

MOLECULAR SEROLOGY PROCEDURES MANUAL

Guidelines for Molecular Serology Body Fluid Assay		
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Guidelines for Molecular Serology Body Fluid Assay

1 Purpose:

- 1.1 Guidelines for body fluid proteomics assay testing to ensure clean laboratory practices, unambiguous sample identification, and relevant control runs.

2 General Procedures:

- 2.1 Lab coat, gloves, and mask must always be worn while in the laboratory. All gowning must be done in the vestibule rooms.
- 2.2 Lab coats can be reused for a period of one week. Afterwards, they should be thrown out. Masks can also be reused for a period of one week.

3 The Body Fluid Assay Workflow Overview

- 3.1 Samples collected from evidence examination are processed through proteomics extraction as warranted.
- 3.2 Samples are quantified using a spectrophotometer.
 - 3.2.1 Low QUANT (i.e., low concentration) samples are those with $<0.2 \mu\text{g}/\mu\text{l}$.
- 3.3 Appropriate volume of each sample is then reduced, alkylated and quenched in preparation for protein digestion.
- 3.4 Samples are digested overnight at 37°C
- 3.5 Samples are then run on the LC-MS for mass spectrometry data collection.
- 3.6 Cleaning solution will be run after each sample runs on the LC-MS instruments.
- 3.7 Sample raw data for is opened through Skyline software.
- 3.8 Sample data from Skyline software is then run with MSDA (Molecular Serology Data Analysis)-Script for data analysis.

4 Sample Batching Guidelines

- 4.1 Extraction batches consist of unknowns and negative control. Quant and digestion batches are the same, but also includes a positive control. LC/MS batches are the same as quant and digestion batches, but also require Peptide Calibration Mixture Standard before and after unknowns and cleaning solutions.

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- 4.1.1 Peptide Calibration Mixture standard is only for QAQC to monitor instrument, will not be looked at by casework analysts.
- 4.1.2 Cleaning Solution is run between unknown samples.
- 4.1.3 Injections should be run in tandem. (i.e., Col1, Col2, Col1, etc.)

5 Sample Nomenclature

5.1 The following naming conventions are required to run the samples through the molecular serology assay and for subsequent data analysis by the MSDA-Script. The goal of this nomenclature is to ensure that sample names are unique identifiers when running the assay and analyzing data. Sample names are introduced at different stages beginning from the start of the assay.

- 5.1.1 Negative control will have “E Neg” in run name.
- 5.1.2 Unknowns will have their designated FBIO identification number in their run names.
- 5.1.3 Positive controls will have “Ext Pos SE”, “Ext Pos SA”, or “Ext Pos BL” for semen, saliva or blood, respectively, in their run names.
- 5.1.4 All samples run through LC/MS will first have the date (YYYYMMDD), followed by a letter identifying batch order (e.g., A, B, C, etc.), an underscore, followed by a three-digit number indicating injection order, followed by an underscore. (e.g., 20220926A_001_)

- 5.1.4.1 Peptide Calibration Mixture Standards will have “PepCal Mix” in their run names.
- 5.1.4.2 Cleaning solutions will have “Cleaning Sol” in their run names.
- 5.1.4.3 Column path designation will follow specifying “Col1” for column one path vs “Col2” for second column path.

6 Repeat Analyses

- 6.1 Samples can be reinjected into the LC-MS if:
 - 6.1.1 Cytochrome c standard targeted marker peptide is not detected at analysis (meaning sample injection may not have occurred).
 - 6.1.2 After interpretation analysis, if an unknown sample is inconclusive, this sample may be re-run at a higher injection volume.
 - 6.1.3 Negative controls show presence of four or more peptides on analysis.
 - 6.1.4 Systemic error where batch failed in one column (all samples run on this path failed).

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- 6.1.4.1 Samples will be rerun in the opposite column path.
- 6.2 If sample(s) undergo repeat injection(s), positive and negative controls from that batch will also be rerun.
- 6.3 When rerunning a sample(s) start at section 3.9 in manual [Body Fluid Identification by Proteomic Mass Spectrometry - Liquid Chromatography & Mass Spectrometer Processing](#).