Body Fluid Identification by Proteomic Mass Spectrometry – Quantitation

1 Purpose

1.1 To quantify extracted proteins to determine the volume of sample needed for trypsin digestion and subsequent analysis by 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.

BSA Standard Dilutions

Tube Label - BSA Concentration	BSA	Diluent (Deionized Water)
2000 µg/ml	183 µl from BSA stock	0 µl
1500 µg/ml	66 µl from **2000 µg/ml**	22 µl
1000 µg/ml	57 µl from **2000 µg/ml**	57 µl
750 µg/ml	30 µl from **1500 µg/ml**	30 µl
500 µg/ml	54 µl from **1000 µg/ml**	54 µl
250 µg/ml	48 µl from **500 µg/ml**	48 µl
125 µg/ml	36 µl from **250 µg/ml**	36 µl
0 µg/ml	0 µl	60 µl

2.4 Retrieve positive control extracts from -80°C freezer and record the lot and identification numbers in LIMS.

2.4.1 Keep all positive control extracts on ice or in a 4°C tube rack.

2.4.2 Label the positive controls with their corresponding LIMS output label. **DO NOT THROW OUT**.

2.5 Prepare positive control dilutions in 1.5 mL microcentrifuge tubes as described below.

Positive Control Dilutions

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

- 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.

Sample Dilutions

Tube Label	Dilution	Extract	Diluent (1% SDC)
Transfer (1X)	**1X**	41 µl	0 µl
5X	**5X**	11 µl of **1X**	44 µl
10X	**10X**	23 µl of **5X**	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.

2.11 Vortex and short spin tubes on benchtop centrifuge before pipetting into microplate.

96 Well Plate Layout

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

2.12 Dispose of Quant Aliquot tubes. Place positive controls extracts into the three designated tube slots in the -20°C extracts storage box.

2.13 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”.

2.14 Mix calculated volumes of Reagents A and B.

2.15 Vortex working solution and pour into reagent reservoir.

2.16 Use a multichannel pipette to add 200 µl of working solution to each well.

2.17 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

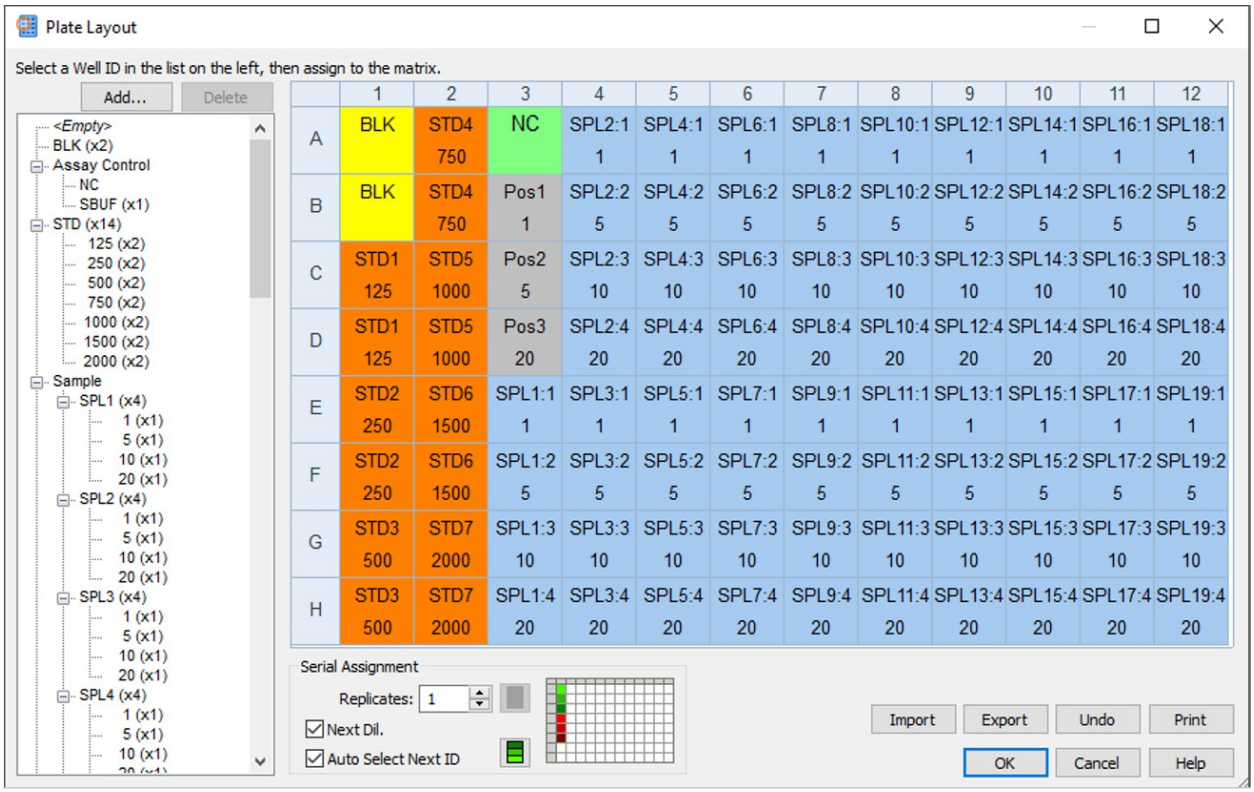
<u>Note:</u> Ensure that there are no other windows of Gen5 or Excel open before you begin.

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 

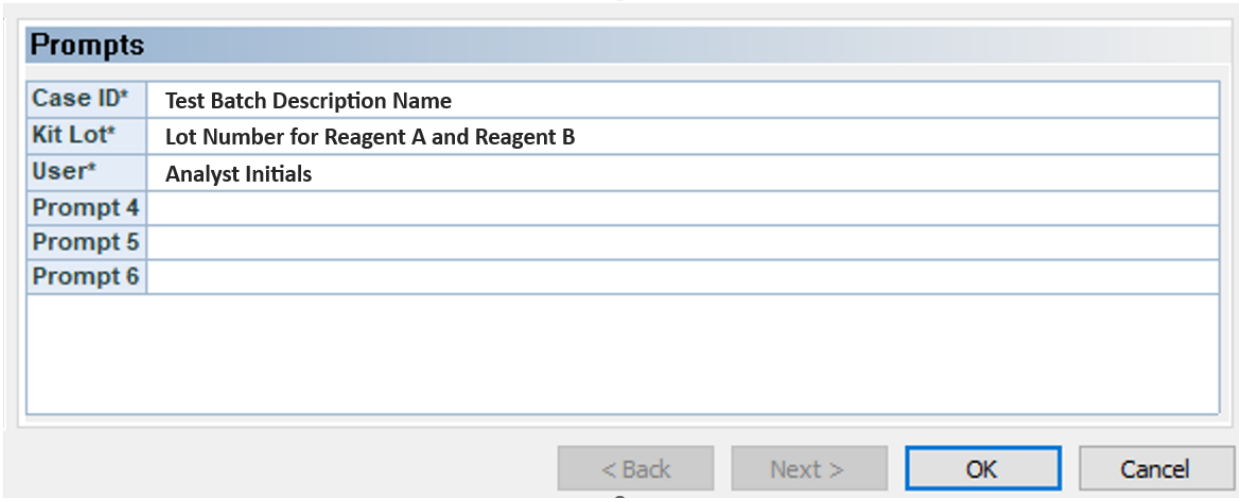
3.4 View 96 well plate layout and ensure it is correct. Add or remove samples on layout as needed.



	1	2	3	4	5	6	7	8	9	10	11	12
A	BLK	STD4 750	NC	SPL2:1 1	SPL4:1 1	SPL6:1 1	SPL8:1 1	SPL10:1 1	SPL12:1 1	SPL14:1 1	SPL16:1 1	SPL18:1 1
B	BLK	STD4 750	Pos1 1	SPL2:2 5	SPL4:2 5	SPL6:2 5	SPL8:2 5	SPL10:2 5	SPL12:2 5	SPL14:2 5	SPL16:2 5	SPL18:2 5
C	STD1 125	STD5 1000	Pos2 5	SPL2:3 10	SPL4:3 10	SPL6:3 10	SPL8:3 10	SPL10:3 10	SPL12:3 10	SPL14:3 10	SPL16:3 10	SPL18:3 10
D	STD1 125	STD5 1000	Pos3 20	SPL2:4 20	SPL4:4 20	SPL6:4 20	SPL8:4 20	SPL10:4 20	SPL12:4 20	SPL14:4 20	SPL16:4 20	SPL18:4 20
E	STD2 250	STD6 1500	SPL1:1 1	SPL3:1 1	SPL5:1 1	SPL7:1 1	SPL9:1 1	SPL11:1 1	SPL13:1 1	SPL15:1 1	SPL17:1 1	SPL19:1 1
F	STD2 250	STD6 1500	SPL1:2 5	SPL3:2 5	SPL5:2 5	SPL7:2 5	SPL9:2 5	SPL11:2 5	SPL13:2 5	SPL15:2 5	SPL17:2 5	SPL19:2 5
G	STD3 500	STD7 2000	SPL1:3 10	SPL3:3 10	SPL5:3 10	SPL7:3 10	SPL9:3 10	SPL11:3 10	SPL13:3 10	SPL15:3 10	SPL17:3 10	SPL19:3 10
H	STD3 500	STD7 2000	SPL1:4 20	SPL3:4 20	SPL5:4 20	SPL7:4 20	SPL9:4 20	SPL11:4 20	SPL13:4 20	SPL15:4 20	SPL17:4 20	SPL19:4 20

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.

3.8 Once plate read is complete, click "Export" 

3.9 Copy and paste results into "**BCA Quant Concentration Template**" excel worksheet into the "**Instrument Output**" tab.

3.10 Input R square value into LIMS

4 Read Plate on Spectrophotometer

4.1 R square value must be equal to or above 0.95 to continue with batch.

4.2 Negative controls are determined by subtracting the background from all sample results. Consequently, the negative controls have a value of zero µg/µl.

4.3 Positive controls must have concentration values for each body fluid as listed below.

Body fluid Type	Lower Limit	Upper Limit
EPosBL	9.86 µg/µl	17.7 µg/µl
EPosSA	0.091 µg/µl	0.524 µg/µl
EPosSE	1.91 µg/µl	3.48 µg/µl

4.3.1 Values outside of this range require quanting a new set of Positive Controls.

4.4 Copy and paste Dilution, Concentration, and Concentration Avg columns from “Result” tab into LIMS.

4.5 Ensure data was properly copied and pasted into LIMS

4.6 Identify the LOW QUANT (low concentration, i.e., samples with <0.2 µg/µl) samples in the interpretation column.