

Laser Microdissection of Products of Conception		
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Laser Microdissection of Products of Conception

1 Initial processing

1.1 The product of conception (POC) can be received in different stages of preparation:

1.1.1 POC scrapings in saline buffer:

- 1.1.1.1 Remove tissue from liquid either by filtration or centrifugation.
- 1.1.1.2 Transfer liquid to 50mL falcon tube.
- 1.1.1.3 Spin sample in a bench top Eppendorf or IEC Centra CL3R at 1000 RPM for 5 minutes.
- 1.1.1.4 Discard liquid supernatant.
- 1.1.1.5 Submit sample to the Histology department for tissue processing, then proceed.

1.1.2 POC fixated and embedded in paraffin blocks:

- 1.1.2.1 Contact histology department and ask them to prepare microscope slides from the paraffin block using the following precautions:
 - Use disposable blades for the microtome and discard after each case.
 - Clean working surface on microtome by wiping with 10% bleach and alcohol before and after each case.
 - Use individual floating chambers for each case.
 - Use uncharged microscope slides.
- 1.1.2.2 The slides then should be stained with hematoxylin and eosin-phloxine (H&E technique) as described in the OCME Histology Procedure Manual. But again during the staining procedure, separate sets of jars have to be used for each case.

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1.1.3 Stained or unstained microscope slides from POC blocks:

1.1.3.1 If the slides are unstained, ask the histology department to stain them as described above. Otherwise proceed with the microdissection technique.

1.1.3.2 **Attention:** for slides that were prepared by a histology laboratory outside of the OCME, foreign DNA not from the mother and the fetus might be present on the slide.

2 PixCell Iie Laser Capture Microdissection

2.1 A trained pathologist has to be present to distinguish decidual tissue from chorionic villi and operate the laser.

2.2 After the slide has been placed on the microscope platform the pathologist will visually identify the area of interest, mark this area for the laser, and activate the laser.

2.2.1 The laser setting is specified in the Arcturus instrument manual.

2.2.2 The Forensic Biology Criminalist needs to be present during the complete procedure to maintain chain of custody of the evidence.

2.3 An area of chorionic villi and an area of maternal tissue should be collected on separate CapSure caps.

2.3.1 The caps can be stored and transported in 50 ml Falcon tubes.

2.3.2 A third unused CapSure cap should be extracted as an extraction negative control.

2.4 Use new scalpel and clean forceps to remove the film from the cap and transfer the film to a fresh 1.5mL microcentrifuge tube containing 500µL of organic extraction buffer, DTT, SDS and Proteinase K as described in the organic extraction manual.