QIASYMPHONY MALE DNA SCREENING PROCEDURE

Background

The Scientific Working Group on DNA Analysis Methods (SWGDAM) provided recommendations to address the high volume of sexual assault kits in an efficient and timely manner in the December 5, 2016 publication titled *"Recommendations for the Efficient DNA Processing of Sexual Assault Evidence Kits"* [1]. Traditionally, serological exams were performed on sexual assault evidence items which are very labor intensive and time consuming and not as sensitive as modern DNA typing kits. SWGDAM has recommended a Direct to DNA approach to replace the typical serological testing with a more sensitive DNA-based technique that has the added benefit of being more efficient than performing serology followed by DNA analysis. Since a portion of the evidence is retained, serology can be performed after DNA testing is complete when necessary. This procedure mimics the recommendations set forth by SWGDAM.

Summary of Procedure

This protocol is to identify the presence of male DNA in samples prior to DNA testing. A portion of the evidence is exposed to Qiagen Buffer ATL, DTT and Proteinase K to digest all cells. The lysate is then purified on the Qiagen QIAsymphony® SP instrument and quantified with Quantifiler® Trio on the Applied Biosystems® 7500 Real-Time PCR System using the HID Real-time PCR Analysis Software v1.2.

Sample Handling

All biological samples and DNA must be treated as potentially infectious. Appropriate sample handling and disposal techniques should be followed. See:

- **Safety Manual**, *Universal Precautions*
- **Quality Assurance Manual**, *General Sample Control and Forensic Sample Preservation Policy*
- **Analytical Procedures Manual**, *Forensic Evidence Handling*

After screening is complete, samples are securely stored at 2-8C.

Warnings and Precautions

Lysis buffer contains chaotropic salt, which is an irritant. Take appropriate laboratory safety measures and wear gloves when handling. This reagent is **not** compatible with disinfecting agents which contain bleach.

Reagent cartridges contain ethanol, isopropanol and guanidine thiocyanate. These substances should be considered flammable, harmful, and irritants.

Improper use of the QIAsymphony® SP may cause personal injuries and/or damage to the instrument. The instrument must only be operated by qualified personnel who have been appropriately trained.

All users MUST read and be familiar with all safety precautions outlined in the first chapter of the QIAsymphony® SP/AS User Manual-General Description.

Damage to the instrument caused by failure to follow these guidelines may result in void of the warranty.

See Quantification of DNA Extracts Using the Applied Biosystems® Quantifiler® Trio DNA Quantification Kit Procedure for relevant warnings and precautions.

Reagents and Materials

QIAsymphony® DNA Investigator Kit (192 reactions*)

- 2 Reagent cartridges
- 2 Enzyme racks
- 2 Piercing lids
- 20mL Buffer ATE
- 20mL Buffer AVE
- 2 X 50mL Buffer ATL
- 3 X 1.4mL Proteinase K
- 310µg Carrier RNA
- 2 Reuse Seal Strips
- 1 Trough cover (for magnetic particles)
- 1 Handbook

60mL Top Elute Fluid
MBG H_2O 200uL
Disposable filter tips (32 tips per tip rack / 4 tip racks per sealed blister pack)
1500uL Disposable filter tips (32 tips per tip rack / 4 tip racks per sealed blister pack)
Sample prep cartridges (28 cartridges pre-racked per unit box)
8-rod covers (12 covers pre-racked per unit box)
Qiagen S-blocks (96 well tray lysates)
Aluminum seal plate covers
Qiagen EMT elution trays and septa strips (96 well elutions)
QIAsymphony® SP sample plate carrier
QIAsymphony® SP elution racks
QIAsymphony® SP Accessory Trough
Pipettes (5mL, 1000µL, 200µL, 40µL, and 10µL)
Pipette tips
Thermomixer-96 well plate and/or single tube
Centrifuge – 96 well plate and/or single tube
Scissors
Eliminase
100% Ethanol
2mL Lo-bind microcentrifuge tubes
QIAGEN Proteinase K
1M Dithiothreitol (DTT)
15mL conical tubes
50mL reagent boats
Permanent Marker/tough tag labels
Bench paper
Kimwipes
Vortex
Micro-centrifuge
Gloves
Lab Coat
Mask
Protective Eyewear

Reagents and Materials – Storage and Handling

All reagents and materials are to be kept under sterile conditions. Store all reagents according to the manufacturers' recommendations.

Do not use reagents beyond the listed expiration dates. Date and initial all reagents when put in use. Record in the **Reagent Log**.

Quality Control

Use of the ***QIAsymphony Male DNA Screening Workbook*** and ***Quantifiler Trio Workbook*** are required for documentation. All information must be completed.

Positive Control

Extraction Positive Control (TF- Optional): Positive control(s) are processed and run with each set of extractions and used to ensure the extraction, amplification and typing procedures are working as expected.

Negative Control

Extraction Negative Control: Reagent negative control(s) are processed and run with each set of extractions and consists of all reagents used in the procedure but contains no DNA sample.

Procedure

When a new QIAsymphony® kit is opened:

- Log the Opened Date and Use By Date
- Dissolve the lyophilized carrier RNA in 1.6mL Buffer ATE (provided in the QIAsymphony® DNA Investigator Kit).
- Transfer 400uL to each of the 2 empty tubes within the included enzyme rack
- Add an additional 1.2mL Buffer ATE to each tube and mix by pipetting up and down several times.
- Store the stock carrier RNA at -20°C for use with the second reagent cartridge/enzyme rack in the kit. Store the enzyme rack at 2-8°C (will be stable for 4 weeks).
- Remove the magnetic-particle trough from the reagent cartridge frame, vortex for at least 3 minutes, and replace it in the reagent cartridge frame before the first use.

Before starting:

- Switch on the QIAsymphony® SP by pushing the blue button on lower left corner. It may take up to 15 minutes to boot up.
- Heat Buffer ATL at 70°C until all precipitate dissolves.
- Pre-warm Thermomixer to 56°C.
- Permanently label the sample tubes/trays and elution tubes/trays with tray/case ID, date and initials.
- Ensure that the magnetic particles are fully re-suspended.
- Place the enzyme rack with the diluted carrier RNA into the reagent cartridge holder.
- If desired elution reagent is MBG H_2O, add appropriate volume of MBG H_2O to accessory trough and place in tip rack position 5 or 12.
- Remove the lids of both carrier RNA tubes and store in the dedicated slots of the gray reagent cartridge holder.
  Before using a reagent cartridge for the first time, place the piercing lid on top of the reagent cartridge (it will be forced down and locked in place by the QIAsymphony® SP during purification).

Caution: The piercing lid is sharp. Take care when placing it onto the reagent cartridge. Make sure to place the piercing lid onto the reagent cartridge in the correct orientation.

- Before using a reagent cartridge for the first time, check that Buffer QSL3 does not contain a precipitate. If necessary, remove the trough containing Buffer QSLE from the reagent cartridge and incubate for 30 minutes at 37°C with occasional shaking to dissolve precipitate. Make sure to replace the trough in the correct position. If the reagent cartridge is already pierced, make sure that the troughs are securely sealed with Reuse Seal Strips and incubate the complete reagent cartridge for 30 minutes at 37°C with occasional shaking in a water bath.

Steps:

1. Cut sample into a 1.7mL microcentrifuge tube labeled by the scribe.

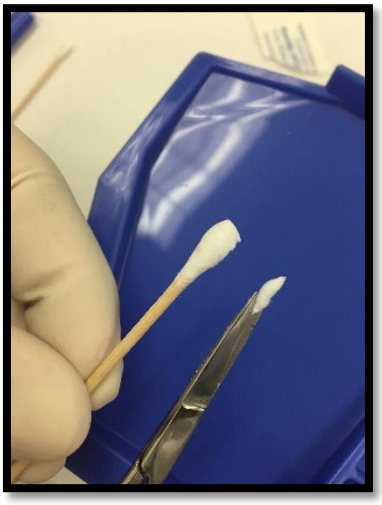
   A scribe's role is to fill out the appropriate inventory sheet electronically and label the sample tubes for the processor. A scribe must be present for the cutting of all samples.

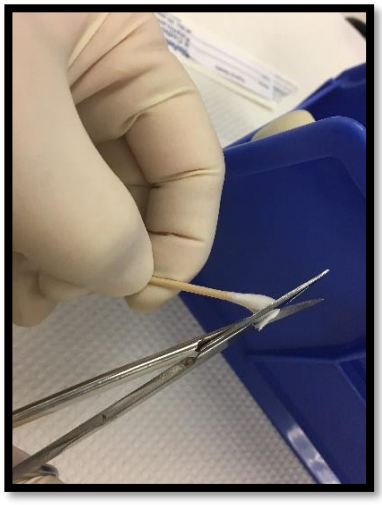
   For swabs, cut up to 1/8 of all swabs. For example, if 4 vaginal swabs are received, cut up to 1/8 of each of the 4 swabs and place all 4 cuttings into the same microcentrifuge tube. See pictures below for cutting ~1/8 of a swab.

   For items other than swabs, cut a portion of the stain. The portion cut for the male screen will depend on the size of the entire stain. As a guide, sample no more than 1/8 of the stain or a 1cm x 0.5cm cutting, whichever is smaller.

   Note: An alternate light source can be utilized to assist in identifying stains on clothing. The acid phosphatase test (AP) can also be utilized to help determine the best stain/ samples to move forward in cases with multiple stains (e.g. >10 stains). Stains with a positive AP test would be male screened before stains with a negative AP test. Stains with a negative AP results may need to be male screened to meet the minimum number of male screened items according to client specifications. The AP test would only apply to cases where semen is expected to be present.

   Upon cutting, the scribe working with the processor logs the date, time, initials of processor, scissors ID, and workstation ID into the corresponding column for Y-Screen cutting in the appropriate inventory sheet.





2. Prepare lysis buffer master mix containing 430uL Buffer ATL, 40uL DTT and 20uL Proteinase K per sample plus an additional 3 samples.

3. Vortex or invert master mix and add 490uL lysis master mix to each sample tube containing substrate or reagent blank.

4. Vortex samples vigorously for 10 seconds and spin down briefly.

5. Incubate samples at 56°C for 45 minutes on a thermomixer with the orbital shaker set to 900RPM.

6. Vortex samples vigorously for 10 seconds and spin down briefly.

7. Transfer the lysate of each sample into the corresponding well of a Qiagen S-block tray, leaving the substrate as dry as possible in the lysate tube. A witness must observe this transfer and initial the appropriate space on the worksheet. Check the appropriate box if the substrate is discarded or retained.

8. Select Log-in on the QIAsymphony® SP touchscreen then choose **Supervisor** and enter the password.

9. Prepare the deck of the QIAsymphony® SP and perform a complete scan prior to starting the run.

Note: If insufficient reagents are loaded on the instrument, the R+C tab on the touchscreen will flash yellow and indicate which reagents are insufficient/missing. Any reagent placed in the accessory trough will be referred to as EtOH.

   a. The Qiagen EMT elution plate base must be labeled with a tough tag on the base prior to the plate being put on the instrument. Label must include: Male screen ID, date, and initials.

Note: The bottom MUST be removed from Qiagen EMT elution plates prior to placing on elution rack.

   b. Open the sample drawer and slide the sample carrier(s) onto the deck. A batch will appear on the touchscreen. Follow the directions on the touchscreen to assign tube type and protocol for each batch of 24 samples to be purified.

   c. Select Queue for each batch and then run.

10. When purification is complete, open the elution drawer and remove the elution rack.

11. The elution tray must also be virtually removed from the QIAsymphony touchscreen. Do this by selecting the elution tray on the touchscreen and select “Remove”.

12. Remove all waste from the QIAsymphony deck and complete cleaning/maintenance procedures.

13. Using a thumb drive collect the *QIAsymphony SP Result File* from the software. This is located in *“Tools”* tab on the main screen. Select “File Transfer” button under the “In-/Output Files” tab, select the “Results File” button and choose “Transfer” button. This will transfer the files to the USB. Using this HTML file, compare the “Input Position” well to the “Output Position” well. This should correspond to the worksheet used to set-up the QIAsymphony and any subsequent worksheet.

    The QIAsymphony SP Result File (HTML file) must be printed post extraction and included in the case file. This file must be checked to ensure the Input [well] position corresponds with the Output [well] position.

Samples

Sample ID	Input position	Type	Liquid-level detection	Output position	Assay control set	Reagent rack (beads + reagents)	Reagent rack (enzymes)	IC tube position/Liquid-level detection	Eluate volume	Validity of result
_01_2000219	A:1	S	N	A:1	CW200_ADV_HE CR21308 ID1604 V1	1,BufferBottle-1	1	n/a	30 µl	unclear, 180047
_02_2000219	B:1	S	N	B:1	CW200_ADV_HE CR21308 ID1604 V1	1,BufferBottle-1	1	n/a	30 µl	unclear, 180047
_03_2000219	C:1	S	N	C:1	CW200_ADV_HE CR21308 ID1604 V1	1,BufferBottle-1	1	n/a	30 µl	unclear, 180047
_04_2000219	D:1	S	N	D:1	CW200_ADV_HE CR21308 ID1604 V1	1,BufferBottle-1	1	n/a	30 µl	unclear, 180047
_05_2000219	E:1	S	N	E:1	CW200_ADV_HE	1,BufferBottle-1	1	n/a	30 µl	unclear,

14. Shut down the instrument by pushing the blue button on lower left corner

15. The samples are now ready to be quantified with Quantifiler® Trio on the Applied Biosystems® 7500 Real-Time PCR System using the HID Real-time PCR Analysis Software v1.2. (See MUFSC Procedure 03.02.06 Quantifiler Trio Quantitation)

Results Interpretation

Analyze controls and samples according to protocol (See MUFSC Procedure 03.02.06 Quantifiler Trio Quantitation)

For the Male DNA screen, the results from the Y (Male) target will be utilized to determine the presence of human male DNA. The results should be analyzed utilizing four decimal places (to the ten thousandth).

Target	Amplicon Length	Dye/Quencher
Y (Male)	75 bases	FAM® /MGB

- For samples with results ≥ 0.0050 ng/uL - report “Male DNA was detected"

- For samples with results below 0.0050 ng/uL - report "Inconclusive for male DNA"
  Unless otherwise specified by the client, samples that would be amplified with Globalfiler™ or PowerPlex® Fusion 6C may stop at Y screen (SAYS) if the Human male results are below 0.001 ng/uL.

- For samples with results of 0.0000 ng/uL - report “No Male DNA was detected"

For samples where sperm cells are expected to be present and male DNA was detected or inconclusive, these samples should proceed to a differential extraction. However, utilizing the variance between the small autosomal target and the Y (Male) target of the male screen results, it may be determined that the male screen extract will produce a male only DNA profile. In this situation and depending on the case scenario, the male screen extract can be utilized for STR typing.

According to our validations, if the small autosomal target is less than the male (Y) target it is expected to generate a male only DNA profile. In addition, If the Male (Y) target is greater than 85% of the small autosomal target it is also expected to generate a male only DNA profile.

For samples that are queued to be amplified using the male screen extract, proceed to “Male Screen Extract Transfer” below.

For samples where non-sperm cells are expected to be present and male DNA was detected or inconclusive OR cases where no male is involved, there are two options:

OPTION 1: STR type the sample by amplifying the male screen extract.

OPTION 2: STR type the sample by taking a second cutting of the sample, extracting with an appropriate protocol and amplifying the sample.

Option 1 is preferred to reduce the amount of sample that is consumed during the testing process.

Option 2 should be considered when the male screen extract quant is too low to expect optimal results.

NOTE: Client specific *technical specifications* should be consulted to determine what samples should move forward within a case.

Male Screen Extract Transfer

If a male screen extract has been queued for amplification based on the results interpretation, the sample is pulled from the elution plate into a labeled Lo-Bind tube and complete the workbook **Male DNA Screening Extract Transfer Worksheet**. A witness must observe this transfer and initial the appropriate space on the worksheet.

Comments on Storage

Non-amplified DNA must be stored separately from amplified product. Store samples at 2-8°C.

Technical Assistance

For information and assistance regarding the performance or applications, contact the Qiagen Technical Service Department 1-800-362-7737.

Reference

1. Recommendations for the Efficient DNA Processing of Sexual Assault Evidence Kits, SWGDAM

Appendix A: Reference Sheet for QIAsymphony

Custom Protocol

CW200ADVHE_CR23153_ID3974	200*µl* lysate with H_2O elution
CW500ADVHE_CR23153_ID3975	500*µl* lysate with H_2O elution
CW200ADVHE_CR22931_ID3666	200*µl* lysate with ATE elution
CW500ADVHE_CR22931_ID3667	500*µl* lysate with ATE elution

Labware

Elution Tray	Deep Well------QIA#19588 *EMTR
Elution Tubes	SAR#72.607 *T1.5 Screw
Lysis Tray- S-Block	Deep Well------QIA#19585 S-Block96
Lysis Tubes	SAR#72.694 T2.0 ScrewSkirt