DNA: Differential Extraction

Sample types

QAS 9.1.1.b

Prior to the organic or robotic extraction of sexual assault samples containing sperm, both the epithelial and sperm cells must be prepared.

Chemicals, reagents, equipment and supplies

Refer to *DNA: Organic Extraction* for a list of chemicals, reagents, equipment, and supplies used in these procedures.

Sample preparation

The following procedure is used to prepare sexual assault samples that have not been pre-screened.

NOTE: If samples have been previously screened, proceed to *Differential lysis*. If samples containing sperm are going directly to Y-STR analysis only, proceed to *Sperm cell lysis*.

Step	Action
1	Cut the swab or fabric substrate into medium size pieces. Suspend the substrate in approximately 0.5 mL to 1.0 mL of sterile water in a sterile microcentrifuge tube.
2	Incubate at room temperature for 30 minutes to rehydrate the sample.
3	Vortex. Agitate the swab or fabric substrate vigorously with a sterile stick to release the cells.
4	Remove the substrate. **Do not** discard the substrate until microscopic analysis is performed to detect spermatozoa. If sperm cells are not visible microscopically, the analyst may agitate the substrate more vigorously and repeat Step 3.
5	Centrifuge the sample in a microcentrifuge for at least one minute on high speed and at room temperature to form a pellet.
6	Without disturbing the pellet, remove and discard all but approximately 50 µL, (or twice the volume of the pellet, whichever is greater) of the supernatant.
7	Resuspend the pellet in the remaining approximately 50 µL by stirring it with a sterile pipette tip.

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DNA: Differential Extraction, Continued

Pellet examination

Use the following procedure to examine resuspended pellets for spermatozoa and epithelial cells.

Step	Action
1	Spot approximately 3 µL of the resuspended sample onto a glass microscope slide and fix the cells to the slide either by incubating in a 60° ± 5°C oven or placing the slide on a heat block until dry.
2	Stain the slide with Nuclear Fast Red Stain (SERI Red Stain) for at least 5 minutes.
3	Wash the slide gently with deionized water until the stain washes off (about 5 seconds).
4	Stain the slide with Picroindigocarmine Stain (SERI Green Stain) for 15 to 30 seconds.
5	Rinse the slide with ethanol (room temperature). Let the slide dry.
6	Add Cytoseal 280 and cover with a cover slip. Examine the slide under 200X magnification. • Epithelial cells will stain green with red nuclei. • Sperm cells will stain red with green tails. – The sperm head stains differentially such that the acrosomal cap stains pink and the sperm cell base stains red.
7	**If sperm cells are…** / **Then ...** detected on the stained slide / proceed to *Differential Lysis* not detected on the stained slide / proceed to one of the following: • *Sample digestion (non-keratinized)* in *DNA: Organic Extraction* • *Non-hair sample preparation* in *DNA: BioRobot® EZ1 and EZ1 Advanced XL Extraction*

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Differential lysis

The following procedure is used to differentially lyse the cells. The volumes of reagents used varies based on the extraction method- either robotic or organic.

Step	Action
1	To the approximately 50 µL of resuspended cell pellet or substrate, add Digest Buffer (the substrate cutting can be included in this step): • robotic extraction: 200 – 300 µL • organic extraction: 500 µL
2	Add 15 µL of Proteinase K Solution. Mix gently.
3	Incubate at 56° ± 1°C for at least one hour to lyse the epithelial cells. **Do not** incubate for more than two hours to minimize lysis of sperm. Remove the substrate cutting, if present. The substrate should be placed in an autoclaved spin basket and returned to the sample tube prior to centrifuging. NOTE: In cases where the sample has been consumed, retain the substrate cutting.
4	Centrifuge the sample for five minutes on high speed and at room temperature. Remove the spin basket, if present.
5	Transfer the supernatant to a new sterile microcentrifuge tube or EZ1 sample tube. This epithelial cell material will be set aside and may be extracted in parallel with the lysed sperm fraction.

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Differential lysis (continued)

<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>6</td><td>Wash the sperm cell pellet as follows.
<table>
<tr><th>Step</th><th>Action</th></tr>
<tr><td>1</td><td>Resuspend the sperm pellet in Digest Buffer and vortex briefly:

• robotic extraction: 200-300 µL
• organic extraction: 500 µL</td></tr>
<tr><td>2</td><td>Centrifuge the sample for five minutes on high speed and at room temperature.</td></tr>
<tr><td>3</td><td>Remove and discard all but 50 µL of the supernatant.</td></tr>
<tr><td>4</td><td>Repeat~~ing~~ Steps 1-3 an additional two to four times to remove residual epithelial cell material</td></tr>
</table></td></tr>
<tr><td>7</td><td>Resuspend the pellet in sterile water and vortex briefly:

• Robotic extraction: 200-300 µL
• Organic extraction: 500 µL</td></tr>
<tr><td>8</td><td>Centrifuge the sample for five minutes on high speed and at room temperature.</td></tr>
<tr><td>9</td><td>Remove and discard all but approximately 50 µL of the supernatant.</td></tr>
<tr><td>10</td><td>Resuspend the sperm pellet in the remaining approximately 50 µL of sterile water by stirring it with a sterile pipette tip.</td></tr>
<tr><td>11</td><td>Verify digestion using the Pellet examination procedure. Grade the sperm observed and record the results on the extraction worksheet.

If epithelial cells remain in your sample, you can repeat the Differential Lysis procedure from Step 1 and incubate for approximately 30 minutes at 56° ± 1°C.</td></tr>
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DNA: Differential Extraction, Continued

Sperm cell lysis

Use the following procedure to lyse the sperm cells. The volumes of reagents used varies based on the extraction method.

Step	Action
1	Add Digest Buffer to the approximately 50 µL resuspended sperm cell pellet or Y-STR substrate: • robotic: 150 µL • Y-STR substrate and robotic extraction: 200 µL • organic: 500 µL
2	Add 20 µL of 1 M Dithiothreitol.
3	Add 15 µL of Proteinase K Solution.
4	Incubate the sample at 56° ± 1°C for at least 60 minutes. If the Y-STR substrate is present, place it in an autoclaved spin basket, return the spin basket to the sample tube, and centrifuge the sample for five minutes at high speed and room temperature. NOTE: If the sample has been consumed, retain the substrate cutting.
5	For organic extraction samples **only**: add 15 µL of Proteinase K to each sperm and reserved epithelial cell fraction. Incubate at 56° ± 1°C overnight.

DNA extraction

For samples that will be extracted using the robot, proceed to *DNA: BioRobot EZ1 and EZ1 Advanced XL Extraction, Set-up and operation of the BioRobot EZ1*.

For samples that will be extracted using the organic method, proceed to *DNA: Organic Extraction, DNA extraction and DNA concentration and wash (Vivacon)*.