4 Reagent Quality Control

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 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 samples. Outdated reagents may be used for training purposes only, but must be clearly marked as such.

4.1 Chemical and Reagent Labels

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

Prepared reagent labels will include the reagent name, initials of preparer and expiration date. In general, most solutions prepared in the Biology section shall expire 1 year from the date of preparation. However, the expiration date of the overall reagent will be no later than the expiration date of the individual reagent with the nearest expiration date (unless otherwise specified). Additional information may be documented in a reagent log. The lot number contains the date prepared and the initials of the preparer, e.g. mmddyyinitials.

4.2 Quality Control Checks

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

- Acid Phosphatase Spot Test
- Alternate Light Source
- Luminol mixture
- Phenolphthalein test kit

The following shall be quality control checked to confirm they are working properly with each new lot received:

- ABAcard® HemaTrace® kits
- Phosphate Buffered Saline (1X)
- SERI Christmas Tree Stain
- SERATEC® PSA Semiquant test devices

4.2.1 Acid Phosphatase (AP) Spot Test

Scope

AP Spot Test solution 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 Spot Test procedure.

Safety

This reagent presents the following hazards: may cause cancer; irritant to eyes, respiratory system and skin; and corrosive (can cause burns). Wear gloves, lab coat, and mask during preparation and use. Broken skin should be covered.

Equipment and Supplies

- AP Spot Test premixed powder – purchased
- Darkened or foil wrapped container for storage
- Scale
- Sterile diH_2O
- Timer
- Known semen sample
- Known non-semen sample

Preparation

1. Measure 10mL of sterile diH_2O in an appropriate container.
2. Add 0.26g of the purchased AP Spot Test premixed powder.
3. Mix until AP Spot Test premixed powder is completely dissolved in water.

   Note: Volume may be smaller or larger than 10mL, but the amount of AP Spot Test premixed powder added should be adjusted accordingly.

Prior to use each day, the prepared AP solution must be subjected to the appropriate in-house quality control test as outlined below:

1. A known semen sample will be used as a positive control. This in-house-prepared sample is typically presented on filter paper.
2. An untreated piece of filter paper or swab moistened with sterile diH_2O will be used as a negative control. Ideally, the negative control substrate mimics your sample substrate as closely as possible.
3. Apply ~ 1 drop of prepared AP solution to both controls.
4. Observe samples for 60 seconds for a color change.
5. The known semen sample must give a strong purple color change indicating a positive result.

6. The untreated filter paper or sterile swab must have no color change indicating a negative result.

The results of each control test must be recorded on every applicable serology worksheet.

Storage, Labeling, and Expiration

Store prepared solution in a darkened or foil wrapped container. Label with reagent name, lot number, and expiration date. Reconstituted reagent is stable and sensitive for one day's use – make solution fresh daily. Store purchased powder frozen. Purchased powder may be used on casework samples up to the expiration date stated on the bottle.

4.2.2 Alternate Light Source

Scope

An Alternate Light Source is specially designed for detection of forensic stains, fibers, and fingerprints. Instructions for use and interpretation are in the Alternate Light Source procedure.

Safety

This instrument presents the following hazards: exposing the skin to the beam of light (directly from the unit) can cause burns and other skin damage. It is essential that proper eye protection be provided. Avoid looking directly at the light source, reflected, or refracted light. Remove all unnecessary reflective surfaces from exam room. Wear goggles, gloves, lab coat, and other proper laboratory attire.

Mini-Crimescope MCS-400 ALS: If the bulb fails (suddenly stops emitting light), it may leak vapors for a few minutes. To be safe, immediately leave the room and let the unit run (fans) for 5-10 minutes until vapors are vented.

Equipment and Supplies

- ALS set at 400 or 455 nm
- Orange and/or yellow glasses
- Known semen sample
- Known non-semen sample

Prior to use each day, the ALS must be subjected to the appropriate in-house quality control test as outlined below:

1. Turn on power switch and ensure the fans are working.
2. Wear the proper glasses.
3. Turn on lamp switch.
4. Darken the examination room.
5. Direct the ALS at the samples.
6. The known semen sample must fluoresce indicating a positive result.
7. The known non-semen sample must not fluoresce indicating a negative result.

When ready to shut down the ALS, turn off lamp switch and wait for the unit to cool down (~5 minutes). Then turn off the power switch.

The results of each control test must be recorded on every applicable serology worksheet.

Storage and Expiration

Store equipment in a clean, dry atmosphere. The bulb should be replaced after 3-3.5 years of non-regular usage.

4.2.3 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. Instruction for use and interpretation are in the Luminol procedure.

Safety

This reagent presents the following hazards: 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 hands after handling. Gloves must be worn during testing. Broken skin should be covered.

Equipment and Supplies

- Luminol powder: 3-aminophthalhydrazide
- Sodium carbonate
- Sodium perborate
- Sterile diH_2O
- Two darkened or foil wrapped spray bottles
- Scale
- Known blood sample
- Known non-blood sample

Preparation (Reagent A)

1. Measure 250 mL of diH_2O.
2. Add 2.5 g of sodium carbonate.
3. Mix until all of the powder is dissolved.
4. Add 0.5 g of luminol powder.
5. Mix until all of the powder is dissolved.

Preparation (Reagent B)

1. Measure 250 mL of diH_2O.
2. Add 6.5 g of sodium perborate**.**
3. Mix until all of the powder is dissolved.

Mix equal volumes of Reagents A and B to create a luminol mixture. The mixture is unstable and must be prepared fresh immediately before use; it is only stable for about one hour.

Prior to use, the luminol mixture must be subjected to the appropriate in-house quality control test as outlined below:

1. Darken the examination room as completely as possible.
2. Spray luminol mixture onto a known blood sample (prepared in-house).
3. Spray luminol mixture onto a known non-blood sample (cotton swab or filter paper).
4. Observe samples for immediate luminescence.
5. The known blood sample must give an immediate luminescence indicating a positive result.
6. The cotton swab or filter paper must not produce luminescence indicating a negative result.

The results of each control test must be recorded on every applicable serology worksheet.

Storage, Labeling, and Expiration

The luminol powder, sodium carbonate and sodium perborate should be stored in a cool, dry, well-ventilated area away from incompatible substances. The expirations for reagents A and B (stored separately) are 8 weeks from date solutions were prepared. If stored, the labels must include, at a minimum, "Luminol, Reagent A" or "Luminol, Reagent B" as appropriate, initials, the date prepared, and the expiration date. Prior to each use, check the reagents for precipitate. If a precipitate has formed in either reagent, ensure that the precipitate is back in solution before use. Luminol mixture should be labeled with the appropriate lot number and expiration date. The mixture will expire one hour after use.

4.2.4 Phenolphthalein Test Kit

Scope

PHT Test Kit Solutions B and C are used for presumptive blood identification by oxidizing phenolphthalin (colorless in a basic solution such as the test reagent) to phenolphthalein (pink) in the presence of heme and hydrogen peroxide. Instructions for use and interpretation are in the phenolphthalein test kit procedure.

Safety

This reagent presents the following hazards:

Solution B: Flammable. Handle with care. Harmful or fatal if swallowed, inhaled, or absorbed through skin. Avoid contact with eyes, skin, and clothing. Light sensitive! Avoid light, heat, sparks, and flame. Keep container tightly closed.

Solution C: Handle with care. May be harmful if swallowed, inhaled, or absorbed through skin. Avoid contact with eyes, skin, and clothing. Avoid heat, sparks, and flame. Keep container tightly closed.

Wear gloves, lab coat, and mask during preparation and use. Broken skin should be covered.

Equipment and Supplies

- Solution B
- Solution C
- Sterile diH_2O
- Sterile cotton swab(s)
- Known blood sample
- Known non-blood sample

Prior to use each day, PHT Solutions B and C must be subjected to the appropriate in-house quality control test as outlined below:

1. Rub a cotton swab moistened with sterile diH_2O on a known bloodstain sample. This swab will be used as a positive control.
2. An untreated cotton swab moistened with sterile diH_2O will be used as a negative control.
3. Apply ~1 drop of Solution B to each swab.
4. Apply ~1 drop of Solution C to each swab.
5. Observe the swabs for a color change.
6. The cotton swab with the known bloodstain sample must give an immediate pink color change indicating a positive result.
7. The untreated cotton swab must have no color change indicating a negative result.

The results of each control test must be recorded on every applicable serology worksheet.

Storage, Labeling, and Expiration

PHT test kits can be stored at room temperature. Each analyst will have an aliquot of Solutions B and C that will be placed in amber bottles stored at room temperature or refrigerated. Each bottle will be labeled with appropriate reagent name, lot numbers, and expiration dates. The kits may be used on casework samples up to the expiration date stated on the bottles.

4.2.5 ABAcard® HemaTrace® Test Devices

Scope

ABAcard® HemaTrace® test kits are used to determine the presence of human blood in suspected blood stains. Instructions for use and interpretation are in the ABAcard® HemaTrace® procedure.

Safety

Wear gloves, lab coat, mask, and other proper laboratory attire. Broken skin should be covered.

Equipment and Supplies

- ABAcard® HemaTrace® test device(s)
- ABAcard® HemaTrace® extraction buffer
- Pipette and pipette tips
- Known blood sample
- Timer

Each new lot of ABAcard® HemaTrace® test kits 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 extraction buffer provided in kit.
2. Add 150µl of dilution to sample well "S" of one test device to be used as a positive control.
3. Add 150 µl of extraction buffer from kit to sample well of second test device to be used as a negative control.
4. Read results up to 10 minutes from application of sample.
5. The 1:10^6 dilution of known human blood must give a positive result. The extraction buffer provided in the kit must give a negative result.

Storage, Labeling, and Expiration

The test devices must be stored at room temperature (below 82ºF) and must remain in the sealed pouch until use. Label each test device with the name of the appropriate sample used. The test kits may be used on casework samples up to the expiration date stated on the box.

4.2.6 Phosphate Buffered Saline (1X)

Scope

PBS is used as an extraction agent for serological stains. Instructions for use and interpretation are in the Phosphate Buffered Saline procedure.

Safety

This reagent presents the following hazards: causes severe eye, respiratory tract and skin irritation. Avoid contact with skin and clothing. Wear gloves, lab coat, and other proper laboratory attire. Broken skin should be covered.

Equipment and Supplies

- OmniPur 10X PBS Premixed Powder
- diH_2O
- Graduated cylinder
- Two sterile containers for storage

Preparation (10X)

1. Measure 1 liter of diH_2O.
2. Add 100g of 10X PBS Premixed Powder.
3. Mix until all of the powder is dissolved.
4. Autoclave solution.

Preparation (1X)

1. Measure 100mL of 10X PBS solution.
2. Measure 900 mL of sterile diH_2O.
3. Mix 10X PBS and sterile diH_2O.

Each new lot of 1X PBS must be subjected to an appropriate in-house quality control test with the lot of SERATEC® PSA Semiquant test currently in use. See SERATEC® PSA Semiquant test quality control procedure for further detail.

Storage, Labeling, and Expiration

1X and 10X PBS solutions can be stored at room temperature. Label each bottle with appropriate concentration, lot number and expiration date. Expiration for both PBS concentrations is one year from the date 10X PBS solution was prepared. Both concentrations of PBS may be used on casework samples up to this expiration date.

4.2.7 SERI Christmas Tree Stain

Scope

SERI Christmas Tree Stain is a Kernechtrot-Picroindigocarmine differential biological stain that assists in scanning slides for the presence of spermatozoa. The test consists of a two stain method which dyes sperm heads red, tails green and epithelium green or blue with red nuclei. Instructions for use and interpretation are in the SERI Christmas Tree Stain procedure.

Safety

This reagent presents the following hazards: May cause irritation upon contact. Toxic. Do not swallow. Do not inhale. Avoid contact with skin and eyes. Wear gloves and proper laboratory attire. Broken skin should be covered.

Equipment and Supplies

- Microscope slide
- Known semen sample
- Pipette and pipette tips
- Heat block
- Christmas Tree Stain - Solution A and Solution B
- Timer
- Tap water
- 95% ethanol
- Compound microscope

Each new lot of SERI Christmas Tree Stain must be subjected to the appropriate in-house quality control test as outlined below:

1. Pipette 10 µl of a neat in-house sample onto a glass slide. Fix smear by heating the slide on the heat block until dry.
2. Stain the slide.
    a. Cover the sample area on the slide with Solution A for 10 minutes.
    b. Wash with tap water by gentle flooding.
    c. Cover the sample area with Solution B for 15 seconds.
    d. Wash with tap water by gentle flooding.
    e. Flood the slide with 95% ethanol and allow the slide to dry for at least 5 minutes on the heat block.
3. Examine the slide for the presence of spermatozoa at 1000X magnification. Sperm heads must be stained red, and any observed tails must be stained green indicating a positive result.

Storage and Expiration

The stain must be stored at room temperature. The stain may be used on casework samples up to the expiration date stated on the bottles.

4.2.8 SERATEC® PSA Semiquant Test Devices

Scope

SERATEC® PSA Semiquant test devices are used to indicate the possible presence of p30 in suspected semen stains. Instructions for use and interpretation are in SERATEC® PSA Semiquant test procedure.

Safety

Wear gloves, lab coat, mask, and other proper laboratory attire. Broken skin should be covered.

Equipment and Supplies

- SERATEC® PSA Semiquant test device(s)
- 1X PBS
- Pipette and pipette tips
- Known semen sample
- Timer

Each new lot of SERATEC® PSA Semiquant 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 1X PBS.
2. Add 120 µl of dilution to sample well of one test device to be used as a positive control.
3. Add 120 µl of 1X PBS to sample well of second test device to be used as a negative control.
4. Read results up to 10 minutes from application of sample.
5. The $1:10^5$ dilution of known semen must give a positive result. The 1X PBS must give a negative result.
6. Mark the stock solution bottle of 1X PBS with all lot numbers of SERATEC® PSA Semiquant test devices quality control checked with that lot of 1X PBS.

Storage, Labeling, and Expiration

The test devices must be stored at room temperature and must remain in the sealed pouch until use. Label each test device with the name of the appropriate sample used. The test devices may be used on casework samples up to the expiration date stated on the sealed pouch.

4.3 Logbook

Reagent preparation logs should include the lot number, the reagent name, the initials of preparer, and the expiration date. Quality control logs for ABAcard® HemaTrace® kits, Phosphate Buffered Saline (1X), SERI Christmas Tree Stain, and SERATEC® PSA Semiquant tests will also contain the test date, signature of the analyst performing the quality control, a second reader signature, if applicable, and date and any supporting documentation necessary to demonstrate the reagent met all of the standards.