Procedure for Body Fluid Unit Quality Control

1.0 Purpose - This procedure specifies the required elements needed for the preparation and quality control measures for reagents used for body fluid identification.

2.0 Scope - This procedure applies to those Forensic Scientists qualified to perform all body fluid identification tests and those Forensic Scientists qualified to perform only presumptive tests for blood.

3.0 Definitions

- **Commercial Reagent** – A commercially produced laboratory reagent designed to conduct a specific forensic test. All commercial reagents shall have an expiration date established by the manufacturer; if no expiration date is provided, the Forensic Biology Section shall establish the expiration date.
  See **5.5** for specific expiration dates. Expiration dates shall be written on bottles/containers by the Section employee who receives the commercial reagent.
- **Critical Reagent** - determined by empirical studies or routine practice to require reliability testing on established samples before use on evidentiary samples. These reagents are listed below:
  Acid Phosphatase Test working solution
  Kernechtrot stain
  Phenolphthalein
  Picroindigocarmine stain
  RSID test kits (blood, semen, and saliva)
- **Negative Control** – A sample which knowingly does not contain the substance being tested for; reagents and substrates used for the test being performed.
- **Positive Control** – A sample which knowingly contains the substance being tested for.

4.0 Equipment, Materials and Reagents

Balance, culture tube, culture tube safety closure, graduated cylinder, magnetic stirrer/hot plate, magnetic stir bar, 3 liter beaker, coffee filter or filter paper, known human blood standard, known human semen standard, known human saliva standard, known animal blood standard, deionized water, round bottom flask, reflux apparatus, reagent storage container, 30 % hydrogen peroxide, picric acid, 100 % ethyl alcohol, acetic acid, sodium acetate, aluminum sulfate, phenolphthalein, zinc dust, indigo carmine, nuclear fast red, sodium hydroxide pellets, alpha-naphthyl phosphate, Fast Blue B (ortho-dianisidine), RSID (Rapid Stain Identification) test kits with associated universal buffer

5.0 Procedure

5.1 Reagent containers and chemicals shall be labeled according to the State Crime Laboratory Procedure for Procurement and Receipt.

5.2 A record shall be maintained for each reagent prepared and/or quality control (QC) checked, reflecting the following:

- Name of the reagent.
- Initials of the analyst who prepared the reagent.
- Date prepared.
- Expiration date.
- Results of the applicable quality control check.
- Lot number of ingredients in the reagent.

- Total volume prepared.

5.3 pH test strip - pH test strips used in the preparation of reagents shall be checked for reliability before use with a standard pH solution close to the desired pH of the buffer or other reagent being prepared.

5.4 QC Check of Critical Reagents

5.4.1 Each critical reagent with a new lot number shall be tested for reliability before being placed into use in forensic casework. A known positive and negative control shall be used for this test.

5.4.2 The QC check shall be performed as stated in the applicable technical procedure.

5.4.3 The test conducted on the positive control must show the appropriate positive results and the test conducted on the negative control must show the appropriate negative result.

5.4.4 Upon successful completion of testing, each bottle shall be labeled QC checked, dated, and initialed and released for forensic testing.

5.4.5 If the correct results are not obtained, the quality control test shall be repeated using a different known positive control.

5.4.5.1 If the correct results are obtained with the second test, refer to **5.4.4**.

5.4.5.2 If the correct results are not obtained with the second test, the critical reagent bearing that lot number shall not be released for forensic testing and a critical reagent with a new lot number shall be prepared or requested from the manufacturer.

5.4.6 Record the lot number, initials, date tested, and quality control results in the applicable chart and in Forensic Advantage (FA).

5.5 Expiration Dates for Commercial Reagents Without Manufacturer-Provided Dates:

5.5.1 The following reagent shall have an expiration date set 5 years from date of receipt or preparation within the Forensic Biology Section:

- Ethyl alcohol, 200 proof, anhydrous, 99.5+%

5.6 Reagents used in the Kastle-Meyer Test Procedure

5.6.1 3% Hydrogen Peroxide Dilution and Expiration

5.6.1.1 Measure out 900 mL of deionized water.

5.6.1.2 Measure out 100 mL of a 30 % stock hydrogen peroxide solution and add it to the water.

5.6.1.3 Mix thoroughly until in solution.

5.6.1.4 Place the solution in a labeled bottle.

5.6.1.5 Record the appropriate preparation information in the applicable chart and in Forensic Advantage (FA).

5.6.1.6 The solution expires 12 months after dilution.

5.6.2 Phenolphthalein preparation and Expiration

5.6.2.1 Measure out and add 1 liter of deionized water to a 3 liter beaker.

5.6.2.2 Measure out and add 40 g of NaOH pellets to the deionized water.

5.6.2.3 Stir on stirring plate with a magnetic stir bar and slight heat until dissolved.

5.6.2.4 Measure out and add 4 g of phenolphthalein to the beaker. Stir until dissolved.

5.6.2.5 Measure out and add 20 g of zinc dust to round bottom flask.

5.6.2.6 Transfer phenolphthalein, NaOH, and water mixture to the round bottom flask.

5.6.2.7 Prepare reflux apparatus and connect round bottom flask.

5.6.2.8 Turn on the water supply to the reflux apparatus. Turn on the transformer and set the voltage to approximately 90 V.

5.6.2.9 Reflux the mixture until the solution is colorless (usually about 4 hours).

5.6.2.10 Remove the mixture from the reflux apparatus and allow cooling to room temperature.

5.6.2.11 Decant the reagent and bring the volume to 1200 mL with ethanol.

5.6.2.12 Add zinc dust to cover the bottom of the amber jar and pour the reagent into the amber jar. Label, date, and initial the container.

5.6.2.13 Zinc used in preparation of the Phenolphthalein reagent shall be disposed of in the trash.

5.6.2.14 After the reagent has been prepared, QC check according to **5.4**.

5.6.2.15 The stock solution expires 6 months after preparation.

5.7 Reagents used in the Sperm Identification Procedure

5.7.1 Kernechtrot Stain Preparation and Expiration

5.7.1.1 Measure out and add 3000 mL of deionized water to an appropriately sized beaker.

5.7.1.2 Measure out and add 150 g aluminum sulfate to deionized water.

5.7.1.3 Stir on stirring plate with a magnetic stir bar until dissolved.

5.7.1.4 Measure out and add 3 g of Nuclear fast red to beaker and stir using slight heat until thoroughly dissolved.

5.7.1.5 If needed, bring to 3000 mL volume with deionized water.

5.7.1.6 Filter the solution using coffee filter or filter paper.

5.7.1.7 Store in a labeled 4 liter container.

5.7.1.8 After the stain has been prepared, QC check according to **5.4**.

5.7.1.9 The solution expires 12 months after preparation.

5.7.2 Picroindigocarmine Stain Preparation and Expiration

5.7.2.1 Measure out and add 2500 mL of deionized water to a 3 liter beaker.

5.7.2.2 Measure out and slowly add 40 g of picric acid to deionized water.

5.7.2.3 Stir on stirring plate with a magnetic stir bar and slight heat until dissolved.

5.7.2.4 Measure out and add 10 g of indigo carmine to beaker. Stir until thoroughly mixed.

5.7.2.5 Bring volume to 3000 mL with deionized water.

5.7.2.6 Using coffee filter or filter paper, filter solution and store in a labeled 4 liter container.

5.7.2.7 After the stain has been prepared, QC check according to **5.4**.

5.7.2.8 The solution expires 12 months after preparation.

5.8 Rapid Stain Identification (RSID) Kits

5.8.1 RSID kits shall be QC checked prior to use in forensic casework according to **5.4**.

5.9 Reagents used in the Acid Phosphatase Test (Walker Test) Procedure

5.9.1 Solution A Preparation

5.9.1.1 Measure out and add 10 mL of deionized water to a beaker.

5.9.1.2 Measure out and add 1.2 g of sodium acetate, anhydrous to beaker.

5.9.1.3 Measure our and add 0.1 g of ortho-dianisidine, tetrazotized (Brentamine Fast Blue B) to beaker.

5.9.1.4 Measure out and add 1 mL of glacial acetic acid to beaker.

5.9.1.5 Stir until thoroughly mixed.

5.9.1.6 Any remaining solution A shall be discarded after the working solution has been prepared.

5.9.2 Solution B Preparation

5.9.2.1 Measure out and add 1.0 mL of deionized water to a beaker.

5.9.2.2 Measure out and add 0.08 g of α-naphthyl phosphate, disodium salt to beaker.

5.9.2.3 Stir until thoroughly mixed.

5.9.2.4 Any remaining solution B shall be discarded after the working solution has been prepared.

5.9.3 Working Solution Preparation and Expiration

5.9.3.1 Measure out an add 10 mL of solution A to beaker.

5.9.3.2 Measure out an add 1 mL of solution B to beaker.

5.9.3.3 Measure out an add 89 mL of deionized water to beaker.

5.9.3.4 Stir until thoroughly mixed.

5.9.3.5 After the working solution has been prepared, it must be QC checked according to **5.4**.

5.9.3.6 Aliquot into 5 mL amber culture tube, or equivalent and cap with safety closure. Label, date, and initial container(s).

5.9.3.7 Acid Phosphatase working solution is light sensitive. If aliquots are not stored in amber culture tubes, the aliquot shall be wrapped with aluminum foil when removed from freezer for use.

5.9.3.8 Prior to use, the working solution must be allowed to come to room temperature.

5.9.3.9 The aliquots expire 1 year after preparation when stored in the freezer.

5.9.3.10 Aliquots removed from freezer for use shall be discarded at the end of each working day.

5.10 Storage

5.10.1 The following reagents must be stored frozen.

5.10.1.1 Acid Phosphastase working solution aliquots.

5.10.2 The following reagents must be stored refrigerated at 4 °C.

5.10.2.1 Alpha Naphthyl Salt.

5.10.2.2 Fast Blue B.

5.10.2.3 Phenolphthalein stock solution.

5.10.2.4 Universal buffers for RSID kits.

5.10.3 The following shall be stored at room temperature.

5.10.3.1 3 % hydrogen peroxide.

5.10.3.2 Kernechtrot Stain.

5.10.3.3 Picroindigocarmine stain.

5.10.3.4 RSID kits.

5.10.4 Individual Forensic Scientists shall obtain personal aliquots of phenolphthalein. These may be stored at room temperature for 1 month.

6.0 Limitations

6.1 See **5.0** for limitations specific to each reagent and/or chemical.

6.2 Quality control tests may be performed on only one new reagent at any given time (i.e., new lots of both Kernechtrot Stain and Picroindigocarmine Stain cannot be quality control checked at the same time).

7.0 Safety

7.1 See safety documents for appropriate safety precautions.

7.2 See MSDS Sheets.

7.3 Picric acid is explosive if allowed to dry. Therefore ensure liquid that picric acid is packaged in remains covering the reagent.

8.0 References

State Crime Laboratory Safety Manual

State Crime Laboratory Quality Manual

Forensic Biology Section Body Fluid technical procedures

9.0 Records

- Completed forms for the verification and preparation of reagents including:
  3 % Hydrogen Peroxide
  Acid Phosphatase working solution
  Kernechtrot stain
  Phenolphthalein
  Picroindigocarmine stain
  RSID test kits

- Forensic Advantage electronic resource manager entries

10.0 Attachments - N/A

Revision History		
Effective Date	Version Number	Reason
09/17/2012	1	Original Document
12/18/2013	2	Header – added issuing authority
02/27/2015	3	3.0, 4.0, 5.10, 5.11 – removed references to Phadebas testing and QC