Male Screen Extraction and Microcon Procedure

Note: If cuttings previously collected, skip to Part A, step 3

Part A: Extraction

Per FB SOP-02 (SAECK Exam) – Multiple swabs collected from the same orifice/area and submitted as one item will be considered one sample. For each item, test a portion of each swab as one sample (i.e. 4 swabs from a vaginal swab submitted; ~1/10 of each of the 4 swabs will be placed in 1 tube). A sample (1/4 of swab) will be taken for any future request of serology testing. The remaining sample will be used for DNA testing if applicable based on the Male Screen Results. Samples taken will be documented on **FB QR-05b SAEK Worksheet for Male Screen**. Please note: depending on the case scenario (i.e possibly non-semen male DNA), a differential or a non-differential request may be made in LIMS if the sample results lead to a DNA EZ1 extraction.

1. Take a small cutting (~1/10) of each swab of the item and place all cuttings of that item into one Spinease tube (Note on tube how many ~1/10 cuttings in the tube).

2. Repeat step 1 for any other items to be tested to generate a set of swab cutting samples for extraction.

3. Document samples and number of swab cuttings on **DNA QR-314 Male Screen Extraction Worksheet.**

4. Alongside the cutting samples, process a male screen positive control (EP-MS), consisting of 10 microliters (µl) of diluted semen pre-aliquoted in a Spinease tube, and a reagent blank (RB), consisting of reagents added to an empty Spinease tube.

5. Add 50 µl of 1N Sodium Hydroxide for each ~1/10 cutting in the tube. Add the 1N Sodium Hydroxide directly onto the swab cutting(s) in each tube. Add 100 µl of 1N Sodium Hydroxide to the male screen positive control (EP-MS). Maximum volume used for any sample is the volume used for the RB (i.e. 2 cuttings = 100 µL).

6. Incubate the Spinease tubes in a thermomixer at 80°C and shaking at 750 rpm for 10 minutes. Verify that the thermometer reads the correct temperature and document on DNA QR-204 Incubation Temperature Log.

7. Remove the tubes from the thermomixer and pulse spin to maximum speed (15,000 rpm) in centrifuge by holding the "short" button for ~15 seconds.

8. Add 2 µl of Glacial Acetic Acid for every 50 µl of 1N Sodium Hydroxide to each tube and vortex for 1-2 seconds to mix.

9. Add 200 µl of Milli-Q purified water for every 50 µl of 1 N Sodium Hydroxide to each tube and vortex ~3 seconds to mix. Pulse spin the set of samples to maximum speed (15,000 rpm) in a centrifuge by holding the "short" button for ~15 seconds.

10. Use 2 µl of each extract in a Quantifiler Trio reaction (see DNA SOP-3, DNA WI-07). If not quantifying for several hours or more, store the extracts at 4°C.

11. Review the Quantifiler Trio results. The positive control Y quant value should be within 5 standard deviations of the aliquot set average (see **DNA QR-312 Male Screen Extraction Positive Control (EP-MS) QC**). The RB and Quant NEG should be 'Undet.' for all quant values. If DNA (≥1pg/ul in SA or LA value, and ≥0.1pg/ul for the Y value) is detected in the Trio negative control or reagent blanks, bring it to the attention of the TL. Depending on the amount detected, the Trio results may be interpreted with caution with TL approval. Detection of less than 1pg/uL in the SA or LA target and/or 0.1pg/ul in the Y target does not need TL notification.

    11.1 Any samples that produce a Y quant value of ≥ 0.0001 ng/µl will be forwarded to DNA for extraction. This is reported as a positive male screen result.

    11.2 Any samples that produce a Y quant value of < 0.0001 ng/µl **AND** an SA value of < 0.001 ng/µl will be forwarded to DNA for extraction. This is reported as an inconclusive male screen result, due to the low quantity of total human DNA.

    11.3 For any samples that produce a Y quant value of < 0.0001 ng/µl **AND** an SA value of ≥ 0.001 ng/µl; perform the following purifying/concentration procedure with a Microcon DNA Fast Flow centrifugal filter (including the RB).

Part B: Microcon

NOTE: Document this step on **DNA QR-313 Male Screen Concentration Worksheet**

1. Assemble a Microcon DNA Fastflow filter cup and tube. Add 400 µl of sterile Milli-Q purified water to the Microcon DNA Fastflow filter cup. Add 100 µl of the Male Screen extract (Sodium Hydroxide and Glacial Acetic Acid diluted with H20) to the cup. Reminder: purify/concentrate the RB associated with the samples.

2. Spin the Microcon device in a centrifuge at 2900 rpm (790 rcf) for ~15 minutes to reduce the 500 µl starting volume to <20 µl, but do not spin the filter cup dry. A second 400 µl Milli-Q water wash may be performed as needed.

3. Invert the filter cup into a clean 1.5 ml tube and spin the remaining volume from the cup into the tube in a centrifuge at 2900 rpm for 2 minutes.

4. Measure the volume with a micro-pipette and bring the sample to a 20 µl total volume by adding sterile Milli-Q purified water.

5. Use 2 µl of each extract in a Quantifiler Trio reaction (see DNA SOP-3, DNA WI-07).

6. Review the Quantifiler Trio results. The RB and Quant NEG should be 'Undet.' for all quant values. If DNA (≥1pg/ul in SA or LA value, and ≥0.1pg/ul for the Y value) is detected in the Trio negative control or reagent blanks, bring it to the attention of the TL. Depending on the amount detected, the Trio results may be interpreted with caution with TL approval. Detection of less than 1pg/uL in the SA or LA target and/or 0.1pg/ul in the Y target does not need TL notification.

    6.1 Any samples that produce a Y quant value of ≥ 0.0001 ng/µl will be forwarded to DNA for extraction. This is reported as a positive male screen result.

    6.2 Any samples that produce a Y quant value of < 0.0001 ng/µl **AND** an SA value that did **not** increase by ≥ 1.5 fold will be forwarded to DNA for extraction. This is reported as an inconclusive male screen result, due to the lack of sufficient

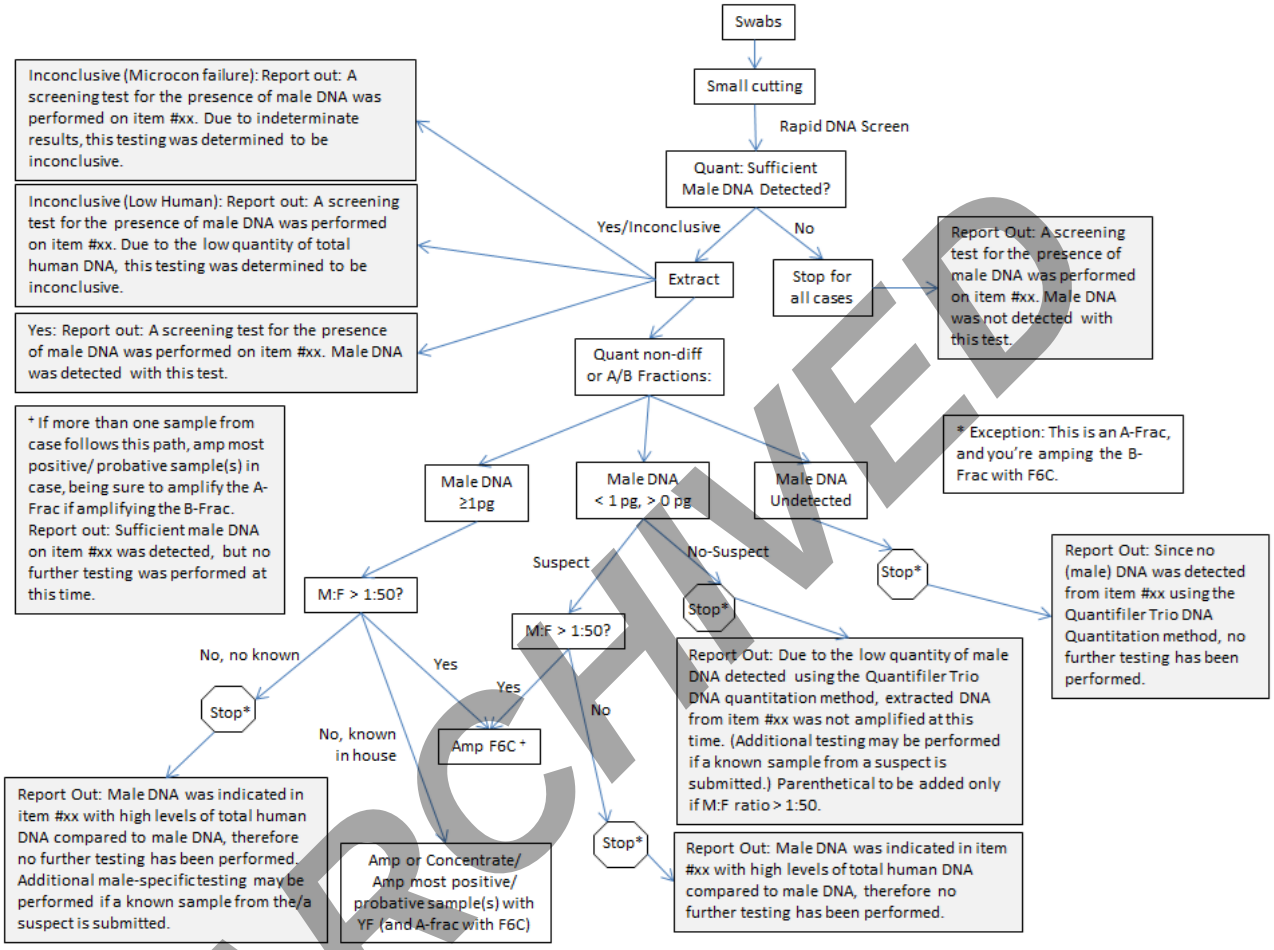
concentration by the Microcon device. The statement will contain the reason as "due to indeterminate results."

6.3 Any samples that produce a Y quant value of < 0.0001 ng/µl **AND** an SA value that **did** increase by ≥ 1.5 fold will be halted for testing. This is reported as a negative male screen result. DNA extraction will not be performed.

QR-313 "Male Screen Concentration Worksheet" is additionally used to flag samples that would need to go through a Microcon step. This worksheet has been validated to follow all guidance in this SOP outlining when a sample is to go through the Microcon step.

After all the samples have been processed, a DNA analyst competent in the male screen procedure will be given the batch to review, determine male screen results, and assign appropriate LIMS requests (differential, non-differential, male negative, knowns, consumption). In addition, the batch will be technically/administratively reviewed by a qualified analyst. This review will be documented on DNA QR-4E. After review, only QR, and worksheets containing results will be photocopied and placed in each case jacket. The original batch paperwork will be scanned to the u-drive, and filed with benchwork batch paperwork. After LIMS requests are made, the case files will be given to Case Management. Analysts or technicians assigned negative samples and/or cases will move those samples virtually and physically to the "Freezer Storage" LIMS storage location.

7. Please see below workflow:



Please note: In General, if multiple samples were sent for DNA extraction, one sample (the most positive/probative) will be forwarded to F6C amplification. However, based on the case scenario (number of perpetrators, consensual partners, different orifices, etc.) two or more samples may be forwarded to F6C amplification. Keep in mind offense, and the potential for CODIS entries when making this determination. If the case scenario is unknown, all samples that qualify for amplification will be amplified.

If a suspect known is available and Y-STR testing is performed, the epithelial-rich fraction will be amplified for autosomal STRs for a quality assurance check.

References:

DNA QR-311 QC Male Screen Extraction Reagents QC: Used to capture QC results of the Male Screen Extraction Reagent Aliquots.

DNA QR-312 Male Screen Extraction Positive Control (EP-MS) QC: Used to capture QC results of a lot of Male Screen Positive Control samples. The Bulk Male Semen Samples will be tracked on a document "BioChemed Semen Samples" that is found on the DNA server in the Male Screen Folder.

DNA QR-313 Male Screen Microcon Worksheet: Used to capture purification and concentration step of the process when needed.

DNA QR-314 Male Screen Extraction Worksheet: Used to capture samples and controls run in a Male Screen Batch. QR will have reagent lot numbers and thermal mixer used.

LIMS:

Forensic Biology Request = "CT 100 – Male screen sample prep" (released at draft complete)

DNA Request = "Male Screen" (released at draft complete)