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

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.

2.3 Pipettes must be wiped down with 10% bleach followed by 70% ethanol before and after each procedure.

3 The Body Fluid Proteomics 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.

3.3 Appropriate volume of each sample is then reduced, alkylated and quenched in preparation for protein digestion.

3.4 Samples are digested overnight at 37ºC

3.5 Samples are then run on the LC-MS for mass spectrometry data collection.

3.6 A 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.

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 After each unknown run, a washing solution "Cleaning Solution" will be run.

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. 20220926**A**_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); this sample may be run at a higher concentration.

6.1.2 After interpretation analysis, an unknown sample is inconclusive; this sample may be run at a higher concentration.

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).

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.