Houston Police Department Crime Laboratory

Standard Operating Procedures

Biology Section

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1 Overview

The Biology Standard Operating Procedure manual (SOP) specifies procedures for routine examinations and analyses of biological evidence for human identification; it is approved for use in the Biology section of the laboratory.

Each approved revision of this manual will be version-controlled and archived for retrieval by date of authority. Only the approved revision in effect at the time of analysis governs the analysis. Any deviation from accepted protocol requires approval as outlined in the Methods section of the quality manual, and must be documented in the case file.

This SOP is only one part of the policies and procedures that govern all work performed by the Biology section. These other relevant documents include, but are not limited to, the:

- Houston Police Department General Orders
- quality manual
- safety manual
- biology training manual

2 Quality Assurance

The quality manual details requirements for the quality assurance program for all sections of the crime laboratory. The Biology Section will follow, in addition, the requirements detailed herein.

2.1 Quality Control

Controls

The following shall be tested to confirm that they are working properly with a positive and negative control the day used:

- AP Test Reagent
- Phenopthalein Solution
- Luminol Reagent
- Alternate light source (ALS)

The staining reagents used to perform spermatozoa examinations (Kernechtrot and Picroindigocarmine) shall be quality checked with each batch prepared to confirm they are working properly.

Substrate controls may be included in body fluid identification tests as appropriate.

Critical Reagents

Critical reagents are those that require testing prior to use on evidentiary samples in order to prevent unnecessary loss of sample. These are itemized in the Critical Reagents Section, and are quality control tested using the forms in the Forms Section.

Chemical and Reagent Labels

Purchased chemicals and reagents will be marked on the container with the date received and/or date opened. An expiration date will be placed on the outer container. In general, the manufacturer's labeling will be followed to determine expiration dates of purchased chemicals and reagents. If no manufacturer information exists for a purchased reagent, it will be considered expired 5 years from the received date.

The prepared reagent label will include the reagent specification, initials of preparer, and date of expiration. In general, most solutions prepared in the DNA laboratory shall expire 1 year from the date of preparation. Additional information may be documented

in a reagent log. The lot number contains the date prepared and the initials of the preparer, eg. Mm/dd/yyinitials.

Equipment Operation Manuals

Equipment operation manuals will be readily available to each examiner approved to use the equipment.

Equipment Calibration and Maintenance Logs

Calibration, maintenance, and repair activities will be recorded in an equipment calibration and maintenance log. The equipment calibration and maintenance log will include at a minimum the date, activity, laboratory personnel performing or overseeing the activity, non-HPD technician(s) performing or overseeing the activity, a record of quality control checks performed to verify operation prior to returning a piece of equipment to casework use.

2.2 Casework Review

All case files and reports will be technically reviewed prior to the release of report or results. All case files and reports will be administratively reviewed. For requirements of the technical and administrative reviews, refer to the "case review-serology" worksheet.

3 Facilities

The Biology laboratory will be designed to provide adequate space and setup to perform casework analysis. General facility requirements are described in the quality manual.

3.1 Work Areas

The Biology section of the laboratory will have space for evidence examination. The tasks performed in this area will include all screening, trace evidence collection (when performed by Biology examiners), body fluid identification testing, and selection and cutting of stains. Microscopy may also be performed in this area.

3.2 Contamination

With the facility provided, setup and procedures will be designed to minimize the potential for DNA contamination. Traffic in and through areas in which testing occurs will be minimized.

Prevention and Decontamination

10% bleach, or DNA Away where bleach may be harmful to instruments or equipment, will be used as to decontaminate utensils and workbenches. Other commercial disinfectants may be used only if they have been demonstrated to destroy or inactivate DNA.

Wear disposable gloves during all testing and reagent preparation. Change gloves frequently and whenever gloves may have become contaminated. Discard gloves when leaving a work area. Centrifuge all liquid to the bottom of closed microcentrifuge tubes before opening. Use sterile, disposable pipet tips and microcentrifuge tubes. Change pipet tips between samples.

In the evidence examination area, clean work surfaces thoroughly with decontaminant prior to and at the end of each evidence examination session. Use disposable bench paper whenever possible and change each time it comes in contact with evidence. Use a clean cutting surface such as a weighing paper or small piece of paper for evidence. Protect supplies of this paper from dust and other particulates or aerosols. Clean instruments (scissors, forceps) with decontaminant between evidence samples. To prevent contamination of other standards or evidence, handle each piece of evidence one at a time. Liquid samples, such as a blood standards, and wet exhibits shall be allowed to air dry in a method that it will not come into contact with other pieces of evidence.

Wear gloves when cleaning glassware. In general, clean glassware with an appropriate soap, e.g., Liquinox or Alconox, and water. Rinse with deionized or distilled water and allow to air-dry inverted, then autoclave. Store glassware when dry.

3.3 Safety

There are biological and chemical hazards in the laboratory. Each lab employee is responsible for familiarity with the Lab Safety Manual. Any incident or condition that occurs in or under the control of the laboratory and that threatens the immediate or future health of any individual must be immediately brought to the attention of the section supervisor and Laboratory Safety Officer. Corrective action related to safety incidents will be defined by the laboratory management.

4 Casework Processing

4.1 Analysis Methods

The Biology section of the laboratory provides body fluid identification (semen and blood). This section also performs the collection and preservation of trace evidence.

4.2 Case Acceptance and Evaluation

Refer to the quality manual for procedures for the submission of evidence into the Crime Laboratory. Before a case is worked, the case will be evaluated. The examiner should be aware of the requested examinations, the reason(s) for the requested analyses, and the quality and quantity of the evidence.

4.3 Evidence Evaluation

Before the case is worked, an evaluation should be made to determine the quality and quantity of the evidence that is going to be analyzed. In order to expedite casework, it is recommended that for cases containing large volumes of evidence (excluding sexual assault kits), 5-10 items of evidence should be screened. Emphasis should be placed on items believed to be of significant evidentiary value after consultation with submitting officials and prosecutors. Of the items screened, it is recommended that a maximum of 5 evidence stains should continue on to DNA analysis. Additional items/stains may be analyzed at a later time. Clothing evidence submitted in a sexual assault case does not have to be examined unless the corresponding sexual assault evidence kit does not reveal any positive probative evidence or there are multiple suspects.

4.4 Evidence Handling

Evidence Requiring Latent Print Examination

Police officers may request that items be analyzed for latent prints. In general criminalistics evidence examinations are done before any latent print examination. Appropriate steps, including consultation with latent print personnel where needed, should be taken to preserve potential latent prints when conducting evidence examination and/or swabbing items for analysis. When the criminalist is finished with the analysis, the evidence is submitted to the Latent Print Lab. When evidence is submitted and

retained by Latent Print personnel, appropriate documentation will be made in the criminalist's report.

Storage of Evidence

Biological evidence must be properly stored to preserve biochemicals assayed in body fluid identifications and DNA typing for current and future analyses. Storage conditions for all types of evidence present must be considered so that none are compromised. In addition to the storage requirements detailed in the quality manual, the following procedures will be followed:

Wet evidence should be dried upon receipt when applicable. Portions of blood standards submitted in liquid form should be dried on stain cards or cloth within 30 days of receipt. Once the sample is in dried form, the liquid blood tube will be placed back in the sexual assault kit and returned to the property room.

Store sexual assault kits in the refrigerator or in a dry area at room temperature once received in the laboratory. Additionally, the kit must be inspected to insure no other liquid specimens are present or that samples such as vaginal swabs are not packaged in airtight containers. Blood and urine specimens requiring toxicological screening will be stored in the refrigerator until ready to be transferred for toxicology analysis.

Blood cases containing small, dry items may be stored at room temperature or frozen depending on available space. Refrigerate or freeze liquid whole blood specimens until a sample is dried on an appropriate substrate. Store larger items such as clothing, bedding, weapons, and other physical evidence containing bloodstains in a dry area at room temperature until examined. Metal objects should be evaluated for fingerprints and/or blood pattern analysis prior to being frozen.

Consumption of Evidence

The evidence quality and quantity will be preserved as much as possible without sacrificing the quality of the analyses. Whenever possible, at least half of the evidence sample will be preserved for possible re-analysis. When this is not possible, appropriate personnel (submitting officer, prosecuting attorney, and/or defense attorney) will be consulted and permission obtained before consumption of evidence.

Chain of Custody

Refer to the Laboratory Quality and Operations Manual for chain-of-custody policies and procedures.

Reference Samples

Whenever possible, reference samples should be collected and retained from suspects, complainants, witnesses, and consensual partners. Oral swabs are the preferred reference

sample for the HPD Crime Lab. In cases of sexual assault, if the oral swabs test positive for semen, the known oral swabs should not routinely be used as a reference sample.

Fingernail Scrapings/Clippings/Swabs

The analyst will process fingernail scrapings by looking for any visible red staining. If red staining is apparent, the analyst will test the stains with a presumptive test for blood and retain any positive scrapings. The analyst will process fingernail swabs for semen when appropriate. Any positive swabs will be retained. The analyst will process fingernail clippings by swabbing the underside of the nail with a sterile swab moistened with sterile water or PBS. Alternatively, the analyst may choose to simply retain the clippings without swabbing them and allow the DNA analyst on the case to perform the swabbing, if DNA analysis is to be performed.

4.5 Documentation

Case Files

Documentation must be in such a form that another qualified examiner or supervisor, in the absence of the primary examiner, would be able to evaluate what was done, interpret the data, and reach a scientific conclusion. Appropriately completed worksheets should be used. In addition to the documentation requirements of the quality manual, the following must be documented in the case file:

Notes that help in the identification of the item of evidence must be included. A written description may suffice for some items, whereas others may need a drawing, sketch, or photograph. This will include documentation of analyst's marking of the evidence.

Significant stains must be drawn or documented in a manner which clearly demonstrates the location, amount, relationship to other stains, degree of degradation, reactions to screening tests, etc. Drawings must be large enough to provide adequate detail.

If needed, the location of the substrate blank control and its relationship to the body fluid stain in question must be clearly documented. The type of material or fabric the stain is on may be significant.

Note any tears, cuts, missing buttons, possible bullet holes, location and length of possible stab holes, soiling condition, and significant foreign material.

Describe the examination procedures, (e.g., "a knife blade and handle were examined at 10× for bloodstains" or "the inside of the pants pockets were examined"), observations made, results of examinations conducted, and conclusions.

Interpretation of Results

Although interpretation of individual tests is discussed in the appropriate protocols, analysis results must be considered together to insure that conclusions take into account

all reasonable possibilities. During analysis, the examiner must continuously monitor results for problems and inconsistencies that may ultimately affect final conclusions. Therefore, careful review of notes and results by the examiner should be undertaken before conclusions are drawn. A supervisor or qualified examiner may assist in the developing of the appropriate conclusions. Slightly different situations may lead to different conclusions. When in doubt, consult with other qualified examiners and/or the Supervisor.

Appendix 4A: Abbreviations

+	positive reaction
neg	negative or no reaction
Ø	none
ALS	alternate light source
AP	acid phosphatase
APP	apparent
BC	barcode
BSC	bloodstain card
BUC	buccal
c̄	containing/contained (c with line over top)
COMPL	complainant
D	depleted
diH_2O	deionized water
DISP	disposition
EF	epithelial fraction
Env	envelope
EVID	evidence
F	frozen
FTC	found to contain
HPS	hospital patient sticker
HT	HemaTrace
Inc	inconclusive
IT	item
K	known standard sample
Micro	sperm search by microscopy
MFG or manuf.	manufacturer
N/A	not applicable
NSO	no stains observed
NTC	no testing conducted
NSTO	no significant trace observed
PHT	phenolphthalein

Q	questioned sample
QNS	quantity not sufficient (for further analysis)
R/B	reddish/brown
RPR	return to property room
SA	sexual assault
SF	sperm fraction
ST	stain
STC	said to contain
SUSP	suspect
TNTC	too numerous to count
Unk	unknown
VAG	vaginal
W/	with
WIF	walk-in freezer

5 Critical Reagents

In order to provide and maintain the quality of the work provided in the Biology section, it is necessary to identify certain reagents as critical. Critical reagents are those that require testing prior to use on evidentiary samples in order to prevent unnecessary loss of sample.

If any new lot of critical reagent does not pass all quality control checks, it may not be utilized in casework. All inconsistencies will be documented and reported to the Section Supervisor. Problems that cannot be resolved must be reported to the manufacturer.

Reagents and supplies that have passed their expiration dates may not be used on casework or database samples. Outdated reagents may be used for training purposes only, but must be designated as such, and must never be used on casework samples.

5.1 Reagents

The following reagents are considered critical reagents: ABACard® p30 Test Devices, ABA Card® Hematrace® Devices.

ABACard® p30 Test Devices

ABACard® p30 test devices are used to determine the presence of p30 in suspected semen stains.

Each new lot of ABACard® p30 test devices must be subjected to the appropriate in-house quality control test as outlined below:

1. Make a $1:10^5$ dilution from a known semen sample in PBS.
2. Add 200 µl of dilution to sample well "S" of the device.
3. Read results up to 10 minutes from application of sample.
4. Repeat test on new device with PBS for negative control.
5. The $1:10^5$ dilution of known semen must give a positive result. The PBS must give a negative result.
6. Mark the solution bottle of PBS with the lot number used for all extractions for that lot.

ABACard® HemaTrace® Test Devices

ABACard® HemaTrace® Test devices are used to determine the presence of human blood in suspected blood stains. These devices are purchased from Abacus Diagnostics [#708424].

Each new lot of ABACard® HemaTrace® test devices must be subjected to the appropriate in-house quality control test as outlined below:

1. Make a $1:10^6$ dilution from a known human blood sample in solution provided in kit or 200 µl of PBS.
2. Add 150 µl of dilution to sample well "S" of the device.
3. Read results up to 10 minutes from application of sample.
4. Repeat test on new device with solution provided in kit or PBS for negative control.
5. The $1:10^6$ dilution of known human blood must give a positive result. The solution provided in the kit or PBS must give a negative result.

5.2 Logbook

A logbook will be maintained for the quality control of all of the critical reagents. This logbook will contain the test date, signature of the analyst performing the quality control, expiration date for lot, lot number, a second reader signature and date and any supporting documentation necessary to demonstrate the reagent met all of the standards listed above.

6 Equipment quality control and maintenance

6.1 Maintenance and Calibration

In order to provide and maintain the quality of the work provided in the DNA section, it is necessary to ensure laboratory equipment is in good working order. Routine quality control and maintenance accomplishes this. The calibration intervals listed below are generally considered to be the minimum appropriate in each case, providing that the equipment is of good quality and of proven stability and the laboratory has both the equipment capability and expertise to perform adequate internal checks. More frequent checks are acceptable. If there is any question concerning the reliability of an instrument or piece of equipment, a maintenance check should be performed immediately.

Full maintenance and calibration records shall be maintained. Documentation includes the numerical result, date of calibration, analyst's signature, and any other relevant observations. The section supervisor and QA Manager are responsible for ensuring all systems are checked and maintained as required.

The following equipment must be maintained and subjected to quality control measures: water baths, pH meters, microcentrifuges, refrigerators/freezers, thermometers, genetic analyzers, pipettes, balances, thermal cyclers (including real-time PCR instruments), hoods, autoclaves, and water filtration system.

6.2 Equipment

Water Bath and Dry Bath

Critical water baths and dry baths are dedicated equipment whose temperature is routinely maintained at 37-100^{o}C for DNA procedures.

Observed Temperature

Observe temperature reading on the thermometer and on the display. The temperature should be within +/- 1 ^{0}C of thermometer temperature. If not within the acceptable range, use the control knobs to adjust the temperature to the acceptable range, or document the correct setting needed to obtain the correct temperature. If the temperature is not stable, repair or replace the water bath or dry bath. Please refer to the equipment manual for specific instructions on temperature adjustment and bath maintenance.

Water Condition

For water baths, the water should be clear and clean with no evidence of bacterial/fungal growth or rust. If the water becomes dirty, discard and clean water bath. Replenish with water.

Maintenance Procedure

The following procedure should be performed as needed:

1. Decant and discard water.
2. Wash inside of water bath with detergent.
3. Rinse well with water.
4. Fill bath with the appropriate quantity of water. Clear bath or another algaecide may be used.

Critical dry baths should be cleaned at least one time per year.

pH Meters

The pH meter comprises a rugged bulb combination electrode with Ag/AgCl reference half-cells. It is suitable for measuring pH in the range of 0 to 14 and equipped with a Thermo-Compensator that provides temperature compensation for pH measurements. The standard pH buffers available for instrument calibration include those at pH 4.00, 7.00, and 10.00.

pH Calibration

The pH meter is calibrated against known pH standards on the day of use with standardized reference buffers. The pH meter must be checked with standardized reference buffers whose pH values bracket the desired sample pH. If, after calibration, the pH meter does not give the correct readings for the reference buffers, the pH meter must be re-calibrated. If the correct reading are still not obtained, consult the technical leader for instructions on scheduling a service call for the instrument.

Consult the equipment manual or the instructions posted next to the pH meter for specific instructions on how to operate and calibrate the pH meter.

If the pH meter exhibits a long stabilization time and significant drift is observed, the electrode may need to be rejuvenated. For electrode rejuvenation, follow the manufacturer's instructions.

Care must be exercised in handling the electrodes since the accuracy of pH measurements is dependent upon the following:

1. Keep the electrodes wet to ensure free flowing junction from the reference electrode.

2. Maintain the reference electrode filling solution at the appropriate level. This is usually up to the filling hole.
3. Cover the filling hole with a sleeve or plug in order to reduce the flow of filling solution during storage.
4. Uncover the filling hole by sliding the sleeve or removing plug during measurement in order to allow the flow of filling solution into the sample.
5. Store the combination electrode in 0.1 M KCl dissolved in pH 4.0 buffer or other suitable electrode storage solution.

Microcentrifuges

Microcentrifuges are bench top, unrefrigerated centrifuges that have been designed for centrifugation of 1.5 ml tubes, test tubes, and Microcon tubes. These microcentrifuges are equipped with fixed angle rotors. The maximum speed is specified in the operations manual for each centrifuge. The relative centrifugal force can be determined as outlined in the manufacturer's instructions, if required.

The microcentrifuges will be cleaned and maintained at least one time per year or as needed. Please consult the appropriate equipment manual for specific instructions on maintenance and operation of the microcentrifuge.

Centrifuges are not to be operated if they have not been installed properly, been partly dismantled, the rotor is not installed securely on the rotor shaft, or proper electrical inspection has not been performed.

Centrifuge housing, rotor chamber, and rotor accessories should be cleaned with neutral cleaning agents (pH 7.0) at least one time per year. All parts must be dry prior to use.

Refrigerators/Freezers

Each refrigerator/freezer used in the laboratory has been shown to be capable of maintaining the optimum temperature range required for storing reagents and samples. A thermometer or digital display for monitoring the temperature has been placed in each unit. The thermometer can sit in oil or water to buffer against rapid temperature swings. Refrigerators/freezers are monitored during working hours to ascertain that they are functioning. Temperature readings will be checked weekly.

The refrigerator maintains optimum temperature with the required range of 0°C to 8°C. The freezer compartment should maintain a temperature below 0°C. If the temperature is not being maintained at the prescribed values, verify that the temperature regulation control is at the proper setting and adjust if necessary. It may also be necessary to reduce over-crowding in an effort to increase air circulation in all compartments. If necessary, a technical representative may need to be called for service or the refrigerator/freezer may need to be replaced if corrective action is not adequate.

Each refrigerator/freezer is maintained in working order and repaired or replaced if necessary. The compartments are kept clean and well organized.

Thermometers

All thermometers must be either calibrated or their accuracy must be verified to ensure the temperature is being accurately measured.

Thermometers (including digital thermometers) must be verified by comparison against a NIST-traceable thermometer. Any deviation from the NIST-traceable thermometer will be noted and all other readings adjusted by the same amount, and in the same direction. If the deviation is greater than 3°C, the thermometer should be serviced or replaced.

Verification must be performed at least once every two years. New thermometers with a certificate of calibration need not be verified for the first two years.

Thermometers are handled carefully to avoid breakage. Thermometers are wiped clean to facilitate easy and accurate readings. It is necessary to examine the thermometers to detect any discontinuities in the mercury column (for glass thermometers). If any discontinuities are detected, repair or replace the thermometer.

Balances

To meet the goal of measuring weights accurately, all analytical balances used in the laboratory must either be calibrated or their accuracy verified against standards.

All analytical balances used in the DNA laboratory must be linear throughout the measurement range. Calibration accuracy will be re-certified by an external vendor on an annual basis.

Analytical balances will be located in a suitable area that is not exposed to extreme heat, radiation, drafts, extreme vibration, or aggressive chemicals. They may not be used in hazardous areas where there is danger of explosion. Balances may not be exposed to extreme moisture over long periods of time. If the balance is transferred to a warmer or cooler area, 2 hours should be allowed for temperature calibration. Balances must be checked whenever they are moved from one location to another.

Hoods

The hood, when used with proper technique, is effective in reducing the potential for exposure of both product and personnel to airborne biological or particulate chemical agents. The laminar flow hood contains a HEPA filter. These hoods will be evaluated for proper airflow annually by an external vendor.

The hood must be re-certified at least once a year and after every filter change or maintenance action or at the operator's discretion.

A qualified technician must certify the cabinet.

No maintenance should be performed on the interior of the cabinet unless the cabinet has been disinfected and known to be biologically clean.

Autoclaves

Autoclaves are used to sterilize solutions, glassware and instruments by subjecting them to high pressure and high heat simultaneously. Autoclaves also may be used to sterilize biohazard trash prior to discarding. Please consult the instructions on the outside of the unit for specific information on operation of the autoclave.

Whenever an item is autoclaved, a small piece of autoclave tape should be adhered to the item, to verify correct functioning of the autoclave. The pressure gauge on the autoclave should read 1.3kg/cm2 and the temperature gauge should read 120^0C during the 30 minute cycle if the autoclave is functioning properly.

If the autoclave is not functioning properly, a qualified service technician may repair the instrument.

Water Filtration System

Water is used in reagents that are prepared in the laboratory. Therefore, it is necessary to ensure only high quality, reagent grade water is being utilized. Generally, 15MΩ-cm or greater at 25°C deionized water is sufficient for the reagents utilized in our laboratory.

Water quality should be checked on a monthly basis. The following procedure will be utilized:

1. Turn on the deionized water
2. Allow water to run for 1-2 minutes
3. Record reading displayed on resistivity monitor on Water Quality sheet
4. If reading is below 15MΩ-cm at 25°C, call for service.
5. If the water filtration system is not functioning properly, a qualified service technician may repair the system.

7 Serology

7.1 Presumptive Blood Tests – PHT

Scope

Catalytic tests for blood are based on the peroxidase-like activity exhibited by the heme group of hemoglobin. For example, colorless phenolphthalin is oxidized to phenolphthalein in the presence of heme and hydrogen peroxide. In a basic solution such as the test reagent, the phenolphthalein is pink. The test is exceedingly sensitive to minute traces of hemoglobin and its derivatives but will produce a false positive reaction in the presence of any of a number of oxidizing substances. Should a color reaction take place, the result only indicates the possible presence of blood; the test is therefore a presumptive test.

Safety

Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Gloves must be worn during testing. Clothing may protect unbroken skin; broken skin should be covered.

Related Forms - Serology Worksheets

Equipment, Materials, and Reagents

- diH2O
- cotton swab(s) or filter paper
- Test reagent – (see Reagents section):
- Phenolphthalin (PHT) solution
- 3% hydrogen peroxide – purchased [Sigma, #H6520 or equal]

Standards, Controls, and Calibration

A positive and negative control must be tested each day of reagent use. An appropriate positive control is a small bloodstain prepared in-house. Appropriate negative controls include a cotton swab, filter paper, or small fabric swatch treated in the manner of the unknown. The positive control should produce an immediate appropriate color change upon addition of the 3% hydrogen peroxide. The negative control should not produce an

immediate color change. The results of each control test must be recorded on every applicable Serology Worksheet.

Procedure

1. Rub or scrape the suspected bloodstain with a cotton swab or piece of filter paper moistened with sterile diH20.
2. Apply approximately one drop of the test reagent (PHT).
3. Observe briefly to identify color change. A green color is often observed when blood is present; any other color change should be documented.
4. Add approximately one drop of 3% hydrogen peroxide.
5. Observe the swab or filter paper for a pink color change indicating a positive reaction.
6. Document the items/areas tested and whether there was or was not a positive reaction after the application of hydrogen peroxide.
7. Discard swab or filter paper by placing it in the biohazard trash.

Interpretation

Appearance of an immediate pink color change after the application of hydrogen peroxide is a presumptive positive result for blood. A negative result indicates that blood is absent or below the detection threshold.

7.2 Presumptive Blood Test – Luminol

Scope

Catalytic tests for blood are based on the peroxidase-like activity exhibited by the heme group of hemoglobin. In the case of luminol, the catalytic oxidation of the substrate compound produces light. Because the reagents are applied as a spray over a large area, the test is primarily used in the visualization of bloodstain patterns. As with similar presumptive blood tests, a number of oxidizing substances will produce a false positive reaction. Should a positive reaction take place, the result only suggests the presence of blood; the test is therefore a presumptive test.

Safety

Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Luminol is an irritant. Sodium perborate and sodium carbonate are toxic and irritating. Avoid breathing dust; do not get in eyes, on skin, or on clothing. Avoid breathing sprayed solution. Wash after handling. Gloves must be worn during testing. Clothing should protect unbroken skin; broken skin should be covered.

Related Forms - Serology Worksheets

Equipment, Materials, and Reagents

- Luminol: 3-aminophthalhydrazide
- Sodium carbonate
- Sodium perborate
- Deionized water (diH2O)
- Spray bottle or dropper

Standards, Controls, and Calibration

A positive and negative control must be tested each day of reagent use. An appropriate positive control is a small bloodstain prepared in-house. Appropriate negative controls include a cotton swab, filter paper, or small fabric swatch treated in the manner of the unknown. The positive control should produce an immediate luminescence upon addition of the luminol reagent. The negative control should not produce luminescence. The results of each control test will be recorded either on the applicable Serology Worksheet or in the examiner's crime scene analysis notes.

The prepared reagents are unstable and must be prepared fresh immediately before use. The components can be measured for transport to the crime scene for preparation prior to use. Reagents A and B can be stored separately refrigerated for up to 8 weeks. If stored, the label must include at a minimum, "Luminol, Reagent A" or "Luminol, Reagent B" as appropriate, initials, the date prepared, and the expiration date on each. Also, prior to next use, check the reagents for precipitate. If a precipitate has formed in either reagent after refrigeration, ensure that the precipitate is back in solution before use.

a) Reagent A
    i) Dissolve sodium carbonate (2.5 g) in diH20 (250 ml).
    ii) Add and dissolve luminol (0.5 g).

b) Reagent B
    i) Dissolve sodium perborate (6.5 g) in diH20 (250 ml).

Procedure

1. Darken the examination room or crime scene area as completely as possible.

2. Mix equal volumes of Reagent A and Reagent B. This mixture is stable for about one hour.

3. Spray mixed reagent and observe for luminescence.

4. Document or photograph luminescent areas.

5. Re-spray as necessary. The ability to further test the stain decreases with increased or repeated spraying. Excessive spraying will cause stains to run. Repeated spraying of non-porous surfaces is not suggested.

6. Dispose of excess solutions in regular sink; flush with water.

Interpretation

Faint to strong luminescence shows oxidation of the luminol reagent and represents a positive presumptive result for blood. Spraying will deposit a light white film on surfaces.

7.3 Human Origin Identification by Hematrace®

Scope

Heme is a part of the hemoglobin molecule that is characteristic of red blood cells found in blood.

The ABACard® HemaTrace® test is a qualitative detection method specifically designed for forensic identification of human blood. Sample is added to a sample well where any detectable human hemoglobin (hHb) present in the sample will bind with mobile monoclonal antihuman Hb antibody which has an attached pink dye particle. The resultant mobile antigen-antibody complex migrates through an absorbent strip to an area where an immobile polyclonal antihuman Hb antibody is bound. The mobile antigen-antibody complex binds to the immobile antibody creating an antibody-antigen-antibody sandwich. Pink dye particles become visible in the area of immobilized antibody. The resultant pink band indicates a positive result. Hemoglobin from the Family Mustelidae (ferrets) and higher primates also gives a positive result. The examiner must evaluate the likelihood of blood from either of these sources being present when this test is used.

Safety

Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Gloves must be worn during use. Clothing may protect unbroken skin; broken skin should be covered.

Related Forms - Serology Worksheets

Equipment, Materials, and Reagents

One Step ABACard® HemaTrace® Test Strips – One per sample or control

Standards, Controls, and Calibration

The lot of ABACard® HemaTrace® test devices must be quality control tested using a known human blood sample positive control and PBS negative control prior to lot use. The results of each lot's quality control test (positive and negative controls), down to the 1:10^6 sensitivity, must be recorded and placed in the Quality Control Manual.

Procedure

1. Allow the sample(s) and control(s) to warm to room temperature if they have been refrigerated.

2. Extract body fluid by placing suspected bloodstain sample into an appropriate microcentrifuge tube along with extraction buffer provided with the kit and allow to sit for 5-30 minutes. If the bloodstain sample is a swab, approximately ¼ of the swab(s) should be cut and used to perform this test. For testing samples other than swabs, take cuttings up to approximately 1 cm^2.

3. For each sample or control, unwrap and label an ABACard® device.

4. Add 150 µl of the appropriate dilution of the extract to the sample well "S" of the device.

5. Read results up to 10 minutes from application of sample.

6. Record the HemaTrace® test results onto the Serology worksheet. Have a 2nd reader verify the test result(s) and initial and date the worksheet.

Interpretation

The appearance of a pink line at the "C" (control) area is expected for all tests and must be present. Absence of the "C" line is an inconclusive result, and the test must be performed a second time. The appearance of a pink line at the "T" (test) area is a positive result and indicates that human Hemoglobin is present.

The absence of a pink line at the "T" area after 10 minutes is a negative result and indicates that human blood is not detected.

7.4 Presumptive Semen Test - AP Spot Test and Map

Scope

Acid phosphatase is found in relatively large quantities in semen and its presence is indicative of the possible presence of semen in a stain. In the following procedure, acid phosphatase is detected by a color-change reaction. Acid phosphatase liberates the phosphate from α-naphthyl phosphate, and the released naphthol combines with tetrazotized o-dianisidine to form a purple azo dye.

The AP test is semi-quantitative. A stronger reaction is more likely to indicate semen. However, because acid phosphatase occurs in other body fluids, most notably vaginal secretions, this is only a presumptive test. The presence of semen in the sample can subsequently be confirmed by the presence of p30 or spermatozoa.

Safety

Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. AP Test Reagent contains a dye that is a suspected carcinogen. The components of the AP Test Reagent are irritants. Avoid contact and inhalation. Gloves must be worn during use. Clothing may protect unbroken skin; broken skin should be covered.

Related Forms - Serology Worksheets

Equipment, Materials, and Reagents

- diH2O
- cotton swab(s) or filter paper
- non-porous support and clips (optional, mapping only)
- AP Test Reagent - See Reagents section

Standards, Controls, and Calibration

A positive and negative control must be tested each day of reagent use. An appropriate positive control is a small semen stain prepared in-house. Appropriate negative controls include a cotton swab, filter paper, or small fabric swatch treated in the manner of the unknown. The positive control should produce a positive result and the negative control should produce negative results, as described in the interpretation section below. The results of each control test must be recorded on each applicable Serology Worksheet.

AP One Step Procedure

1. Take a small blotting or swabbing of the unknown stain using a diH_20-moistened piece of filter paper or cotton swab.

2. Apply one or two drops of the AP Test Reagent.

3. Observe the blotting or swabbing for up to 60 seconds for a color change.

4. Discard used filter paper or swab by placing it in the biohazard trash.

AP Map Procedure

1. Spread the garment, sheet, shirt, panties, towel or other item flat. Fasten the item as necessary to a piece of plastic-covered wood, cardboard, or other non-porous support using clips.

2. Moisten a sheet or swatch of filter paper with sterile diH_2O.

3. Lay the moist filter paper over the item or area of the item to be tested. Press the paper firmly against the item at all points of the item's surface. Allow approximately 30 seconds to 5 minutes for any possible acid phosphatase to transfer to the paper.

4. Mark the position of the paper on the item.

5. Remove the sheet of filter paper.

6. Spray or drop AP test reagent on the surface that was pressed to the item.

7. Observe the treated filter paper for up to 60 seconds for a color change.

8. After interpretation, draw or otherwise document the areas and whether there is or is not a positive reaction.

9. Discard used filter paper by placing it in the biohazard trash.

Interpretation

The appearance of a purple color within 60 seconds indicates the presence of acid phosphatase and is a positive presumptive test for semen. Grade the reaction according to the time it takes for the color to appear as follows:

- 0-15 seconds: 4+
- 16-30 seconds: 3+
- 31-45 seconds: 2+
- 46-60 seconds: 1+
- No change, or color change after more than 60 seconds: negative.

The AP map procedure reflects the size and shape of the acid phosphatase containing stains. A negative result indicates acid phosphatase is absent or below the detection threshold.

7.5 p30 Identification

Scope

The cells that line the ducts of the prostate make a protein known as p30 or prostate-specific antigen (PSA). The protein is secreted into seminal fluid to a concentration of approximately 0.24-5.5 mg/ml1, and its detection confirms the presence of semen.2

The ABACard p30 test is a qualitative detection method specifically designed for forensic identification of semen. Sample is added to a sample well where any detectable p30 present in the sample will bind with mobile p30 antibody which is attached to a pink dye particle. The resultant mobile antigen-antibody complex migrates through an absorbent strip to an area where immobile p30 antibody is bound. The mobile antigen-antibody complex binds to the immobile antibody creating an antibody-antigen-antibody sandwich. When the p30 concentration in the sample exceeds 4 ng/ml (value reported by manufacturer), pink dye particles become visible in the area of immobilized antibody. The resultant pink band indicates a positive result.

Safety

Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Gloves must be worn during use. Clothing may protect unbroken skin; broken skin should be covered.

Related Forms - Serology Worksheets

Equipment, Materials, and Reagents

- OneStep ABACard® p30 Test strips – one per sample or control
- PBS – see Reagents section

Standards, Controls, and Calibration

The lot of ABACard p30 test devices must be quality control tested using a known semen sample and reagent blank (PBS) prior to lot use. The results of each lot's quality control test (positive and negative controls) must be recorded and placed in the Quality Control Manual.

Procedure

1. Allow the sample(s) and control(s) to warm to room temperature if they have been refrigerated.

2. Extract body fluid. See the Body Fluid Stain Extraction Procedure.

3. For each sample or control, unwrap and label an ABACard device.

4. Add 200 µl of the appropriate dilution of the extract to the sample well "S" of the device.

5. Read results up to 10 minutes from application of sample.

6. Samples that produce a negative result but that show strong positive acid phosphatase activity must be diluted to an appropriate dilution and re-tested (to ensure the High Dose Hook Effect is not occurring).

7. Record the p30 results onto the Serology Worksheet. Have a 2nd reader verify the test result(s) and initial and date the worksheet.

Interpretation

The appearance of a pink line at the "C" (control) area is expected for all tests and must be present. Absence of the "C" line is an inconclusive result, and the test must be performed a second time. The appearance of a pink line at the "T" (test) area is a positive result and indicates that the p30 concentration in the applied solution is at least 4 ng/ml, equivalent to approximately 1:1,000,000 dilution; however, our laboratory validation showed the sensitivity to be 1:100,000. This confirms the presence of semen in the sample. The absence of a pink line at the "T" area after 10 minutes is a negative result and indicates that semen is absent, below the detection threshold, or above the high dose threshold.

7.6 Spermatozoa Examination

Scope

Spermatozoa (sperm) detected on a smear submitted in a kit, in a stain, or on a swab, confirm the presence of semen. For spermatozoa examination, material from a stain or swab is fixed to a microscope slide, labeled, stained, and examined under the microscope for cells with sperm characteristics. This procedure uses nuclear fast red (also known as Kernechtrot) and picroindigocarmine, also called "Christmas Tree" stain..

Human sperm are flagellated with a total length of about 50 μm. The sperm cell head generally is oval, flattened at the anterior end, with dimensions about 4.6 μm × 2.6 μm × 1.5μm. The procedure outlined below differentially stains spermatozoa to aid in their identification.

Safety

Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Gloves must be worn during testing. Clothing should protect unbroken skin; broken skin should be covered.

Related Forms - Serology Worksheet

Equipment, Materials, and Reagents

- microscope slide
- heat block
- known sperm slides – optional, prepared in-house
- nuclear fast red (NFR) – purchased [Sigma, #N8002 or equal] or prepared-see Reagents section
- picroindigocarmine (PIC) – purchased or prepared, see Reagents section
- 95% ethanol (Reagent Alcohol, Absolute)

Standards, Controls, and Calibration

Staining reagents must be quality control tested to ensure proper performance. Using a known semen standard, the stained slide should show the sperm heads to be stained red and the tails to be stained green.

Procedure

Prepare a smear from a stain or swab (start with step 1) or use a smear provided in the sexual assault evidence kit (start with step 3).

1. Extract body fluid. See the Body Fluid Stain Extraction procedure.
2. Pipet a small portion of the pelleted cellular debris from the body fluid extract onto a glass slide OR put a drop of sterile water on the slide and smear the sample on the slide to transfer cellular material. Fix smear by heating the slide on the heat block.
3. Stain the slide.
    a. Cover the sample area on the slide with NFR for 15 minutes.
    b. Wash with tap water by gentle flooding.
    c. Cover the sample area with PIC for up to 15 seconds.
    d. Wash with tap water by gentle flooding.
4. Fix by gently flooding the slide with 95% ethanol, and allow to dry.
5. Examine the slide for the presence of spermatozoa at a minimum of 400X magnification. A search of the entire slide is required before negative results for spermatozoa can be reported. A minimum of 1 sperm head should be identified to confirm the presence of semen.
6. Indicate on the worksheet either an exact or approximate number of sperm seen on the slide. More than 25 sperm may be recorded as too many to count. If too much cellular material and/or debris is present on the slide for the analyst to make a determination about the presence or absence of sperm, the following procedure may be performed:
    a. Centrifuge the sample to pellet any cells. Remove and save supernatant for p30 analysis.
    b. Wash the cell pellet 1x with 500ul of sterile water or PBS, pipetting the pellet up and down or vortexing.
    c. Centrifuge again to pellet any cells.
    d. Resuspend in 50 to 300ul of sterile water or PBS, again by pipetting up and down or vortexing.
    e. Use the new suspension to make another slide, repeating steps 2-5.

Interpretation

Sperm heads stain red and the tails stain green by this procedure. Identification may be confirmed without flagellum when 1 head is viewed. If the slide contains too much cellular material or debris for the analyst to determine the presence of absence of sperm, the analyst may then report the result as "inconclusive".

7.7 Presumptive Semen Test - Alternate Light Source

Scope

The Alternate Light Source (ALS) lamps are specially designed for detection of forensic stains, fibers, and fingerprints. These lamps provide intense light of specific wavelengths, usually through a hand-held wand.

Most dried semen stains on cloth are detectable visually because their color, off-white or yellow, is different from that of the material on which the semen has been deposited. On many substrates, however, semen stains are not readily visible. Under ultraviolet; (UV, <400 nm) or intense blue (450 nm) light, semen stains fluoresce. When irradiated with an ALS, semen stains typically appear fluorescent.

Untreated, dry bloodstains do not show significant fluorescence. However, luminescence can be induced chemically with Luminol (see Presumptive Blood - Luminol procedure).

Safety

Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Gloves must be worn during evidence handling. Clothing may protect unbroken skin; broken skin should be covered. Alternate light sources produce intense light, and some are capable of producing light in the ultraviolet range (below 400 nm). Do not look into the light wand. Wear UV glasses when using wavelengths below 400 nm.

Related Forms - Serology Worksheets

Equipment, Materials, and Reagents

- Alternate light source (ALS) set at 450 nm.
- orange and/or yellow glasses

Standards, Controls, and Calibration

An appropriate semen stain positive control (which will fluoresce) and a non-semen negative control (which will not fluoresce) must be performed with each use.

Procedure

1. Turn on the lamp and allow to warm up for a minimum of 5 minutes.
2. Put on the glasses.
3. Direct the ALS at the evidence.
4. Document areas of apparent fluorescence on worksheet and include in case folder**.**

Interpretation

Semen and saliva stains typically appear fluorescent under ALS.

7.8 Body Fluid Stain Extraction

Scope

Possible body fluid stains are removed from the substrate and dissolved for testing. The clear extract is used for p30 testing. It is also used to collect cellular debris from a stain for a spermatozoa examination. Extraction of DNA is described in different protocols and should not be confused with a body fluid stain extraction.

Safety

Body fluids and extracts may contain infective agents. Use universal precautions during evidence handling. Follow instructions for reagent preparation. Gloves must be worn during testing. Clothing may protect unbroken skin; broken skin should be covered.

Related Forms - Serology Worksheets

Equipment, Materials, and Reagents

- microcentrifuge tube
- spin basket
- fine forceps
- microcentrifuge
- vortex

- sterile diH_2O
- PBS - see Reagents section

Standards, Controls, and Calibration

See p30 testing and spermatozoa identification procedures.

Procedure

1. In a microcentrifuge tube, soak a portion of the dried body fluid stain (up to approximately 1 cm^2) or swab (approximately ¼ of swab(s)) in approximately 250 µl of PBS for 30 minutes - 24 hours, refrigerated. Alternately, the sample may be placed at room temperature in a sonicating water bath for 5-15 minutes.
2. Samples may be vortexed briefly.
3. The extract may be used for p30 testing. The cellular debris, collected by centrifugation for 3 minutes, may be used to make a microscopic sperm slide.
4. Store the extract refrigerated.

Interpretation

See p30 testing and spermatozoa identification procedures.

7.9 Trace Evidence Collection

Scope

This procedure may be used where necessary for the preservation of trace evidence prior to forensic biology analysis.

Safety

Observe standard laboratory precautions.

Related forms - Serology Worksheets

Equipment, Materials, and Reagents

- butcher paper
- tweezers
- plastic zipper bags

- sheet protectors
- clear packing tape
- spatula
- glassine or paper fold envelopes
- stereomicroscope or magnifying glass

Standards, Controls, and Calibration

Collection room doors should remain closed at all times. The rooms will be vacuumed and the floors damp mopped at least once per week when in use.

Procedure

Generally, for any method used, a clean sheet of butcher paper will be used on the table for each evidence package opened. For any method used, tables and tools will be cleaned with bleach solution before each use and between opening of each evidence package to remove any extraneous debris.

Evidence from victim(s) and suspect(s) will be examined in separate rooms to prevent cross contamination. The examiner will change lab coats between the examination of items from victim(s) and suspect(s). If separate rooms are not available, items from victim(s) and suspect(s) can be processed in the same room after a period of 7 days.

There are several options available for ensuring that trace evidence is properly collected/stored/preserved. These are as follows:

1. Visual examination: locate and remove trace evidence from the evidence item manually or with tweezers. Recovered trace will be placed in a suitable labeled container (zipper bags, vials, paper folds, or on tape) to prevent loss or contamination of the sample.
2. Tape Lifting: clear tape is pressed over the area potentially bearing trace evidence. This application is repeated until all areas of interest are covered. New tape should be used once the tape in current use loses its stickiness. Tape(s) will be placed on the inside surface of a clean cut open zipper bag or a sheet protector. The bag/protector will be labeled as to its contents.
3. Scraping: the evidence is either held or placed on a table rack over a clean sheet of butcher paper. A spatula is used to scrape the evidence in a downward motion until all areas of interest have been covered. Any debris is collected from the butcher paper and placed in a suitable container (zipper bags, vials, or paper folds) and labeled.

Notes should be made in the case folder as to whether trace evidence was collected. Examiners should not make any attempt to identify on their worksheets what was collected (i.e. hair, fiber, etc) unless they also possess current training in the disciplines of Trace Evidence.

Place all collected trace evidence in the original evidence container with the item from which it was collected.

8 Reagents

8.1 Acid Phosphatase (AP) Test Reagent

Scope

AP test reagent undergoes a color change (Brentamine reaction) in the presence of acid phosphatase, which is found in highest concentration in semen. Instructions for use and interpretation are in the AP Test protocol.

Specification

Acid phosphatase (AP)

Safety

α-Naphthyl acid phosphate, o-Dianisidine, sodium acetate and Aerosol are irritants. Avoid contact and inhalation. o-Dianisidine is a suspected carcinogen. Avoid contact and inhalation. Acetic acid causes burns and is extremely destructive to tissues of the upper respiratory tract, eyes, and skin. Use only in a chemical fume hood. Wear gloves, lab coat, and goggles during preparation. Gloves must be worn during use. Clothing may protect unbroken skin; broken skin should be covered.

Equipment and Supplies

- Graduated cylinder or conical tube with lid
- darkened or foil wrapped container for storage
- scale and weighing paper
- spatula

Reagents

All the chemicals necessary for brentamine blue spot test are provided as a mixed powder. Just add water as listed below to obtain a buffered (pH 5.0) reagent containing brentamine dye and alpha napthyl phosphate substrate.

- diH_2O
- AP test reagent - purchased

Procedure

1. Measure .26 g of the AP test reagent.
2. Add AP test reagent and 10 ml of diH_2O into cylinder or tube.
3. Shake until test reagent is completely dissolved in the water.

Testing, Storage, Expiration, and Disposal

Label with Specification above, initials, date prepared, and expiration date. Store in a darkened or foil wrapped container. Store refrigertated when not in use, for up to 5 days.

Dispose in regular sink, flushing with water.

8.2 Nuclear Fast Red Solution (NFR)

Scope

Nuclear Fast Red (or Kernechtrot) solution is used with picroindigocarmine in the staining of microscopic slides for spermatozoa examination.

Specification

Nuclear Fast Red solution (NFR)

Safety

Aluminum sulfate and Nuclear Fast Red are irritants and may be harmful by inhalation, ingestion, or skin absorption. Solid reagents must be handled in a chemical fume hood. Gloves must be worn during preparation and testing. Clothing should protect unbroken skin; broken skin should be covered.

Equipment and Supplies

- hot plate
- stir plate
- funnel and filter paper

Reagents

- aluminum sulfate $Al_2(SO4)_3$•n(H_2O)
- Nuclear Fast Red
- diH_2O

Procedure

1. Dissolve aluminum sulfate (5 gm) in hot diH_2O (100 ml).
2. Add Nuclear Fast Red (0.1 gm) and stir to dissolve.
3. Allow to cool. Filter.

Testing, Storage, Expiration, and Disposal

Minimum labeling includes specification above, initials, date prepared, and expiration date. NFR can be stored refrigerated or at room temperature for one year. Dispose in regular sink, flush with water.

8.3 Picroindigocarmine (PIC)

Scope

PIC solution is used with Nuclear Fast Red in the staining of microscopic slides for spermatozoa examination.

Specification

Picroindigocarmine (PIC)

Safety

Picric acid is flammable, toxic, and explosive when dry. Keep container tightly closed, and check at least every 6 months for sufficient water. Avoid contact with metals; forms very sensitive explosive metallic compounds. Contact of picric acid with concrete floors may form the friction-sensitive calcium salt. Indigocarmine is an irritant and may be harmful by inhalation, ingestion, or skin absorption. Reagents must be handled in a chemical fume hood. A lab coat and goggles must be worn during preparation. Gloves must be worn during preparation and testing. Clothing should protect unbroken skin; broken skin should be covered.

Equipment and Supplies

- stir plate

Reagents

- indigocarmine

- picric acid
- diH_2O

Procedure

Dissolve indigocarmine (1 gm) in picric acid solution (300 ml).

Testing, Storage, Expiration, and Disposal

Minimum labeling includes specification above, initials, date prepared, and expiration date. PIC can be stored refrigerated or at room temperature for one year. Dispose in regular sink, flush with copious amounts of water.

8.4 Phenolphthalein (PHT) Solution

Specification

Phenolphthalin (PHT) solution

Scope

PHT solution is used for presumptive blood identification because it oxidizes to pink phenolphthalein in the presence of heme and hydrogen peroxide.

Safety

PHT is an irritant. PHT may be carcinogenic and may cause reproductive disorders. Potassium hydroxide is corrosive. Ethanol is highly flammable. Avoid contact with or inhalation of powdered zinc.

Solid reagents must be handled in a chemical fume hood. Goggles and protective clothing must be worn during preparation. Gloves must be worn during preparation and use. Clothing may protect unbroken skin during use; broken skin should be covered.

Equipment and Supplies

- fume hood
- hot plate
- dark bottle for storage

Reagents

- phenolphthalein
- potassium hydroxide
- diH_2O
- powdered zinc
- 95% ethanol (Reagent Alcohol, Absolute)

Procedure

1. Dissolve phenolphthalein (2.0 g) and potassium hydroxide (20 g) in 100 ml diH_2O.
2. Heat with powdered zinc (20 g) until solution is colorless (2-3 hours).
3. Decant solution into dark bottle with a small amount of zinc. This is the PHT stock solution.
4. Prepare PHT working solution by mixing one part PHT stock solution with four parts ethanol.

Testing, Storage, Expiration, and Disposal

Minimum labeling includes specification above, initials, date prepared, and expiration date. Store PHT stock and working solutions refrigerated. PHT stock solution can be stored refrigerated for one year. PHT working solution can be stored refrigerated for one year. Positive and negative controls must be tested day of use.

Powdered zinc in contact with limited amounts of water liberates hydrogen, an extremely flammable gas. Store dry, and keep residues thoroughly wet until disposal. Either 1) carefully dry zinc residue in a chemical fume hood and dispose in a container to keep dry or 2) dispose in a container to keep very wet. Do not dispose of damp residue in the trash where generated heat could cause a fire.

Discard PHT working solution in sink, flush with water. Dispose of PHT stock solution in sink, flush with copious amounts of water.

8.5 Phosphate Buffered Saline (PBS)

Scope

Phosphate-buffered saline is used as an extraction agent for serological stains. A 1X solution consists of 137 mM sodium chloride, 2.7 mM potassium chloride, and 10 mM phosphate buffer (Na_2HPO_4 / KH_2PO_4) at pH 7.4.

Specification

Phosphate-Buffered Saline (PBS)

Safety

Gloves are recommended for preparation and use.

Equipment and Supplies

- Stir Plate

Reagents

- 10X PBS - purchased
- diH_2O

Procedure

Mix 100 mL 10X PBS with 900 mL diH_2O.

Testing, Storage, Expiration, and Disposal

Minimum labeling includes specification above, initials, date prepared, and expiration date. PBS can be stored refrigerated for one year. Discard PBS in regular sink, flush with water.

9 Reports

The notes and other documentation must support the conclusions of the examiner. The laboratory report must communicate both the analytical results and the conclusions of the examiner, conveying the essence of what he or she would say if asked for an expert opinion in court. Decisions may be made by police officers, attorneys and the courts based on the report alone without examiner clarification, so the report should be able to stand alone.

The report must contain the information required in the quality manual and DAB specifications including, but not limited to, case identifier, description of evidence, results and/or conclusions, date issued, and signature and title of the responsible person. Report results of all items; note if any items listed were not analyzed.

Examples of typical casework reports will be provided to examiners, to ensure consistency of reports released from the laboratory. However, not every situation can be represented in example reports, and unusual circumstances may require wording variations that accurately reflect the findings. When in doubt, consult another qualified examiner or supervisor.

9.1 Chain of Custody and Disposition of Evidence

List all barcodes on the evidence packaging in the chain-of-custody section of the report, including items that were not opened, analyzed, or inventoried. Report the disposition of all items as well.

9.2 Blood and Semen Examinations

Report the results of any presumptive tests performed, including ALS, AP, and PHT, as well as any confirmatory tests. If presumptive tests are positive but confirmatory tests are not performed, this fact should be reported.

For some items, such as clothing, it may be useful to report what area of the item was tested.

9.3 Trace Collection and Preservation

Report items from which trace evidence has been collected, and how/where that trace evidence has been stored. As trace evidence is often collected but not examined, instructions should be included in the report as to requesting trace analysis.

9A Activity Report guidelines for counting Serology examinations

For statistical purposes, laboratory cases are coded according to the Uniform Crime Reporting Statistics. For reports, code 01 is listed as "Attempted Murder", but it is included with "Murder" whenever statistical reports are generated.

Information related to the number of examinations performed during each analysis can be documented on the "Serology Examination Count Sheet" form. The following is a summary of how totals for this form should be calculated:

Category	Included in Count Sheet totals
Number of Items	Number of items listed on the report
Samples Analyzed	Number of items inspected/retained for evidentiary value (This would not include items labeled for identification purposes only)
Total Examinations	Sum of the number of exams listed below
ALS exams	Number of items viewed with ALS (excluding controls)
AP / PHT	Number of swabs/stains tested (excluding controls)
Sperm searches	Number of samples viewed
p30 / HemaTrace	Number of cards tested
Trace collection/observation	Number of items with trace collected or trace observed
Stereoscopic examination	Number of items viewed with a stereomicroscope
Bloodstain cards	Number of cards made
Swabbings/scrapings/cuttings	Number of swabs/scrapings taken by analyst; Number of cuttings retained

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