FORENSIC BIOLOGY
SEROLOGY MANUAL

Approving Authority:
Eugene Y. Lien, Technical Leader, Serology Operations

Working version as of 09/01/14

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Highlighted sections indicate a new revision to that procedure

General Guidelines

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.

Revision History:
March 24, 2010 – Initial version of procedure.

Processing of Postmortem Specimens

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. The LIMS system will automatically update the PM bin's chain of custody once the PM bin's custody has transferred from the Evidence Unit to the Forensic Biology Personnel..

   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 record 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 record the chain of custody.
   - Scan the included chain of custody to a PDF document, and incorporate into the LIMS system. The original is given back to the Evidence Unit.
   - Scan the manifest to a PDF document, and incorporate into the LIMS system. Discard the original in a red biohazard waste container.
   - Sort the manifests by borough and set aside.

3. For discrepancies or problems with the inventory, refer to **"Section E: Troubleshooting"** and proceed as specified.

4. Fill out the PM documentation for each bin. The LIMS system will automatically create the chain of custody for each sample, and record the packaging and processing as the analyst unpacks the postmortem evidence and exemplar samples.

5. 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.

6. 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)

7. Spray the inside of the red bins with disinfectant and let air dry. 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 LIMS system. Wear gloves when handling the bloodstain cards. Handwrite the ME # if unable to generate labels. Initial each bloodstain card prepared.

   The preparer of the bloodstain cards must initial and date each card.

2. 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 record the witnessing setup in the documentation.

3. 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) Date the stain card was prepared
   d) LIMS stain card ID

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 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.

5. Use a transfer pipette to make four stains for each bloodstain card, filling in the four circles on each card with blood.

6. Re-cap non-vouchered PM blood vials and discard in the plastic biohazard "sharps" container.

7. Allow the bloodstain cards to dry overnight in the hood with the exhaust fan running. Document that the stain cards are being stored in the hood.

8. 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.

9. 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. Document the cards' new storage location.

10. 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.

    The electronic chain of custody will document the transfer between storage locations and Criminalists.

11. CLEAN THE BIOLOGICAL SAFETY CABINET (refer to Quality Control Procedure #QC125 in the Quality Assurance/Quality Control Manual).

C. Assignment of case numbers

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

1. Gather all appropriate documentation. The daily case census sheets are available electronically through the MEANS system (see Appendix I). The autopsy case worksheets are available electronically through the Document Archiving system (see Appendix II).

2. Compare each autopsy case documentation 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 documentation 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)

4. **For cases that will be assigned an FB case number:** Check the database to determine if FB case numbers have been assigned to the ME numbers.

   a. If the database has a FB # for the ME #, the PM samples will be signed into the pre-existing case numbers.

   b. If the database does not have a FB # for the ME #, review and assign the PM samples an FB case number. Enter the appropriate information into the database. Create a new case folder by obtaining a manila folder with the FB case number.

   Upon electronically assigning a FB # to the ME #, LIMS will create a unique PM number for each specimen.
   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.

5. 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 to document the chain of custody for these items to reflect their final location.

6. Give the FB cases to the evidence sign-in supervisor.

7. 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

8. **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.

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; unscanable label	Criminalist I: For an unlabeled specimen, do not process; record the deviation and notify supervisor. Store questionable samples in designated refrigerated area. For an unscanable label, process as long as the ME number is legible. 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.

Problem	Recommended Action
Unreadable but scannable barcode label	Criminalist I: Scan barcode and generate new label. Use new label to confirm ME# with manifest and place label on staincard. Continue with processing.
Specimen collected but not listed on manifest	Criminalist I: Record the deviation 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: Record the deviation 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: Record the deviation, 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: Record the deviation and continue with processing, and notify supervisor. Criminalist III/IV: Contact the respective borough Deputy ME.

Problem	Recommended Action
Container not sealed with black ties	Criminalist I: Record the deviation, continue with processing, and notify supervisor. Criminalist III/IV: Notify Dan Stevelman.
Broken blood vials/ Blood vial with a detached rubber stopper	Criminalist I: Record the deviation 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: Record the deviation 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: Record the deviation and 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: Record the deviation, continue with processing, and notify supervisor. Criminalist III/IV: Contact ME who performed the autopsy and ask for a bone sample.

Problem	Recommended Action
Blood vial labeled for HIV testing (or paperwork for HIV testing included)	Criminalist I: Do not process; Record the deviation and notify supervisor. Store questionable samples in designated refrigerated area. Criminalist III/IV: Return items to the Manhattan morgue.
RNAlater® samples: liver, spleen, and heart and/or requisition forms	Criminalist I: Do not process; record the deviation 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 OCME 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.

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

   If a sample is available, retrieve it from EU, and process the sample. Store the stain card in the appropriate retained storage location. Update all appropriate databases. 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. 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.

10. 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.

11. Fill out an OCME autopsy specimen chain of custody documentation 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.

12. 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.

13. 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.

14. Place sample, court order, and other appropriate paperwork in the kit.

15. 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.

16. 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.

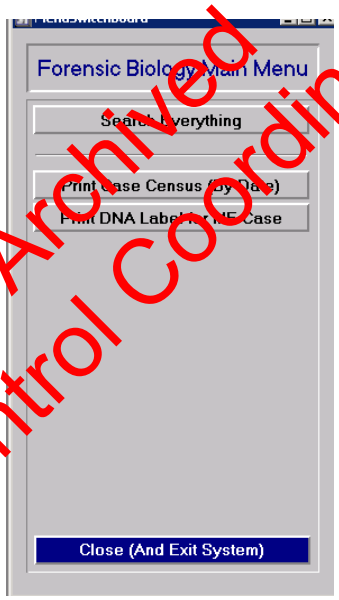
APPENDIX I: DataEase MEANS (Forensic Biology Version)

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

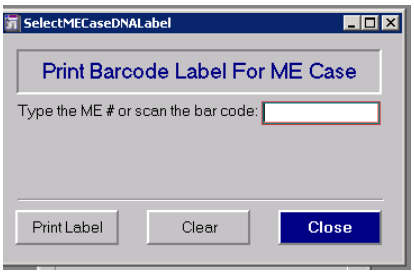
A. Printing Barcode Labels

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):



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.

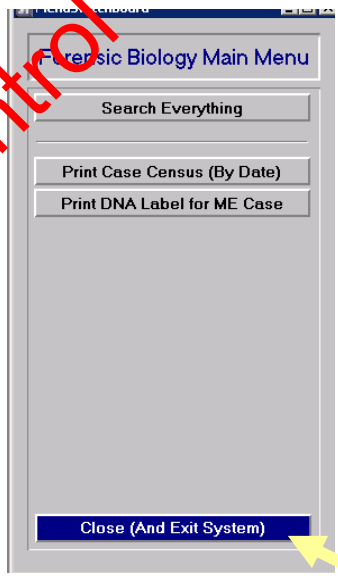
8. Log out as soon as you are done. Failure to log out prohibits other users from accessing the program (See Section B for logging out).

B. Logging Out of MEANS:

1. Select "Close" to exit from each menu open.

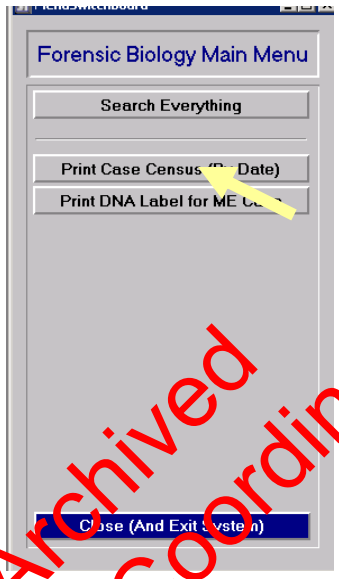
2. Select "Close (and exit system)" (pictured below) to quit out of MEANS.

Note: Do not use the "×" on the upper-right corner to close out of menus in MEANS.

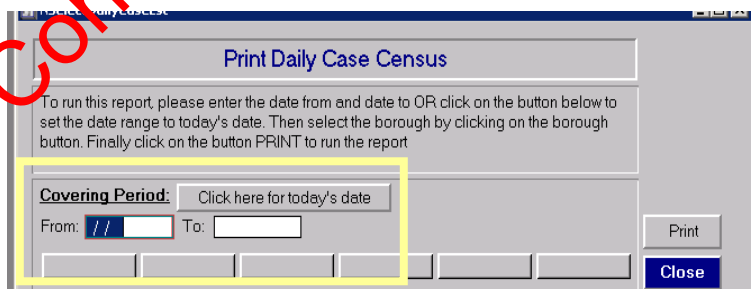


C. Printing Daily Case Census Sheets

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.

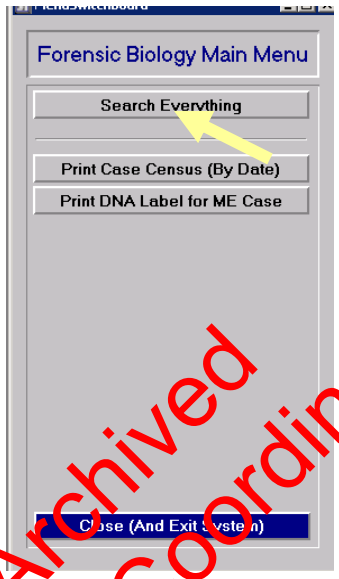


2. Select the borough (Brooklyn, Queens, Manhattan, Bronx, or Richmond) by clicking on the desired borough button.

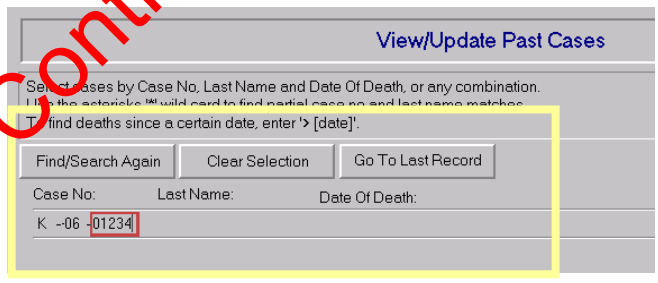
3. Select "Print" to run the report.

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 5-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."

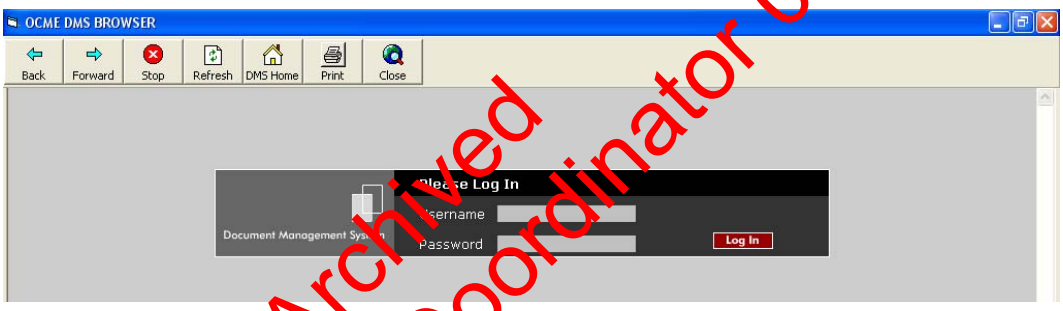
5. To review more cases, click on "Clear Selection" and repeat steps 2-4.

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

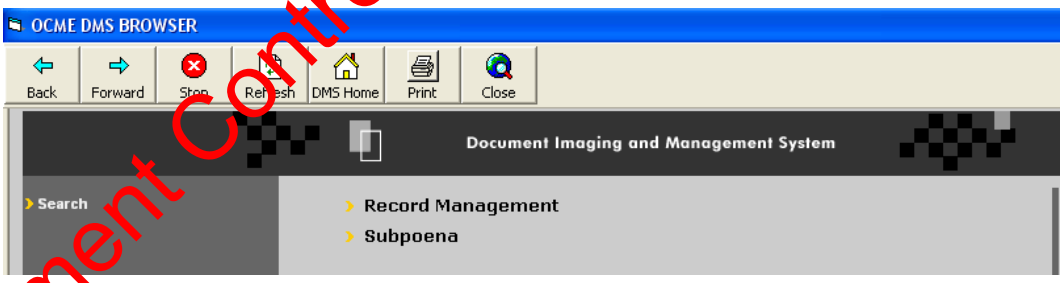
Note: A user must obtain access rights from DoITT in order to use MEANS. DoITT will issue the username and password.

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."

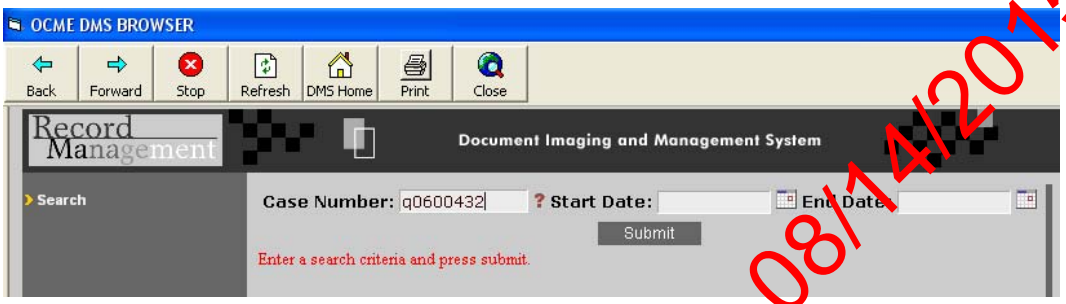


2. The "Document Imaging and Management System" main screen (pictured below) will appear:



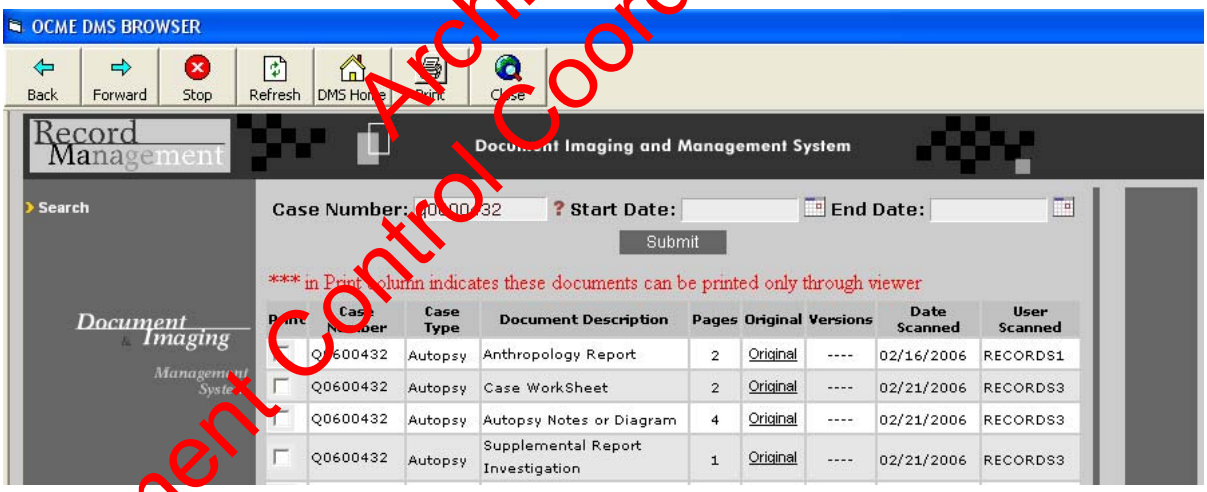
3. Double click on "Record Management".

4. 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:

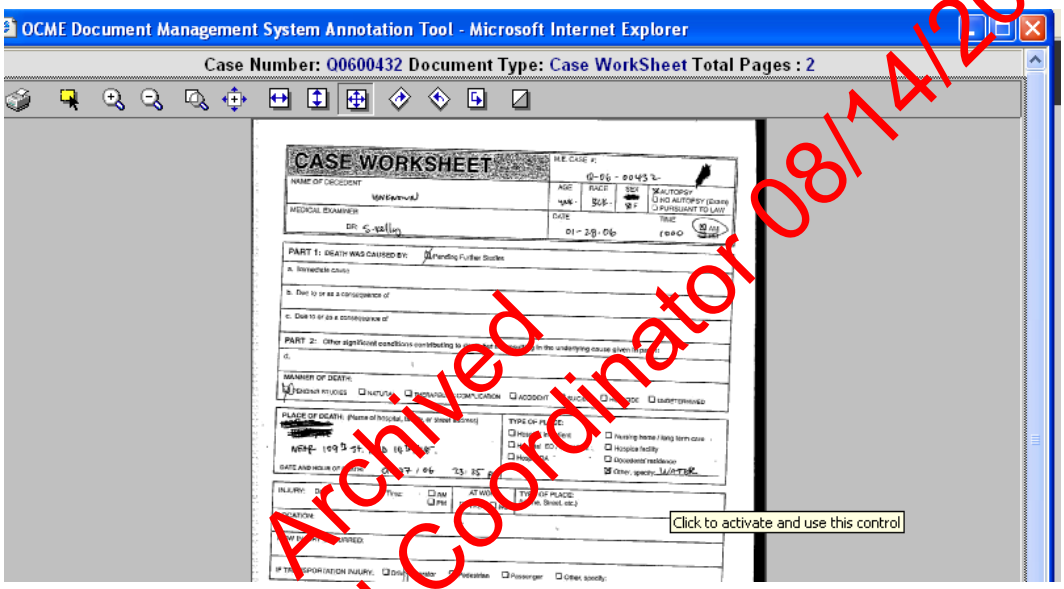


5. Click on the "Submit" button (hitting "enter" will not work.)

6. The following screen will appear:



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.

Bloodstain Preparation from Whole Blood

Staincards are prepared from all vouchered blood samples and from post-mortem blood samples:

1. Take custody of the blood vials awaiting bloodstain preparation.

2. Prepare the UltraSTAIN™ cards by affixing a pre-printed FB case number sticker (if available) and writing in the following:

   - 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. The witness must confirm that the processor is handling the correct blood vial and stain card BEFORE the stain is made. After each stain is made, the witness must initial the stain card and the evidence packaging worksheet.

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, and record the storage location for the chain of custody.

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.

Revision History:

March 24, 2010 – Initial version of procedure.
July 16, 2012 – Specific terminology was removed and replaced with generic terminology to accommodate LIMS.

Kastle-Meyer (KM) Presumptive Testing for Blood

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.

Revision History:
March 24, 2010 – Initial version of procedure.

Acid Phosphatase Presumptive Test for Semen

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.

Revision History:
March 24, 2010 – Initial version of procedure.

Amylase Diffusion Presumptive Test for Saliva

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.

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 (dH_2O) from purchased amylase.

   A. Prepare 1mL of 20 units/10uL amylase by adding the appropriate amount of amylase standard to dH_2O. 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 dH_2O to each of 4 microcentrifuge tubes. Then transfer 100uL of your 20 unit standard into one of the tubes containing 900uL of dH_2O. 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:

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

Use this value in the equation C1 x V1 = C2 x V2 where C1 and C2 are concentrations of solutions 1 and 2, while V1 and V2 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 units amymase/uL)(x uL) = (20 units/10uL)(1000uL)

Solving for x = 77uL 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 documentation with the tube labels.

2. Fill wells according to the Amylase Diffusion documentation (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. Save the file as a JEPG and upload to the LIMS system for the related Amylase assay.

6. Measure the diameter of the clear areas and record on the documentation.

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.

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 the amylase documentation.

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 the amylase documentation.

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
FOURCHET	PERINEAL
INTROITUS	PERINEUM
LABIA MAJORA	PERIORAL
LABIA MINORA	PERIVAGINAL
LIPS	VESTIBULE
MOUTH	VULVA

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

BUTTOCKS	INGUINAL
CHEEK	INNER THIGH
CHIN	MONS VENEVIS (mons pubis)
GROIN	PENIS
SCROTUM	

AMYLASE DIFFUSION PRESUMPTIVE TEST FOR SALIVA		
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Revision History:

March 24, 2010 – Initial version of procedure.
July 16, 2012 – Specific names of worksheets were removed and replaced with generic terminology to accommodate LIMS.

Slide Preparation for Spermatozoa Searches

There are two methods to prepare slides for spermatozoa searches. Either may be used:

1. **Mashing**

   A. Cut 1.0 x 1.0 mm of the sample and place it on a clean microscope slide.

   B. Add a drop of distilled water.

   C. Tweeze apart sample until fibers are in a thin even layer across the slide.

   D. Fix sample to the slide by heating on a hot-plate (approximately 5 to 10 seconds).

   E. Stain slide using the Christmas Tree Staining procedure.

2. **Pipettete Tip/Test Tube Extraction**

   A. Using the pipette tip/test tube method, extract 1.5 x 1.5 mm samples in 50uL of distilled water for 30 minutes at room temperature.

   B. Centrifuge sample for 2 minutes.

   C. Pipette pellet onto microscope slide.

   D. Fix sample to the slide by heating on a hot-plate (approximately 5 to10 seconds).

   E. Stain slide using the Christmas Tree Staining procedure.

Revision History:
September 17, 2012 – Initial version of procedure.

Christmas Tree Stain for Spermatozoa

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 to10 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).

Revision History:
March 24, 2010 – Initial version of procedure.

Seratec® PSA Semiquant and a-Amylase Tests

A. Sample Preparation and Antigen Extraction (for both tests):

1. Make a 1/4 cutting for swabs, or ~ 3 mm x 3 mm for stains.

2. Add the cuttings to separately labeled 1.5 mL Eppendorf tubes.

3. Add 0.5 mL Phosphate Buffered Saline (PBS) solution to each sample. Record the PBS lot number.

4. Place the tubes on the Thermomixer. Shake at 300 RPM at room temperature (25°C) for 5 to 30 minutes.

Note: The same extract may be used for both Seratec® PSA Semiquant and Seratec® α-Amylase testing.

B. Seratec® PSA Semiquant Testing:

1. Record the cassette lot number. Remove the cassette from the foil pouch and label the cassette. The provided dropper may be discarded. *Do not use a cassette if the foil pouch has been opened.*

2. Prepare a 0.5 dilution by adding 100 µL of each extract to 100 µL PBS in a separately labeled Eppendorf tube.

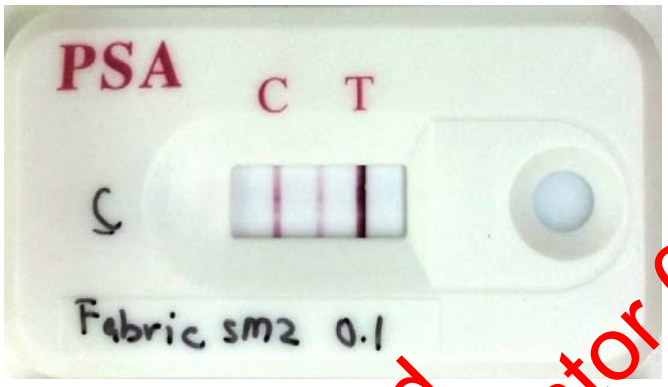
3. Aliquot the full 200 µL of each 0.5 dilution into the test chamber of a new Seratec® PSA Semiquant card.

4. Read results at **10 minutes**. Record the results for the Internal Standard and Control by indicating positive or negative. Record the results for the test region by indicating positive or negative.

Note: Positive results may be indicated if the lines appear prior to 10 minutes; however, negative results can only be indicated after 10 minutes.

5. Interpretation of overall Seratec® PSA Semiquant Test:

a. Positive (three lines):



i. A line appears in both the Internal Standard and Control regions, and

ii. A positive line appears within the test region. *Note: weak or strong positive may be indicated within the exam notes.*

b. Negative (two lines):

i. Line appears in both the Internal Standard and Control regions, and

ii. No line appears in the test region.

c. Fail:

i. A line does not appear in either the Internal Standard and/or Control regions.

ii. An incomplete line appears in the Internal Standard, Control, and/or Test Region.

An example of an incomplete line at the Test Region

If a test fails, the test must be repeated by performing Steps 1-5 in Section B, "Seratec® PSA Semiquant Testing", on a new Seratec® PSA Semiquant card.

C. Seratec® α-Amylase Testing:

1. Record the cassette lot number. Remove the cassette from the foil pouch and label the cassette. The provided dropper may be discarded. *Do not use a cassette if the foil pouch has been opened.*

2. Aliquot 200 µL of each extract (no dilution) directly into the test chamber of the Seratec® α-Amylase card.

3. Read results at **10 minutes**. Record the results for the Control by indicating positive or negative. Record the results for the test region by indicating positive or negative.

   Note: Positive results may be indicated if the lines appear prior to 10 minutes; however, negative results can only be indicated after 10 minutes.

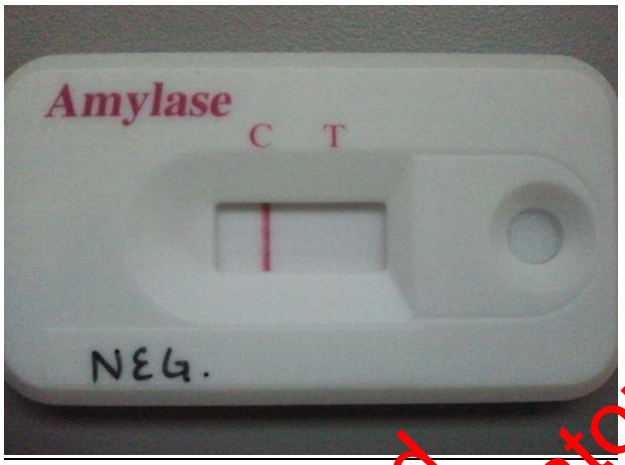
4. Interpretation of overall Seratec® α-Amylase Test:

   a. Positive (two lines):

   i. A line appears in Control region, and

   ii. A positive line appears within the test region. *Note: weak or strong positive may be indicated within the exam notes.*

b. Negative (one line):



i. A line appears in Control region, and

ii. No line appears in the test region.

c. Fail:

i. A line does not appear in the Control region.

ii. An incomplete line appears in the Control, and/or Test Region.

If a test fails, the test must be repeated by performing steps 1-4 in section C, Seratec® α-Amylase Testing, on a new Seratec® α-Amylase card.

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AMYLASE

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