

FORENSIC BIOLOGY SEROLOGY MANUAL

Approving Authority:
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Procedure	Effective Date	Revision History
General Guidelines	3/24/2010	Initial Version of Procedure
Processing of Postmortem Specimens	3/24/2010	Initial Version of Procedure
Bloodstain Preparation from Whole Blood	3/24/2010	Initial Version of Procedure
Kastle-Meyer (KM) Presumptive Test for Blood	3/24/2010	Initial Version of Procedure
Acid Phosphatase Presumptive Test for Semen	3/24/2010	Initial Version of Procedure
Amylase Diffusion Presumptive Test for Saliva	3/24/2010	Initial Version of Procedure
Christmas Tree Stain for Spermatazoa	3/24/2010	Initial Version of Procedure
P30 ELISA	3/24/2010	Initial Version of Procedure
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AMYLASE DIFFUSION PRESUMPTIVE TEST FOR SALIVA

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Preparing Amylase Plate

1. Prepare starch-containing agarose gel by adding the ingredients listed below:

100 ml batch size (enough for 2 plates)

1.0g Sigma Type I agarose

0.1g potato starch

100mL amylase gel buffer

To dissolve, mix and boil this solution. Allow to slightly cool. Pour 40mL each into a 10 x 10cm disposable Petri dishes. Avoid air bubbles as much as possible. Scale up batch size when necessary.

2. Punch wells in the gel using the suction tube apparatus, leaving at least 1.5cm between wells. Use Amylase sheet as a template.
 - Make sure that the holes that you create are completely clean of agar debris and residual liquid. This can be ensured by punching each hole twice in succession.
 - Following this protocol and with an accurate dispersion of agarose will guarantee an adequate amount of space for the loading of 10uL each of standard, control, or sample into each well.
3. Use Parafilm® around the lid/bottom joint to seal the amylase plates.
4. Store in a 4°C refrigerator upside-down (resting on the lid) to avoid condensation on the gel.
5. Pre-made plates are good for one week.

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Preparing Standards/Samples

1. Extract approximately a 5 x 5mm stain or a portion of a swab in 100uL deionized water for 30 minutes at room temperature using the pipette tip and test tube method. For samples that have been analyzed with P30 ELISA, use the extracts prepared in that procedure.
2. Prepare α -amylase standards containing 0.02 and 0.002 units each per 10 uL of deionized water (dH₂O) from purchased amylase.
 - A. Prepare 1mL of 20 units/10uL amylase by adding the appropriate amount of amylase standard to dH₂O. The appropriate amount of amylase standard to add is determined by the QC of the current lot of amylase. See example calculation below.
 - B. Continue to prepare the remaining 2, 0.2, 0.02, and 0.002 unit standards by doing ten-fold serial dilutions. This is easily accomplished by first adding 900uL of dH₂O to each of 4 microcentrifuge tubes. Then transfer 100uL of your 20 unit standard into one of the tubes containing 900uL of dH₂O. This is your 2 unit standard. Continue making the remaining dilutions in the same manner.

When doing serial dilutions, make sure to mix each standard well before each subsequent transfer. Use a fresh unplugged pipette tip for each transfer.

Sample calculation:

Given a specific activity of 870 units amylase/mg total protein (from vendor) with a total protein concentration of 30 mg/mL, then:

$$\frac{870 \text{ units amylase}}{\text{mg total protein}} \times \frac{30 \text{ mg total protein}}{\text{mL of solution}} \times \frac{1 \text{ mL}}{1000 \text{ uL}} = 26.1 \text{ units amylase/uL}$$

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Use this value in the equation $C_1 \times V_1 = C_2 \times V_2$ where C_1 and C_2 are concentrations of solutions 1 and 2, while V_1 and V_2 are volumes of solutions 1 and 2. In this case, solution 1 is the vendor amylase stock solution while solution 2 is the 20 units standard in preparation:

$$(26.1 \text{ units amylase/uL})(x \text{ uL}) = (20 \text{ units/10uL})(1000\text{uL})$$

Solving for $x = 77\text{uL}$ of vendor amylase stock solution
 1000uL (total volume) – 77uL (amylase stock solution) = 923uL of dH_2O .

Loading/Incubation/Staining of Amylase Plates

1. Have a witness verify the Amylase Worksheet with the tube labels.
2. Fill wells according to the Amylase Diffusion Worksheet (10 uL each well) with standards, negative control (deionized water), and samples. The first two wells are reserved for the 0.02U and 0.002U amylase standard, the negative control is added to the third well, and the remaining wells are filled with samples.
3. Incubate 5-8 hours at 37°C or 12-16 hours at room temperature; keep the plate in a humid chamber to avoid drying.
4. Pour a 0.01N (100-fold dilution of a 1N stock) iodine solution onto the gel; clear areas indicate regions of amylase activity. **Do not over stain the plate.** Do this by monitoring the plate as it is staining; pour off the iodine solution when a sufficient amount of staining has occurred so that all the standards are clearly visible.
5. Photograph the results via the Mideo System. Ensure there is a scale in the photograph.
6. Measure the diameter of the clear areas and record on the worksheet.

Entire items (blood spatter patterns, etc.) can be tested for amylase. Prepare a large plate on a bordered glass plate (scale up reagents) and allow to solidify; bring item (or area of item) into contact with the gel for 5 minutes. Follow steps 3-6 above to visualize any amylase pattern.

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Interpretation of Results

The values of diffusion for the 0.02 and 0.002 unit standards should fall in the ranges of 7-15 and 4-10 mm, respectively. In addition, the amount of diffusion of the 0.02 unit standard must be greater than that of the 0.002 unit standard.

The interpretation of amylase results depends on the source of the sample:

1. Body cavity swabs (e.g., vaginal and anal) are positive if the diameter is equal to or greater than the diameter of the 0.02 U standard. Designate as orifice (“O”) on amylase worksheet.
2. Samples not from a body cavity (e.g., penile swabs, cigarette butts, cups, etc.) are positive if the diameter is equal to or greater than the 0.002U standard. Designate as external (“E”) on amylase worksheet.
3. The location from which a “dried secretion” swab is taken affects the interpretation. Swabs taken essentially from a body cavity or similar place (e.g., introitus, etc.) are interpreted as if the sample is from a body cavity. Other locations (e.g., breast, thigh, penis, etc.) may need to be interpreted differently.

Below is a general list of common sample types and designation as **body cavity/orifice** samples:

ANUS	OUTSIDE/OUTER ANUS
EXTERNAL GENITALIA (female)	OUTSIDE/OUTER VAGINA/L
EXTERNAL VAGINA/L	PERIANAL
FOURCHE	PERINEAL
INTROITUS	PERINEUM
LABIA MAJORA	PERIORAL
LABIA MINORA	PERIVAGINAL
LIPS	VESTIBULE
MOUTH	VULVA

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Below is a general list of common sample types and designation as **external** samples:

BUTTOCKS
CHEEK
CHIN
GROIN
SCROTUM

INGUINAL
INNER THIGH
MONS VENEVIS (mons pubis)
PENIS

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ACID PHOSPHATASE PRESUMPTIVE TEST FOR SEMEN

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GENERAL

An Acid Phosphatase test is a presumptive test for semen. It may be performed directly on a cut out portion of a stain, an extract of a stain, or a “wipe” of the stained material. A wipe may be made using a piece of filter paper, thread, or swab. Wet the wipe with water, then rub over the stained area while still wet.

CONTROLS

Analysts using Acid Phosphatase test reagents must test each lot/aliquot of reagent at least once per day, using positive and negative controls, before any evidence items are tested. The results of this test shall be recorded in the case notes. Semen must be used as a positive control. A drop of deionized water may be used for the negative control. If controls do not pass, inform the Quality Assurance Team immediately.

PROCEDURE

1. Apply a drop of the Alpha-Naphthyl Phosphate reagent; wait 60 seconds.
If a purple color occurs at this point, the testing results should indicate “inconclusive.”
2. Apply a drop of the Fast Blue B reagent. An immediate purple color is a positive reaction.

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BLOODSTAIN PREPARATION FROM WHOLE BLOOD		
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Staincards are prepared from all vouchered blood samples and from post-mortem blood samples:

1. Find the case files and obtain the blood vials; sign the chain of custody forms.
2. Prepare the UltraSTAIN™ cards by affixing a pre-printed FB case number sticker (if available) and writing in the following:
 - Subject's name
 - Date
 - Initials of person preparing the stain
 - FB number, if no sticker is available

Wear latex gloves when handling these cards.

3. Preparation of the bloodstain **must** be witnessed by another laboratory staff member. After each stain is made, the witness must initial the stain card and the evidence packaging worksheet. The witness must confirm that the processor is handling the correct blood vial and stain card **BEFORE** the stain is made.
4. Prepare stains one at a time. Staining of the cards and the opening of liquid blood samples **MUST** be performed under a biological safety cabinet with the exhaust fan operating. It is advisable that a new KimWipe™ be used to open each vial stopper. Make sure a blood tube is closed before preparing the next stain.
5. Fold back the paper "flap" and make four stains on the card, placing the blood in the outlined areas. Use four drops of blood per area; apply the drops slowly, allowing them to soak in. This will prevent appreciable transfer to the paper "flap".
6. Bring down the paper "flap", turn the entire card over, and allow it to air-dry upside down. The stain cards must be allowed to dry overnight before storage.
7. Package the air-dried stains into a 4x6" KAPAK™ bag. Heat seal the KAPAK™, the person sealing the bag must date and initial the bag. Store at room temperature. Continue the chain of custody to reflect their final location.

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8. **CLEAN THE BIOLOGICAL SAFETY CABINET (refer to QC Procedure #QC125 of the Quality Assurance/Quality Control Manual).**

9. Place all case files that contain **any** sexual assault evidence in the designated area so that they may be processed. Place all cases files that contained any evidence from the NYPD or DA's office back from where they were retrieved (either "cases to be called on," "cases to be assigned," or the assigned analyst). Place all remaining case folders in the Forensic Biology office so that they may be filed.

10. Disposal of blood and blood vials:

For non-vouchered blood, the remainder of the liquid blood and the blood vial will be discarded immediately. Purple-topped vials **must** be discarded in a plastic BIOHAZARD "sharps" container.

For vouchered blood, the remainder of the liquid blood is discarded into bleach immediately after making the bloodstain card. The empty vial rinsed with 10% bleach. The empty vial is packaged for return to the Evidence Unit.

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CHRISTMAS TREE STAIN FOR SPERMATOZOA		
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The nuclear material within the cell is stained red by the Nuclear Fast Red stain. Sperm heads are usually well differentiated with the acrosome staining significantly less dense than the distal region of the head. Epithelial membranes and sperm tails are stained green by the Picric Indigo Carmine (PIC) stain; nuclei inside epithelial cells appear purple. Yeast cells also stain red, however the stain is uniform throughout the cell and extends into polyp-like structures that are occasionally seen in yeast.

Reagents: Nuclear Fast Red and Picric Indigo Carmine

1. Fix cells to the slide by heating (approximately 5 to 10 seconds).
2. Cover cell debris with Nuclear Fast Red stain and allow to sit for at least 10 minutes.
3. Wash away the nuclear fast red with deionized water.
4. Add PIC stain to the still-wet slide; allow to sit for no more than 30 seconds.
5. Wash away the PIC stain with ethanol.
6. Place slide over a heat source to complete drying.
7. Examine the slide at 100X or 400X (don't use immersion oil).

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GENERAL GUIDELINES		
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1. The procedures within this Serology Procedures Manual are intended to support the processes outlined in the Evidence Examination Procedure in the Evidence and Case Management Manual.
2. In general, screening tests and/or confirmatory tests are used to identify physiological fluids such as blood, semen, and saliva prior to further analysis.
3. All reagents are available pre-made and are quality control checked, where possible. Do not make your own or use supplies that have not been quality control checked. If reagents are needed, contact the Quality Assurance Unit for assistance.

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KASTLE-MEYER (KM) PRESUMPTIVE TESTING FOR BLOOD

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GENERAL

A Kastle-Meyer test may be performed directly on a cut out portion of a stain, an extract of a stain, or a “wipe” of the stained material. A wipe may be made using a piece of filter paper, thread, or swab. Wet the wipe with water, then rub over the stained area while still wet.

CONTROLS

Positive and negative controls must be used to test each lot/aliquot of reagent at least once per day and before any evidence items are tested. Blood must be used as a positive control. A drop of deionized water may be used for the negative control. If controls do not pass, inform the Quality Assurance Team immediately.

PROCEDURE

1. Apply a drop of KM reagent if using a wipe. If performing directly on a cut out portion of a stain, use enough until sample is covered. Observe any color change.

A normal color reaction is a greenish/gray tint with the presence of possible blood.

A PINK COLOR HERE IS DUE TO THE PRESENCE OF AN OXIDIZING AGENT (e.g., a chemical oxidant), NOT BLOOD. If a pink color occurs at this point, the testing results should indicate “inconclusive.”

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

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P30 ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA)		
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Solutions: Phosphate buffered saline (PBS)
PBS-Casein (PBS with 0.02% w/v casein)

Standards: P30 antigen [prostate specific antigen (PSA)] and phosphate buffered saline

Plates: Immulon II microELISA plates (microtiter plate)

Antibodies: Mouse monoclonal anti-human PSA (prostate specific antigen, P30)
Rabbit polyclonal anti-human PSA
Goat anti-rabbit IgG alkaline phosphatase conjugate
Mouse IgG1, Kappa chain (MOPC 21, mouse myeloma protein)

Note: Store all antibodies at 4°C.

SOLUTION PREPARATION

Phosphate buffered saline (PBS):

1. To prepare 1 liter, dissolve 5 tablets in 1 liter of deionized water. This can be stored at 4°C for up to 2 weeks.
2. On label write initials of preparer, the date of make (DOM) and the date of discard (DOD)

For each plate or pair of plates you will need 1 bottle (1 liter).

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

If preparing from Frozen Casein Aliquots:

1. Thaw casein at room temperature.
2. Dilute 20mL of casein stock solution to 1 liter of PBS.

If preparing from a Bottle of Liquid Stock Casein (Refrigerated):

1. Shake the bottle well.
2. Using a graduated cylinder, take 20mL of casein stock solution and dilute it into 1 liter of PBS.

One plate uses approximately 500mL.

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PLATE PREPARATION:

COATING THE PLATES

1. Prepare an appropriate dilution of mouse monoclonal anti-human PSA by adding the antiserum to PBS as determined by the QC of the current lot of antiserum. Each plate requires about 5mL of diluted antiserum. **Always make dilutions in glass, not in plastic.**
2. Prepare a 1/8000 dilution of MOPC by adding 10uL MOPC to 80 mL PBS. **Always make dilutions in glass, not in plastic.** This is enough for 16 plates. For 4 plates add 2.5uL MOPC to 20mL PBS.
3. Coat the plate as shown in the diagram. Use 100uL of the appropriate solution per well.

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

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

4. Cover plates with Parafilm® and label “Coating” with initials and date. Incubate overnight (approximately 17-20 hours) at 4°C.

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BLOCKING THE PLATES:

1. Prime/Rinse washer (See Rinsing and Priming procedure below.)
2. Use the “Block 1” program to aspirate contents of well. (This will wash each plate twice and fill the wells with wash buffer) Let the filled plate sit at room temperature for 15-20 minutes (see Block 1 procedure).
3. Use the “Block 2” program to aspirate the final wash (See Block 2 procedure).
4. Tap plate upside-down on paper towels to remove excess liquid.
5. Plates that are not used immediately should be wrapped in Parafilm® and stored at 4°C; they can be stored for up to 2 weeks.

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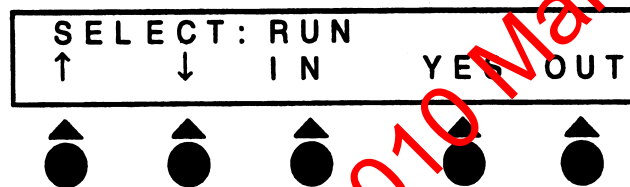
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Instructions for Bio-Rad 1575 washers

The Bio-Rad 1575 plate washer has a cover that can be swiveled to an opened or closed position.

Rinsing and priming

1. The on/off switch is located on the right-hand side of the lower back of the washer. Turn plate washer on.
2. After initialization (concurrent with a quick flash of a Bio-Rad version #), the **main menu** appears and looks like this:



The circles with the triangles above represent the soft keys that you will be pressing to access various functions on the machine. In this menu, soft keys corresponding to “**In**” and “**Out**”, when pressed instruct the plate washer to move the plate holder in and out of the washer, respectively. Soon after turning on the plate washer, the plate holder automatically sets itself in the out position.

3. Fill the **Rinse** bottle with deionized water, close the lid, and attach the tube with the blue colored line to the top of the container. Also, make sure that the stopper connected to the yellow and red colored lines is snugly connected to the **Waste** bottle (if not, the plate washer will not aspirate which can result in flooding of the plate holder).
4. Prime the washer with deionized water (from the **Rinse** bottle). Do this by pressing the very first soft key corresponding to the upward arrow in the **main menu**. Now you will see the **Prime/Rinse** menu. Press the soft key corresponding to “**YES**” and the washer will proceed to prime itself. This step is important to ensure proper vacuum pressure and for the washer to dispense correct volumes. *Always make sure to re-prime the washer whenever changing the blue colored tube from the **Wash** bottle to the **Rinse** bottle and vice versa.*
5. Fill the **Wash** bottle with PBS-casein solution. Remove the blue-striped tube from the **Rinse** bottle and attach it to the **Wash** bottle. Repeat the priming procedure 1 time using the PBS-casein solution from the **Wash** bottle. You are now ready to run the plate washer.

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Running plate washer programs

BLOCK 1

1. To start the blocking procedure, insert a coated plate into the plate washer lengthwise, so that plate well #A1 is located on the upper right portion of the plate
2. Proceed with the first portion of the blocking procedure in which the contents of the wells are aspirated, washed 2 times, and then filled with PBS-casein. The program that does this is called **BLOCK1**.
3. To access the **BLOCK1** program, press the soft key that corresponds to “**YES**” while in the main menu. You will now be in the **run menu**. While in the run menu, scroll to the **BLOCK1** program by pressing the soft keys that correspond to the upward and downward arrows.
 - Before proceeding, you should see “**RUN: BLOCK1**” on the first line of the display. Press the “**YES**” key.
 - You will then see “**LAST STEP 12**” on the first line of the display. Press the “**YES**” key again.
 - Usually you will then get one more prompt (“**CONNECT THE WASH R9**”) on the first line of the display. Press the “**YES**” key once again.

The program will then run on its own, leaving the wells filled at the end of the program.

4. Incubate the plate for 15-20 min. at room temperature as described in the P30 protocol.

BLOCK 2

5. If you have removed your plate for the incubation step, place plate back into the plate washer by using the “**IN**” and “**OUT**” keys from the main menu. The last step of the blocking procedure requires aspiration of the wells. This is achieved by the **BLOCK2** program. Access the **BLOCK2** program from the **run menu** as described in step 3 for the **BLOCK1** program. Before proceeding, you should see “**RUN: BLOCK2**” on the first line of the display. Run the **BLOCK2** program by pressing the “**YES**” key.

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WASH

6. The washing program required for the P30 run has been named **“WASH”**. The **WASH** program can be accessed from the **run menu** as described for the **BLOCK1** program above. Before running the **WASH** program, make sure that **“RUN: WASH”** appears on the first line of the display. Press the **“YES”** key to run the **WASH** program. This program will wash/aspirate the plate three times with PBS-casein.
7. When finished using the plate washer, remove plate from the washer. Replace it with the “test” plate, a dummy wash plate that is provided at each plate washing station.
 - Remove the blue colored tubing from the **WASH** bottle and attach it to the **RINSE** bottle.
 - Prime the machine with deionized water; access the priming command from the **main menu** as described above.
 - Run the **WASH** program so that the lines are thoroughly flushed with deionized water.
8. Turn off plate washer. Discard liquids from the **WASH** and **WASTE** bottles and rinse thoroughly with water. Loosely replace covers onto these bottles and leave them near the plate washer.
9. When the plate washer sits idle for about 10 min., it will display a **“PLEASE RINSE”** message. When you are ready to use the washer again, press the **“YES”** key to return to the **main menu**.

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SAMPLE AND STANDARDS PREPARATION:

Prepare samples and standards on the day of use.

- 1. Stains/swabs** Using the pipette tip/test tube method, extract 2.5 x 2.5 mm samples in 100uL of PBS for 30 minutes at room temperature. Centrifuge. Prepare a 1/25 dilution by adding 20uL sample extract to 480uL wash buffer (PBS-casein).

Cover and refrigerate the remainder of the sample extract until the ELISA is complete and/or ready for amylase analysis.

- 2. Standards** Prepare a 2ng/ml standard by first preparing a 10ng/mL solution followed by a 5-fold dilution of this solution as follows:

- 10 ng/mL: 50uL P30 (10ng/mL) + 5.0mL PBS-casein
- 2 ng/mL: 1000uL of 10ng/mL P30 standard + 4.0mL of PBS-casein

SAMPLE AND STANDARD APPLICATION:

1. Have a witness verify the worksheet and tube order. Apply PBS, PBS-casein, standards and samples as shown in the diagram. Use 100 uL of the appropriate solution per well.

	1	2	3	4	5	6	7	8	9	10	11	12
A	PBS	W	2ng	S2	S4	S6	S8	S10	S12	S14	S16	S18
B	PBS	W	2ng	S2	S4	S6	S8	S10	S12	S14	S16	S18
C	PBS	W	2ng	S2	S4	S6	S8	S10	S12	S14	S16	S18
D	PBS	W	2ng	S2	S4	S6	S8	S10	S12	S14	S16	S18
E	PBS	W	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19
F	PBS	W	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19
G	PBS	W	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19
H	PBS	W	S1	S3	S5	S7	S9	S11	S13	S15	S17	S19

PBS = phosphate buffered saline

W = PBS-casein

S = samples

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2. Cover the plates with Parafilm® and incubate at room temperature for 1 hour. Prime/Rinse washer.
3. Use the “Wash” program to aspirate contents of wells. Make sure that there is no excess buffer remaining in the wells by gently tapping the plate upside-down on papertowels.
4. For each plate, prepare a 1/1000 dilution of rabbit polyclonal anti-PSA by adding 10 uL antiserum to 10 mL PBS. For 2 plates add 20 uL antiserum to 20 mL PBS.
5. Apply 100 uL of the 1/1000 dilution of rabbit anti-human polyclonal anti-PSA to each well of columns 2-12. Apply 100 uL of PBS to each well of column 1.
6. Cover the plates with Parafilm® and incubate the plates at room temperature for 1 hour.
7. Use the “Wash” program to aspirate contents of wells. Make sure that there is no excess buffer remaining in the wells by gently tapping the plate upside-down on papertowels.
8. For each plate, prepare a 1/3000 dilution of goat anti-rabbit IgG alkaline phosphatase conjugate by adding 4uL antiserum to 12mL PBS. For 2 plates add 8uL antiserum to 24mL PBS.
9. Apply 100 uL of the 1/3000 dilution of goat anti-rabbit alkaline phosphatase conjugate to each well of columns 2-12. Apply 100 uL of PBS to each well of column 1.
10. Cover the plates with Parafilm® and incubate at room temperature for 1 hour. During the incubation, aliquot 20mL of alkaline substrate buffer (ASB) and allow to come to room temperature, about 30 minutes. For each two plates, dissolve one 20mg p-nitrophenyl phosphate tablet (PNPP) in the 20mL of ASB. Store in a dark place until needed.
11. Use the “Wash” program to aspirate contents of wells. Make sure that there is no excess buffer remaining in the wells.
12. Apply 100 uL of PNPP substrate solution to each well of columns 1-12.
13. Cover the plates with Parafilm and incubate for 1 hour at 37°C. Uncover and read at 405nm.

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READING THE PLATE

BioRad Benchmark and 680 XR Plate Reader

1. Turn on the computer and allow the *Windows* software to boot up. Turn on the Benchmark or 680 XR plate reader and let it warm up. For the 680 XR plate reader, type in the password "00000" and hit the **Enter** soft key to start the program. This will take approximately 3 minutes following an initial self-diagnosis that requires about 1 minute. When the plate reader has warmed up, its screen should read as follows:

<u>Benchmark</u>	<u>680 XR</u>
PLATE READING:	01: Forensic-130
M=2:405, R=4:655	M405 (2) R655 (4)
Mixing = ON (05s)	Shake: 5 Low
Incu.= OFF	

2. From this point on, you will be controlling the plate reader from the computer it is attached to.
 - Double click on the Forensic-p30 **Microplate Manager 5.2.1** shortcut icon.
 - The screen for the plate readers should now read **Remote Mode**
3. A protocol window will appear. The default settings should read as follows:

Reader:	Benchmark	Model 680 XR
Read speed:	N/A	Fast Read
Reading Parameters:	Dual	Dual
Measurement Filter:	405	405
Reference Filter:	655	655
Dual Wavelength Operation:	Subtract	Subtract
Incubator On:	deselected	deselected
Mix Speed:	N/A	Low
Initial Wait:	0 sec	0 sec
Mix Time:	5 sec	5 sec

4. Place your microtiter plate into the plate reader.

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5. Click **Run** located at the upper right corner of the protocol window. The plate analysis takes about 20 seconds.
6. When the analysis is finished the **Raw Data** window appears. Save the Raw Data onto the network by doing the following:
 - Go to **File** from the main menu and select **Export**.
 - Change the following:
 - i. **Save In:** box to read **M:\FBI BIOLOGY_MAIN\P30ELISA\RAW_DATA folder.**
 - ii. **Save As Type:** box to **Tab delimited (.txt)**
 - iii. **File Name** to your plate name.
Name plates using the date (**MMDDYY**) and plate letter (i.e. **021210A**).
The plate letter is written on the top right corner of the plate.
 - Click **Save**.

Printing Raw Data:

1. On the **Benchmark** plate reader, press the **START/STOP** soft key once and then the **PAGE(+)** key three times, and then press **ENTER**. The raw data will print on thermal paper using the built in thermal printer. (Note: The raw data can also be printed using an external network printer by going to **File** and selecting **Print**.) Write on the printout your initials, date and plate name.
2. The **680 XR** plate reader does not have a built in thermal printer so the raw data has to be printed using an external network printer by going to **File** and selecting **Print**. Write on the printout your initials, date and plate name.
3. When you are finished with the Microplate Manager software, go to **File** in the main menu and select **Exit**. Click **NO** when prompted to save changes to your plate.
4. When done, shut down the computer and turn off the plate reader.

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Spreadsheet calculations

1. Open the *Microsoft Excel* program by double clicking on the “P30XL” template worksheet in the **M:\FBI BIOLOGY_MAIN\P30ELISA\WRKSHEET** folder.
2. The P30 spreadsheet will appear (this a **READ-ONLY** file; it cannot be altered). Two lettered A and B will be at the bottom of the page. Using the mouse, click once on the **B** tab.
3. The cursor should be at the **A1** box position. If not, position the cursor in the **A1** block using the mouse (simply click once on the **A1** box; if it is not visible, use the scroll bars/buttons on the right and bottom sides of the screen to bring that box into view).
4. Pull down the **Data** menu and move the arrow to the **Import External Data** menu. Click once on **Import Data...**. The **Select Data Source** window will pop up. Click once on the box directly to the right of **Look in:** and then click once on **M:\FBI BIOLOGY_MAIN\P30ELISA\WRKSHEET**. (NOTE: This may be out of view. Simply scroll up using the small scroll bar in the right of the window.)
5. You should now be able to see icons representing the raw data files that were generated by the plate reader. Double click on the file that needs to be examined (or click once to highlight the file and press **ENTER**). This window will now close and the spreadsheet window will remain be open. Press **Finish**.
6. The **Import Data** window will pop up. The **Existing Worksheet** option should have a black dot to the left and **=\$A\$1** should appear in the window below it. Press **OK**. The saved data from the plate reader should appear on the page in the upper left corner.
7. Below the data are a series of bold headings for the following: name, date, plate (number), QC # (for QC purposes only), and the sample series (3E-H, 4A-D, 5E-H, and so on). Using the mouse, click on the box to the immediate right of each heading and enter with the keyboard the appropriate information.

NOTE:

- a) The date **MUST** be entered in the following format: **June 21, 20YY** (*Y is the last digits of the year*)
- b) **Plate:** refers to the plate number (*i.e., A, B, etc.*)

8. Enter the sample series in the following manner: **FBYY-XXXXX vaginal**

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9. When all the information has been entered, click once on the **A** tab at the bottom of the page with the mouse. All the information should now be in the pre-made template. Print the page by pulling down the **F**ile menu and clicking once on **P**rint.... When the **PRINT** window appears, click once on the **P**rint... button (or press **ENTER**).
10. The file should now be saved. Pull down the **F**ile menu once more. Click once on **S**ave **A**s....
11. Click on the **F**ile **n**ame: window and enter the correct filename. The filename should be the date entered as such: **06210XA** (where **A** designates the **first** plate, if more than one and **X** designates the last digit of the year). Also check that the file is being saved in the proper location; "**Wrksheet**" should be present in the **S**ave **i**n window. If not, click on the arrow directly to the right of the **S**ave **i**n window. Save file in the "**Wrksheet**" folder via this path:

M:\FBI BIOLOGY_MAIN\P30ELISA\WRKSHEET
12. To do calculations for a second plate, close the file by pulling down the **F**ile menu and clicking once on **C**lose. Repeat steps 2 through 12.
13. When you are finished, exit the program by pulling down the **F**ile menu and click once on **E**xit.

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CALCULATION OF P30 ELISA RESULTS

Manual calculations

The calculations are done automatically by transferring the data from the microtiter plate reader to an *Microsoft Excel* spreadsheet set up to perform them. If necessary, the calculations can be done manually as follows:

1. Subtract the mean value for column 1 from each value in all remaining columns.
2. Calculate the plate threshold value (PT) of the plate:

Determine the average (AVE) and standard deviation (standard deviation = $s = s^{1/2}$) (SD) of the values in column 2.

PT value = $2(\text{AVE}) + 3(\text{SD})$
3. Subtract the PT value from the values in columns 3-12.
4. For all standards and samples, calculate the average of the duplicate samples for both the MOPC and monoclonal PSA coated wells.
5. Subtract the MOPC averages from their corresponding monoclonal PSA averages. The remaining value is the P30 ELISA result.

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Example:

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

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

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

Average of column 2 values: 0.089

Standard deviation of column 2 values: 0.020

Plate threshold = $2(0.089) + 3(0.020) = 0.238$

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

A3 0.000	
B3 0.000	average of A3 and B3 = 0.000
C3 0.118	
D3 0.087	average of C3 and D3 = 0.103
E3 0.028	
F3 0.034	average of E3 and F3 = 0.031
G3 0.473	
H3 0.609	average of G3 and H3 = 0.541

P30 ELISA results:

$$\begin{aligned}\text{Sample 1} &= (\text{AVE C3/D3}) - (\text{AVE A3/B3}) \\ &= (0.103) - (0.000) \\ &= 0.103\end{aligned}$$

$$\begin{aligned}\text{Sample 2} &= (\text{AVE G3/H3}) - (\text{AVE E3/F3}) \\ &= (0.541) - (0.010) \\ &= 0.531\end{aligned}$$

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INTERPRETATION OF RESULTS

The interpretation of P30 results depends on the source of the sample:

1. Body cavity swabs (e.g., oral, vaginal, and anal) are positive if the result is greater than the 2ng standard.
2. Samples not from a body cavity (e.g., panties, etc.) are positive if the result is greater than 0.05 absorbance units.
3. The location from which a “dried secretion” swab is taken will affect the interpretation. Swabs taken essentially from a body cavity or similar place (e.g., introitus, etc.) are interpreted as if the sample is from a body cavity. Other locations (e.g., breast, thigh, etc.) may need to be interpreted differently.
4. If P30 results are close to the 2ng level (for body cavity swabs) or the 0.05 level (for other samples), a slide should be prepared from the sample and a sperm search done. A general guideline is that sperm searches should be performed when the P30 value is >70% of the 2ng cutoff.
5. Off scale P30 values are indicated by “HIGH” in the results table of the P30 ELISA spreadsheet. All “HIGH” values are interpreted as positive results.

Revision History:

March 24, 2010 – Initial version of procedure.

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A. Receipt of postmortem specimens

This task should be performed reasonably soon after a batch of samples arrives in the laboratory. The assigned Criminalist I will report to the postmortem (PM) processing supervisor, and perform any and all tasks related to PM processing. Criminalist I's assigned to the Exemplar rotation will be responsible for PM exemplar processing, and witnessing of the PM bloods.

1. Specimens from all five boroughs are delivered to the laboratory in sealed red plastic containers.

Note: if samples arrive late in the day, inventory red bins (Step 2) and store samples in a refrigerator. Samples will be processed the next day.

2. To inventory the contents of the red plastic containers proceed with the following:
 - Inventory each container separately. **(Check for completeness and make a note of any discrepancies. Report any discrepancies to the PM supervisor.)**
 - Compare the plastic tags with serial numbers to the serial numbers written on the chain of custody.
 - The person on the rotation must sign the chain of custody.
 - Copy the chain of custody. The copy is kept by the X-team and the original is given back to the Evidence Unit.
 - Make a copy of the manifest for our records and discard the original in a red biohazard waste container.
 - Sort the manifests by borough and set aside.
 - **Write down the container number, initial and date the manifest (see below).**

CONTAINER#: 12

Container received in Forensic Biology and opened by MB on 3/4/06
(Forensic Biologist Initials) (Date)

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3. For discrepancies or problems with the inventory, refer to **“Section E: Troubleshooting”** and proceed as specified.
4. Fill out a PM Sample Batch, Blood Processing and Chain of Custody Sheet (referred to as the Batch Chain from hereon) for each borough and list the date received.

Record the ME numbers in numerical order, indicate if blood was received (circle Y or N), use the abbreviations found on the form to list all the other items received in the Additional Samples column, and tally up the total number of samples received (see below).

Borough: Manhattan

Date Received: 3/4/06

Item	Medical Examiner #	PM blood received	ADDITIONAL SAMPLES (see abbreviations below)	Total # of Samples
1	M06-00163	(Y) N	HH, OS, VS, AS, O - debris	6
2	M06-00172	Y (N)	SB	1
3	M06-00184	(Y) N		1

HH:head hair, PH: pubic hair, LF:left hand fingernails, RF:right hand fingernails, SAK: sexual assault kit, OS:oral swabs, VS:vaginal swabs, AS:anal swabs, PS:penile swabs, BS:bladder swabs, ST:soft tissue/muscle, SB:skeletal remains/bone, O:other – specify

- 4a. Complete the Chain of Custody section of the Batch Chain. If the PM samples are **processed on the same day the samples are received and put away the following day**, the chain of custody will reflect this (see below):

CHAIN OF CUSTODY

ITEM(S)	RECEIVED FROM	RECEIVED BY	now FB #:	DATE
1-3	sealed red container	M. Shernaw	n/a	3/4/06
1-3	M. Shernaw	Fbiology Storage	n/a	3/4/06
1-3	Fbiology Storage	B. Donner	----	3/5/06
1-3	B. Donner	Fbiology Storage	----	3/5/06

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4b. If PM samples are **not processed on the same day the samples are received**, the chain of custody will reflect this (see below):

CHAIN OF CUSTODY

ITEM(S)	RECEIVED FROM	RECEIVED BY	now FB #:	DATE
1-3	sealed red container	M. Shernaw	n/a	3/4/06
1-3	M. Shernaw	Fbiology Storage	n/a	3/4/06
1-3	Fbiology Storage	B. Donner	----	3/5/06
1-3	B. Donner	Fbiology Storage	----	3/5/06
1-3	Fbiology Storage	B. Donner	----	3/6/06
1-3	B. Donner	Fbiology Storage	----	3/6/06

Use the Comments section to document damaged packaging or other unusual observations. (Ex: If a sample was not received in a purple top tube, list affected ME # and the color top tube that had been used.)

5. Enter the inventoried PM samples from the Batch Chains into the Post-Mortem Inventory spreadsheet. This step can be performed before or after PM samples are processed. Go to **M:\FBI BIOLOGY_MAIN\PERSONS\PM'S\PM'S_DB**. Select the appropriate worksheet tab based on the year. Important: enter data after the last entry (start from the bottom), do not sort the database, and always remember to save.

Enter the ME #, date samples received, "blood" or "no blood", and additional samples (using abbreviations listed on the Batch Chain). Do not fill out the other categories unless instructed (see below).

FB #	ME#	Date Received by FB	Blood	Addtl. PM Sample (use codes)	Location
	B06-00001	1/3/2006	blood	SB (bone)	
	B06-00003	1/3/2006	blood	PH, HH	

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6. Staple the Batch Chains to the respective manifest and put aside until processing.
7. Ensure that the PM items all have barcode labels and are stored in an appropriate container (See Table 1).

If items are not packaged properly, repackage according to the table below. Seal the package with Evidence Tape or using a heat-sealer for the 4x6" KAPAK™ bag, except where indicated. Initial and date all seals. Note: the evidence tape should not obscure the ME # on the barcode label.

Table 1

Sample	Packaging
Bloodstain cards	4x6" KAPAK™ bag (seal KAPAK bag)
Hair, Nails, Trace Evidence*	Coin envelopes placed into 4x6" KAPAK™ bag (do not seal KAPAK bag)
Oral, vaginal, anal, penile, and bladder swabs*	Coin envelopes placed into 4x6" KAPAK™ bag (do not seal KAPAK bag)
Bone	Plastic specimen container
Muscle or soft tissue	Plastic specimen container or 15 ml Falcon tube

* Store samples from the same ME # in the same KAPAK bag. Do not seal the bag.

8. Once inventoried and processed, store samples in the appropriate storage area (See Table 2).

Table 2

Room Temperature (20°C)	Refrigerator (4°C)	Freezer (-20°C)
- Bloodstain cards - Fingernails - Hair - Other Trace Evidence	- Oral, vaginal, anal, penile, and bladder swabs - SAK - Samples in RNAlater®	- Bone - Muscle or Soft Tissue - Product of conception (POC)

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9. Spray the inside of the red bins with disinfectant and let air dry. Place a blank manifest into each container. Set the red containers aside in the designated area for pick up.

B. Postmortem bloodstain processing (non-vouchered bloods)

1. Make the ME barcode labels for the bloodstain cards using the MEANS program (see Appendix I-A). Wear gloves when handling the bloodstain cards. Handwrite the ME # if unable to generate labels. Initial each bloodstain card prepared.
2. Fill out the Blood Processing section of the Batch Chain (see below). Make sure that only those item numbers for which blood was received are listed here. The preparer of the bloodstain cards must initial and date each card. Leave the Witnessed by field blank.

<p>Blood processing:</p> <p>Stain cards from the above bloods # <u>1</u> and # <u>3</u> were made on Date: <u>3/4/06</u> By analyst: <u>M. Shernaw</u> Witnessed by: _____.</p>
--

3. The setup of the bloods and bloodstain cards must be witnessed by another laboratory staff member. That person must confirm that the order of the blood vials in the rack match the order of the prepared bloodstain cards. The witness will then initial the bloodstain cards, and initial the Witnessed By field of the Batch Chain (see Figure 6).
4. The bloodstain cards should have the following information prior to processing:
 - a) ME case number (on affixed label or handwritten)
 - b) Initials of the person preparing the stain
 - c) Initial of the witness
 - d) Date
5. Prepare stains one at a time. Staining of the cards and the opening of liquid blood samples MUST be performed under a biological safety hood with the exhaust fan operating. A new KimWipe™ should be used to open each vial stopper. Make sure the blood vial is closed before preparing the next bloodstain card.

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6. Use a transfer pipet to make four stains for each bloodstain card, filling in the four circles on each card with blood.
7. Re-cap non-vouchered PM blood vials and discard in the plastic biohazard “sharps” container.
8. Allow the bloodstain cards to dry overnight in the hood with the exhaust fan running.
9. Package the air-dried stains into a 4x6” KAPAK™ bag. Seal the bag with evidence tape or using a heat sealer. Initial and date the seal.
10. Organize the bloodstain cards by borough and in ME # order. Add the cards to the appropriate yellow borough bin located on the bench where they are temporarily stored until a supervisor has had a chance to review the cards.
11. Bloodstain cards of ME cases that have been assigned FB #'s by a supervisor will be labeled with the FB # and transferred to the red bin on the bench. Cards of ME cases that will not be assigned an FB # are transferred to the blue borough bins. The transfer of cards reviewed by the supervisor are placed to their appropriate long-term storage locations by the assigned Criminalist III on PM Processing:
 - Cards with FB #'s are stored numerically by FB # in the designated bloodstain card box.
 - Cards without FB # are stored numerically by borough and ME # in the designated bloodstain card box.
12. **CLEAN THE BIOLOGICAL SAFETY CABINET (refer to Quality Control Procedure #QC125 in the Quality Assurance/Quality Control Manual).**

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C. Assignment of case numbers

This task should be performed by the PM supervisor or trained supervisor.

1. Retrieve the daily case census sheets and autopsy case worksheets for all five boroughs. The paperwork is typically sent in envelopes addressed to the “Serology Department” except for loose paperwork retrieved from the ID Unit. The daily case census sheets are available electronically through the MEANS system (see Appendix D). The autopsy case worksheets are available electronically through the Document Archiving system (see Appendix II).
2. Organize the daily case census sheets in chronological order. For each borough the daily case census sheet is a list of the autopsies performed by the ME for that day. Attach the respective autopsy worksheets to the daily case census sheets. Compare each autopsy case worksheet with the manifest and the specimens received to ensure that all of the specimens designated for Forensic Biology have been received. See Section E. Troubleshooting if there are discrepancies.
3. Screen all the paperwork for potential Forensic Biology cases. The following types of cases should be assigned an FB case number:
 - Homicides
 - Any case in which sexual assault evidence (SAK or orifice/penile swabs) has been collected
 - Any case in which a Forensic Biology test is requested via email, phone, or noted on the manifest. Note: Hemoglobin, thrombophilia, and sickle cell cases are assigned an MG # and not an FB #. Contact the Molecular Genetics group.
 - Any unknown body with PM samples requiring DNA identification (must verify the victim is still unknown by checking MEANS or the ID Unit)
 - Any case in which evidence from the NYPD or DA’s office has been submitted
 - POC/fetus (only if criminal activity is involved)

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4. **For cases that will be assigned an FB case number:** Separate the autopsy worksheets from the others. Check the Access database to determine if FB case numbers have been assigned to the ME numbers.
 - a. If Access has an FB # for the ME #, the PM samples will be signed into the pre-existing case numbers. Retrieve any existing case folders.
 - b. If Access does not have an FB # for the ME #, assign the PM samples the next available FB case number. Enter the appropriate information into the FB logbook and Access. Create a new case folder by obtaining a manila folder with the FB case number.
5. Fill out a chain of custody for the PM sample(s). Numerically list each specimen in the evidence submitted section. Give each specimen a unique PM number (i.e.- blood is usually designated "PM 1" and sexual assault kits are usually designated "PM 2"). In the chain of custody section, enter the date the specimens were received from Forensic Biology storage.

Exception: For Missing Persons cases (unknown victim), the PM sexual assault evidence (PM SAK or PM orifice/penile swabs) should be placed on a separate chain of custody from the other PM samples.
6. On the corresponding Batch Chain, indicate which ME # was signed over to an FB file. Make a copy of the Batch Chain and place it behind the chain of custody in the case file. Both should be affixed to the left side of the folder.
7. Autopsy case worksheets are affixed to the right side of the folder.
8. PM SAK and PM orifice/penile swabs must be signed over to the Evidence Unit so that they may be processed. All other specimens must be placed in retained storage. Continue the chain of custody for these items to reflect their final location.
9. Give the FB cases to the evidence sign-in supervisor.

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10. All other cases are not assigned an FB case number. These would include cases where the Manner of Death is:

- Pending Studies (possible homicides, i.e.- CUPPI, case unknown pending police investigation)
- Natural
- Therapeutic Complication
- Accident/Motor vehicle accidents (MVA's) *which are under investigation* (i.e.- hit and run)
- Suicide
- Undetermined
- Or any case which involves child abuse or suspected child abuse

11. **For cases that will NOT be assigned an FB case number:** File the daily case census sheets and respective autopsy worksheets in chronological order for archival purposes. After 30 days, discard the paperwork. Electronic copies are available through MEANS and DMS.

D. Discarding postmortem items

Refer to the table below regarding storage and discarding of blood and non-blood items:

Table 3:

	Bloodstain?	Non-Blood?	Discard?
FB cases	Y	Y	Retain all indefinitely.
Non-FB cases	Y	Y	Discard non-blood after 6 months; discard bloodstain after 5 years.
	N	Y	Discard non-blood after 5 years.
	Y	N	Discard bloodstain after 5 years.
POC/Fetus (criminal activity)	n/a	Y	Retain a small piece and discard the remainder.

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Prepare a manifest for discarding postmortem samples. The manifest must reflect:

- ME #
- Samples that are discarded
- Date of discard
- The individual discarding the samples.

A copy of the manifest will be filed with Batch Chain for the sample being discarded. The original manifest will be filed in a binder for discarded postmortem samples.

E. Troubleshooting

Problem	Recommended Action
Unlabeled specimen; unscannable label	<p>Criminalist I: For an unlabeled specimen, do not process; make a note on the Batch Chain and notify supervisor. Store questionable samples in designated refrigerated area.</p> <p>For an unscannable label, process as long as the ME number is legible.</p> <p>Criminalist III/IV: Narrow down possible ME by process of elimination. Contact ME who performed the autopsy to request an additional sample. If not available, retrieve sample from Department of Toxicology.</p>
Unreadable but scannable barcode label	<p>Criminalist I: Scan barcode and generate new label in MEANS. Use new label to confirm ME# with manifest and place label on stainer card. Continue with processing.</p>

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Problem	Recommended Action
Specimen collected but not listed on manifest	Criminalist I: Make a note on the Batch Chain and continue with processing. Criminalist III/IV: Confirm what samples were collected by the ME who performed the autopsy.
Specimen not collected but listed on manifest	Criminalist I: Make a note on the Batch Chain and notify the supervisor. Criminalist III/IV: Contact ME who performed the autopsy to request an additional sample. If not available retrieve sample from Department of Toxicology.
Blood vial labeled "Hospital Blood" and/or has the ME # written on the hospital label	Criminalist I: Make a note on the Batch Chain, continue with processing, and notify supervisor. Criminalist III/IV: Verify on the autopsy worksheet that ME submitted hospital blood. If so, do nothing. If not, contact ME who performed the autopsy to inform them of the situation and attempt to retrieve sample in a purple top tube.
Missing manifest	Criminalist I: Handwrite manifest, make a note on the batch chain of custody, continue with processing, and notify supervisor. Criminalist III/IV: Contact the respective borough Deputy ME.
Container not sealed with black ties	Criminalist I: Make a note on the Batch Chain, continue with processing, and notify supervisor. Criminalist III/IV: Notify Dan Stevelman.

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Problem	Recommended Action
Broken blood vials/ Blood vial with a detached rubber stopper	Criminalist I: Make a note on the Batch Chain and notify supervisor. Criminalist III/IV: Contact ME who performed the autopsy to request an additional sample. If not available, retrieve sample from Department of Toxicology.
Blood vial with a non-purple stopper	Criminalist I: Make a note on the Batch Chain and continue with processing. Criminalist III/IV: Contact ME who performed the autopsy to inform them of the situation and attempt to retrieve sample in a purple top tube.
Blood that appears to be decomp fluid, grayish in color, or clotted	Criminalist I: Make a note on the Batch Chain, continue with processing, and notify supervisor. For blood clots, smear clot onto the stain card. Discard leftover blood clot properly. Criminalist III/IV: Contact ME who performed the autopsy and ask for a bone sample.
Blood labeled "decomp" on blood vial or autopsy case worksheet	Criminalist I: Make a note on the Batch Chain, continue with processing, and notify supervisor. Criminalist III/IV: Contact ME who performed the autopsy and ask for a bone sample.
Blood vial labeled for HIV testing (or paperwork for HIV testing included)	Criminalist I: Do not process; make a note on the Batch Chain and notify supervisor. Store questionable samples in designated refrigerated area. Criminalist III/IV: Return items to the Manhattan morgue.

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Problem	Recommended Action
RNAlater® samples: liver, spleen, and heart and/or requisition forms	Criminalist I: Do not process; make a note on the Batch Chain and notify supervisor. Place samples in designated refrigerated area. Criminalist III/IV: Notify the Molecular Genetics group to pick up samples and sign Batch Chain.
Incorrect or no sample submitted for decomposed victim or a case for FB	Criminalist III/IV: Contact ME who performed the autopsy and ask for an appropriate sample (long bone, rib, etc.) Retrieve sample from Toxicology as a last resort.

F. Civil paternity requests

Do not accept any phone calls from family members. Direct all phone calls to the Legal Department.

1. A paternity request is initiated with an email from the Legal Department indicating the family plans to have DNA paternity testing done and to place any specimens on hold.
2. Check the PM database to determine the following:
 - A. Was a sample collected?
 - B. What type of PM sample is available (blood, hair, etc.)?
 - C. Is this an FB or non-FB case?
 - D. Verify subject's name with autopsy sheet (See Appendix II, Section A for viewing autopsy sheet in DMS).
3. Locate the appropriate PM sample and verify that you have the correct PM sample and subject name. Place PM sample into paternity bin for FB case # assignment.
4. Send a "reply to all" email answering all of the questions listed above in #2. List all samples in FB custody. Indicate if there is an inconsistency between the subject's name listed in the email from the Legal Department and what is listed in the autopsy sheet.

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5. If no sample is available in FB, contact the Toxicology Department for a potential sample.

If a sample is available, retrieve it, make a chain of custody, and process the sample.

Store the stain card in the appropriate retained storage location. Update the PM_DB and the paternity database. Retain the email requesting a specimen from the Toxicology Department and your reply. Place PM Sample into the Paternity Bin for FB Case Number assignment.

6. FB will be contacted by the Legal Department when a paternity kit has arrived for the subject. Retrieve the kit.
7. Locate the appropriate FB case file & sample.
8. Open kit and discard any glass containers for liquid blood in the sharps container.
9. Sign the Chain of Custody found in the FB file when retrieving samples from FB Storage.
10. Submit a quarter of the PM sample for testing. If PM sample appears to be decomposed, submit half of the sample. (Example- If four circles are stained, submit one circle. If the bloodstain is decomp fluid, submit two circles.) Do not send the entire sample; a minimum of 50% of the sample should be retained. If the testing laboratory or family is requesting the entire item, verify this with the Legal Department and proceed as advised.
11. Submit the portion of stain card in a coin envelope labeled with the subject name, ME #, and any other relevant information. Submit a portion of the tissue or bone sample in a plastic, puncture- and leak-proof container labeled as described previously. Seal, initial, and date packaging. Return unused sample to their original storage location and sign the Forensic Biology Chain of Custody.
12. Fill out an OCME autopsy specimen chain of custody documentation (found at M:\FBIOLGY_MAIN\FORMS\PM\PrivateLabCOC.doc) and shipping paperwork. Refer to the autopsy sheet for information regarding the subject's age, race, time of death, and medical examiner who performed the autopsy (Refer to DMS for a scanned image of the autopsy sheet).

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13. If requested, have autopsy specimen chain of custody notarized. Consult with the PM Blood Processing Supervisor for a list of Public Notaries within the agency.
14. Make copies of the paperwork and save the sender's receipt from the shipping envelope. File the relevant paperwork in the FB file. Update the paternity database.
15. Place sample, court order, and other appropriate paperwork in the kit.
16. Seal and place kit in appropriate area to be sent. Call the appropriate shipping company to arrange pick-up, as needed. Record the confirmation number in FB file.
17. Email the original contact and inform them that the kit will be picked up. Include the confirmation number. File the email with the relevant paperwork in the FB file.

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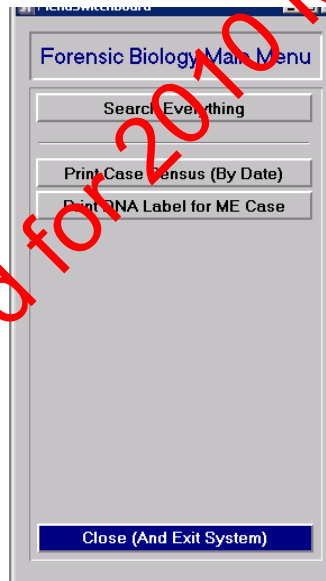
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APPENDIX I: DataEase MEANS (Forensic Biology Version)

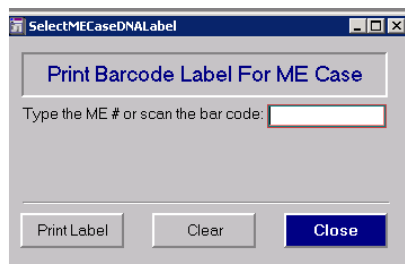
Note: A user must obtain access rights from DoITT in order to use MEANS. DoITT will issue the username and password.

A. Printing Barcode Labels

1. Double-click on the MEANS icon on desktop
 - a. Enter login name.
 - b. Enter password.
 - c. Make sure that “CSC” is selected for the field “Log on to:”
2. The MEANS “Forensic Biology Main Menu” screen (pictured below) will appear:



3. Select “Print DNA label for ME Case”. The “Print Barcode Label for ME Case” will appear (pictured below):



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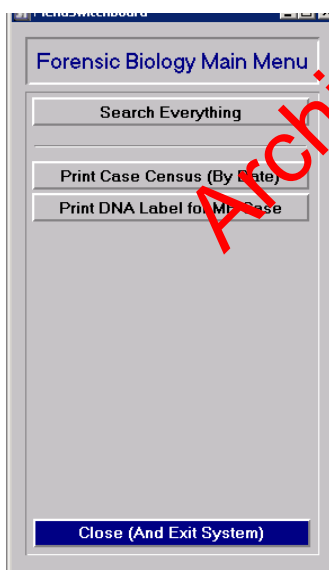
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4. To print a label, either type in the ME# without spaces or hyphens or scan the barcode from the labels on the manifest sheet.
5. Click on “Print Label.”
6. Click on “OK” to print.
7. To print a different label, select “Clear” and repeat steps #4-#6. You must clear the ME# otherwise the previous label will be reprinted.
8. Log out as soon as you are done. Failure to log out prohibits other users from accessing the program (See Section B for logging out).

B. Logging Out of MEANS:

1. Select “Close” to exit from each menu open.
2. Select “Close (and exit system)” (pictured below) to quit out of MEANS.

Note: Do not use the “x” on the upper-right corner to close out of menus in MEANS.

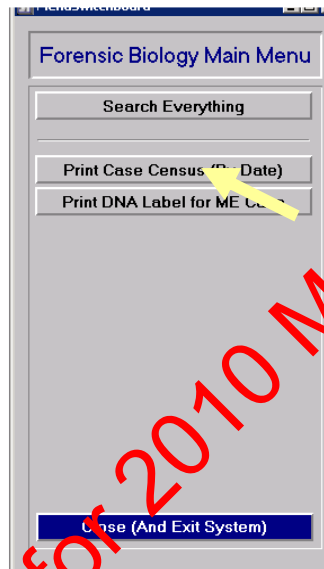


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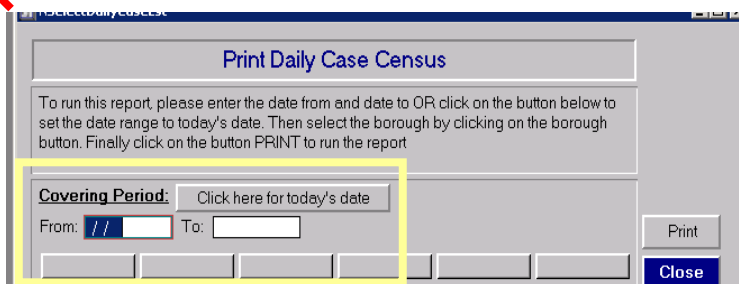
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C. Printing Daily Case Census Sheets

1. Select “Print Case Census (By Date)” from the Forensic Biology Main Menu (pictured below):



- a. The “Print Daily Case Census” screen will appear (pictured below). Enter a “From” date and a “To” date. For the current, select the “Click here for today’s date” button.



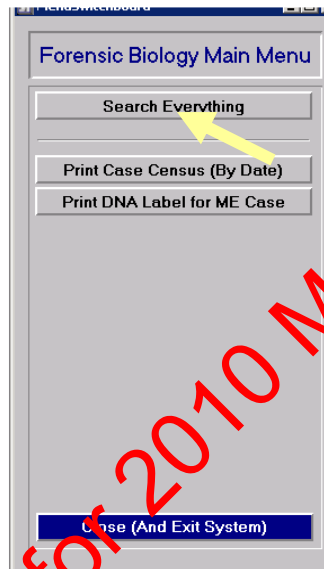
2. Select the borough (Brooklyn, Queens, Manhattan, Bronx, or Richmond) by clicking on the desired borough button.
3. Select “Print” to run the report.

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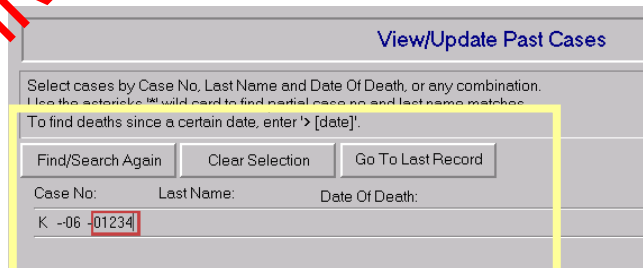
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D. Resolving Issues Using MEANS

1. Select “Search Everything” from the MEANS Forensic Biology Main Menu (pictured below):



2. On the “View/Update Past Cases” (pictured below), type in the ME # using borough, year, and 3 digit ME #.



3. If the ME # is not available, type in last name and/or date of death in the proper fields.
4. Click on “Find/Search again.”
5. To review more cases, click on “Clear Selection” and repeat steps 2-4.

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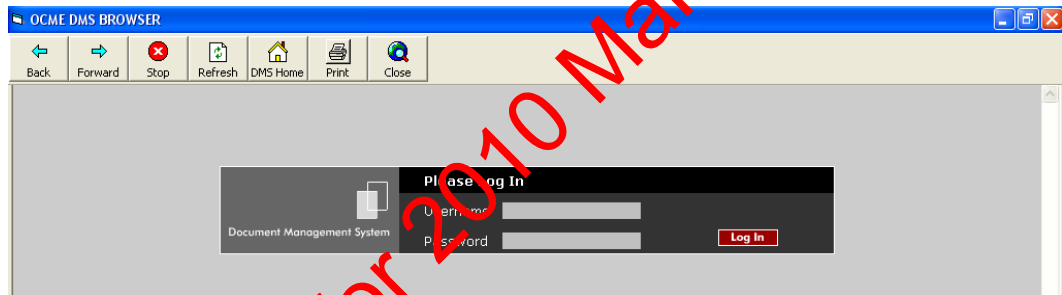
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APPENDIX II: DMS (Document Imaging and Management System) Browser

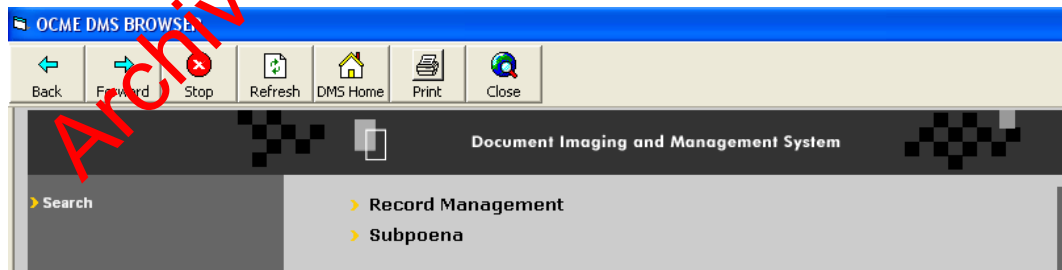
Note: A user must obtain access rights from DoITT in order to use MEANS. DoITT will issue the username and password.

A. Printing out Autopsy Worksheets

1. Double click on OCME DMS Browser icon on desktop
 - a. Enter username.
 - b. Enter password.
 - c. Click “Log In” button or hit “Enter.”



2. The “Document Imaging and Management System” main screen (pictured below) will appear:

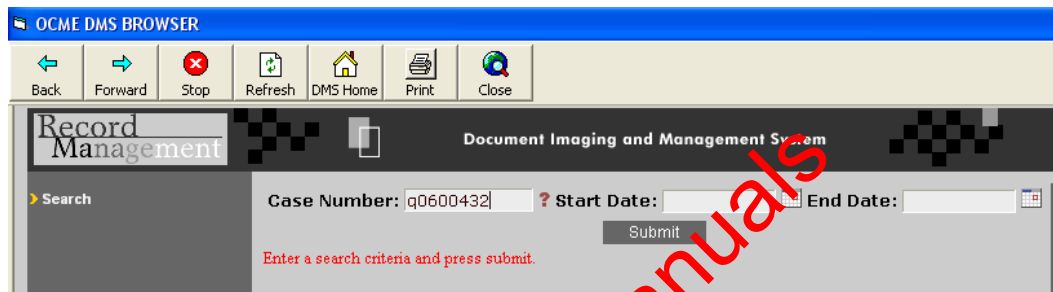


3. Double click on “Record Management”.

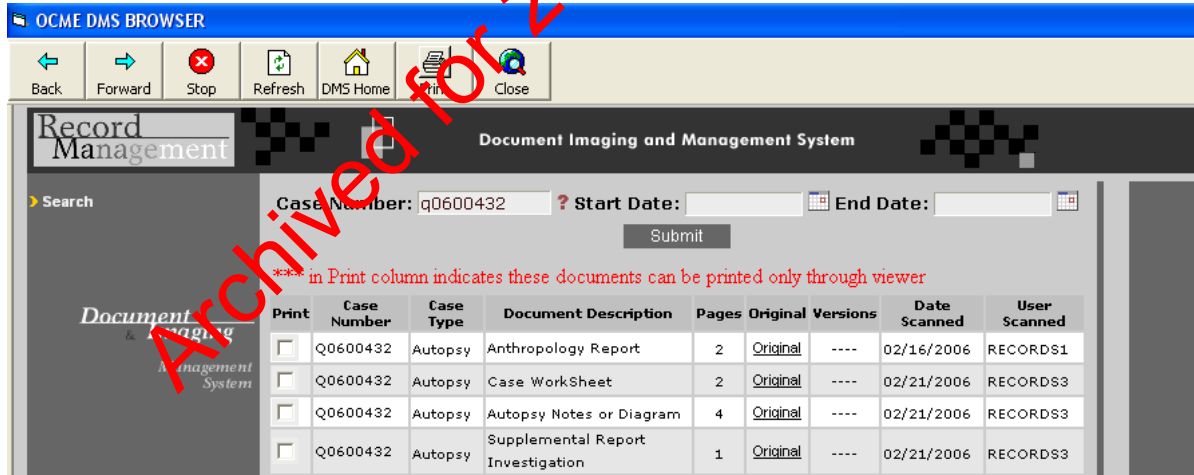
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- Enter Medical Examiner case number in the field called “Case Number” in the following format: if the ME # is Q06-00432, enter q0600432 (no hyphens or spaces). See example below:



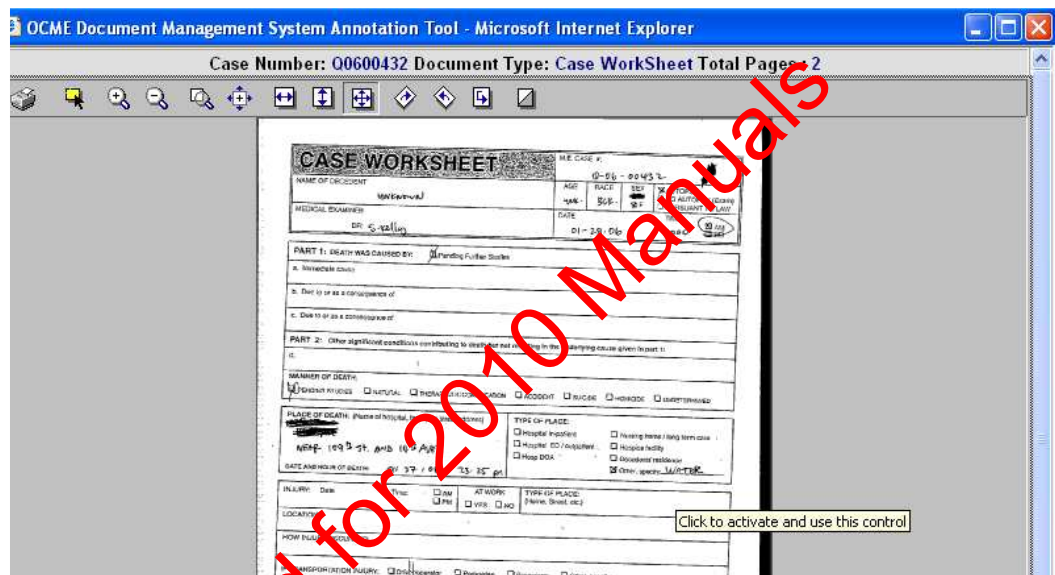
- Click on the “Submit” button (hitting “enter” will not work.)
- The following screen will appear:



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7. Look for “Case Worksheet”; this is a scanned .pdf image of the autopsy worksheet.
 - a. To obtain a copy, click on “Original.”
 - b. The autopsy worksheet will open up in an Internet Explorer window (pictured below):



- c. Double click on the printer icon to obtain a printed copy of the autopsy worksheet. Click “X” to close the window.
 - d. Go back to the main login screen and double click the red “Log Out” button. Then click the “Close” icon at the top of the screen.

Revision History:

March 24, 2010 – Initial version of procedure.

Controlled versions of Department of Forensic Biology Manuals only exist electronically on the Forensic Biology network. All printed versions are non-controlled copies.

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