focusing"**, J. For. Sci. Soc. 28(4): 219-226 (1988).

POPULATION STATISTICS REFERENCES

Buckleton, J.S. et al., **"A stratified approach to the compilation of blood group frequency surveys"**, J. For. Sci. Soc. 27(2): 103-112.

Lai, L.Y. and R.J. Goetz, **"Phosphoglucomutase and haptoglobin phenotypes amony Australian caucasions"**, J. For. Sci. Soc. 28(3): 191-198 (1988).

Selvin, S. and B.W. Grunbaum, **"Genetic marker determination in evidence bloodstains: the effect of classification errors on probability of non-discrimination and probability of concordance"**, J. For. Sci. Soc. 27(1): 57-64 (1987).

Walsh, K.A.J. and J.S. Buckleton, **"A discussion of the law of mutual independence and its application to blood group frequency data"**, J. For. Sci. Soc. 28(2): 95-98 (1988).