

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 be worn while performing casework. 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 Each batch will consist of the unknowns, a positive control for each body fluid, negative controls (ENEG), Peptide Calibration Mixture standard runs before and after unknown runs and the 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.
 - 5.1.1 All samples run through the assay 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_)
 - 5.1.1.1 Peptide Calibration Mixture Standards will have “PepCal Mix” in their run names.
 - 5.1.1.2 Unknowns will have their designated FBIO identification number in their run names.
 - 5.1.1.3 Cleaning solutions will have “Cleaning Sol” in their run names.
 - 5.1.1.4 Positive controls will have “Ext Pos SE”, “Ext Pos SA”, or “Ext Pos BL” for semen, saliva or blood, respectively, in their run names.
 - 5.1.1.5 Negative control will have “E Neg” in run name.
 - 5.1.2 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](#).

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