

SER: Sexual Assault Sample Preparation

Principle Items of evidence from sexual assaults are examined for the presence of bodily fluids including semen and saliva. This procedure outlines the series of tests performed to identify bodily fluids and determine which samples are suitable for subsequent DNA analysis.

Chemicals, reagents, equipment and supplies Refer to *SER: Spermatozoa – Microscopic Examination*, *SER: Seminal Fluid – Acid Phosphatase Spot Test*, *SER: Seminal Fluid – ABACard p30 Immunoassay Test*, and *SER: Saliva – SALIgAE Test for Amylase* for a list of chemicals, reagents, equipment, and supplies used in this procedure.

Sample preparation Use the following procedure to prepare sexual assault samples from kits or samples that have not been pre-screened. For samples that have previously been screened, proceed to the appropriate DNA extraction method based on the serology results.

Step	Action
1	Place one whole swab or stain cutting into a sterile microcentrifuge tube.
2	Add 300 µL of TE Buffer and incubate at room temperature for 30-60 minutes on the ThermoMixer.
3	While the substrate is incubating, label the appropriate number of microscope slides and SALIgAE vials, if needed.
4	Place the substrate in a spin basket, place the spin basket into the original tube, and centrifuge for 5 minutes to form a cellular pellet.
5	For samples being tested for semen, label a new microcentrifuge tube.

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SER: Sexual Assault Sample Preparation, Continued

Sample
preparation
(continued)

Step	Action								
6	<p>One sample at a time, remove the spin basket and perform the appropriate tests.</p> <table> <tr> <th>If the sample is being tested for...</th><th>Then ...</th></tr> <tr> <td>Semen and saliva</td><td> <ul style="list-style-type: none"> • transfer 240 µL of the supernatant to the new tube • transfer 30 µL of supernatant to a SALIgAE vial (Refer to SER: Saliva – SALIgAE Test for Amylase) • record the SALIgAE results on the <i>Sexual Assault Examination Worksheet</i> </td></tr> <tr> <td>Semen only</td><td> <ul style="list-style-type: none"> • transfer 270 µL of the supernatant to the new tube </td></tr> <tr> <td>Saliva only</td><td> <ul style="list-style-type: none"> • transfer 30 µL of the supernatant to a SALIgAE vial (Refer to SER: Saliva – SALIgAE Test for Amylase) • remove and discard 240 µL of supernatant • record the results on the <i>Sexual Assault Examination Worksheet</i> </td></tr> </table>	If the sample is being tested for...	Then ...	Semen and saliva	<ul style="list-style-type: none"> • transfer 240 µL of the supernatant to the new tube • transfer 30 µL of supernatant to a SALIgAE vial (Refer to SER: Saliva – SALIgAE Test for Amylase) • record the SALIgAE results on the <i>Sexual Assault Examination Worksheet</i> 	Semen only	<ul style="list-style-type: none"> • transfer 270 µL of the supernatant to the new tube 	Saliva only	<ul style="list-style-type: none"> • transfer 30 µL of the supernatant to a SALIgAE vial (Refer to SER: Saliva – SALIgAE Test for Amylase) • remove and discard 240 µL of supernatant • record the results on the <i>Sexual Assault Examination Worksheet</i>
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7	For all samples, re-suspend the cell pellet in the remaining 30 µL of supernatant and transfer 2 µL to a microscope slide.								
8	Dry the microscope slide for a minimum of 10 minutes in a 60° oven or on a heat block.								
9	Stain and examine the slide. See SER: Spermatozoa – Microscopic Examination . Record the results on the <i>Sexual Assault Examination Worksheet</i> .								

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SER: Sexual Assault Sample Preparation, Continued

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(continued)

Step	Action						
10	For samples being tested for semen: <table> <tr> <th>If sperm are...</th><th>Then ...</th></tr> <tr> <td>detected</td><td>discard the tube containing the supernatant</td></tr> <tr> <td>not detected</td><td> <ul style="list-style-type: none"> • open an ABACard p30 test device and label an AP spot plate • using the p30 disposable pipette, collect the supernatant • add 1 drop of the supernatant to the AP spot plate (Refer to <i>SER: Seminal Fluid – Acid Phosphatase Spot Test</i>) • expel the remaining supernatant into the ABACard p30 test device (Refer to <i>SER: Seminal Fluid – ABACard p30 Immunoassay Test</i>) • discard the disposable pipette • record the results on the <i>Sexual Assault Examination Worksheet</i> </td></tr> </table>	If sperm are...	Then ...	detected	discard the tube containing the supernatant	not detected	<ul style="list-style-type: none"> • open an ABACard p30 test device and label an AP spot plate • using the p30 disposable pipette, collect the supernatant • add 1 drop of the supernatant to the AP spot plate (Refer to <i>SER: Seminal Fluid – Acid Phosphatase Spot Test</i>) • expel the remaining supernatant into the ABACard p30 test device (Refer to <i>SER: Seminal Fluid – ABACard p30 Immunoassay Test</i>) • discard the disposable pipette • record the results on the <i>Sexual Assault Examination Worksheet</i>
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Determining the appropriate DNA extraction method

Use the following procedure to determine which DNA extraction method is appropriate for each sample based on the screening results. Based on case information and analyst discretion, some samples may not proceed to extraction.

NOTE: As needed, samples may be refrigerated for short term storage pending DNA analysis.

If...	Then ...
All screening results are negative	<ul style="list-style-type: none"> • DNA extraction is optional. • Fabric cuttings may be discarded. • If only one swab of the sample type was collected, retain the swab: <ul style="list-style-type: none"> – remove the swab from the spin basket and place it into the original sample tube to allow the swab to soak up the remaining liquid and cell pellet – dry the sample – place the tube(s) in an envelope to be itemized and returned to the original submission • If more than one swab of the sample type was collected, the swab may be discarded.
Spermatozoa are detected	<ul style="list-style-type: none"> • Remove the substrate from the spin basket and place it in the original sample tube. Proceed to <i>DNA: Differential Extraction</i>. • If few sperm cells are detected, an additional untested swab from the kit may be added to the tube for combined extraction.
Seminal fluid is detected (positive for AP and/or p30)	<ul style="list-style-type: none"> • Extraction is optional for the sample. • Remove the substrate from the spin basket and place it into the original sample tube. Proceed to <i>DNA: Differential Extraction</i> or <i>DNA: BioRobot EZ1 Advanced XL Extraction</i>. • If an extraction is not conducted, retain the swab following the steps outlined above.

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SER: Sexual Assault Sample Preparation, Continued

**Determining
the appropriate
DNA extraction
method**
(continued)

If...	Then ...
Amylase is detected	<ul style="list-style-type: none">Remove the substrate from the spin basket and place it into the original sample tube. Proceed to DNA: BioRobot EZ1 Advanced XL Extraction.
Only nucleated epithelial cells are detected	<ul style="list-style-type: none">Extraction is optional for the sample.Remove the substrate from the spin basket and place it into the original sample tube. Proceed to DNA: BioRobot EZ1 Advanced XL Extraction.If an extraction is not conducted, retain the swab following the steps outlined in the section of the table for “If all screening results are negative.”