

MOLECULAR SEROLOGY PROCEDURES MANUAL

Body Fluid Identification by Proteomic Mass Spectrometry – Extraction		
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Body Fluid Identification by Proteomic Mass Spectrometry – Extraction

1 Purpose

- 1.1 To extract protein from samples in order to identify specific body fluids markers by liquid chromatography and mass spectrometry.

2 Protein Extraction Procedure

- 2.1 Procure a 4°C tube rack or bucket filled with ice.
- 2.2 Obtain labeled cuttings in 1.5 ml microcentrifuge tubes and place in an ice bucket or 4°C tube rack. Compare your sample labels and tube tops to the input sample list in LIMS and confirm that you have the correct samples.
 - Note: Sample size for the extraction should be approximately ¼ of a cotton swab or a 3x3 mm cutting of a stain.
- 2.3 Obtain a 1.5 ml microcentrifuge tubes for your extraction negative. Label it with the associated input LIMS label and tube top label.
 - Note: You will need **one** Extraction Negative for every **nineteen** samples in the batch.
- 2.4 Obtain two 1.5 ml microcentrifuge tubes for each sample for subsequent processing.
- 2.5 Label with the associated output LIMS label and tube top label.
- 2.6 Write a Q on one tube to designate it the Quant Aliquot (1X) tube and an EX on the other to designate it the Extraction tube.
- 2.7 **Label WITNESS**: Have a witness verify that correct tubes are present in the set and the labels on input tubes match labels on output tubes.
- 2.8 Turn refrigerated centrifuge on. Ensure it is set to 4°C.
- 2.9 Retrieve the 1% SDC extraction solution from 4°C refrigerator. Record its identification number in LIMS.
- 2.10 Add 200 µl of 1% SDC to each of the samples and ENEG.
- 2.11 Incubate at room temperature (~22°C) for 30 minutes at 1,000 RPM in a thermomixer. Record instrument and temperature in LIMS.

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- 2.12 Spin tubes in a refrigerated centrifuge (set to 4°C) at 18,000 g for 30 minutes. Record instrument and temperature in LIMS.
- 2.13 Transfer supernatants to corresponding Extraction tubes, discard the sample tube.
- 2.14 Pipette 41 µl from Extraction tubes into Quant Aliquot tubes, for protein quantification.
- 2.15 Store Extraction and Quant Aliquot tubes at -20°C.