

M-VAC® SAMPLE COLLECTION**30.1 PURPOSE**

To provide guidelines for the collection and preservation of samples using the M-Vac® system.

A. Theory/Background:

The M-Vac® system is a DNA sample collection method that utilizes wet-vacuum principles to rinse and capture cellular material. A sterile buffer is sprayed onto the surface of an item while a vacuum is created around the dispensed buffer to collect the buffer and dislodged cellular material into a collection bottle. The cellular material is filtered through a disposable filter device and deposited onto a sterile filter for future DNA extraction.

B. Limitations:

The spray is approximately 1" in width, and the sample collection head is approximately 1.34" wide. Passes with the collection head should overlap by approximately 30%.

When performing a sample collection, the collection head should only be used in a motion parallel to the handle, not perpendicular.

30.2 RESPONSIBILITY

Personnel qualified to perform Forensic Biology duties.

30.3 SAFETY

Use appropriate measures for the proper handling of biohazardous materials and hazardous chemicals according to GL-2 (Safety Manual).

30.4 DEFINITIONS/ABBREVIATIONS

- 30.4.1 LIMS: Laboratory Information Management System
- 30.4.2 PPE: Personal Protective Equipment
- 30.4.3 QRW(s): Quality Record Worksheet(s); Appendix 1
- 30.4.4 SEC/SEC100: Support Equipment Case
- 30.4.5 SRS: Surface Rinse Solution
- 30.4.6 M-Vac®: Microbial Vacuum

30.5 MATERIALS

- A. M-Vac® SEC 100 unit
- B. SRS
- C. Extension tubing
- D. Separation unit
- E. Sampling head
- F. Tray (glass or metal)
- G. Benchkote or other liquid barrier sheets
- H. Filter unit
- I. Pre-filter
- J. Pre-filter vacuum shield
- K. Collection bottle
- L. Chest harness
- M. Scalpel
- N. Forceps
- O. Scissors
- P. Sterile microcentrifuge tubes

30.6 GENERAL NOTES

- 30.6.1 The M-Vac® collection procedure will be performed at the discretion of the examiner, with input from the Unit Lead(s) when applicable, based on the submitting agency requests, case information, and the condition of the evidence.
- 30.6.2. If a body fluid stain or stain portion can be easily cut from the substrate, that is generally the preferred method of sample collection and/or submission to DNA.
- 30.6.3 Refer to FB SOP-01 for instruction on cleaning utensils and laboratory areas, PPE, and for additional instruction on evidence examination including but not limited to documentation, collection, preservation, sterile microcentrifuge tube labeling, verification, sub-itemization, transfers/storage, and LIMS.
- 30.6.4 For additional information and guidance see the SEC 100 User Guide and FB SOP-23 (Equipment Maintenance).

30.7 PROCEDURE

When performing M-Vac® sample collection and filtration, aspects associated with sample collection and containment (including but not limited to collection bottle(s) and filter housing) will be labeled appropriately.

30.7.1 M-Vac® SEC set-up:

Each port is a point of potential contamination; the handling and connection of any lines and tubing should be performed carefully to avoid touching the exposed ends of the connection points.

- 30.7.1.1 Document the use of the M-Vac® on the appropriate QRW(s) and equipment log sheet(s).
- 30.7.1.2 Clean the exterior housing of the SEC with 10% bleach or stabilized bleach followed by ethanol.
- 30.7.1.3 Remove the SRS from the overwrap and hang the solution bag on the hook on the pressure chamber door.
- 30.7.1.4 Remove the M-Vac® sampling head and separation unit from the overwrap. Tighten the lid of the separation unit (tighten, release, tighten) and place it in the designated holder. Ensure that the switch is pulled back to the off position on the sampling head, and place the sampling head in the designated holder (the configuration of both holders may be adjusted as needed).
- 30.7.1.5 Remove the extension tubing from the overwrap.
 - 30.7.1.5.1 Attach the solution line fitting to the fitting on the collection unit. Gently attach the vacuum tubing to the port on the collection unit.
 - 30.7.1.5.2 Break off the cover from the SRS port. Push and twist to connect the spiked fitting of the tubing to the bag port. Connect the vacuum side of the tubing to the SEC.
 - 30.7.1.5.3 Extension tubing may be used for multiple sample collections.
- 30.7.1.6 Close the pressure chamber door until it is locked shut by the hinged latch.
- 30.7.1.7 Turn on the SEC.
- 30.7.1.8 Turn on the solution pressurization. The solution is pressurized when the low pressurization indicator light turns off.

30.7.2 Sample collection:

If there could be runoff or pooling of the buffer during the sample collection process, place the item in a clean tray for sample collection.

- 30.7.2.1 Turn the vacuum switch to on.
- 30.7.2.2 With the vacuum pump on, retighten the lid of the separation unit. During sample collection, the separation unit should be maintained and/or secured in a manner that ensures it is always upright during sampling (i.e. placed back into the holder on the SEC or placed in the chest harness).
- 30.7.2.3 Place the sampling head against the surface to be sampled so that it is flush with the surface, keeping all flexible feet in light even contact with the surface, as the item permits.
- 30.7.2.4 In general, unidirectional multi-pass sampling will be employed:
- 30.7.2.4.1 The sample spray is turned on while pulling the sample head over the collection area. The spray is turned off at the end of the stroke.
 - 30.7.2.4.2 Repeat, covering the same area with the sample spray off, performing at least two vacuum only post-spray passes.
 - 30.7.2.4.3 Move the head to the next sample path, and repeat, attempting to overlap the previous sample area by approximately 30%.
 - 30.7.2.4.4 Repeat, until the desired area has been sampled.
 - 30.7.2.4.5 Return to the initial sample area and repeat steps 30.7.2.4.1 – 30.7.2.4.4, for a total of two wet passes and a minimum of four vacuum only passes for each sample path.
 - 30.7.2.4.6 Collect any pooled or runoff liquid from the tray using vacuum only passes as needed.
- 30.7.2.5 If the sample area is small, sample until a minimum of 30mL has been collected.
- 30.7.2.6 If the sample area is large, additional collection bottles may be needed to complete the sample collection.
- 30.7.2.6.1 Turn off the vacuum.
 - 30.7.2.6.2 Unscrew the bottle from the separation unit and cover with a lid.
 - 30.7.2.6.3 Screw a new bottle onto the separation unit.
- 30.7.3 Removing/replacing the M-Vac® separation unit and sampling head.

- 30.7.3.1 Turn off the vacuum and solution pressurization.
- 30.7.3.2 Unscrew the bottle from the separation unit and cover with a lid.
- 30.7.3.3 If replacing with a new separation unit and sampling head:
 - 30.7.3.3.1 Remove the used separation unit and place it on a clean surface.
 - 30.7.3.3.2 Clean the holders as appropriate and place the new separation unit and sampling head on the holders.
 - 30.7.3.3.3 Disconnect extension tubing at the used separation unit and connect to the new separation unit as previously described.
 - 30.7.3.3.4 Properly dispose of the used separation unit and sampling head.
- 30.7.3.4 If not replacing with a new separation unit, disconnect extension tubing and properly dispose of the used separation unit and sampling head.

30.7.4 Replacing the SRS

- 30.7.4.1 Turn off the solution pressurization to depressurize the solution pressurization chamber.
- 30.7.4.2 Remove and discard used SRS.
- 30.7.4.3 Install the new SRS bag as previously described.

30.7.5 Filtering a sample

If the sample appears to contain debris, pre-filtering may be necessary. See 30.7.6.

- 30.7.5.1 Remove the vacuum filter unit from the overwrap. Attach the vacuum tubing to the vacuum filter (the end of the tubing that was previously connected to the separation unit).
- 30.7.5.2 Remove the lid on the filter. Maintain the vacuum filter in an upright position during the filtration process.
- 30.7.5.3 Turn on the vacuum.
- 30.7.5.4 Swirl the sample in the bottle and then slowly pour the sample into the vacuum filter.

- 30.7.5.5 Continue vacuum pressure until all of the solution has passed through the filter. If it is taking longer than 20 minutes for the liquid to pass through the filter, see section 30.7.7 below.
- 30.7.5.6 Turn off the vacuum and release the pressure.
- 30.7.5.7 The filtrate can be used to rinse the collection bottle (1-3 times). Remove the filtrate collection bottle from the bottom of the concentration filter and pour it back into the original sample collection bottle to rinse this bottle. If a pre-filter apparatus was used, it should also be rinsed.
- If the appropriate sampling head is still connected, it can be used to dispense fresh buffer for rinsing.
- 30.7.5.8 Replace the filtrate collection bottle (if removed) and repeat steps 30.7.5.3 through 30.7.5.6.
- 30.7.5.9 Properly dispose of the filtrate.
- 30.7.5.10 Allow the filter to dry in the filter housing, possibly overnight.
- 30.7.6 Pre-filtering samples
- 30.7.6.1 Remove the pre-filter and vacuum shield from their respective overwrap.
- 30.7.6.2 Attach the vacuum tubing (the end of the tubing that was previously connected to the separation unit) to the vacuum shield. The vacuum shield may need to be replaced during the filtration process if liquid is observed in the shield.
- 30.7.6.3 Connect the pre-filter to the vacuum shield.
- 30.7.6.4 Turn on the vacuum. Maintain the pre-filter system in an upright position during the filtration process.
- 30.7.6.5 Swirl the sample in the bottle and then slowly pour the sample into the pre-filter.
- 30.7.6.6 Continue vacuum pressure until all the solution has passed through the filter, or the conical (pre-filtration collection) tube needs to be emptied. See 30.7.6.6 below.
- 30.7.6.7 Turn off the vacuum and release the pressure.
- 30.7.6.8 The conical (pre-filtration collection) tube may need to be emptied multiple times throughout the pre-filtration process. Depending on the overall volume, the eluent may be poured directly into the vacuum filter unit, or a collection bottle.

30.7.6.9 Properly dispose of the used prefilter unit (once rinsed) and vacuum shield.

30.7.7 Filtering slow samples

30.7.7.1 Turn off the vacuum filter (leave the power to the SEC unit on).

30.7.7.2 As the system is sealed, the vacuum pressure will be maintained, and the sample should continue to slowly pass through the filter.

30.7.7.3 When the vacuum has dropped below 16inHg or when the sample has completely filtered, release the vacuum by disconnecting the vacuum hose.

30.7.7.4 Reconnect the vacuum hose and turn the vacuum back on.

30.7.7.5 Repeat until the filtration is complete.

30.7.7.6 If the filtration process appears to have slowed considerably or stopped, additional filter(s) may be used for the remaining sample.

30.7.7.7 If using the filtrate when rinsing the original collection bottle (and other supplies), use a small amount.

30.7.8 Post collection

30.7.8.1 The submission/item will be allowed to dry prior to repackaging. Store/transfer to an appropriate location (such as a hood) while drying overnight.

30.7.8.2 Preparing the filter

30.7.8.1.1 Once dry, excise the filter from the housing using a scalpel and cut in half. Each filter half will be placed into one sterile microcentrifuge tube.

30.7.8.1.2 Properly dispose of the used filter unit.

30.7.9 Filter(s) submitted by an outside agency

30.7.9.1 Cut and prepare filter(s) as described in 30.7.8.2

30.7.9.2 Testing of reddish-brown staining, if observed on a filter, will be determined on a case-by-case basis, based on the information and/or request(s) received with the case.

30.7.10 QC procedure: SRS Buffer

Approved by Director: Dr. Guy Vallaro

Prior to use with casework samples, a portion of SRS buffer will be provided to the DNA Section for QC purposes according to DNA QR-356 (M-vac Buffer QC).

30.7.10.1 SRS buffer will be dispensed directly into a sterile microcentrifuge tube, using new tubing and a new collection head.

30.7.10.2 If the appropriate results are not obtained, the buffer QC may be repeated with a new bag of the same lot of SRS buffer, new tubing and a new collection head.

30.7.11 Discard/replace buffer and consumables according to the manufacturer's expiration date. Manufacturer's expiration dates with only the month and year indicated expire the last day of the month indicated.

30.8 REFERENCES

30.8.1 M-Vac® SEC 100 User Guide Rev. J

30.8.2 M-Vac® SEC 100 User Guide Rev. L

30.8.3 GL-2 (Safety Manual)