Body Fluid Identification by Proteomic Mass Spectrometry -Quantitat					
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Body Fluid Identification by Proteomic Mass Spectrometry - Quantitation

1 Purpose

1.1 To quantify extracted proteins in order to identify specific body fluids on evidence samples using liquid chromatography and mass spectrometry.

2 Protein Quantitation Procedure

2.1 Retrieve the reagents for protein quantitation and record the lot and identification numbers in LIMS.

1% SDC at 4°C			
Bovine Serum Albumin (BSA), 2 mg/mL at 4°C			
Deionized Water			
Pierce™ BCA Reagent A			
Pierce™ BCA Reagent B			

- 2.2 Turn on Mini-Shaker and set to 37°C.
- 2.3 Prepare the BSA standards in 1.5 mL microcentrifuge tubes as described below. Vortex and short spin tubes on benchtop centrifuge before making subsequent dilutions.

Tube Label	BSA Concentration	BSA	Diluent (Deionized Water)
A	2000 μg/ml	183 µl from BSA stock	0 μ1
В	1500 μg/ml	66 μl from tube A	22 μ1
C	1000 μg/ml	57 μl from tube A	57 μl
D	750 μg/ml	30 μl from tube B	30 μ1
Е	500 μg/ml	54 μl from tube C	54 μ1
F	250 μg/ml	48 μl from tube E	48 μ1
G	125 μg/ml	36 μl from tube F	36 μ1
Н	0 μg/ml	0 μ1	60 μ1

- 2.4 Retrieve positive control extracts from -80°C freezer
 - 2.4.1 Keep all positive control extracts on ice or on a frozen tube rack.
 - 2.4.2 Write your initials on all positive control extract tubes, as you will continue to use the same tube for the remainder of the procedure. **DO NOT THROW OUT.**
- 2.5 Prepare positive control dilutions in 1.5 mL microcentrifuge tubes as described below.

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Positive Control	Extract	Diluent (1% SDC)
Saliva 1X	30 μ1	0 μ1
Semen 5X	6 μl	24 μ1
Blood 20X	2 μ1	38 μ1

- NOTE: Each microplate will only hold a total of **nineteen** unknown samples.
- 2.6 Retrieve Quant aliquot tubes from -20°C freezer.
- 2.7 Prepare sample dilutions in separate 1.5 ml microcentrifuge tubes, labeled 5X, 10X and 20X as described in the table below. Vortex and short spin tubes on benchtop centrifuge before making subsequent dilutions.

Tube Label	Dilution	Extract	Diluent (1% SDC)
Transfer (1X)	1X	41 µÎ	0 μ1
5X	5X	11 μl of 1X	44 µl
10X	10X	23 μl of 5 X	23 μl
20X	20X	15 μl of 10X	15 μl

- 2.8 **WITNESS:** Confirm the sample names by reading the tube top labels and LIMS INPUT sample ID. Tube top labels should be re-witnessed at the load plate screen.
- 2.9 Pipette 25 µl of Standards in duplicate into the 96-well microplate beginning at wells A1 through H2. Pipette 25 µl Eneg and Pos Controls beginning at wells A3 through D3. See example layout below.
 - 2.9.1 Vortex and short spin tubes on benchtop centrifuge before pipetting into microplate.
- 2.10 Pipette 25 µl of all sample dilutions (1X through 20X) into the 96-well microplate beginning at wells E3 through H12. Continue pipetting in this order until all sample dilutions are loaded. See example layout below.
 - 2.10.1 Vortex and short spin tubes on benchtop centrifuge before pipetting into microplate.

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	1	2	3	4	5	6	7	8	9	10	11	12
	H	D		Sample 2	Sample 4	Sample 6						
A	[0 μg/ml]	[750 µg/ml]	Eneg	Tube 1X	Tube 1X	Tube 1X						
	H	D	Saliva	Sample 2	Sample 4	Sample 6						
В	[0 μg/ml]	[750 µg/ml]	Positive Ctrl	Tube 5X	Tube 5X	Tube 5X						
	G	С	Semen	Sample 2	Sample 4	Sample 6						
C	[125 µg/ml]	[1000 µg/ml]	Positive Ctrl	Tube 10X	Tube 10X	Tube 10X						
	G	С	Blood	Sample 2	Sample 4	Sample 6						
D	[125 µg/ml]	[1000 µg/ml]	Positive Ctrl	Tube 20X	Tube 20X	Tube 20X						
	F	В	Sample 1	Sample 3	Sample 5							
Е	[250 µg/ml]	[1500 µg/ml]	Tube 1X	Tube 1X	Tube 1X							
	F	В	Sample 1	Sample 3	Sample 5							
F	[250 µg/ml]	[1500 µg/ml]	Tube 5X	Tube 5X	Tube 5X							
	E	A	Sample 1	Sample 3	Sample 5							
G	[500 µg/ml]	[2000 µg/ml]	Tube 10X	Tube 10X	Tube 10X							
	E	A	Sample 1	Sample 3	Sample 5							
Н	[500 µg/ml]	[2000 µg/ml]	Tube 20X	Tube 20X	Tube 20X							

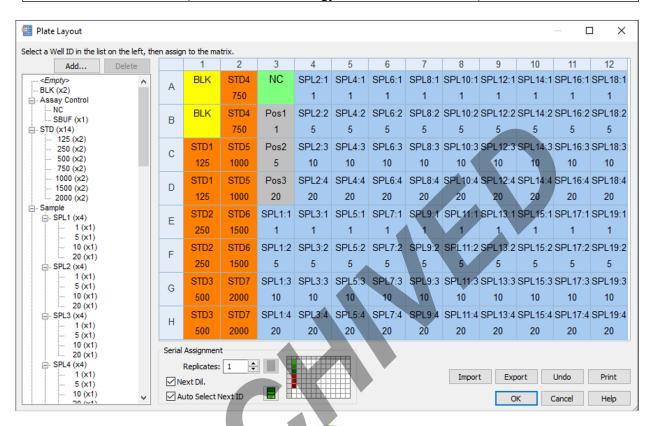
- 2.11 Dispose of Quant Aliquot tubes. Place positive controls extracts into previous Quant Aliquot tube slots in -20°C storage box.
- 2.12 Determine the volume of working solution you will need. In LIMS, select BCA Kit Reagent A and BCA Kit Reagent B and click "calculate amount".
- 2.13 Vortex working solution and pour into reagent reservoir.
- 2.14 Use a multichannel pipette to add 200 µl of working solution to each well.
- 2.15 Incubate for 30 minutes on the Mini-Shaker at 37°C while shaking at 200 RPM. Record instrument and temperature in LIMS.

3 Read Plate on Spectrophotometer

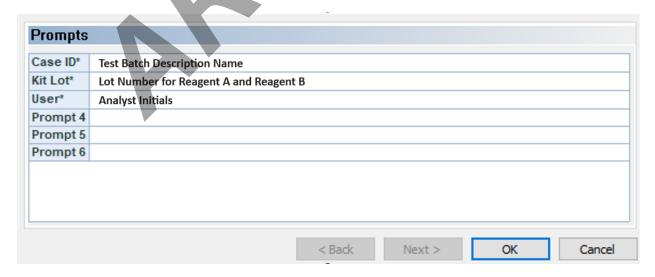
Note: Ensure that there are no other windows of Gen5 or Excel open before you begin.

- 3.1 Click on Gen5.3 10 software on desktop.
- 3.2 Select "BCA Casework" as experiment protocol and click OK.
- 3.3 Click "Plate Layout" icon
- 3.4 Ensure layout is correct, add samples if needed.

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- 3.5 Click "Create Experiment and Read Now"
- 3.6 Input information as prompted and click OK.



3.7 Insert plate in correct alignment when prompted and click OK.

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- Once plate read is complete, click "Export" 3.8
- Copy and paste results into "BCA Quant Concentration Template" excel worksheet into the 3.9 "Instrument Output" tab.
- 3.10 Input R square value into LIMS
- 3.11 R square value must be equal to or above 0.95 to continue with batch.
- 3.12 Copy and paste Dilution, Concentration, and Concentration Avg columns from "Result" tab into LIMS.
- 3.13 Ensure data was properly copy and pasted onto LIMS
- Identify the LOW QUANT (low concentration, i.e., samples with <0.2 μg/μl) samples in the 3.14 interpretation column.

Qualtrax template 040621